
**INTERNATIONAL JOURNAL OF ADVANCES IN
PHARMACY, BIOLOGY AND CHEMISTRY****Research Article****COMPARISON BETWEEN NATURAL *AAMBIL*
PRODUCTION WITHOUT AND WITH CURD****Pruthviraj Jagannath Khade, Niteen Vinay Phirke***P. G. Department of Microbiology, Sant Gadge Baba Amravati University,
Amravati, India - 444602.**ABSTRACT**

For sorghum, the fermented grain food product like *aambil*, *dhirade*, *popadum* etc. are widely produced and consumed in *Varhad-Khandesh* region. Aim of this study is to isolate different microflora and check the aroma and taste between naturally occurring *aambil* fermentation with and without curd. The fermentation process was checked by change in pH, production of gas in the form of bubbles on surface of media, odour and turbidity of media. In the present study, total 10 lactic acid bacterial isolates were obtained from 5 sets of sorghum. The isolates were separated according to cultural, morphological and biochemical tests. Out of ten, two resembled like *Lactobacillus* species (P1), two resembled like *Streptococcus* species (P2), four resembled with *Pediococcus acidilactici* strains (P3), and two resembled like *Leuconostoc mesenteroides* (P4). The fermentation of *aambil* with curd gives better taste and aroma as compared to without curd.

Keywords: *Varhad-Khandesh* region, sorghum, *aambil*, Lactic acid bacteria.**INTRODUCTION**

Sorghum is a tropical plant belonging to the family of Poaceae is one of the important crops of Africa, Asia and Latin America¹. It is a staple food grain grown in many tropical and subtropical areas of the Africa and Asia, because it can grow in prolonged absence of rain². India maintains its position among the top three producers of the crop, globally. Sorghum is produced in both as the summer and winter crop i.e. *Kharif* and *Rabi* crop in the country. Indian production however around an average of 9 million metric tons but since last few years a slow downfall in the production as well as in the area covered for sorghum production has been observed. Fermentation is a metabolic process that converts sugar to acids, gases. It is carried by yeasts and bacteria, in case of lactate fermentation, it also occurs in oxygen starved muscle cells. Fermentation is one of the oldest and most economical methods of producing and preserving food. In addition fermentation provides a natural way to reduce the volume of the material to be transported to destroy undesirable components to enhance the nutritive value and appearance of the food to reduce the

energy required for cooking and to make a safer product. For sorghum, the fermented grain product like *aambil*, *dhirade*, *popadum* etc. are widely produced and consumed in the *Vidarbha* region. The microbial flora responsible for the fermentation of steeped sorghum grains is yet to be established such products in the climate of *Vidarbha* region. *Aambil* is commonly made in *Vidarbha* region of Maharashtra. *Aambil* is made in *Ganesh ustav* as well as *Gauri pujan*, which shows that *aambil* has traditional as well as cultural value. *Aambil* is used as breakfast in *Bhandara* region as porridge. Most bacterial fermentations produce lactic acids. Many of the indigenous fermentation products of cereals are valued for the taste and aroma active components produced and are used as seasonings and condiments³.

Lactic acid bacteria are a group whose main characteristic is the fermentation of carbohydrates with the production of lactic acid. Originally, this group included four genera of great importance in the production of foods: *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus*⁴. In the present work study on *aambil* fermentation using sorghum floured based fermentation batter and we are tried to isolate

and characterized lactic acid bacteria from fermentation batter and comparison between naturally occurring *aambil* production with and without curd taste and aroma.

MATERIALS AND METHODS

Materials

MRS agar (deMan, Rogosa and Sharpe), MRS broth. Composition of MRS media^{5,6} is shown in table no.1.

Methods

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 washed by sterile distilled water 2 to 3 times. Then this sorghum was mixed with sterile distilled water in ratio of 1:2 (dry w/v). Then this mixture was incubated at 30⁰C temperature for 12h in sterile covered flask. Then this sorghum is allowed to dry in shade for 12h, after drying the sorghum grains are rubbed and rubbing followed by grinding it gives endosperm rich starch flour. This starch flour mix with water and allowed to ferment overnight with and without curd. During fermentation samples were aseptically withdrawn for its physicochemical and microbiological analysis. Then isolation of bacteria or microbes on preferable cultivated media from fermented batter followed by

characterization of these bacteria. Isolated bacteria/microbes were preserved.

Source of isolates

A total 5 fermentation sets were subjected for study and analysis.

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 plate were selected and stock cultures were prepared for further analysis. All these isolates were further characterized by standard biochemical test according to Bergey's manual of determinative bacteriology⁷

RESULTS AND DISCUSSION

The lactic acid content was increased with fermentation this due to lactic acid bacteria used soluble sugar as their main carbon source to produce energy. Due to lactic acid production, pH dropped down from 6.8 to 4. The fermentation process was checked by change in pH, production of gas in the form of bubbles on surface of media odour and turbidity of media. Total 10 isolates were isolated from 5 sets of sorghum. The isolates were separated according to morphological and biochemical test into lactic acid bacteria such as *Lactobacillus* spp., *Streptococcus* spp., *Pediococcus* spp. and *Leuconostoc mesenteroides*.

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
Agar	12
pH (at 25 °C)	6.5 ± 0.2

Table 2
Morphological, Cultural and Biochemical Characterization

No. of Isolates	Test	P1(2)	P2(2)	P3(4)	P4(2)
Cultural Characteristics	Colour of colony	White	Pale yellow	White	Pale yellow
	Colony shape	Circular	Circular	Circular	Circular
	Margin	Entire	Entire	Irregular	Entire
	Elevation	Convex	Convex	Convex	Convex
	Density	Opaque	Opaque	Opaque	Opaque
Morphological characteristics	Cell shape	rods	Cocci in chain	Cocci in tetrads	Cocci in chain or pairs
	Gram reaction	+	+	+	+
	Motility	NM	NM	NM	NM
	Endospore formation	NS	NS	NS	NS
Biochemical characteristics	Catalase	-	-	-	-
	Oxidase	-	-	-	-
Growth at	15^oC	+	-	-	+
	45^oC	+	+	+	+
	pH 3.9	+	-	+	+
Growth in Nacl	2%	+	+	+	+
	5%	-	+	-	+
	10%	-	-	-	-
Sugar fermentation	Lactose	+	+	+	+
	Xylose	+	+	-	+
	Maltose	+	+	+	-
	Fructose	+	+	+	+
	Dextrose	+	+	-	+
	Galactose	+	+	+	-
	Raffinose	+	+	+	+
	Trehalose	+	-	+	-
	Melibiose	+	+	+	+
	Sucrose	+	+	+	+
	L-Arabinose	-	+	+	-
	Mannose	+	+	+	-
	Insulin	+	+	+	-
	Sodium gluconate	+	+	+	+
	Glycerol	+	+	+	+
	Salicin	+	+	+	+
	Dulcitol	+	+	+	+
	Inositol	+	+	+	+
	Sorbitol	+	+	+	+
	Mannitol	+	+	+	+
	Adonitol	+	+	+	+
	Arabitol	-	+	+	+
	Erythritol	+	+	-	+
	Alpha-Methyl-D-glucoside	+	+	+	+
	Rhamnose	+	+	+	+
	Cellobiose	+	-	-	+
	Melezitose	+	+	+	+
	Alpha-Methyl-D-mannoside	+	+	+	+
	Xylitol	+	+	+	+
	ONPG	-	-	-	-
Esculin hydrolysis	-	+	-	+	
D-Arabinose	+	+	+	+	
Citrate utilization	-	-	-	+	
Malonate utilization	-	-	-	-	
Sorbose	+	+	+	+	
Probable Microorganism	<i>Lactobacillus</i> species	<i>Streptococcus</i> species	<i>Pediococcus acidilactici</i>	<i>Leuconostoc mesenteroides</i>	

Where NM = Non Motile, NS = Non Spore forming, (-) = Negative, (+) = Positive.

Out of ten, two resembled like *Lactobacillus* species (P1), two resembled like *Streptococcus* species (P2), four resembled with *Pediococcus acidilactici* strains

(P3), and two resembled like *Leuconostoc mesenteroides* (P4). The isolates P1, P2, P3 and P4 were Gram positive short rod, Gram positive cocci in chain, Gram positive cocci in tetrads or pairs, and Gram positive cocci in chains or pairs respectively. The isolates species were different in their cultural and biochemical characteristics (Table 2). Catalase and oxidase test for all bacterial isolates were negative. The lactic acid bacterium was the dominating microorganism at the end of fermentation. The other organisms were not detected after 24 to 48h due to increased acidity of media by lactic acid bacteria. Danova *et al.*⁸ isolated *Lactobacillus* strains from Koumiss is a slightly alcoholic fermented milk beverage. Osuntoki and Korie⁹ isolated *Lactobacillus* strains from five indigenous fermented foods i.e. *ogi*, *ogi baba*, *wara*, *kunnu* and *ugba* out of which *wara* is a dairy based food while the others are not dairy based. The bacteria were isolated on MRS agar and identified on the basis of Gram stain reaction, cellular morphology, biochemical tests and carbohydrate utilization. The inoculated culture will pass through a number of phases. Generally, after inoculation for some period, the process witnessed a considerably long lag phase of no growth of microbes serving as a time for adaptation should be minimised for a commercial fermentation and this may be achieved by using a suitable efficient inoculum. Following this period, the growth rate increased gradually to achieve a maximally constant logarithmic or exponential growth phase¹⁰. The sorghum if cultivated using microbial inputs regime¹¹; tastes better in *aambil* preparation. In the present study starch flour mix with water and allowed to ferment overnight with and without curd, the curd containing *aambil* gives better taste because curd containing cells grow at a constant, maximum rate and this period is known as the log phase.

CONCLUSION

In this study we tried to make *aambil* as a good and tasty sorghum fermented food product. Due to the log phase curd containing *aambil* gives good taste as compared to without curd. The outcome of this study is that the naturally isolated species and curd containing species were responsible for microbial fermentation of *aambil* giving the typical sour flavour, good texture and aroma and improves nutritional quality of *aambil* and inhibited the pathogenic microorganism due to lactic acid bacteria.

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