Fishery Technology 52 (2015) : 34 - 41

Minimisation of Fermentation Period of *Shidal* from Barbs (*Puntius* spp.)

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Abstract

Fermented barb (*Puntius* spp.) is a semi-fermented solid fish product of north-east India which is a popular means of fish preservation and product development in the region. Its normal fermentation period is 4-6 months which restricts the production only to 2 batches per year. In the present study, fortnightly samples from traditional *shidal* fermenting vessels (*mutka*) (PC) and laboratory prepared *shidal* (L) were analyzed to identify an appropriate stage and time of fermentation for harvesting *shidal* with desired quality. A total of 5 fermentation stages were characterized. Bacillus, Staphylococcus and Micrococcus were the predominant genera of bacteria. Biochemical parameters such as pH, titratable acidity and proximate composition combined with sensory analysis suggested that good quality *shidal* can be harvested after a fermentation period of 75 days (stage 3) under traditional fermentation system at room temperature (30±5ºC) and from this stage onwards the quality of *shidal* was best at 120 days of fermentation with highest sensory scores, dark red brown colour, typical *shidal* odour, pH 5.7±0.8 and titratable acid 1.78%. *Shidal* had excellent nutritional properties including 33-34% moisture, 30-31% protein, about 18-19% fat and 11-13% ash content.

Keywords: Bacteria, *shidal*, fermented fish, fermentation stage, north-east India

Introduction

Fermented fish play an important role in addressing protein malnutrition in south-east Asian countries including north-eastern region of India. There are many fermented fish products in this region developed traditionally which have cultural and enthnic specificity to the various tribes and other communities of the region. *Shidal* is a salt-free, semi-fermented solid product produced from freshwater barbs (*Puntius* spp.) locally known as *puthi* or *puti* (Muzaddadi & Basu, 2003). It is consumed normally as *Shidal*-chutney which is a highly relished culinary dish by many tribal and non-tribal communities of north-east India. There are several similar products in different north-eastern states such as *shidal* and *hidal* in Assam, *sepaa* and *shidal* in Tripura, Nagaland and Arunachal Pradesh and *ngari* in Manipur. Similar products have also been reported in Bangladesh as *chepa shutki* (Mansur et al., 2000: Nayeem et al., 2010). *Hentak* and *ngari* are prepared from sun-dried freshwater fish in Manipur, India (Sarojnalini & Vishwanath, 1994). *Tungtap,* another similar product of Meghalaya has been reported to be good source of protein (40.6%) (Murugkar & Subbulakshmi, 2006; Thapa et al., 2004). Bacterial diversity associated with this product has also been reported (Rapsang et al., 2011; Rapsang & Joshi, 2012). In Nagaland, fermented fish products are prepared by keeping washed fish inside bamboo pipe and fermenting over the fireplace (Mao & Odyuo, 2007). Hastening of fish sauce fermentation process could be achieved by the use of proteolytic bacteria *viz.*, *Brevibacterium linens* and *Micrococcus* spp. (Putro, 1993). Muzaddadi & Basu (2012) standardized the method of traditional fermented fish production in north-east India. For the preparation, the semi-dried fish are tightly packed in specially made earthen pots, locally called *mutka* and sealed with fish-paste, plant leaves and fine clay and subsequently kept for fermentation at room temperature. It is a naturally fermented product with no food additives/preservatives or starter culture. However, the process is time consuming, taking about 4-6 months. The present study aims identify and characterize the fermentation stages under traditional fermentation process in

Received 15 January 2014; Revised 02 July 2014; Accepted 12 September 2014

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order to avoid over fermentation and quality loss as well as to identify the resident bacterial population, their occurrence and relationship with the quality of the product. This study is expected to help the *shidal* producers to optimally ferment fish, thus avoiding economic loss due to quality loss and to reduce the time of fermentation.

Materials and Methods

Traditional *shidal* samples were collected in triplicates from six different production centers at Kamalghat and Agartala, Tripura, India (PC) at 15 days intervals during August to December 2012. Samples were collected aseptically in sterile polyethylene bags immediately after opening the *mutka*. *Shidal* was also prepared in the laboratory following the traditional method (Muzaddadi & Basu, 2012) and fermented at room temperature for 120 days (L) and sampling was carried out fortnightly. But the *mutka* used was smaller in size and it was covered with fine cover paste of fish ground in a mixer grinder. A destructive sampling pattern was followed *viz.,* the *mutka* once opened was not used further to ensure equal fermenting environment before every sampling. Three samples from each *mutka, viz.,* top (T), center (C) and bottom (B) were collected in order to represent whole environment inside the *mutka*.

Samples from both sources were analyzed to monitor microbial population and the occurrence of various bacterial species during fermentation process. The stages of fermentation were characterized for earmarking the best quality *shidal* that correlated to the dynamics of resident bacterial flora, pH, titratable acidity, and sensory attributes such as texture, colour, appearance, odour and surface stickiness (Table 1).

For microbiological study, 25 g fish sample was homogenized in a sterile stomacher bag for 2 min with 225 ml of sterile diluent (0.1% peptone + 0.8% physiological saline) using a stomacher blender (Seward stomacher 400 circulator, England). Serial dilution of the samples were used for bacteriological studies. For sensory evaluation and biochemical studies, required amount of sample was collected in sterile Petri plates.

Total Plate Count (TPC) and Total Fungal Count (TFC), were carried out by spread plate method on surface dried Soybean Casein Digest Agar (SCDA) (MU290, Himedia, India) and Rose Bengal Chloramphenical agar (M640, Himedia, India) plates respectively (APHA, 1995). *E. coli*, *Staphylococcus* and *Micrococcus* were counted using USFDA method (USFDA, 2001). *Micrococcus* were enumerated by method given by Baker (1984) and Faller & Schleifer (1981). The colonies were purified onto Tryptose soya agar (TSA) plates and further subjected to Gram staining, indole test, methyl red (MR), Voges Proskauer (VP) tests and citrate utilization test. Modified Mannitol-egg yolk-polymyxin (MYP) agar (M1139, Himedia, India) was used for isolation and enumeration of *Bacillus* spp. (USFDA, 2001). *Enterococcus*/*Streptococcus* counts were estimated by using Blood Agar with Strepto Supplement (FD031, Himedia, India) and Slanetz and Bartley medium (M612, Himedia, India) (CDC, 2010).

Moisture, protein, ash, acid insoluble ash, total fat content, pH, free fatty acid, titratable acid level and non-protein nitrogen (NPN) were estimated following standard AOAC methods (AOAC, 2001). α-amino nitrogen was determined as per Pope & Stevens, 1939. Total Volatile Base Nitrogen (TVB-N) was determined by Conway's micro-diffusion method

Table 1. Sensory characteristics of good quality *Shidal*

Attributes	Characteristics	Sensory Score*
Appearance	Moist with very soft surface, sticky on touch	9
Colour	Dark reddish brown	9
Texture	Moderately hard texture	9
Odour	Typical strong *Shidal* odour	9
pH	5.7 ± 0.8	-
Titratable acid level	1.78 ± 0.05 (%)	-
TPC	$10^3 - 10^6$ (cfu g^{-1})	-

*All traits measured on ten-point scale with 1 being least and 10 being the most.

(Conway, 1947), Peroxide Value (PV) by the method given by Jacob (1958) and Thiobarbituric Acid (TBA) number as per Tarladgis et al. (1960).

Sensory studies of *shidal* were carried out by an expert panel of 10 judges on a 10 point hedonic scale. Sensory characteristics included general appearance, texture, odour and colour. The overall acceptability was calculated by taking arithmetic average from the score-sheet.

Statistical analysis was done by performing one way ANOVA and Duncan, multiple range test used for comparisons using SPSS-15.0 (2005). All bacteriological counts were converted to $\log_{10}$ cfu g^{-1} for statistical analysis.

Results and Discussion

Changes in TPC and fungal count during fermentation of *Shidal* prepared in production center (PC) is showed in Table 2. These samples showed moderately high values of total plate count during the during September to October (6-7.5 log CFU g^{-1}) (Fig. 2). Perhaps, ambient temperature (25-35°C) was one of the reasons for this higher count. Counts changed significantly ($p<0.05$) during winter (November and December) with moderate stability (5.5 to 6.5 log cfu g^{-1}). The TPC of the fermented fish showed an increasing trend for the first 90 days and thereafter the count decreased. This was a clear indication of establishment of fermenting bacteria and elimination of those bacteria that were incapable of growing in the substrate under prevailing environment. The raw material dry *Puntius* spp. and fermented fish for the first 15 days were dominated by *S. aureus*, *Micrococcus* spp., *Bacillus* spp., *E. coli* and *Streptococcus*, *S. aureus* and *Micrococcus* spp. were found throughout the fermentation period, while *Bacillus* spp. was present up to 45 days of fermentation. The total counts of bacteria towards the end of the fermentation period mostly composed of *S. aureus* and *Micrococcus* spp. The findings of Tanasupawat et al. (1991) who isolated *Staphylococcus* spp. from fermented fish support the present observation.

Fungal count was significantly different ($p<0.05$) up to 75 days of fermentation within the samples, while the count did not change significantly thereafter. Near anaerobic condition inside the *mutka* may be one of the reasons for low fungal growth. The initial lower count may be due to the vigorous sun-drying processes before packing of mutka. A higher count was recorded in the top layers followed by the center and bottom layers (Fig. 1). The cover paste in the mouth of the mutka might have favoured the bacterial proliferation. *Shidal* prepared in the laboratory (L) also showed similar dynamics of bacterial proliferation as illustrated in Fig. 2. Here, the count showed significant difference after 45 days of fermentation with a faster and consistent growth up

Table 2. Total Plate Count (TPC), Total Fungal Count (TFC) and predominant bacteria in traditional *Shidal* from production centers (PC) during fermentation (August - December) (mean ± SD, n=6)

Fermentation Period (days)	TPC (Log cfu g^{-1})	TFC (Log cfu g^{-1})	Predominant bacteria
0	3.3 ± 0.2^{ab}	3.3 ± 0.1^{a}	A, B, C, D, E
15	4.0 ± 0.3^{b}	4.1 ± 0.2^{c}	A, B, C, D, E
30	4.9 ± 0.3^{c}	5.1 ± 0.4^{d}	A, B, C
45	5.0 ± 0.2^{bc}	6.8 ± 0.2^{cd}	A, B, C
60	5.4 ± 0.4^{bc}	4.2 ± 0.2^{c}	A, B
75	**7.9 ± 0.3^{d}**	**2.6 ± 0.3^{b}**	**A, B**
90	8.1 ± 0.3^{e}	2.4 ± 0.2^{b}	A, B
105	6.3 ± 0.2^{bcd}	2.4 ± 0.2^{b}	A, B
120	6.0 ± 0.2^{bc}	2.4 ± 0.2^{b}	A, B

* Means in a column with the same superscript letters are not signiûcantly different ($p>0.05$)

Predominant bacteria (15-30% of TPC): (A). *S. aureus*, (B). *Micrococcus* spp. (C). *Bacillus* spp., (D). *E. coli*, (E). *Streptococcus*/ *Enterococcus*

Bold row indicates "*Shidal* attained acceptable quality"

to 105 days of fermentation (8 log cfu g^{-1}). A stable physical environment with a room temperature of 30±5ºC in the laboratory, fineness of cover paste (as fish crushing was done with a grinder, instead of traditional wooden grinder), smaller size of *mutka* (8 kg capacity compared to traditional *mutka* with 30-40 kg capacity) may be some of the reasons for the consistent growth of bacteria.

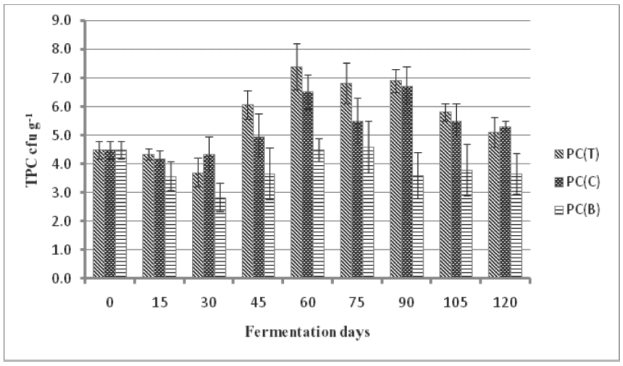

Fig. 1. Dynamics of TPC in the Top (T), Center (C) & Bottom (T) of mutka for samples collected from Production Center (Kamalghat) during August to December, values are mean ± SD (error bars), n=6. Bars with asterisks denote no significant difference (p>0.05) with best quality shidal

TPC, biochemical parameters (titrable acid level and pH), sensory scores and physical characteristics of different fermentation stages have been summarized in Table 3. It was observed that fish remained almost unfermented (R0) up to 15 days and fermentation process started only after 30 days of treatment (R1). This period may be termed as incubation period during which the resident fermenting bacteria over grew the common bacteria. The use of hard textured raw material and sun-drying before processing, perhaps, are the reasons that prolonged the incubation period. These activities either provided an unfavourable growth environment or reduced the initial count of bacteria. The titratable acid level and pH of the products also did not show significant difference ($p<0.05$). Although the sensory scores of the product of 45 days and 60 days fermentation showed significant differences, the TPC, titratable acidity, pH and physical characteristics had no significant differences ($p>0.05$) and therefore these products were kept under same category, *viz.*, R2. Stage R3 showed significant differences in respect of TPC, titratable acidity, sensory scores and physical characteristics. This stage may be termed as the highest fermentation activity period in which

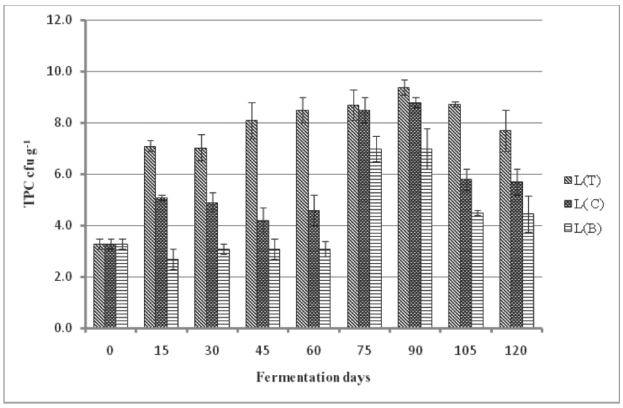

Fig. 2. Dynamics of TPC in the Top (T), Center (C) & Bottom (T) of *mutka* for Laboratory preparation, values are mean ± SD(error bars), n=6. Bars with asterisks denote no significant difference (p>0.05) with best quality *shidal*.

shidal first attained its characteristic colour, odour and texture. This stage extended to another 15 days characterized by highest microbial count (8.1 log cfu g^{-1}) and slight reduction in pH. Thus, at the end of this stage (R3), *shidal* may be harvested in order to minimize the economic loss to the producers due to extended fermentation period. However, the quality still improved after 105 days of fermentation and the product entered into the last stage R4 and it extended up to 120 days. In this stage, the bacterial counts decreased which indicates stationary and declining phases of bacterial growth dynamics. The titratable acidity increased significantly and the pH dropped to less than 6. In this stage, product scored highest sensory scores (8.5-9.5) with ideal characteristic colour, odour and texture. However, immediately after this stage *shidal* might enter the stage of undesirable quality due to over fermentation, loss of volatile flavouring components and extremely soft texture.

Nutritional properties of both the samples were almost similar with protein content of (30-31%), fat content of (18-19%) low moisture of (33-34%) and ash content (11.7-13.8%) (Table 4). This indicates nutritional soundness of the product. Protein and fat content did not differ significantly ($p>0.05$), however moisture, ash, acid insoluble ash, salt and NPN had significant differences ($p<0.05$). *Shidal* L, had lower moisture content, ash content, acid insoluble ash, salt, NPN, TVB-N and free alpha amino acid. The moisture loss may be due to the small size of mutka and the indices of protein breakdown products such as NPN and free alpha amino acid showed lesser

Table 3. The characteristics of different fermentation stages in *Shidal* preparation (mean ± SD, n=3)

Ripening (days)	*TPC (Log cfu g^{-1})	*Titratable acid level (%)	*pH	*Sensory score (Overall acceptability)	Physical Characteristics distinction	Stages with
0	3.3 ± 0.2^{ab}	0.11 ± 0.02^{b}	6.9 ± 0.2^{b}	3.0 ± 0.2^{a}	Dry with hard surface, silvery colour	R0 (Raw material)
15	4.0 ± 0.3^{b}	0.12 ± 0.02^{b}	6.5 ± 0.3^{a}	3.4 ± 0.2^{a}	Moist hard surface, hard texture, no colour change, no characteristic *shidal* smell	R0 (Almost like raw material)
30	4.9 ± 0.3^{c}	0.23 ± 0.02^{a}	6.4 ± 0.3^{a}	3.6 ± 0.2^{b}	Moist with moderately soft surface, hard texture, almost no colour change, no characteristic *shidal* smell	R1 (Initiation of fermentation)
45	5.0 ± 0.2^{bc}	0.26 ± 0.01^{a}	6.4 ± 0.2^{a}	4.2 ± 0.3^{c}	Moist with moderately soft surface, silvery white colour not completely disappears, moderately hard texture	R2 (fermentation active period)
60	5.4 ± 0.4^{bc}	0.21 ± 0.01^{a}	6.3 ± 0.2^{ab}	5.2 ± 0.3^{d}	Moist with moderately soft surface, silvery white colour disappears, moderately hard texture, mild putrefied smell	R2 (marginally acceptable)
75	7.9 ± 0.3^{d}	0.51 ± 0.02^{bc}	6.3 ± 0.2^{ab}	6.4 ± 0.3^{cd}	Soft and sticky surface, colour changes to pale brown, characteristic *shidal* smell moderately prominent, texture starts softening	R3 (highest fermentation activity period, acceptable quality)
90	8.1 ± 0.3^{e}	0.71 ± 0.02^{c}	6.1 ± 0.4^{b}	7.6 ± 0.2^{cd}	Moist with soft and sticky surface, colour changes to pale brown, characteristic *shidal* smell moderately prominent, texture starts softening	R3 (Ripened, average quality)
105	6.3 ± 0.2^{bcd}	1.28 ± 0.5^{d}	5.9 ± 0.5^{b}	8.5 ± 0.2^{de}	Moist and sticky surface, dark reddish brown colour, characteristic strong *shidal* smell, moderately soft texture with shape of the fish intact	R4 (Ripened, Good Quality *shidal*)
120	6.0 ± 0.2^{bc}	1.78 ± 0.5^{b}	5.7 ± 0.8^{b}	9.4 ± 0.2^{e}	Moist and sticky surface, dark reddish brown colour, characteristic strong *shidal* smell, moderately soft texture with shape of the fish intact	R4 (Ripened, Best Quality *shidal*)

*The products having means in a column with the same superscript letters are not significantly different ($p<0.05$).

protein degradation in *shidal* L than in *shidal* PC. Use of clean water and hygiene practices in laboratory reduced the resident bacterial load, i.e. 3.5 log CFU g^{-1} (Fig. 2), while the use of pond water along with unhygienic packaging environment in the production centers harboured higher initial bacterial load *viz.,* about 4.8 log cfu g^{-1} (Fig. 1). Thus, the higher bacterial load in *shidal* PC may be one the reasons for greater protein degradation. The higher content of acid insoluble ash and salt in *shidal* PC is another index that showed the admixture of sand like particles during processing which is comparatively less in *shidal* L. On the other hand, the indices of lipid oxidation such as PV, FFA and TBA did not show significant differences in both *shidal* PC and *shidal* L. However, FFA and TBA indicated higher lipid oxidation in *shidal* L than in *shidal* PC. This may be attributed to small size of *mutka* in *shidal* L that had higher exposure to air and less compactness of fish layers inside the *mutka.*

Based on the scores given by the judges under sensory analysis (Table 5), fermentation stages could be categorized into 5 distinct stages as R0, R1, R2,

Table 4. The biochemical properties of *shidal* from production centers (PC) and *shidal* prepared in laboratory (L), (mean ± SD, independent sampled *t*-test p<0.05, n=3)

Biochemical properties	*Shidal* (PC)	*Shidal* (L)	*P Value*
Moisture (w/w %)	34.02±0.90	33.02±0.50	0.02
Ash (w/w %)	13.8±0.18	11.8±0.12	0.003
Protein (w/w %)	31.06±0.05	30.06±0.15	0.06
Fat (w/w %)	18.87±0.42	18.77±0.52	0.07
Acid-insoluble Ash (w/w%)	0.53±0.02	0.23±0.12	<0.001
Salt (w/w %)	0.72±0.17	0.42±0.15	<0.001
Non-protein Nitrogen (w/w%)	5.49±0.24	4.49±0.14	0.03
Free Alpha Amino Nitrogen (w/w mg%)	60.67±4.67	55.67±3.17	0.06
Total Volatile Base Nitrogen (mg%)	223.67±3.28	123.67±4.28	0.02
Peroxide Value (milliequivallent peroxide oxygen 1000 g^{-1})	17.03±0.32	16.22±0.42	0.07
Free Fatty Acid (% as oleic acid)	20.62±0.17	22.62±0.47	0.05
Thiobarbituric Acid Number (mg malonaldehyde 1000 g^{-1})	2.21±0.64	3.11±0.34	0.06

Table 5. Sensory scores of *Shidal* on 10 point hedonic scale (mean ± SD, n=10) with 120 days fermentation period

Fermentation Period (days)	Appearance*	Colour*	Cooked Taste*	Texture*	Odour*	Overall Acceptability*	Product stage
0	3.3 ± 0.4^{a}	3.1 ± 0.5^{a}	2.9 ± 0.6^{a}	3.0 ± 0.3^{a}	2.6 ± 0.2^{a}	3.0 ± 0.2^{a}	R0
15	3.5 ± 0.2^{a}	3.6 ± 0.4^{a}	3.2 ± 0.2^{a}	3.0 ± 0.2^{a}	3.5 ± 0.7^{a}	3.4 ± 0.2^{a}	R0
30	3.7 ± 0.1^{b}	3.6 ± 0.3^{b}	3.2 ± 0.5^{b}	3.7 ± 0.3^{b}	3.9 ± 0.1^{b}	3.6 ± 0.2^{b}	R1
45	4.1 ± 0.5^{c}	4.3 ± 0.6^{c}	4.1 ± 0.4^{c}	3.8 ± 0.3^{bc}	4.7 ± 0.2^{c}	4.2 ± 0.3^{c}	R2
60	$5.1\pm0.5c^{d}$	5.1 ± 0.2^{d}	5.2 ± 0.8^{d}	5.3 ± 0.5^{d}	5.2 ± 0.4^{de}	$5.2\pm0.^{d}$	R2
75	**6.3 ± 0.3^{cd}**	**6.2 ± 0.1^{cd}**	**6.3 ± 0.1^{cd}**	**6.9 ± 0.3^{cd}**	**6.3 ± 0.4^{cd}**	**6.4 ± 0.3^{cd}**	**R3**
90	7.6 ± 0.2^{cde}	7.8 ± 0.3^{cd}	7.4 ± 0.5^{cdf}	7.9 ± 0.1^{cd}	7.3 ± 0.3^{cd}	7.6 ± 0.2^{cd}	R3
105	8.2 ± 0.2^{de}	8.5 ± 0.1^{def}	8.7 ± 0.5^{de}	8.6 ± 0.7^{de}	8.4 ± 0.6^{de}	8.5 ± 0.2^{de}	R4
120	***9.4 ± 0.2^{e}***	***9.5 ± 0.5^{e}***	***9.4 ± 0.3^{e}***	***9.1 ± 0.4^{e}***	***9.7 ± 0.1^{e}***	***9.4 ± 0.2^{e}***	***R4***

*All traits measured on ten-point scale with 1 being least and 10 being the most. The products having means in a column with the same superscript letters are not significantly different (p<0.05).
Bold row indicates "*Shidal* attained acceptable quality", bold and italicized indicates best quality attained.

R3 and R4. All sensory attributes such as appearance, colour, coked taste, texture, odour and overall acceptability showed acceptable scores (6 and above) after 75 days of fermentation which was termed as R3. This stage may be treated as crucial, since from this stage onwards *shidal* could attain good scores. The scores increased with increased period of fermentation and maximum (9.5) was observed only at the end of fermentation period. Sensory scores are described with help of radar graph in Fig. 3. The dotted line in the graph (R3) shows the line of acceptability as the fermentation period proceeds from the center to the periphery of the graph.

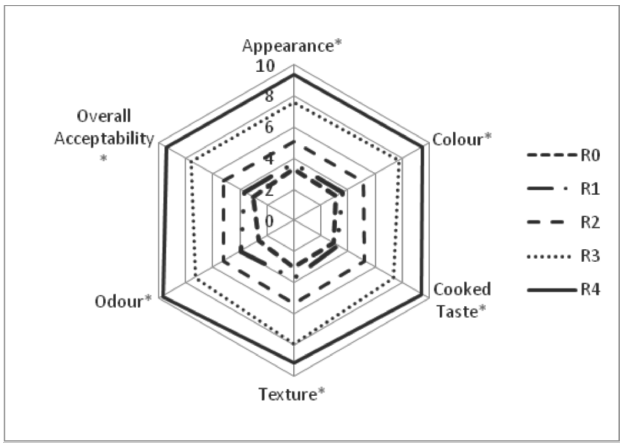

Fig. 3. The sensory scores of differnet fermentation stages

The present study indicated that even though the best quality *shidal* may be procured after 4 months of fermentation for commercial operation 2½ months fermentation is sufficient, so that more batches can be produced for marketing.

Acknowledgements

The author is highly grateful to Ministry of Food Processing Industries, Govt. of India and Central Agricultural University for providing budgetary allocation and other support for conducting the research.

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