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Research paper

Comparative studies on *Bhatooru* fermented with traditional inoculum (*malera*) and standard starter cultures

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Abstract

Bhatooru is a cereal based traditional fermented food of Himachal Pradesh prepared by fermentation of wheat flour dough by sourdough starter '*malera*'. *Bhatooru* was prepared in laboratory by using yeast and lactic acid bacteria or combination of these microorganisms as starter. *Bhatooru* fermentations carried out by the combination of starters (C1 and C2) had higher protein content than other samples. Total sugar level in these samples decreased and reducing sugar level increased during fermentation. However, the starch content in these samples decreased. With the progress in fermentation, the amylase and protease activity also increased in almost all the samples except control where very little activity was observed from 0-10 h.It was observed that B-vitamin content (thiamin, riboflavin, nicotinic acid and cyanocobalamin) in samples were higher as compared to the control. Out of various yeast strains used, *Saccharomyces cerevisiae* inoculated samples showed higher vitamin content. Thiamin content was relatively higher in samples prepared from *Lactobacillus* strains and combination of starters. Increase in essential amino acid contents (methionine, phenylalanine, threonine, lysine and leucine) was observed in the *bhatooru* dough prepared by using combination of starters.

Keywords: *Bhatooru*, comparative study, co-starter, Fermented food, Starter,traditional,*Lactobacillus*, Yeast.

Introduction

Indigenous fermented foods are produced at the household level in majority of countries of the world. Most traditional fermented products are made by natural fermentations carried out in a non-sterile environment. The specific environmental conditions cause a gradual selection of microorganisms responsible for the final product. However, the method is difficult to control and hence, there is risk of accompanying microflora that may cause spoilage of products making these unsafe for consumption (Battcock and Azam Ali, 1998).

As most of the fermentation processes used in developing countries do not use standard inocula or extrinsic cultures, these processes could be improved by using starter cultures (Holzapfel, 2002). The microorganisms isolated from traditional fermentation processes could be used as starter culture for the production of improved products with large quantities of vitamins and amino acids, thus, helping to improve the nutritional value of food with better health benefits. A strong starter may reduce fermentation times, minimize nutritional losses, avoid contamination with pathogenic and toxicogenic bacteria and molds and minimize the risk of pathogenic microflora causing off-flavor (Haard, 1999). Lactic acid bacterial fermentation is used to improve sensory and nutritional properties of foods (Anderson, 1988) and to obtain products of high and consistent qualities, the fermentation process has to be controlled using tailormade starter culture (Cooke *et al.*,

1987).This approach would improve the shelf-life of the products quality thereby reducing microbial risks associated with the traditional food processing methods (Agarry *et al.*, 2010).

In the present study, we have tried to improve the quality of *Bhatooru* (traditional fermented food of Himachal Pradesh) by fermenting *Bhatooru* dough with different starters cultures isolated from fermented foods as well as known strains obtained from Microbial Type Culture Collection (MTCC), Chandigarh and compared the various parameters with the dough prepared with *malera* (traditional inoculum).

Material and Methods

Microorganisms

Lactic acid bacteria used for these studies were *Lactobacillus brevis* MTCC 1750 purchased from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh and *Lactobacillus amylovorus* MTCC 8129, earlier isolated from fermented food *seera* in our laboratory, identified and deposited at MTCC Chandigarh. Yeast strains used in these studies were *Saccharomyces cerevisiae* MTCC 36 purchased from MTCC Chandigarh, and *Cryptococcus laurentii*, *Saccharomyces cerevisiae* and *Torulospora delbrueckii* strains isolated from *siddu, bhatooru* and *seera* samples earlier in our laboratory. *Lactobacillus* strains were maintained on Man Rogosa Sharpe (MRS) agar slants and yeasts were maintained on YMEA slants. Following strains (106 cfu/g) designated as Y1:*Saccharomyces cerevisiae*, Y2: *Cryptococcus laurentii*, Y3: *Torulospora delbrueckii*, SC: *Saccharomyces cerevisiae*, L1: *Lactobacillus plantarum*, L2: *Lactobacillus brevis*, C1: C-starters (*S. cerevisiae* + *L. plantarum* + *L. brevis*), C2: Co-starters (*T. delbrueckii* + *L. plantarum* + *L. brevis*) were used for different experiments during fermentation of *bhatooru*.

Preparation of dough

Wheat flour (*Shakti Bhog* brand) was used for different dough preparations. Dough was prepared by mixing 500 g of wheat flour, 300 ml of distilled water and the inoculum of lactic acid bacteria and yeasts (106cfu/g) in a beaker covered with aluminum foil and kept at 250C for 10 h of fermentation. Dough samples were drawn periodically at every 2 h and immediately frozen for determining pH, acidity, protein, carbohydrate, reducing sugars, starch, amylase, protease, vitamins and amino acids as per standard methods.(Raganna, 1986) Fermentation using traditional inocula or *malera* (as control) has also been studied for various parameters.

Sensory analysis

Bhatooru prepared by using different starter cultures were organoleptically evaluated using a score range of 1 (bad) to 7 (good) and 3.5 (moderate) taking traditionally made *bhatooru* as a control *(*Joshi,2006).

Statistical analysis

The experimental data generated were analyzed adopting standard statistical procedures. Statistical significance was accepted at p value was equal to or less than 0.05.

Results and Discussion

pH and titrable acidity

A significant ($p< 0.01$) decrease in pH was observed in all the samples with a significant ($p< 0.01$) increase in titrable acidity as compared to the control (Table 1 and 2). However, decrease in pH was higher in costarters as compared to other samples. In dough samples inoculated with *Lactobacillus*, decrease in pH was more pronounced than the samples inoculated with yeast strains only. The marked decrease in pH with simultaneous increase in titrable acidity in fermentations carried out by using starter cultures may be related to fermentation of carbohydrates and production of lactic acid by lactic acid bacteria. This acidity might have possibly enhanced the sensory quality attributes of product making it more preferable to the product made by traditional inoculum (*malera*). Similar trend was observed in fermentation of *Kunun-zaki* (a Nigerian fermented cereal beverage) by using starter culture (Agarry *et al.*, 2010).

Protein content

The protein content during *bhatooru* fermentation with various starters was assayed at an interval of 2 h up to 10 h and the results are summarized in Table 3. Initially upto 4 h of fermentation, there was no significant increase in protein content, however, after 4 h of fermentation there was significant ($p< 0.01$) increase in protein content in all the dough samples. The samples fermented with co-cultures of yeast and bacteria exhibited maximum increase but least increase was observed in dough fermented with only yeasts (Y1and Y2). However no significant increase in protein content has been observed in control. Oyarekua (2011) has observed a significant increase in protein content and crude fiber during production of fermented pigeon pea flour.

Total sugars

The total sugars during fermentation of *bhatooru* with different starters were estimated and the results are shown in

Table 1: pH of dough inoculated with various combinations of starter cultures during the course of *bhatooru* fermentation

Fermentation time (h)	Y1	Y2	Y3	S C	L1	L2	C1	C2	Control
0	6.30±0.026	6.28±0.036	6.25±0.045	6.26±0.060	6.26±0.036	6.23±0.036	6.23±0.026	6.20±0.060	6.54±0.055
2	6.16±0.020	6.23±0.036	6.21±0.036	6.22±0.036	6.20±0.045	6.18±0.043	5.93±0.045	5.97±0.060	6.50±0.072
4	6.05±0.030	6.15±0.036	6.14±0.026	6.12±0.037	5.88±0.043	6.02±0.036	5.57±0.050	5.68±0.040	6.47±0.070
6	6.00±0.026	6.05±0.026	6.01±0.050	5.97±0.051	5.67±0.036	5.74±0.043	4.73±0.043	4.98±0.036	6.40±0.036
8	5.91±0.020	5.90±0.026	5.86±0.043	5.84±0.036	5.25±0.065	5.30±0.036	4.45±0.043	4.39±0.045	6.37±0.052
10	5.87±0.036	5.78±0.026	5.69±0.050	5.57±0.043	4.75±0.055	4.71±0.026	4.20±0.030	4.20±0.010	6.28±0.052

Results are shown as mean of three replicates (± standard deviation)

Table 2: Titrable acidity (%) of dough inoculated with various combinations of starter cultures during the course of *bhatooru* fermentation

Fermentation time (h)	Y1	Y2	Y3	S C	L1	L2	C1	C2	Control
0	0.028±0.002	0.034±0.002	0.035±0.002	0.035±0.001	0.034±0.001	0.032±0.002	0.036±0.002	0.036±0.002	0.019±0.002
2	0.036±0.001	0.037±0.001	0.037±0.002	0.037±0.002	0.038±0.002	0.038±0.001	0.044±0.001	0.044±0.002	0.019±0.001
4	0.040±0.002	0.038±0.001	0.038±0.002	0.038±0.002	0.045±0.002	0.040±0.002	0.046±0.002	0.045±0.001	0.027±0.001
6	0.040±0.002	0.040±0.002	0.040±0.002	0.042±0.002	0.050±0.001	0.045±0.001	0.072±0.003	0.070±0.001	0.027±0.002
8	0.042±0.001	0.042±0.001	0.044±0.001	0.045±0.002	0.058±0.001	0.054±0.002	0.099±0.002	0.100±0.002	0.036±0.002
10	0.045±0.002	0.045±0.001	0.045±0.002	0.050±0.002	0.072±0.002	0.072±0.003	0.110±0.002	0.110±0.004	0.036±0.002

Results are shown as mean of three replicates (± standard deviation)

Table 3: Protein content (% w/w in dry matter) of dough inoculated with various combinations of starter cultures during the course of *bhatooru* fermentation

Fermentation time (h)	Y1	Y2	Y3	S C	L1	L2	C1	C2	Control
0	13.5±0.26	13.6±0.36	13.4±0.26	13.5±0.45	13.6±0.36	13.8±0.26	13.5±0.36	13.4±0.26	13.3±0.36
2	13.2±0.3	14.1±0.26	12.2±0.17	13.7±0.17	13.8±0.26	13.4±0.20	13.8±0.10	13.4±0.20	13.3±0.26
4	14.7±0.52	14.9±0.2	14.4±0.26	14.7±0.36	14.2±0.26	14.4±0.17	14.9±0.26	14.8±0.36	13.4±0.36
6	15.8±0.26	15.7±0.43	15.4±0.36	16.2±0.26	16.3±0.20	15.4±0.40	15.9±0.36	16.4±0.26	13.9±0.26
8	16.2±0.26	15.6±0.4	16.0±0.26	16.8±0.2	17.0±0.26	16.1±0.26	17.3±0.26	17.0±0.17	14.3±0.36
10	15.7±0.3	14.8±0.26	16.2±0.34	16.5±0.26	16.6±0.26	16.8±0.26	18.3±0.34	18.2±0.20	13.9±0.10

Results are shown as meanof three replicates (± standard deviation)

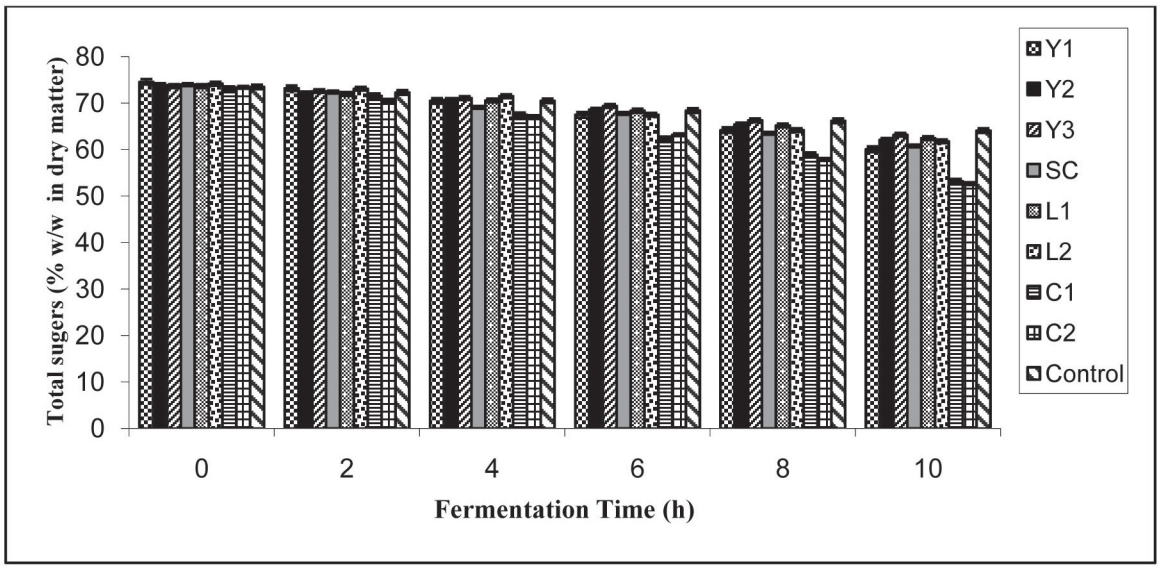

Figure 1: Total sugars (% w/w in dry matter) of dough inoculated with various combinations of starter cultures during the course of *bhatooru* fermentation (See Material and Methods for codes)

Figure 1. As the fermentation progressed, the total sugar content of the dough decreased due to the growth of fermentative organism. The utilization of total sugar was more in case of *bhatooru* prepared with co-starters, however little decrease was observed in case of dough fermented with pure starters or control.

Reducing sugars

The level of reducing sugars in dough during fermentation with different starters was determined and the results are shown in Figure 2. The reducing sugar levels in various samples increased from 0 h to 8 h and at 10 h of fermentation a slight decrease in reducing sugar level was observed. Mosha and Svanberg (1983) have reported the hydrolysis of starch and oligosaccharides present in the substrates of fermentation resulting in an increase in reducing sugars due to the activity of amylases present in cereal grains or secreted by microorganisms. The decrease in reducing sugars with prolonged fermentation was attributed to their utilization by fermenting microflora as was observed by Daeschel *et al.* (1987).

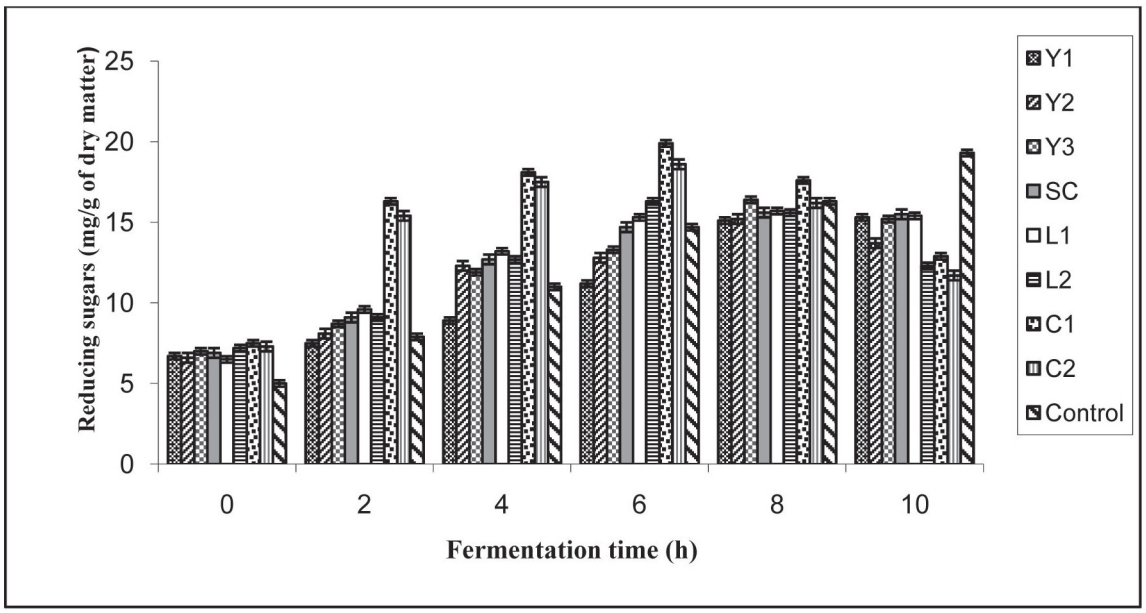

Figure 2: Reducing sugars (mg/g dry matter) of dough inoculated with various combinations of starter cultures during the course of *bhatooru* fermentation (For Codes,see Material and Methods)

Starch content

The starch content in dough fermented with various starters was assayed at an interval of 2 h till 10 h and the results are presented in Figure 3. The starch content in various samples decreased significantly ($p < 0.01$) from 0-10 h (from 69.1%, 67.2%, 67.7%, 69.2, 70.1, 70.2%, 69.9%, 68.5% and 67.1% to 59.2%, 58.9%, 58.3%, 55.6%, 54.2%, 52.9%, 46.5%, 47.0 and 66.3% in Y1, Y2, Y3, S C, L1, L2, C1, C2 and control respectively).

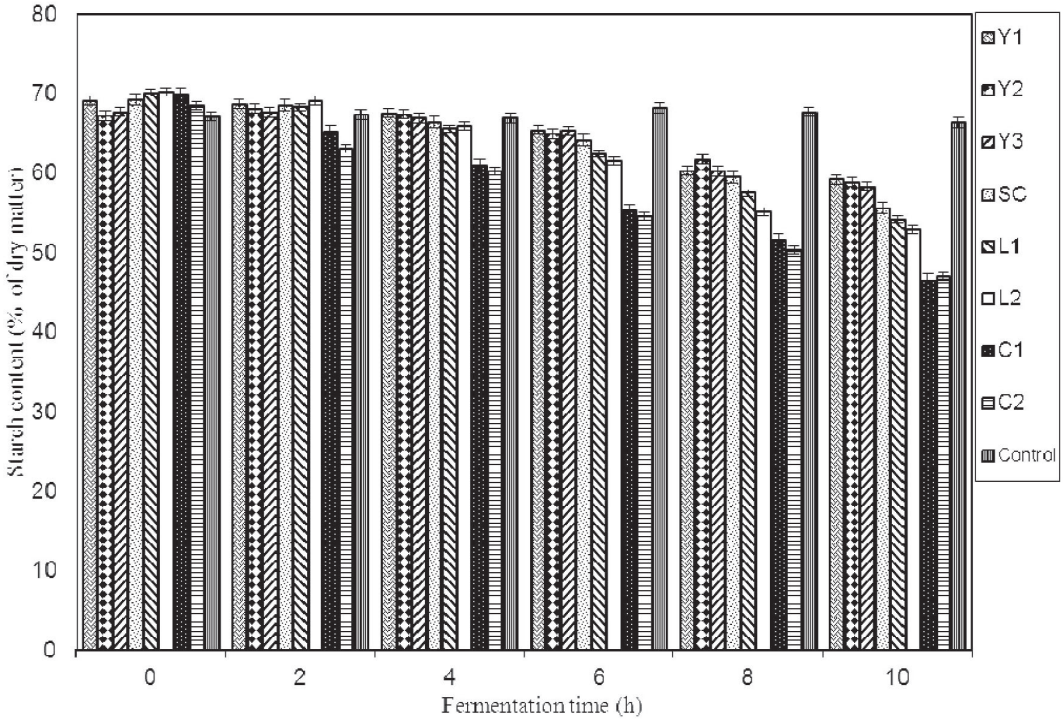

Figure 3: Starch content (%) of dough inoculated with various combinations of starter cultures during the course of *bhatooru* fermentation (For Codes,see Material and Methods)

The starch content in control did not change significantly. The amylase present in substrate and that produced during fermentation may be responsible for hydrolysis of starch to produce maltose and glucose which are utilized by bacteria and yeasts for their growth.

The starch, fiber and reducing sugar contents have been reported to decrease during fermentation of starchy substrates (Soni and Marwaha, 2003). A continuous degradation of starch during natural fermentation *of bushera* with concomitant increase in maltose and glucose has been recorded by Muyanja *et al.* (2004).

Amylase and protease activity

The samples of dough prepared by using various starters were assayed for amylase activity at an interval of 2 h till 10 h and results are given in Figure 4.

In all the dough samples, amylase activity increased up to 6 h and then showed a decrease. The decrease in the amylase activity may be due to the production of acids, thus lowering the pH of the dough. Katina *et al.* (2005) reported the reduction in amylolytic enzymes with the decline in pH in the sourdough.

The proteolytic activity in different dough samples was measured up to 10 h of *bhatooru* fermentation with different starters and the results are shown in Figure 5. As the fermentation progressed, the protease activity also increased in almost all the samples except control where very little activity was observed from 0-10 h. A significant ($p<0.01$) increase in proteolytic activity was observed in the dough fermented with cocultures. In 6 h, protease activity increased from 0.58-11.5 U/g, 0.60-12.07 U/g on dry weight basis in dough fermented with C1 and C2 respectively. This increased proteolytic activity during the fermentation may lead to hydrolysis of wheat protein to enhance free amino acids content in *bhatooru*. However, protease activity decreased after 6 h of fermentation.

Vitamin and amino acids

The B-vitamins which tend to increase significantly ($p<0.01$) during food fermentations were analyzed in dough prepared from various starters and the results are summarized in Fig. 6. The B-vitamin content (thiamine, riboflavin, nicotinic acid and cyanocobalamin) in all the samples were found to be higher than the control. Out of the various yeast strains used, *Saccharomyces cerevisiae* inoculated samples had maximum vitamin content. Fermentations, which involve yeasts and particularly those in which the yeasts are consumed along with the substrate, tend to be enriched in the water-soluble B vitamins (Steinkraus, 1998). Thiamin content was relatively

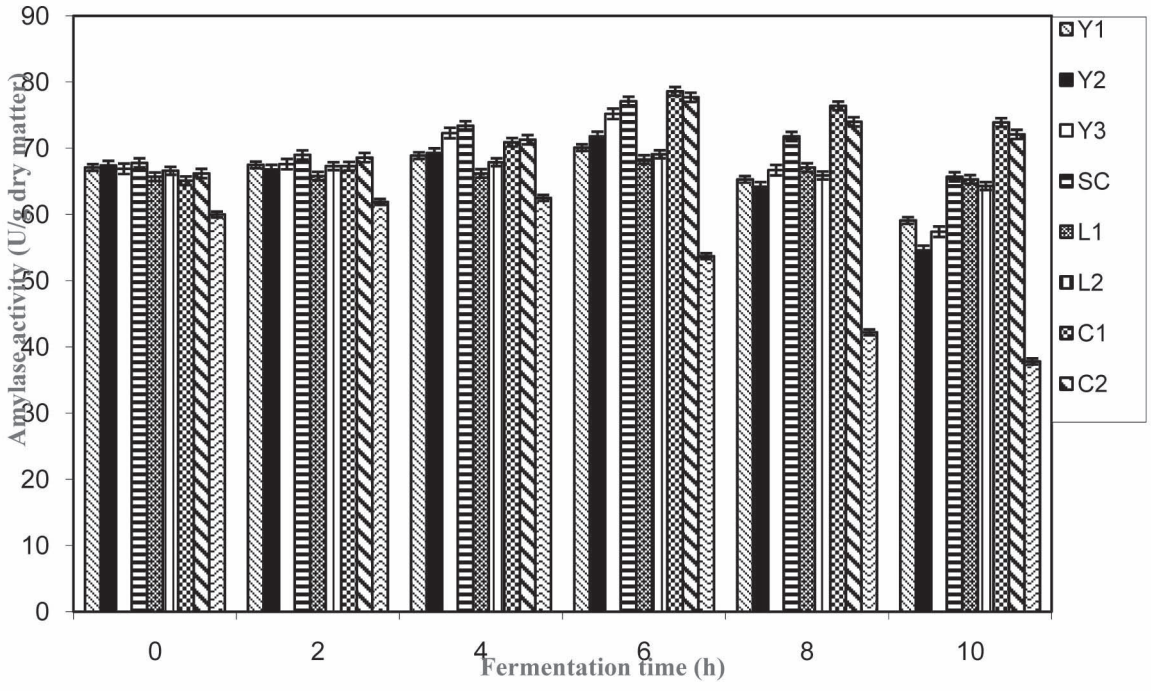

Figure 4: Amylase activity (U/g dry matter) in *bhatooru* dough prepared from different starter cultures (For Codes,see Material and Methods)

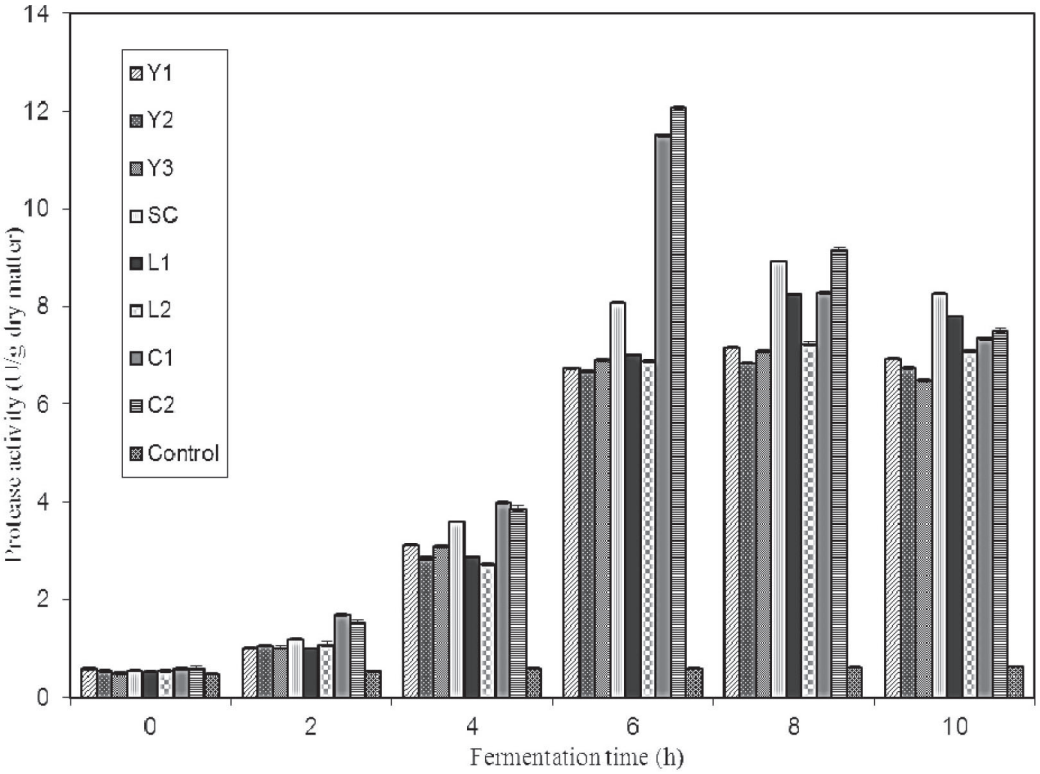

Figure 5: Protease activity (U/g dry matter) in dough fermented with different starters (For Codes,see Material and Methods)

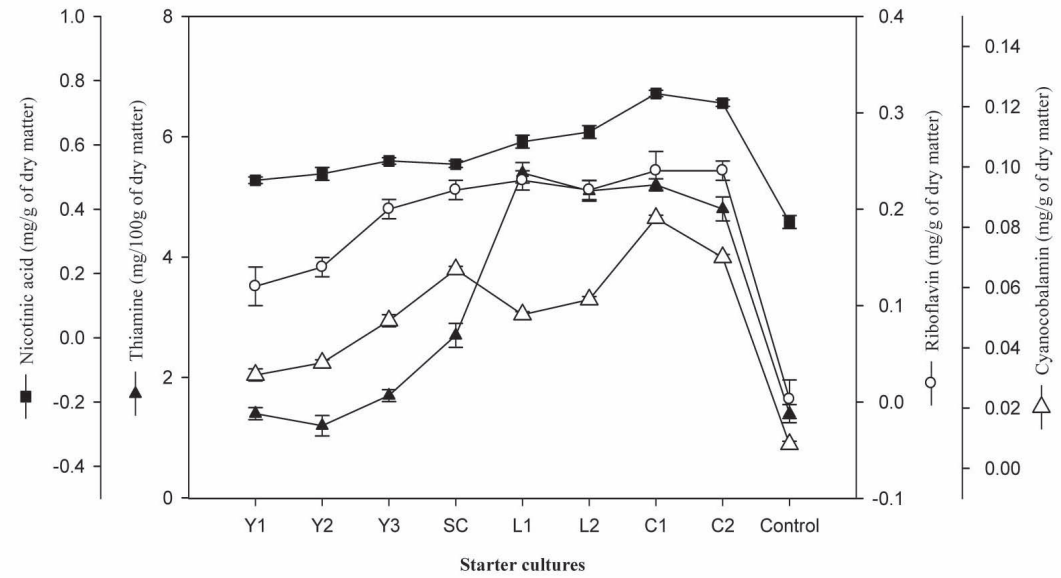

Figure 6: Vitamin content in dough prepared from different starter cultures (For Codes,see Material and Methods)

higher in samples prepared from *Lactobacillus* strains and co-starters.

Since the level of yeasts in traditional fermentations is significantly lower than the bacteria (Sandhu *et al.*, 1986; Soni *et al.*, 1986) the possibility of yeast enrichment has been investigated by experiments in which common yeast isolated from traditionally fermented *dosa* batters were used (Soni and Sandhu, 1999). *Saccharomyces cerevisiae, Debaromyces hansenii, Hansenula anomala, Trichosporon beigelii* and *Torulopsis candida* were used to inoculate the *idli* batters without eliminating the natural bacterial flora of the ingredients and a significant increase in vitamin B1 and B2 has been observed (Soni, 1987; Soni and Sandhu, 1989).

The amino acid content of dough fermented with different yeasts, lactic acid bacteria and costarters as well as control was determined and the results are summarized in Figure 7. The increase in amino acids in dough fermented with yeasts especially *Saccharomyces cerevisiae* showed the desired contribution of this yeast during fermentation. Soni and Arora (2000) reported a significant increase in free amino acids in *idli* batter fermentation enriched with *S. cerevisiae, Torulopsis candida, Hansenula anomala, Debaromyces hansenii* and *Trichosporon beigelli* as compared to that of control.

During the studies on improvements of Indian *dosa* batters, Soni and Sandhu (1989) reported that *S. cerevisiae* enriched fermentations were found to be the best in terms of degree of leavening, final level of amylases, reducing sugars, free amino acids, total proteins, vitamin B1 and B2 and organoleptic characteristics. Addition of *Saccharomyces cerevisiae* along with natural bacterial flora of the ingredients is the best method for standardizing *idli* fermentations in terms of improved sensory quality , leavening and nutritional constituents (Soni *et al.*, 1986).

Sensory analysis of bhatooru prepared by using different starter cultures

Bhatooru prepared by using co-starters were organoleptically evaluated best in quality as compared to *bhatooru* prepared from yeast or lactic acid bacteria alone (Table 4). Since the values of nutritional constituents and organoleptic characteristics were maximum in various yeast-enriched samples and in samples prepared by using co-starters, it could be concluded that standardization of fermentation process by choosing suitable yeast, especially *S. cerevisiae* in combination with *Lactobacillus* sp. or with the natural bacterial flora can play a significant role in further improving the nutritional quality and acceptability of foods. A combination of *Torulospora delbrueckii* and *S. cerevisiae* were reported to produce doughs with good stability and proofing qualities (Jenson, 1998). Similar study using dominant lactic acid bacteria (*Lactobacillius plantarum, L. fermentum* and *Lactococcus*

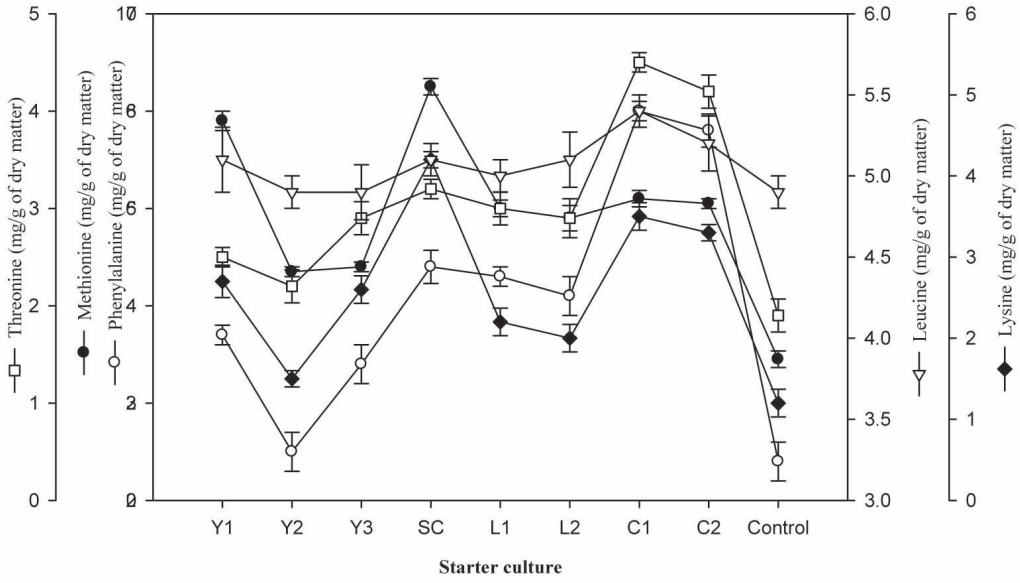

Figure 7: Amino acid content in dough fermented with different starters (For Codes,see Material and Methods)

Table 4: Sensory analysis of *bhatooru* prepared from different starters

Starter cultures	Appearance	Taste	Aroma	General acceptability
Y1	5.5±0.26	3.0±0.26	3.4±0.17	3.9±0.2
Y2	2.3±0.1	1.2±0.17	2.0±0.2	2.0±0.17
Y3	4.9±0.2	2.4±0.26	3.3±0.17	3.7±0.3
SC	5.7±0.1	3.2±0.17	3.7±0.2	4.0±0.1
L1	5.8±0.17	3.5±0.17	4.2±0.2	4.3±0.17
L2	5.4±0.2	3.6±0.1	4.2±0.1	4.1±0.17
C1	6.9±0.2	4.2±0.17	6.4±0.3	6.2±0.1
C2	6.8±0.26	4.1±0.2	6.3±0.17	6.1±0.17
Control	5.2±0.1	3.9±0.1	2.1±0.17	2.0±0.1

Results are shown as mean of three replicates (± standard deviation)

lactis) has shown that use of starter culture in the production of *Kunun-zaki* has affected the sensory and nutritional qualities of the product positively (Agarry *et al.*, 2010).

Conclusions

Based on chemical, biochemical and organoleptic studies of *bhatooru* dough/*bhatooru* prepared by using different starters, it has been found that best *bhatooru* (in terms of increased protein content, vitamins, essential amino acids, good taste, aroma, appearance and overall acceptability) was obtained by using costarters i.e. C1 ((*S. cerevisiae* + *L. plantarum* + *L. brevis*) and C2 (*T. delbrueckii* + *L. plantarum* + *L. brevis*). So use of these costarters for carrying out fermentation can be emphasized to enhance the nutritional value of fermented foods. Further the use of an appropriate starter for fermentations can produce certain bioactive components that can beneficially affect the health of the consumer.

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