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Optimization of traditional processing of *Selroti*, a popular cereal-based fermented food

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Selroti is a popular fermented rice food in the Himalayas. Pure culture strains of lactic acid bacteria and yeasts, previously isolated from naturally fermented *selroti* batters, were tested singly or in combination for their ability to ferment rice flour to produce *selroti*. Sensory evaluations were carried out in order to choose the best culture combinations. *Selroti* batters produced using a mixture of pure culture strains of *Leuconostoc mesenteroides* BS1:B1 and *Saccharomyces cerevisiae* BA1:Y2 at 28° C for 4 h had organoleptically scored the highest acceptability. This was also correlated by decrease and increase in pH and acidity of the fermenting batters, respectively from 0 h to 4 h. The consumers' preference trial showed that *selroti* batter prepared by a mixture of *Leuc. mesenteroides* BS1:B1 and *S. cerevisiae* BA1:Y2 was more acceptable than *selroti* batters prepared by conventional method. *Selroti* prepared by using a consortium of starter cultures had advantages over the traditional method.

Keywords: *Selroti*, starter culture, cereal fermentation, LAB, optimization.

Introduction

Selroti is an ethnic fermented cereal-based staple food of the Himalayan regions of India, Nepal and Bhutan[1,2]. It is ring shaped, spongy, pretzel-like and deep-fried food and is consumed in religious festivals and special occasions. During traditional method of preparation, rice is soaked, pounded, mixed with sugar, butter, milk and spices, kneaded into batter and is left to ferment naturally for 6-10 h. The fermented batter is squeezed and deposited as continuous ring onto hot edible oil and fried until golden brown and is served as confectionary bread[3]. Lactic acid bacterial species of *Leuconostoc mesenteroides*, *Enterococcus faecium*, *Pediococcus pentosaceus* and *Lactobacillus curvatus* and yeasts *Saccharomyces cerevisiae*, *Saccharomyces kluyveri*, *Debaryomyces hansenii*, *Pichia burtonii* and *Zygosaccharomyces rouxii* were isolated from naturally fermented batters of *selroti*[4]. The present paper is aimed to study optimization of the traditional processing method using a consortium of pure strains of lactic acid bacteria (LAB) and yeasts, previously isolated from naturally fermented batters of *selroti*, in order to minimize the production time and improve the quality of the product.

Materials and methods

Selection of starter culture(s)

Pure strains of LAB and yeasts, previously isolated from naturally fermented batters of *selroti*[4], were tested singly or in combination for their ability to ferment rice flour to produce *selroti*. Different starter cultures and their combinations used were starter A- cells of *Leuconostoc mesenteroides* BS1:B1; starter B (1:1:1:1) - mixture of cells of all LAB strains (*Enterococcus faecium* BS1:B2; *Lactobacillus curvatus* BP:B1; *Leuc. mesenteroides* BS1:B1; *Pediococcus pentosaceus* BG:B2); starter C- cells of *Saccharomyces cerevisiae* BA1:Y2; starter D (1:1:1:1:1) - mixture of cells of all yeasts strains (*Debaryomyces hansenii* BR1:Y4; *Pichia burtonii* BG1:Y1; *S. cerevisiae* BA1:Y2; *S. kluyveri* S3:Y3; *Zygosaccharomyces rouxii* S1:Y6); starter E (1:1) - mixture of B and D (LAB and yeasts) mentioned above; and starter F (1:1) - mixture of cells of *Leuc. mesenteroides* BS1:B1 and *S. cerevisiae* BA1:Y2.

Preparation of consortium of starter culture(s)

A loop-full of LAB culture was inoculated in 5 ml de Man, Rogosa and Sharpe (MRS) broth (HiMedia,

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Mumbai, India) and incubated overnight at 30° C. The 1 ml of each culture was centrifuged (Biofuge pico, Heraeus, Germany) at 8,000 *g* for 5 min, the supernatant was discarded, 1 ml of sterile distilled water was added to the pellet, cells were resuspended and again centrifuged at 8,000 *g* for 5 min. Cells were again suspended in 1 ml sterile distilled water. This procedure achieved an inoculum size containing 10^8 cfu/ml of individual strain and was checked as viable count in MRS agar (HiMedia) plates. Similarly, a loopful of different yeast cultures were inoculated in 5 ml YM broth (HiMedia) separately and incubated overnight at 28° C. These cultures were centrifuged and washed as described previously and an inoculum, containing 10^5 cfu/ml of individual strain, was made. Selection of inoculum size was based on the maximum microbial load of LAB (10^8 cfu/ml) and yeast (10^5 cfu/ml) in naturally fermented *selroti* batters[4].

Preparation of selroti batters at laboratory

Rice (*Oryza sativa* L.) local variety 'attey' was purchased from Gangtok market in Sikkim. *Selroti* batter was prepared in the laboratory following the traditional method[3] based on the combination of three popular methods being practiced at different places in Sikkim. One kg of rice was sorted, washed and soaked overnight at ambient temperature (20-22° C). Water was decanted from the soaked rice, pounded and sieved to get rice flour. The rice flour was thoroughly mixed with 250 g of wheat flour (refined), 250 g sugar, 100 g butter and 25 g powdered spices including large cardamom (*Amomum subulatum* Roxb.), cloves (*Syzygium aromaticum* Merr.), coconut (*Cocos nucifera* L.), fennel (*Foeniculum vulgare* Mill), nutmeg (*Myristica fragrans* Houtt.) and cinnamon (*Cinnamomum zeylanicum* Breyn.). Boiled cow milk was added to the mixture and kneaded into soft dough. Equal volumes of batter were distributed in 11 sterile 500 ml Duran bottles and were loosely capped, labeled and incubated at 28° C. Successional studies were carried at every 1 h interval within a range of 0 to10 h.

Sensory evaluation of *selroti* prepared by starter culture

Pounded rice along with all ingredients mentioned above was mixed thoroughly and soft dough was made as described earlier. Equal volumes (nearly 350 ml) of batter were distributed in 14 sterile 500 ml Duran bottles with screw-caps. *Selroti* batter samples were inoculated with six different combinations of starter cultures (A-F) containing 1 ml each of LAB and yeast inocula per 100 g of batter. Inoculated samples were mixed thoroughly by sterile spatula, loosely capped, labeled and incubated at 28° C. Fermenting batters were taken out at 4 h and 6 h and deep-fried in hot edible oil and were served to 10 judges for sensory evaluation with score rate of 1 as bad (hard texture) and 5 as excellent (soft texture). Ready-made *selroti* purchased from vendor's shop in Gangtok was considered as control with scoring rate of 3, moderate.

pH and acidity

The pH and acidity of the fermenting batters were determined at 0 h, 4 h and 6 h. Ten g of sample were mixed with 20 ml CO_2-free distilled water in a blender for 1 min and the pH of the slurry was determined directly[5] using a digital pH meter (Model 361, Systronics, India) calibrated with standard buffer solutions (Merck). Acidity of sample was calculated by titrating the filtrates of blended 10 g sample in 90 ml CO_2-free distilled water with 0.1 N NaOH to end point of phenolphthalein (0.1 % w/v in 95 % ethanol)[5].

Sensory evaluation

Sensory properties of product were evaluated in terms of aroma, taste, texture, colour and general acceptability[6]. *Selroti* batters were prepared in the laboratory following the traditional method as described above. Fermenting batters were taken out in every hour and fried in hot edible oil. *Selroti* prepared from every hour fermenting batters were served to 10 judges for sensory evaluation with score rate of 1 as bad (hard texture) and 5 as excellent (soft texture); *selroti* purchased from market was considered as control with scoring rate of 3, moderate.

Consumers' preference trial

Freshly fried *selroti* purchased from Gangtok market as well as fried *selroti* prepared from batters made in the laboratory by using a mixture of selected isolates were served to 50 consumers who were familiar with *selroti*. The 9-point Hedonic scale ranging from like extremely (9) to dislike extremely (1) was used.

Statistical analysis

The data, representing the means scores ±SD of three sets of experiments, were analysed by determining standard deviation (SD), standard error of measurement (SEM) and analysis of variance (ANOVA)[7].

Table 1—Sensory evaluation of *selroti* batter prepared during natural fermentation

Fermentation time (Hour)	Attribute				
	Aroma	Taste	Texture	Colour	General acceptability
0	1.2 ± 0.5^{e}	1.2 ± 0.1^{de}	2.6 ± 0.6^{a}	2.0 ± 0.1^{b}	1.2 ± 0.5^{gd}
1	1.2 ± 0.5^{e}	1.2 ± 0.1^{de}	2.6 ± 0.6^{a}	2.0 ± 0.1^{b}	1.2 ± 0.5^{gd}
2	1.2 ± 0.5^{e}	1.4 ± 0.5^{ce}	2.6 ± 0.6^{a}	2.0 ± 0.1^{b}	1.4 ± 0.6^{fd}
3	1.8 ± 0.5^{de}	1.4 ± 0.6^{ce}	2.8 ± 0.5^{a}	2.0 ± 0.1^{b}	1.6 ± 0.6^{ed}
4	2.0 ± 0.7^{cfe}	2.4 ± 0.6^{ae}	2.8 ± 0.5^{a}	2.0 ± 0.1^{b}	2.2 ± 0.5^{cdefg}
5	2.2 ± 0.5^{bdf}	2.6 ± 0.8^{abcd}	3.0 ± 0.6^{a}	2.0 ± 0.1^{b}	2.6 ± 0.6^{bcdef}
6	2.8 ± 0.5^{af}	2.6 ± 0.8^{abcd}	3.0 ± 0.4^{a}	2.4 ± 0.6^{ab}	3.0 ± 0.1^{abcd}
7	3.0 ± 0.7^{ab}	2.6 ± 1.1^{abcd}	3.8 ± 0.5^{a}	2.8 ± 0.5^{ab}	3.0 ± 0.1^{ab}
8	3.6 ± 0.6^{a}	4.0 ± 0.6^{a}	4.0 ± 0.4^{a}	3.8 ± 0.5^{a}	4.0 ± 0.7^{a}
9	3.6 ± 0.6^{a}	3.2 ± 1.3^{ab}	4.0 ± 0.6^{a}	3.8 ± 0.8^{a}	3.6 ± 0.9^{ab}
10	3.0 ± 1.0^{ab}	1.8 ± 1.1^{be}	3.8 ± 0.4^{a}	2.8 ± 0.8^{ab}	1.8 ± 0.7^{d}

Data represents the mean scores (±SD) of ten judges.Values bearing different superscripts in each column differ significantly (p<0.05).Market *selroti* was used as control (score 3), score 1, bad/hard; score 5, excellent/soft.

Results and discussions

Sensory properties of naturally fermented *selroti*

Batters prepared during natural fermentation of *selroti* from 0-10 h were collected aseptically, and deep-fried in edible oil to make *selroti* at laboratory, and were subjected to sensory evaluation by 10 judges (Table 1). There was no significant (p<0.05) difference in aroma attribute of *selroti* prepared until 3 h. There was significant (p<0.05) difference in aroma score of *selroti* prepared at 8 to 9 h. No significant (p<0.05) difference in texture of *selroti* prepared during 0-10 h was observed. Similarly, no significant (p<0.05) difference in colour attribute of *selroti* prepared until 5 h fermentation period was observed, however, the significant (p<0.05) increase was seen after 5 h till 9 h. In general acceptability, *selroti* batter prepared at 8 h showed significantly (p<0.05) highest score. *Selroti* prepared at 8 h following the traditional method had soft texture, sweet taste and aroma, significantly (p<0.05) acceptable to judges (Table 1). It is generally noted that a soft texture and sweet-taste, with golden brown colour fried *selrori* is considered the best to the consumers. Yeasts play vital role in production of many traditional fermented foods mostly enhancing sensory quality of the foods[8,9]. Yeasts associated with *selroti* fermentation might have enhanced sensory quality of the product.

Sensory evaluation of *selroti* prepared by starter culture

The batter was prepared following the traditional method as described above. About 350 g of batter was equally distributed in sterile 500-ml Duran bottles with screw caps. Each batter was inoculated with 1ml of the starters (A-F) per 100 g of batter either singly or in combinations as described before; mixed thoroughly by a sterile spatula or incubated at 28° C. *Selroti*, prepared from different batter samples incubated for 4 h and 6 h were deep-fried in hot edible oil and served to 10 judges for sensory evaluation. There was a significant (p<0.05) decrease and increase in pH and acidity of the fermenting batters, inoculated by starter culture(s), respectively from 0 h to 6 h. There was no significant (p<0.05) difference in all sensory attributes of *selroti* prepared by different combinations of pure cultures starters except starter F, a mixture of *Leuc. mesenteroides* and *S. cerevisiae* (Table 2). Organoleptically, *selroti* prepared from 4-h fermented batter inoculated with starter F scored significantly (p<0.05) highest in taste, aroma, texture and general acceptability. *Selroti* prepared from the batter supplemented with starter B, starter D and starter E strains had undesirable sweet sour taste and unpleasant acidic flovour due to high acid content, which were unacceptable to consumers. *Selroti* prepared from batters fermented by a mixed starter culture of *Leuc. mesenteroides* BS1:B1 and *S. cerevisiae* BA1:Y2 for 4 h, had desirable sweet taste, typical *selroti* flavour, soft texture, thus significantly (p<0.05) acceptable to judges.

Traditionally, the use of standard starter culture is not a practice in the Himalayas except in alcoholic beverage production[10]. However, optimization of the traditional processing of some naturally fermented foods of the Himalayas using starter cultures has been

Table 2—Sensory evaluation of *selroti* batter prepared using selected starter cultures

	Fermentation time (Hour)	Attribute				
		Aroma	Taste	Texture	Colour	General acceptability
A	4	3.0 ± 0.6[a]	2.8 ± 0.7[b]	2.7 ± 0.7[a]	3.2 ± 0.7[a]	2.9 ± 0.6[b]
	6	2.7 ± 1.3[a]	2.8 ± 1.1[b]	3.7 ± 1.0[a]	3.3 ± 0.7[a]	2.8 ± 1.1[b]
B	4	2.9 ± 0.6[a]	2.8 ± 0.7[b]	3.3 ± 0.8[a]	2.2 ± 0.8[b]	2.9 ± 0.6[b]
	6	3.1 ± 0.6[a]	2.9 ± 0.6[b]	2.6 ± 1.0[a]	3.1 ± 0.6[a]	2.9 ± 0.6[b]
C	4	3.0 ± 0.7[a]	3.1 ± 0.7[a]	3.8 ± 0.8[a]	3.2 ± 0.3[a]	3.2 ± 0.6[b]
	6	2.7 ± 0.6[a]	2.7 ± 0.7[b]	2.6 ± 0.9[a]	3.3 ± 0.6[a]	2.9 ± 0.8[b]
D	4	2.9 ± 0.6[a]	2.9 ± 1.0[b]	2.7 ± 0.8[a]	2.2 ± 0.8[b]	2.8 ± 0.8[b]
	6	2.4 ± 0.6[a]	2.9 ± 0.6[b]	3.3 ± 0.8[a]	3.2 ± 0.5[a]	2.8 ± 0.8[b]
E	4	2.5 ± 0.9[a]	2.1 ± 1.0[b]	1.9 ± 1.2[b]	2.3 ± 0.9[b]	2.1 ± 1.0[b]
	6	2.7 ± 0.5[a]	2.1 ± 1.2[b]	2.0 ± 1.1[b]	2.6 ± 0.7[a]	2.2 ± 1.1[b]
F	4	4.0 ± 1.0[a]	4.6 ± 1.1[a]	4.0 ± 1.1[a]	3.9 ± 0.7[a]	4.8 ± 1.0[a]
	6	3.1 ± 1.0[a]	3.4 ± 1.1[a]	3.8 ± 1.1[a]	3.7 ± 0.8[a]	3.3 ± 1.3[b]
G	4	2.8 ± 0.8[a]	3.3 ± 0.8[a]	3.3 ± 0.7[a]	3.3 ± 0.7[a]	3.2 ± 0.8[b]
	6	3.7 ± 1.0[a]	3.8 ± 1.2[a]	4.0 ± 0.9[a]	3.9 ± 0.9[a]	3.2 ± 1.0[b]

Market *selroti* was used as control (score 3), score 1, bad/hard; score 5, excellent/soft.Data represents the means scores ±SD of 10 judges. Values bearing different superscripts in each column differ significantly ($p<0.05$).A, *Leuconostoc mesenteroides* BS1:B1. B (1:1:1:1), all strains of LAB (*Enterococcus faecium* BS1:B2; *Lb. curvatus* BP:B1; *Leuc. mesenteroides* BS1:B1; *Pediococcus pentosaceus* BG2:B2). C, *Saccharomyces cerevisiae* BA1:Y2. D (1:1:1:1:1), all strains of yeasts (*Debaryomyces hansenii* BR1:Y4; *Pichia burtonii* BG1:Y1; *Saccharomyces cerevisiae* BA1:Y2; *Saccharomyces kluyveri* S3:Y3; *Zygosaccharomyces rouxii* S1:Y6). E (1:1), mixture of B and D (LAB + Yeasts) mentioned above. F (1:1), mixture of *Leuc. Mesenteroides* BS1:B1 and *S. cerevisiae* BA1:Y2.G, without inoculum.

reported[11,12,13]. None of the strains combinations of *E. faecium, Lb. curvatus, P. pentosaceus, D. hansenii, P. burtonii, S. kluyveri,* and *Z. rouxii,* used as starters could produce organoleptically acceptable *selroti* product. The principle requirements of the strains are rapid production of CO_2 from maltose and glucose, and generation of good bread flavour[14], which were performed by both isolates (*Leuc. mesenteroides* and *S. cerevisiae*) in *selroti* batters[4]. *Leuc. mesenteroides* BS1:B1 was selected as a starter culture based on its heterofermentative property, superior technological properties such as acidifying ability, antagonistic properties and high enzymatic profiles than most of the other genera[4]. *S. cerevisiae* BA1:Y2 was selected based on vigorous fermentative property and a wide spectrum of enzymes[4].

Consumers' preference trial

Selroti batter was prepared by using a consortium of *Leuc. mesenteroides* BS1:B1 and *S. cerevisiae* BA1:Y2 at 28° C for 4 h, deep-fried, and served freshly to 50 consumers from different places for consumers' preference trial. *Selroti* batter prepared in the laboratory by a mixture of cell suspension of *Leuc. mesenteroides* BS1:B1 and *S. cerevisiae* BA1:Y2 as starter was more acceptable than *selroti* batters prepared by conventional method. Market *selroti* was liked extremely (score, 9) by 8 %, very much (score, 8) by 24 % and moderately (score, 7) by 68 %, whereas *selroti* prepared from batters fermented by a pure cultures mixture of LAB + yeast (*Leuc. mesenteroides* + *S. cerevisiae*) was liked extremely by 46 %, very much by 48 % and moderately by 6 % of the consumers. Sridevi et al.[15] also demonstrated that *idli* batter prepared by using a mixture of LAB and yeasts had higher sensory scores.

Selroti prepared by using a starter culture had advantages over the traditional method, which resulted in a shorter fermentation time that eliminates the chance of growth of contaminants, hygienic conditions, maintaining consistency with better quality and flavour. The final product is not always consistent in natural fermentation; the use of a mixed starter culture could provide more consistent fermentations and products of higher quality[16]. Modern starter cultures are selected, either as single or multiple strains, especially for their adaptation to a substrate or raw material, for example cereals, milk, meat, legumes, roots, and tubers[17,18]. Commercial starter cultures of the yeast-bacterial combinations are now available for sourdough production[19]. Though, optimised process condition is

always superior and advantageous than the conventional method, however, replacement of natural and easily operated traditional technology may be difficult to change for the producers or rural populace[20].

Conclusion

Consortium of pure strains of LAB and yeasts, previously isolated from naturally fermented batters of *selroti* were tested singly or in combination for their ability to ferment rice flour to produce *selroti*. *Selroti* batters produced using a mixture of pure culture strains of *Leuc. mesenteroides* BS1:B1 and *S. cerevisiae* BA1:Y2 at 28° C for 4 h had organoleptically scored the highest acceptability. *Selroti* batters prepared by a mixed starter cultures had many advantages over the conventionally prepared products.

References

1. Tamang J P, *Himalayan Fermented Foods: Microbiology, Nutrition & Ethnic Values*. (CRC Press, New York) 2010.
2. Tamang J P, Tamang N, Thapa S, Dewan S, Tamang B M, Yonzan H, Rai A K, Chettri R, Chakrabarty J & Kharel N, Microorganisms and nutritional value of ethnic fermented foods and alcoholic beverages of North East India. *Indian J Trad Knowl,* **11** (1) (2012) 7-25.
3. Yonzan H & Tamang J P, Traditional processing of *Selroti* - a cereal-based ethnic fermented food of the Nepalis. *Indian J Trad Knowl,* **8** (1) (2009) 110-114.
4. Yonzan H & Tamang J P, Microbiology and nutritional value of selroti, an ethnic fermented cereal food of the Himalayas. *Food Biotechnol,* **24** (3) (2010) 227-247.
5. AOAC, Official methods of analysis, 15th edition. Association of Official Analytical Chemists, Virginia, 1990.
6. Meilgaard M, Civille G V & Carr B T, *Sensory Evaluation Techniques*. (CRC Press, Florida) 1990.
7. Snedecor G W & Cochran W G, *Statistical Methods*, 8th edn. (Iowa State University Press, Ames) 1989.
8. Boekhout T & Robert V, *Yeasts in Food.* (Woodhead Publishing Ltd., Verlag) 2003.
9. Tamang J P & Fleet G H, Yeasts diversity in fermented foods and beverages, in *Yeasts Biotechnology: Diversity and Applications*, edited by T. Satyanarayana & G. Kunze (Springer, New York) 2009, 169- 198.
10. Thapa S & Tamang J P, Product characterization of kodo ko jaanr: fermented finger millet beverage of the Himalayas. *Food Microbiol,* **21** (2004) 617-622.
11. Tamang J P & Nikkuni S, Selection of starter culture for production of kinema, fermented soybean food of the Himalaya. *World J Microbiol Biotechnol,* **12 (**1996) 629-635.
12. Tamang J P, Development of pulverised starter for *kinema* production. *J Food Sci Technol,* **36** (5) (1999) 475-478.
13. Tamang B & Tamang J P, *In situ* fermentation dynamics during production of *gundruk* and *khalpi*, ethnic fermented vegetables products of the Himalayas. *Indian J Microbiol,* **50** (Suppl 1) (2010) S93-S98.
14. Decock P & Cappelle S, Bread technology and sourdough technology. *Trends in Food Sci Technol,* **16** (2005) 113-120.
15. Sridevi J, Halami P M & Vijayendra S V N, Selection of starter cultures for idli batter fermentation and their effect on quality of idlis. *J Food Sci Technol,* **47(**5) (2010) 557–563.
16. Gardner N J, Savard T, Obermeier P, Caldwell G & Champagne C P, Selection and characterization of mixed starter cultures for lactic acid fermentation of carrot, cabbage, beet and onion vegetable mixtures. *Int J Food Microbiol,* **64** (3) (2001) 261-275.
17. Buchenhüskes H J, Selection criteria for lactic acid bacteria to be used as starter cultures in various food commodities. *FEMS Microbiol Rev,* **12** (1993) 253-272.
18. Holzapfel W H, Giesen R, Schillinger U & Lücke F K, Starter and protective cultures, in *Food Preservatives*, 2nd edn, edited by N J Russell & G W Gould (Kluwer Academic/Plenum Publishers, New York), 2003, 291-319.
19. De Vuyst L, Vrancken G, Ravyts F, Rimaux T & Weckx S, Biodiversity, ecological determinants, and metabolic exploitation of sourdough microbiota. *Food Microbiol,* **26** (2009) 666–675.
20. Holzapfel W H, Use of starter cultures in fermentation on a household scale. *Food Control,* **8** (5 & 6) (1997) 241-258.