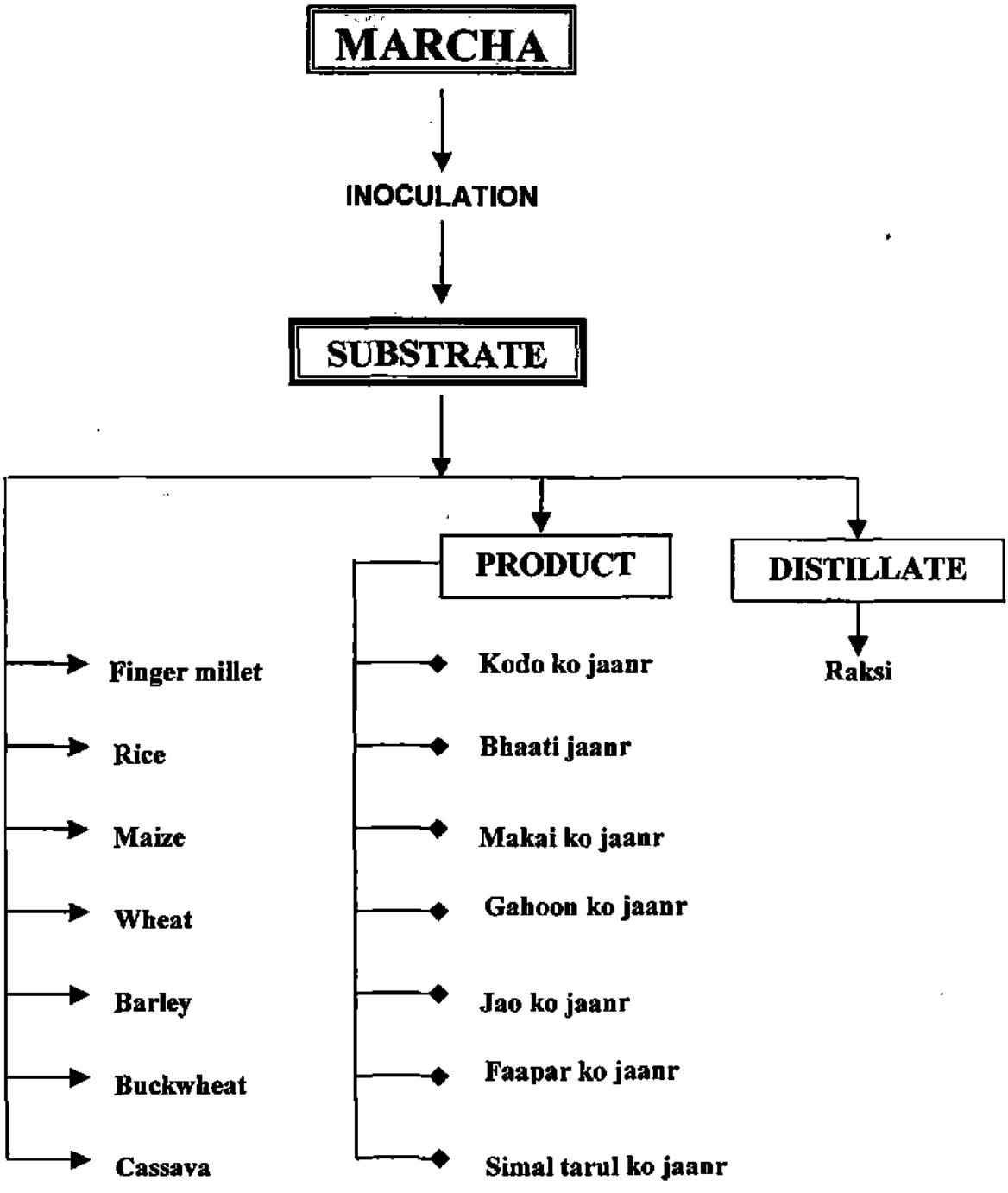
4. RESULTS

4.1. SURVEY ON FERMENTED BEVERAGES

Survey was conducted using questionnaire in the Darjeeling hills and Sikkim. The traditional methods of preparation, equipment used, mode of consumption, feeding frequency, socio-economy and ethnical importance of indigenous fermented beverages (Table 1) were documented. Survey data showed that in rural areas majority of people prepared indigenous fermented beverages (57.6 % the Darjeeling hills and 76.7% in Sikkim) for home consumption.

Table 1: Traditional fermented beverages of the Darjeeling hills and Sikkim

Product	**Substrate**	**Consumption rate (%)**	
		The Darjeeling hills	**Sikkim**
Marcha (starter)	Rice, wild herbs, spices		
Common Alcoholic Beverages:			
Kodo ko jaanr	Finger millet	95.0	85.0
Bhaati jaanr	Rice	55.0	40.0
Makai ko jaanr	Maize	45.0	35.0
Gahoon ko jaanr	Wheat	15.0	25.0
Lesser-known Alcoholic Beverages:			
Simal tarul ko jaanr	Cassava tuber	< 10.0	< 10.0
Jao ko jaanr	Barley	< 10.0	< 10.0
Faapar ko jaanr	Buck wheat	< 10.0	< 10.0
Distilled liquor:			
Raksi	Starchy substrates	85.0	60.0


MARCHA
INOCULATION
SUBSTRATE
PRODUCT
DISTILLATE
Raksi
Finger millet
Rice
Maize
Wheat
Barley
Buckwheat
Cassava
Kodo ko jaanr
Bhaati jaanr
Makai ko jaanr
Gahoon ko jaanr
Jao ko jaanr
Faapar ko jaanr
Simal tarul ko jaanr

4.2. MARCHA

Marcha is the traditionally prepared mixed dough inocula used as a starter culture for production of various indigenous alcoholic beverages. Marcha is a dry, round to flattened, creamy white to dusty white, solid ball like starter ranging from 1.9 cm to 11.8 cm in diameter with the weight ranging from 2.3 g to 21.2 g (Plate 1).

4.2.1. Synonym of marcha

Marcha is a Nepali word. Different ethnic communities of this region call it by their own dialect such as *khesung* by Limboo, *bharama* by Tamang, *bopkha* or *khabed* by Rai, *phab* by Bhutia, and also by the Tibetan, and *buth/thanbum* by the Lepcha.

4.2.2. Traditional method of preparation

A traditional method of marcha preparation practiced by the Limboo women in Aho village in East Sikkim was cited. During marcha preparation, glutinous rice (*Oryza sativa* L.) is soaked in water for 6-8 h at ambient temperature. Unheated soaked rice is crushed in a foot-driven heavy wooden mortar by a pestle. In 1 kg of grinned rice, ingredients added include roots of 'guliyo jara' or 'chitu' (*Plumbago zeylanica* L.), 2.5 g; leaves of 'bheemsen paate' (*Buddleja asiatica* Lour), 1.2 g; flowers of 'sengreknna' {*Vernonia cinerea* (L.) Less}, 1.2 g; ginger, 5.0 g; red dry chilli, 1.2 g; and previously prepared marcha as a mother culture, 10.0 g. The mixture is then made into paste by adding water and kneaded into flat cakes of varying sizes and shapes, and placed individually on the ceiling floor made up of bamboo stripes above the kitchen, bedded with fresh fronds of ferns, locally called 'pire uneu'

{*Glaphylopteriolopsis erubescens* (Wall ex Hook.) Ching}, and covered with dry ferns (Plate 2 & 3) and jute bags. These are left to ferment for 1-3 days, the longer period being used under the colder condition. Completion of fermentation is indicated by distinct alcoholic and ester-aroma and puffy/swollen appearance of marcha. Finally, cakes of marcha are sun dried for 2-3 days (Fig 1). Marcha is stored at room temperature and in dry place for more than a year.

In Jhosing and Tibuk villages in North Sikkim, root-barks and flowers of wild herbs locally called 'marcha jar' (*Polygala arillata* Buch. Hum.) are mixed and grinded with soaked water during marcha preparation.

Rice
↓
Soaked in water (6-8 h)
↓
Crushed in foot-driven heavy wooden mortar
↓
← Mixed with herbs, spices and previously prepared marcha powder (~ 1 %)
↓
Made into paste and kneaded into flat cakes
↓
Wrapped in fern leaves, covered by jute bags
↓
Fermented (25-35° C, 1-3 d)
↓
Sun dried (2-3 d)
↓
Marcha

Fig 1. Flow sheet of marcha preparation in East Sikkim

Plate 1. Marcha of varying sizes

Plate 2. Marcha preparation by the Limboo woman in Aho village.

Plate 3. Kneaded dough of marcha are incubated in fern leaves.

Plate 4. Marcha is sold in Gangtok market.

4.2.3. Socio-economy

Marcha is produced at household level in few villages in the Sikkim Himalayas (Table 2 and Fig 2) exclusively by the rural women belonging to the Limboo, Rai and the Lepcha. Men can help women in collection of wild herbs and pounding the herbs during marcha preparation. This art of technology is protected as hereditary trade and passes from mother to daughters.

Table 2. Important marcha-making villages in the Sikkim Himalayas

Village	Location	Altitude (Feet)	Linked Market	Sample code	Dominant marcha-maker
The Darjeeling Hills:					
Nor Busty	Darjeeling	2300	Bijanbari, Darjeeling	MN	Rai and Limboo
Kashyong	Kalimpong	4500	Algarah, Kalimpong, Rhenock	MK	Limboo and Rai
Mangzing	Kalimpong	1800	Kalimpong, Lava, Gorubathan	MM	Limboo, Rai and Lepcha
Sikkim:					
Jhosing	North	3200	Magan, Gangtok	MJ	Limboo
Tibuk	North	3120	Magan, Gangtok	MT	Limboo
Chhejo	West	6800	Geyzing, Legsep	MC	Limboo
Lingchom	West	5000	Geyzing, Yoksum	ML	Limboo
Salghari	South	4150	Jorethang	MS	Limboo
Barnyak	South	4000	Namchi, Ravangla	MB	Limboo and Rai
Aho	East	3630	Gangtok, Pakyong	MA	Limboo
Kopchey	East	3510	Rhenok, Rongli	MKo	Limboo

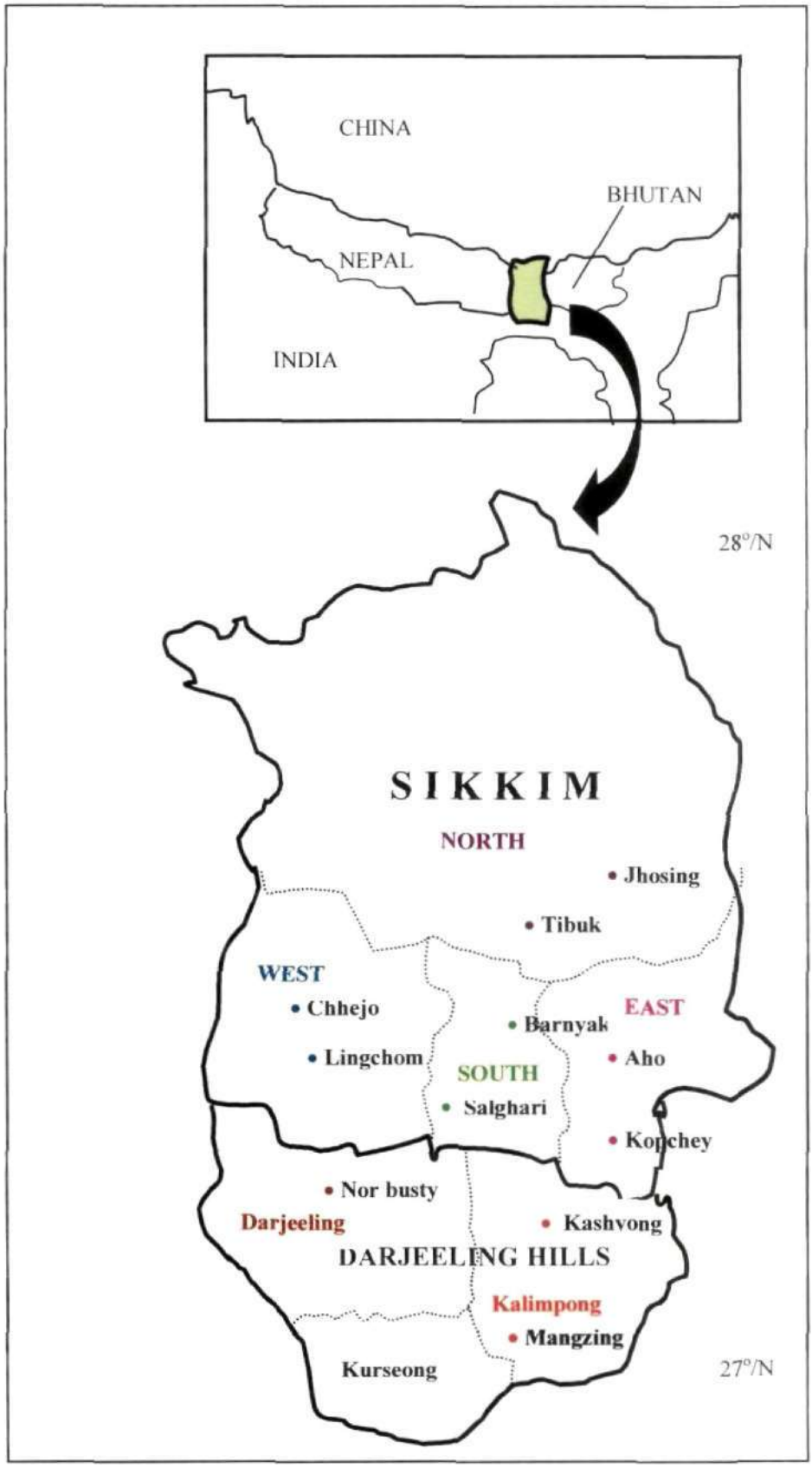


Fig 2. Location map of marcha-making villages in the Sikkim Himalayas.

Some people are economically dependent upon this product. Marcha is sold in local markets, called "haats" (Plate 4). Cost of marcha depends on its size. Small-sized marcha (~2.0 cm) costs about 0.50 paise each, while the large-sized (~12 cm) marcha is sold at Rs.5.00 per piece.

4.2.4. Similar Product

Marcha is similar to ragi of Indonesia, nuruk of Korea, bubod of the Philippines, loogpang of Thailand and chiuyueh of China. These starter cultures are used to prepare alcoholic beverages from starchy substrates.

4.2.5. Microorganisms

Sixty-six samples of marcha were collected from main source of marcha-making villages of the Darjeeling hills and Sikkim as shown in Table 2. Samples were analysed for microbial load (Table 3). Mould population in marcha was detected at the level of 10^6 cfu/g, whereas the loads of yeasts and lactic acid bacteria were 10^8 cfu/g and 10^7 cfu/g, respectively. Total viable count was at the level of 10^8 cfu/g. Out of 733 strains of microorganisms isolated from marcha samples, 152 isolates were filamentous moulds, 321 were yeasts and 260 were lactic acid bacteria.

Table 3. Microbial load of marcha collected from marcha-making villages

Source	× 10^7 cfu/g dry weight			
	Mould	Yeast	LAB	Total Viable Count
Nor Busty	0.2 (0.1-0.3)	3.3 (2.8-3.8)	3.5 (2.8-4.1)	6.9 (5.3-9.8)
Kashyong	0.2 (0.1-0.3)	5.6 (4.6-6.7)	3.7 (1.6-6.5)	9.1 (5.9-11.8)
Mangzing	0.2 (0.1-0.3)	0.7 (0.5-0.9)	0.3 (0.2-0.4)	1.4 (0.6-2.3)
Jhosing	0.3 (0.1-0.2)	8.1 (5.8-10.4)	4.7 (1.4-7.7)	15.0 (14.0-18.0)
Tibuk	0.1 (0.08-0.14)	23.0 (15.0-29.0)	13.0 (6.0-18.0)	37.0 (34.0-40.0)
Chhejo	0.2 (0.1-0.2)	9.7 (6.3-11.0)	0.5 (0.3-0.7)	12.0 (8.0-16.0)
Lingchom	0.3 (0.1-0.4)	6.7 (6.3-13.0)	1.0 (0.3-1.2)	10.0 (7.0-15.0)
Salghari	0.2 (0.1-0.3)	21.0 (15.0-26.0)	16.0 (4.0- 30.0)	28.0 (24.0-33.0)
Barnyak	0.08 (0.05-0.1)	18.0 (15.0-21.0)	7.8 (6.8-8.5)	29.0 (24.0-33.0)
Aho	0.3 (0.2-0.3)	15.0 (13.0-18.0)	18.0 (16.0-20.0)	34.0 (31.0-36.0)
Kopchey	0.15 (0.1-0.2)	1.0 (0.4-1.8)	2.0 (0.9-3.6)	3.5 (1.8-5.3)

LAB, lactic acid bacteria
Data represent the means of 6 samples from each source. Ranges are given in parentheses.

4.2.5.1. Characterisation and identification of moulds

On the basis of morphology and presence or absence of stolon and rhizoids, all 152 strains of filamentous moulds isolates from each source were classified into two genera: *Mucor* (94 strains) and *Rhizopus* (58 strains) belonging to family Mucoraceae (Table 4).

Table 4. Selection of representative strains of moulds isolated from marcha[a]

Source	Number of strains[b] isolated	Stolon and rhizoids	Grouped strains	Representative strains
Nor Busty	12	Absent	6	MN:Mu1
		Present	6	MN:Rh1
Kashyong	14	Absent	8	MK:Mu2
		Present	6	MK:Rh2
Mangzing	7	Absent	7	MM:Mu1
Jhosing	13	Absent	8	MJ:Mu1
		Present	5	MJ:Rh1
Tibuk	20	Absent	10	MT:Mu1
		Present	10	MT:Rh1
Chhejo	16	Absent	8	MC:Mu1
		Present	8	MC:Rh2
Lingchom	10	Absent	10	ML:Mu2
Salghari	16	Absent	8	MS:Mu3
		Present	8	MS:Rh2
Barnyak	10	Absent	10	MB:Mu1
Aho	18	Absent	10	MA:Mu1
		Present	8	MA:Rh3
Kopchey	16	Absent	9	MKo:Mu4
		Present	7	MKo:Rh1

[a]Number of samples was six from each source.

[b]All strains had aseptate mycelia.

Table 5. Characteristics of representative strains of moulds isolated from marcha

Strain	Sporangium		Sporangiospore		Identification
	Shape	Diameter (µ m)	Shape	Diameter (µ m)	
MM:Mu1	Globose, borne circinately	30-68	Ellipsoidal to oval	5.6-7.2	*Mucor*
MJ:Mu1	Globose	18-52	Ellipsoidal to oval	4.8-7.2	
MA:Mu1	Globose, borne circinately	34-92	Ellipsoidal to oval	4.2-5.6	
MC:Mu1	Globose	28-80	Spherical, ellipsoidal to oval	2.4-5.6	
MS:Mu3	Globose, borne circinately	32-80	Ellipsoidal to oval	4.5-5.6	
MJ:Rh3	Globose to sub-globose	72-144	Round to ellipsoidal	4.0-8.0	*Rhizopus*
MA:Rh3	Globose	52-116	Round	2.0-5.0	
MC:Rh2	Globose to sub-globose	44-120	Round to ellipsoidal	5.0-8.0	
MS:Rh1	Globose to sub-globose	72-148	Round to ellipsoidal	4.0-8.5	
MKo:Rh2	Globose	76-160	Round	3.0-6.0	

Following the taxonomical keys of Schipper (1976, 1984) and Hesseltine (1991), representative strains MM:Mu1 (IMI 375454), MA:Mu1 and MS:Mu3 which had repeatedly branched sporangiophores with many sporangia borne circinately (Plate a) were identified as *Mucor circinelloides* forma *circinelloides* van Tieghem. Representative strains MJ:Mu1 (IMI 375452) and MC:Mu1 were identified *Mucor* sp. close to *M. hiemalis* sensu lato. Sporangial walls of these isolates were

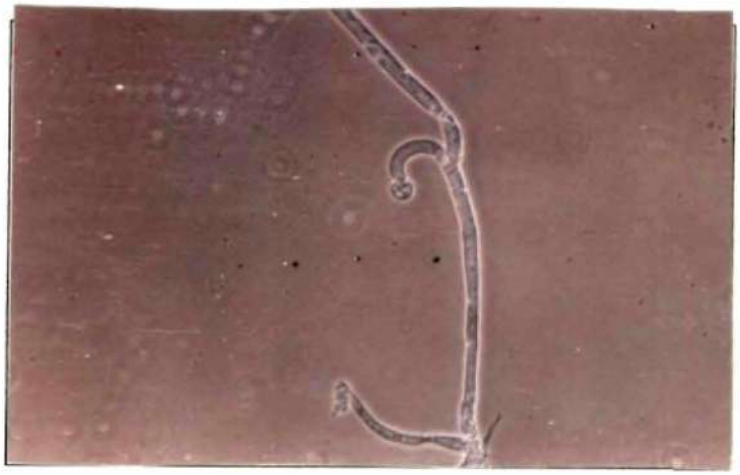


Plate (a). *Mucor circinelloides* forma *circinelloides* MJ:Mu1 (PDA, 3 d, 28° C), isolated from marcha, showing circinately borne sporangium in phase contrast micrograph (× 480).

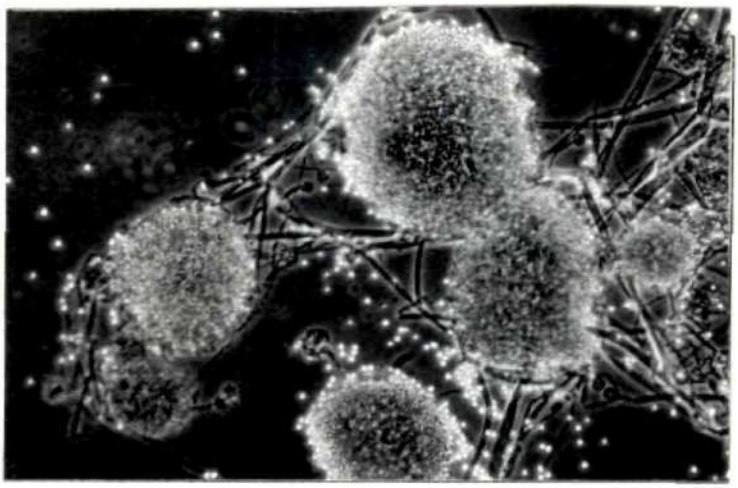


Plate (b). *Mucor circinelloides* forma *circinelloides* MJ:Mu1 (PDA, 7 d, 28° C), isolated from marcha, showing deliquesced sporangia with sporangiospores in phase contrast micrograph (× 250).

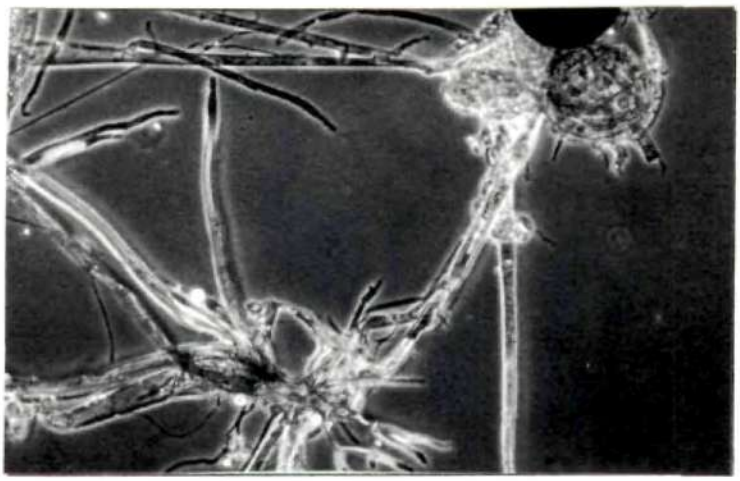


Plate (c). *Rhizopus chinensis* MJ:Rh3 (PDA, 7 d, 28° C), isolated from marcha, showing rhizoids, sporangiophores and sporangia in phase contrast micrograph (× 250).

mostly deliquesced (Plate b). Representative strains MJ:Rh3, MC:Rh2 and MS:Rh1 were identified as *Rhizopus chinensis* Saito (Plate c). Representative strains MA:Rh3 (IMI 375453) and MKo:Rh1 were identified as *Rhizopus stolonifer* variety *lyococcus* (Ehrenb.) Stalp. & Schipper. Confirmation of identity of these strains was done at International Mycological Institute, Surrey, U.K. Prevalence of *Mucor* and *Rhizopus* was 100 % and 73 %, respectively in sixty-six samples collected from eleven different marcha-making villages in the Sikkim Himalayas.

4.2.5.2. Characterisation and identification of yeasts

Four genera of yeasts were selected on the basis of colony, cell morphology, vegetative reproduction and type of ascospore among 321 yeasts isolates (Table 6a & b).

Table 6a. Selection of representative strains of yeasts isolated from marcha of the Darjeeling hills

Source	Number[a] of strains isolated	Colony	Cell Shape	Mycelium	Ascospore	Grouped strains	Representative strains
Nor Busty	30	Ds	O-Cy	True	Hat-shaped	10	MN:YD3
		Ss	O-E	Pseudo	Hat-shaped	8	MN:YP1
		Ss	O-E	Pseudo	Globose	6	MN:YS1
		Fs	O-E	–	–	6	MN:YC2
Kashyong	32	Ds	O-Cy	True	Hat-shaped	10	MK:YD1
		Ss	O-E	Pseudo	Hat-shaped	10	MK:YP1
		Ss	O-E	Pseudo	Globose	6	MK:YS5
		Fs	O-E	–	–	6	MK:YC1
Mangzing	33	Ds	O-Cy	True	Hat-shaped	15	MM:YD3
		Ss	O-E	Pseudo	Hat-shaped	10	MM:YP2
		Ss	O-E	Pseudo	Globose	8	MM:YS1

[a]Number of samples was six from each source. All isolates reproduced by multilateral budding.

Ds, dusty surface; Ss, smooth surface; Fs, fringed surface; O-Cy, Oval to cylindrical; O-E, Oval to ellipsoidal.

Table 6b. Selection of representative strains of yeasts isolated from marcha of Sikkim

Source	Number[a] of strains isolated	Colony	Cell Shape	Mycelium	Ascospore	Grouped strains	Representative strains
Jhosing	26	Ds	O-Cy	True	Hat-shaped	12	MJ:YD1
		Ss	O-E	Pseudo	Hat-shaped	8	MJ:YP1
		Ss	O-E	Pseudo	Globose	6	MJ:YS2
Tibuk	35	Ds	O-Cy	True	Hat-shaped	15	MJ:YD2
		Ss	O-E	Pseudo	Hat-shaped	12	MT:YP2
		Ss	O-E	Pseudo	Globose	8	MT:YS1
Chhejo	29	Ds	O-Cy	True	Hat-shaped	10	MC:YD2
		Ss	O-E	Pseudo	Hat-shaped	8	MC:YP1
		Ss	O-E	Pseudo	Globose	6	MC:YS1
		Fs	O-E	–	–	5	MC:YC1
Lingchom	28	Ds	O-Cy	True	Hat-shaped	10	ML:YD2
		Ss	O-E	Pseudo	Hat-shaped	7	ML:YP1
		Ss	O-E	Pseudo	Globose	6	ML:YS7
		Fs	O-E	–	–	5	ML:YC1
Salghari	26	Ds	O-Cy	True	Hat-shaped	12	MS:YD2
		Ss	O-E	Pseudo	Hat-shaped	8	MS:YP1
		Ss	O-E	Pseudo	Globose	6	MS:YS1
Barnyak	24	Ds	O-Cy	True	Hat-shaped	10	MB:YD1
		Ss	O-E	Pseudo	Hat-shaped	8	MB:YP2
		Ss	O-E	Pseudo	Globose	6	MB:YS2
Aho	32	Ds	O-Cy	True	Hat-shaped	12	MA:YD1
		Ss	O-E	Pseudo	Hat-shaped	8	MA:YP2
		Ss	O-E	Pseudo	Globose	6	MA:YS2
		Fs	O-E	–	–	6	MA:YC3
Kopchey	26	Ds	O-Cy	True	Hat-shaped	10	MKo:YD2
		Ss	O-E	Pseudo	Hat-shaped	8	MKo:YP1
		Ss	O-E	Pseudo	Globose	8	MKo:YS3

[a]Number of samples was six from each source. All isolates showed by multilateral budding. Ds, dusty surface; Ss, smooth surface; Fs, fringed surface; O-Cy, Oval to cylindrical; O-E, Oval to ellipsoidal.

Sugar fermentation and assimilation tests of randomly selected representative strains of yeasts were carried out (Table 7). Following the taxonomical keys described by Kreger-van Rij (1984) and Kurtzman and Fell (1998), representative strains MC:YD2 and MKo:YD2 had dusty and dry surfaced colonies with horn-like projections made up of many strands of mycelia when grown on agar plates. There were 2 to 4 hat-shaped ascospores per ascus. All of them fermented starch and assimilated sucrose. These strains were identified as *Saccharomycopsis fibuligera* (Lindner) Klöcker (Plate d). Representative strains MN:YP1 and MJ:YP1 had glistening surfaced colonies on agar plates, showed 1 to 4 hat-shaped ascospores per ascus and could not ferment starch. Asci were deliquescent. They were identified as *Pichia anomala* (E.C. Hansen) Kurtzman (Plate e). Representative strains MK:YS5 and MS:YS1 had smooth surfaced colonies, showing globose ascospores and fermented starch. They were identified as *Saccharomyces cerevisiae* Meyen ex Hansen (Plate f). Representative strains MN:YC2 and ML:YC1 showed fringe-surfaced colonies without ascus and ascospore and fermented only glucose and trehalose. They were identified as *Candida glabrata* (Anderson) Meyer et Yarrow (Plate g).

Prevalence of all strains of yeasts in marcha samples was 100 % except *Candida glabrata* showing only 45 % in sixty-six samples analysed.

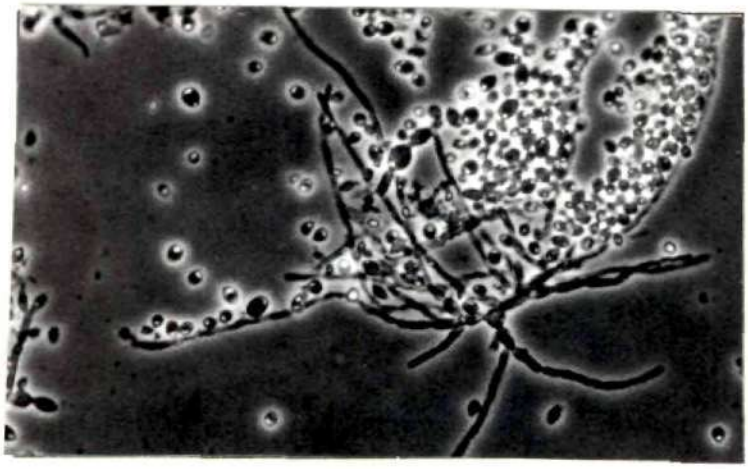


Plate (d). *Saccharomycopsis fibuligera* MC:YD2 (YM agar, 5 d, 28° C), isolated from marcha, showing oval to cylindrical cells with true mycelia in phase contrast micrograph (× 480).

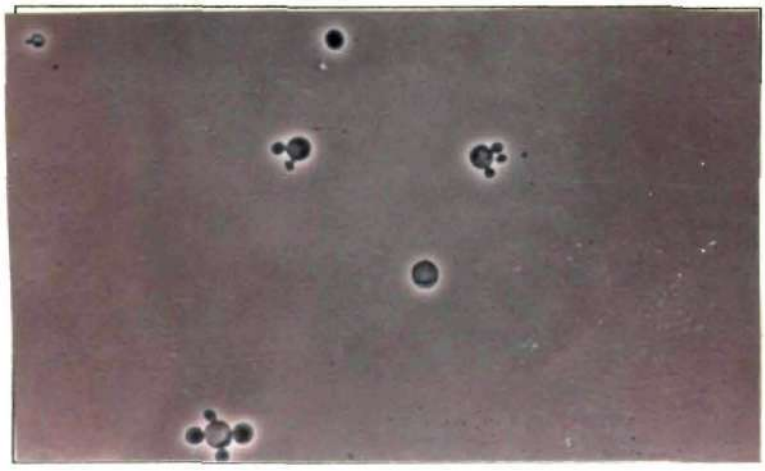


Plate (e). *Pichia anomala* MN:YP1 (YM agar, 3 d, 28° C), isolated from marcha, showing oval cells with multilateral budding in phase contrast micrograph (× 480).

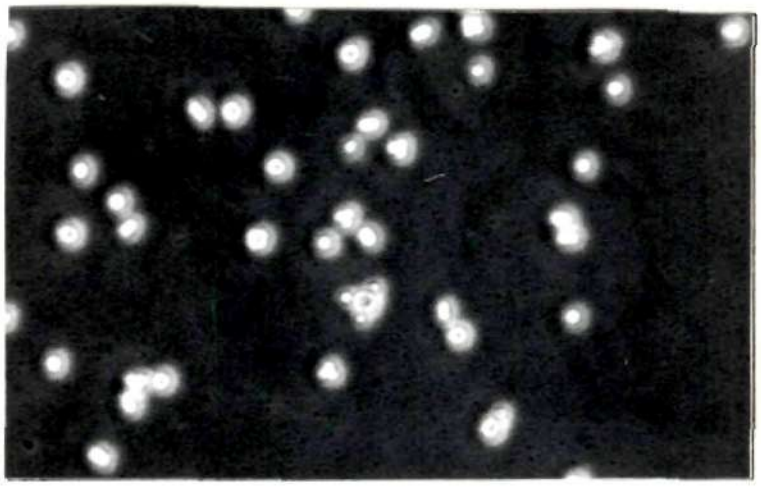


Plate (f). *Saccharomyces cerevisiae* MK:YS5 (Ascospore agar, 7 d, 28° C), isolated from marcha, showing globose ascospores in phase contrast micrograph (× 480).

Table 7. Characteristics of representative strains of yeasts isolated from marcha

Parameter	MC:YD2	MKo:YD2	MN:YP1	MJ:YP1	MK:YS5	MS:YS1	MN:YC2	ML:YC1
Cell width (μm)	2.0-5.0	2.0-5.0	1.6-4.5	1.6-3.2	1.6-4.5	1.6-4.0	0.8-2.4	0.6-2.7
Cell length (μm)	3.0-7.0	3.0-7.0	1.9-5.4	1.9-5.4	2.0-5.0	2.0-5.0	1.6-4.0	1.3-3.7
Nitrate reduction	–	–	+	+	–	–	–	–
Growth at 37^0 C	+	+	+	+	+	+	+	+
Sugar fermentation:								
Glucose	+	+	+	+	+	+	+	+
Galactose	–	–	–	–	+	+	–	–
Lactose	–	–	–	–	–	–	–	–
Maltose	+	+	+	+	+	+	–	–
Raffinose	–	+	+	+	+	+	–	–
Sucrose	+	+	+	+	+	+	–	–
Starch	+	+	–	–	+	+	–	–
Trehalose	–	–	+	–	–	–	+	+
Sugar assimilation:								
Arabinose	–	–	+	$+_w$	–	–	–	–
Cellobiose	+	+	+	+	–	–	–	–
Galactose	–	–	+	+	+	+	–	–
Glycerol	–	–	–	–	–	–	–	$+_w$
Inositol	+	–	–	–	–	–	–	–
Lactose	–	–	–	–	–	–	–	–
Maltose	+	+	+	+	+	+	–	–
Melibiose	+	+	–	–	+	+	–	–
Mannitol	–	+	+	+	–	–	–	–
Rhamnose	–	–	–	–	–	–	–	–
Raffinose	+	+	+	+	+	+	–	–
Sucrose	+	+	+	+	+	+	–	–
Starch	+	+	+	+	+	+	–	–
Trehalose	–	–	+	+	+	+	+	+
Xylose	–	–	–	+	–	–	–	–
Identification	*Saccharomycopsis*		*Pichia*		*Saccharomyces*		*Candida*	

+, positive; –, negative; $+_w$, weak positive

4.2.5.3. Characterisation and identification of bacteria

Out of 260 lactic acid bacteria strains isolated from sixty-six samples of marcha, 195 strains were coccii-tetrads and 65 strains were non-sporeforming rods (Table 8).

Table 8. Selection of representative strains of LAB isolated from marcha

Source	Number[a] of strains isolated	Cell Shape	Gas from glucose	NH_3 from arginine	Grouped strains	Representative strains
Nor Busty	35	Coccus	–	+	20	MN:C1
		Rod	+	–	15	MN:R5
Kashyong	20	Coccus	–	+	20	MK:C7
Mangzing	30	Coccus	–	+	20	MM:C3
		Rod	+	–	10	MM:R1
Jhosing	30	Coccus	–	+	20	MJ:C2
		Rod	+	–	10	MJ:R2
Tibuk	15	Coccus	–	+	15	MT:C1
Chhejo	15	Coccus	–	+	15	MC:C7
Lingchom	30	Coccus	–	+	20	ML:C3
		Rod	+	–	10	ML:R8
Salghari	20	Coccus	–	+	20	MS:C7
Barnyak	25	Coccus	–	+	15	MB:C9
		Rod	+	–	10	MB:R4
Aho	25	Coccus	–	+	15	MA:C1
		Rod	+	–	10	MA:R5
Kopchey	15	Coccus	–	+	15	Mko:C1

[a]Number of samples was 6 from each source

[b]All isolates were Gram-positive, catalase-negative, non-sporeformers and non-motile

All isolates of lactic acid bacteria were Gram-positive, non-sporeforming, non-motile, catalase negative and facultative anaerobes; they did not hydrolyse casein, gelatin and starch (Table 9a). Representative strains MN:C1, MM:C3, MA:C1 and MKo:C1 were coccii in tetrads, grew well in 4 % and 6.5 % NaCl but not in 18 % NaCl, produced no gas from glucose. Phenotypic and sugar fermentation characterisation of coccii representative strains MK:C7, MJ:C2, MT:C1, and MS:C7 were identical to strains MN:C1 whereas strains MC:C7, MC:C3, and MB:C9 were identical to MA:C1. All rod-shaped lactic representative strains MN:R5, MJ:R2, MB:R4 and MA:R5 produced gas from glucose. Rod-shaped lactic strain MM:R1 was identical to MN:R5 whereas strain ML:R8 was identical to MA:R5. Following sugar fermentation pattern of isolates using API 50 CHL system (Table 9b) and the taxonomical keys of Sneath *et al.* (1986) and Wood and Holzapfel (1995), coccii-tetrads were identified as *Pediococcus pentosaceus* Mees (Plate h). *P. pentosaceus* is closely related to *P. acidilactici* due to hydrolysis of arginine, but is distinguished from *P. acidilactici* due to fermentation of maltose and trehalose, and no growth at 50° C. Rod strains were identified as heterofermentative *Lactobacillus bifermentans* Kandler, Schillinger and Weiss (Plate i) due to similar characters such as gas from glucose, no hydrolysis of arginine, growth at 15° C but no growth at 45° C, fermented ribose, D-xylose and L-arabinose. However, *Lb. bifermentans* has been placed in Group B (facultative heterofermentative lactobacilli) by Hammes and Vogel (1995).

Prevalence of *P. pentosaceus* was 100 %, whereas that of *Lb. bifermentans* was only 54 % in sixty-six samples analysed.

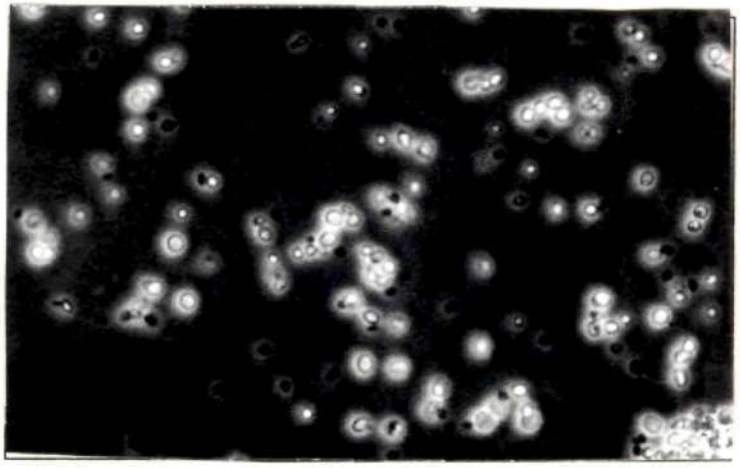


Plate (g). *Candida glabrata* MN:YC2 (YM agar, 3 d, 28° C), isolated from marcha, showing oval to ellipsoidal cells in phase contrast micrograph (× 480).

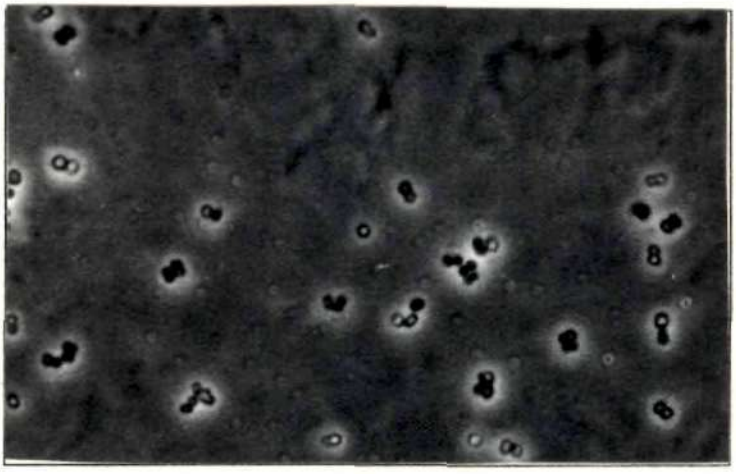


Plate (h). *Pediococcus pentosaceus* MA:C1 (MRS agar, 3 d, 30° C), isolated from marcha, showing coccoid cells in tetrads in phase contrast micrograph (× 1200).

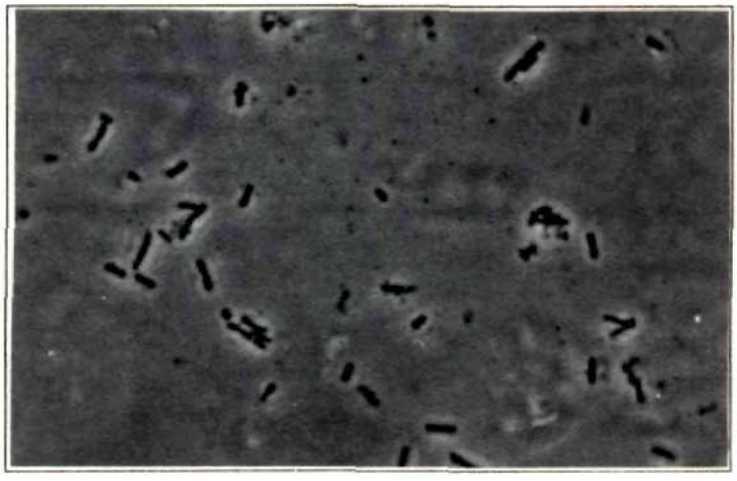


Plate (i). *Lactobacillus bifermentans* MA:R5 (MRS agar, 3 d, 30° C), isolated from marcha, showing non-sporeforming rod cells in phase contrast micrograph (× 1200).

Table 9a. Phenotypic characterisations of representative strains of LAB isolated from marcha

Parameter	MN:C1	MM:C3	MA:C1	MKo:C1	MN:R5	MJ:R2	MB:R4	MA:R5
Cell shape	Ct	Ct	Ct	Ct	R	R	R	R
Cell diameter ((µm)	0.2-0.7	0.2-0.6	0.2-0.6	0.4-0.7				
Cell width (µm)					0.2-0.3	0.2-0.3	0.2-0.3	0.2-0.3
Cell length (µm)					0.8-2.3	0.8-2.2	1.0-2.0	1.0-2.4
Anaerobic growth	+	+	+	+	+	+	+	+
Hydrolysis of:								
Casein	–	–	–	–	–	–	–	–
Gelatin	–	–	–	–	–	–	–	–
Arginine	+	+	+	+	–	–	–	–
Starch	–	–	–	–	–	–	–	–
Indole production	–	–	–	–	–	–	–	–
Nitrate reduction	–	–	–	–	–	–	–	–
Growth in NaCl:								
4.0 %	+	+	+	+	+	+	+	+
6.5 %	+	+	+	+	+	+	+	+
10.0 %	+	+	+	+	+	+	+	+
18.0 %	–	–	–	–	–	–	–	–
Growth in pH:								
4.2	+	+	+	+	+	+	+	+
7.5	+	+	+	+	+	+	+	+
8.5	+	+	+	+	+	+	+	+
Growth at:								
15° C	+	+	+	+	+	+	+	+
45° C	–	–	–	–	–	–	–	–

Ct, coccii-tetrad; R, rod in chain with 2-3 cells

Table 9b. Sugar fermentation of LAB strains using API 50 CHL system

Parameter	MN:C1	MM:C3	MA:C1	MKo:C1	MN:R5	MJ:R2	MB:R4	MA:R5
Glycerol	+	+	–	–	–	–	+	–
Erythritol	+	+	–	–	–	–	–	–
D-Arabinose	+	+	–	–	–	–	–	–
L-Arabinose	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	+
L-Xylose	–	–	–	–	–	–	–	$+_w$
Adonitol	+	+	–	–	–	–	+	–
β-Methyl-D-Xyloside	–	–	–	–	–	–	$+_w$	–
Galactose	+	+	+	+	+	$+_w$	–	$+_w$
D-Glucose	–	–	+	+	+	–	+	+
D-Fructose	–	–	+	+	+	+	+	+
D-Mannose	–	$+_w$	+	+	–	–	+	–
L-Sorbose	$+_w$	–	–	–	–	–	–	–
Rhamnose	+	+	–	–	–	–	+	–
Dulcitol	+	+	–	–	–	–	–	–
Inositol	–	–	–	–	–	–	–	–
Mannitol	–	–	–	–	–	–	–	$+_w$
Sorbitol	+	+	–	–	–	–	–	–
α-Methyl-D-Mannoside	+	+	–	–	–	$+_w$	+	–
α-Methyl-D-Glucoside	–	–	–	–	$+_w$	$+_w$	+	+
N-Acetyl-Glucosamine	+	+	+	+	$+_w$	–	+	+
Amygdalin	+	+	+	+	–	–	–	–
Arbutin	–	–	–	–	–	–	–	–
Esculin	–	–	–	–	–	–	–	–
Salicin	$+_w$	–	$+_w$	–	–	–	–	–
Cellobiose	+	+	+	+	–	–	–	–
Maltose	+	+	+	+	+	+	+	$+_w$
Lactose	–	–	–	+	–	–	–	–

Parameter	MN:C1	MM:C3	MA:C1	MKo:C1	MN:R5	MJ:R2	MB:R4	MA:R5
Melibiose	–	–	–	–	$+_w$	$+_w$	+	$+_w$
Sucrose	–	–	–	–	–	–	–	–
Trehalose	+	+	+	+	–	–	–	–
Inulin	–	–	–	–	–	–	–	–
Melezitose	–	–	–	–	–	–	–	–
Raffinose	–	–	–	–	–	–	–	–
Starch	–	–	–	–	–	–	–	–
Glycogen	–	–	–	–	–	–	–	–
Xylitol	–	–	–	–	–	–	–	–
Gentiobiose	+	+	+	+	–	–	–	–
D-Turanose	–	–	–	–	–	–	–	–
D-Lyxose	–	–	–	–	–	–	–	–
D-Tagatose	+	+	+	+	–	–	–	–
D-Fucose	–	–	–	–	–	–	–	–
L-Fucose	–	–	–	–	–	–	–	–
D-Arabitol	–	–	–	–	–	–	–	–
L-Arabitol	–	–	–	–	–	–	–	–
Gluconate	–	–	–	–	$+_w$	$+_w$	–	–
2-Keto-Gluconate	–	–	–	–	$+_w$	$+_w$	–	–
5-Keto-Gluconate	–	–	–	–	$+_w$	$+_w$	–	$+_w$
Identification	*Pedicoccus*				*Lactobacillus*			

4.2.6. Composition of marcha

Marcha is slightly acidic in nature containing pH 5.58 with 0.1 % acidity. Marcha contained 14 % moisture and 1.4 % ash (Table 10).

Table 10. Proximate composition of marcha

Parameter	Kashyong	Salghari	Jhosing	Aho	Chhejo
pH	5.77 (5.76-5.78)	6.20 (6.16-6.24)	5.28 (5.27-5.29)	5.43 (5.43-5.43)	5.20 (5.18-5.22)
Acidity (%)	0.10 (0.10-0.10)	0.10 (0.10-0.10)	0.10 (0.10-0.11)	0.10 (0.10-0.10)	0.12 (0.11-0.13)
Moisture (%)	15.3 (14.6-15.8)	13.9 (12.6-14.2)	16.2 (13.3-16.8)	13.3 (12.2-13.6)	14.0 (12.3-15.0)
Ash (%)	1.8 (1.2-2.0)	2.6 (2.3-2.7)	1.0 (0.8-1.3)	0.9 (0.7-1.2)	0.9 (0.7-1.3)

Data represent the means of 5 samples from each source. Ranges are given in parentheses.

4.2.7. Enzymatic activities of isolates

Enzymatic profiles of selected strains of moulds, yeasts and lactic acid bacteria isolates of marcha were assayed using API zym (bioMérieux, France) galleries (Table 11). Marcha isolates showed relatively weak esterase and lipase activities as compared with phosphatase activities. Preliminary screenings of amylolytic activities of all isolates of marcha were tested in starch agar plates. On the basis of amylolytic activity (showing >1.0 mm hydrolysis zone in starch agar plate), 5 strains of *Rhizopus* spp., 6 strains of *Mucor* spp., 7 strains of *Saccharomycopsis fibuligera*, 5 strains of *Pichia anomala*, 5 strains of *Saccharomyces cerevisiae* and 4 strains of *Candida glabrata* were selected for α-amylase and glucoamylase assays (Table 12). None of the lactic acid bacteria showed amylolytic activity, hence they were not selected for amylolytic enzyme assay. *Saccharomycopsis fibuligera* MS:YD4 showed highest liquefying activity (α-amylase) and *Rhizopus chinensis* MJ:Rh3 showed highest saccharifying activity (glucoamylase).

Table 11. Enzymatic profiles using API zym system of representative strains of microorganisms isolated from marcha

Enzyme	Activity (nanomoles)								
	A	B	C	D	E	F	G	H	I
Control (without enzyme)	0	0	0	0	0	0	0	0	0
Phosphatase alcaline	≥40	≥40	0	5	5	20	≥40	0	0
Esterase (C4)	0	0	5	5	5	5	10	5	0
Esterase Lipase (C8)	0	≥40	5	5	0	10	20	5	0
Lipase (C14)	0	0	0	0	0	0	0	0	0
Leucine arylamidase	≥40	≥40	≥40	≥40	≥40	≥40	≥40	≥40	≥40
Valine arylamidase	0	5	5	5	5	0	0	≥40	≥40
Cystine arylamidase	0	5	0	0	0	0	0	5	5
Trypsin	0	0	0	0	0	0	0	0	0
Chymotrypsin	0	0	0	0	0	0	0	0	0
Phoshatase acid	≥40	5	≥40	5	5	10	10	5	5
Napthol-AS-BI-phosphohydrolase	≥40	≥40	5	10	10	10	10	5	5
α-galactosidase	0	0	0	0	0	0	20	10	0
β-galactosidase	0	0	0	0	0	0	0	≥40	0
β-glucuronidase	0	0	0	0	0	0	0	0	0
α-glucosidase	5	5	0	0	0	0	0	≥40	0
β-glucosidase	≥40	≥40	0	0	0	0	0	≥40	5
N-acetyl-β-glucosaminidase	0	0	0	0	0	0	0	0	10
α-mannosidase	10	0	0	0	0	0	0	0	0
α-fucosidase	0	0	0	0	0	0	0	0	0

A = *Saccharomycopsis fibuligera* MS:YD4; **B** = *Pichia anomala* MN:YP1; **C** = *Saccharomyces cerevisiae* MJ:YS2:Y2; **D** = *Mucor* sp. (close to *M. hiemalis*) MJ:Mu1; **E** = *Mucor circinelloides* MM:Mu1; **F** = *Rhizopus chinensis* MJ:Rh3; **G** = *Rhizopus stolonifer* var *lycoccus* MKo:Rh1; **H**= *Lactobacillus bifermentans* MA:R5; **I** = *Pediococcus pentosaceus* MA:C1

Data represent the means of 2 replicate sets.

Table 12. Amylolytic activities of selected strains isolated from marcha

Strain	α-amylase (U/ml)	Glucoamylase (U/ml)
Rhizopus stolonifer var *lycococcus* MKo:Rh1	5.3	71.3
Rhizopus stolonifer var *lycococcus* MA:Rh3	2.8	59.3
Rhizopus chinensis MT:Rh1	0.7	67.9
Rhizopus chinensis MJ:Rh3	1.5	96.3
Rhizopus chinensis MK:Rh1	0.4	87.4
Mucor sp (close to *M. hiemalis*) MJ:Mu1	0.7	37.0
Mucor circinelloides ML:Mu1	0.4	16.3
Mucor circinelloides MS:Mu1	1.8	15.4
Mucor sp (close to *M. hiemalis*) MC:Mu1	1.6	8.9
Mucor circinelloides MM:Mu2	1.2	11.7
Mucor circinelloides MA:Mu7	1.6	24.0
Saccharomycopsis fibuligera MK:YD1	4.5	40.2
Saccharomycopsis fibuligera MKo:YD2	4.2	47.0
Saccharomycopsis fibuligera MA:YD4	4.0	81.5
Saccharomycopsis fibuligera MS:YD4	6.6	43.1
Saccharomycopsis fibuligera MC:YD3	5.6	63.3
Saccharomycopsis fibuligera MA:YD5	5.3	46.0
Saccharomycopsis fibuligera MN:YD1	4.8	62.8
Pichia anomala MC:YP3	1.4	45.7
Pichia anomala MN:YP1	2.4	30.8
Pichia anomala MA:YP2	4.3	23.1
Pichia anomala MA:YP3	2.0	37.0
Pichia anomala MA:YP8	2.2	24.5
Saccharomyces cerevisiae MJ:YS1	4.1	18.8
Saccharomyces cerevisiae MJ:YS2	5.6	24.5
Saccharomyces cerevisiae MS:YS2	4.6	24.5
Saccharomyces cerevisiae MA:YS3	1.2	9.1
Saccharomyces cerevisiae MA:YS7	0.8	11.7
Candida glabrata MS:YC2	1.2	19.4
Candida glabrata MS:YC4	1.6	27.1
Candida glabrata MS:YC5	1.7	35.8
Candida glabrata MS:YC6	1.3	3.1

4.3. KODO KO JAANR

Kodo ko jaanr is the most common fermented beverage prepared from dry seeds of finger millet [*Eleusine coracana* (L) Gaertn.], locally called 'kodo' in the Darjeeling hills and Sikkim. Finger millet is sown in June and is harvested in December (Plate 5). Some indigenous local varieties of finger millet of these regions are 'mudke', 'nangrey', 'fyakre', 'nangkatwa', etc. Hybrid varieties of finger millet such as PR-202, HR-374 and VL- 101 have also been introduced to these regions. Finger millets, grown in these regions, are mostly utilized for preparation of alcoholic beverages. Non-fermented dough of grounded millet is also consumed as baked bread commonly called kodo ko roti, which is a traditional food.

4.3.1. Synonym of kodo ko jaanr

Jaanr is common name for all alcoholic beverages in Nepali. Different ethnic groups call it by their own dialect such as *mandokpenaa thee* by Limboo, *sampicha ummaak* by Rai, *naarr paa* by Gurung, *saangla chi* by Tamang, *chirs shyaabu* by Sunwar, *paadaare haan* by Magar, *gyaar chyyaang* by Sherpa, *minchaa chhyaang* by Bhutia, and *mong chee* by Lepcha.

4.3.2. Traditional method of preparation

During traditional method of kodo ko jaanr preparation, seeds of finger millet are cleaned, washed and cooked for about 30 min in an open cooker. Excess water is drained off and spread on a mat made up of bamboo locally called mandro for cooling. Powdered marcha (1 to 2 %) is sprinkled over

cooked seeds (Plate 6), mixed thoroughly and packed in a bamboo basket lined with fresh fern, locally called 'thadre unioon' *(Thelypteris erubescens* Well ex Hook.) or banana leaves, then covered with sack clothes, and kept for 2-4 days at room temperature for saccharification. During saccharification sweet aroma is emitted out and the saccharified mass is transferred into an earthen pot or into specially made bamboo basked called 'septu' and made it air-tight and fermented for 3-4 days during summer and 5-7 days in winter at room temperature (Fig 3).

Finger millet
↓
Cleaned and Washed
↓
Cooked
↓
Excess water drained off
↓
Spread on bamboo mat for cooling (20 –25° C)
↓
← added marcha (~ 2 %)
↓
Mixed
↓
Packed in heaps and saccharified for 2-4 d
↓
Transferred into a closed earthen pot
↓
Fermented (25-30° C, 3-7 d)
↓
Kodo ko jaanr

Fig 3. Flow sheet of kodo ko jaanr preparation in East Sikkim

Good quality of jaanr has sweet taste with mild alcoholic flavour (Plate 7). Prolonged fermentation makes the product bitter in taste and more alcoholic. Sour taste and unpleasant flavour of jaanr is unacceptable to consumers.

4.3.3. Mode of consumption

Kodo ko jaanr is consumed in an unique way in the Himalayan regions. About 200-500 g of kodo ko jaanr is put into a vessel called toongbaa (Plate 8 & 9) and lukewarm water is added up to the edge of the toongbaa. After 10-15 min, milky white extract of kodo ko jaanr is sipped through a narrow bamboo straw called pipsing having a hole in a side near the bottom to avoid passing of grits (Plate 10). Water can be added 2-3 times after sipping up the extract. Guests are served with toongbaa along with fried meat or pickles. Alternately, thick milky white liquid pressed from the kodo ko jaanr is filtered using a filter called chhapani under pressure. Such liquor is believed to be good tonic for ailing persons and post-natal women. After consumption, grits of kodo ko jaanr are used as fodder for pigs and cattle. This is a good example of total utilization of substrate as food and fodder.

Feeding frequency of kodo ko jaanr has been summarised in Table 13. About 70 % of people consume kodo ko jaanr daily in rural areas of the Sikkim Himalayas. Per capita daily consumption of kodo ko jaanr extract in the Darjeeling hills and Sikkim is 5.1 ml and 6.5 ml, respectively.

Plate 5. Finger millets "kodo" field in West Sikkim

Plate 6. Kodo ko jaanr preparation by the Bhutia woman. Cooked millets are placed on *mandro* (bamboo-made mat) and powdered marcha is added.

Plate 7. Finger millets seeds and kodo ko jaanr

Plate 8. Fermented grits of finger millets are filled in *toongbaa.*

Plate 9. *Toongbaa*: (a) silver-lined wooden *toongbaa*; (b) with lid; (c) bamboo-made *toongbaa*; (d) straw called *pipsing*

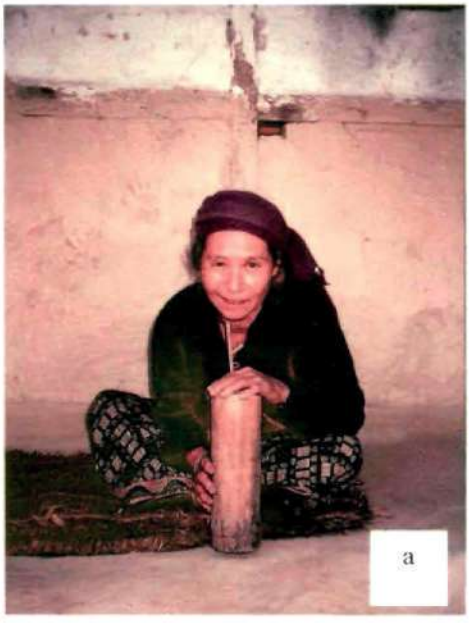
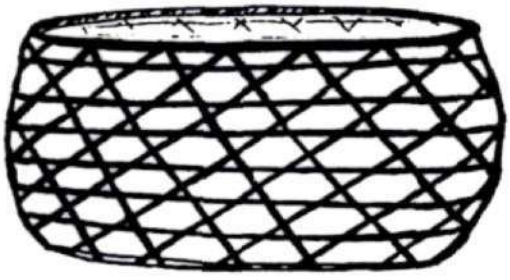
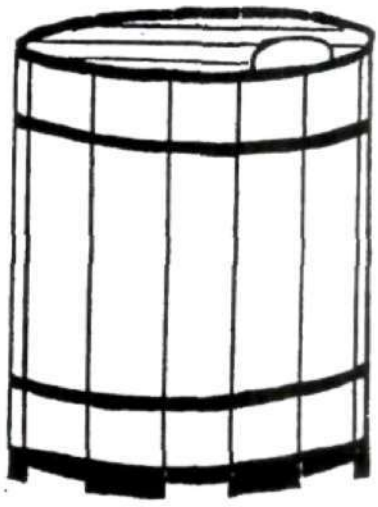


Plate 10. (a) Kodo ko jaanr extract is sipped through the *pipsing* (a) by Rai woman in bamboo-made *toongbaa* and (b) by Lepcha man in wood-made *toongbaa* in Sikkim.

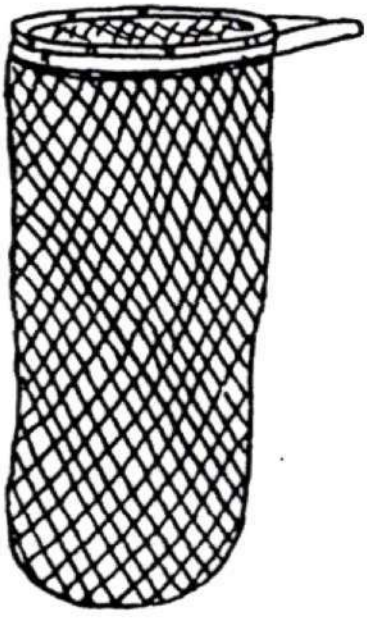


a



b

Plate 11. (a) Bamboo-made *septu* and (b) wood-made *septu*



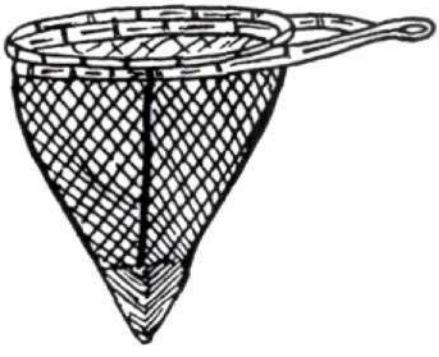


Plate 12. Chappani made up of bamboo stripes

bamboo straw called *pipsing*. *Toongbaa* is made up of wood or bamboo or sometime earthenware (Plate 9 a-c). Usually wooden toongbaa is decorated with silver lining and is provided with a lid.

Pipsing: It is a narrow straw, made up of bamboo having a hole in opposite sites near the bottom to avoid passing of grits during sipping of jaanr from *toongbaa* (Plate 9 d).

4.3.5. Ethnical Importance

In the Sikkim Himalayan regions social activities require provision and consumption of appreciable quantities of alcoholic beverages by the 'matwali' castes meaning alcohol drinkers of the non-Brahmin Nepali ethnic community mostly Limboo, Rai, Gurung, Magar, Tamang, Sunwar, Newar and Sherpa; the Bhutia and the Lepcha communities. Ethnical importance of fermented beverages was documented during survey and noted in discussion chapter.

4.3.6. Microorganisms

Forty samples of kodo ko jaanr, collected from different places of the Darjeeling hills and Sikkim, were analysed for microbial load (Table 14). Average load of yeasts and total viable counts was detected at the level of 10^7 cfu/g whereas the population of lactic acid bacteria was comparatively less (~10^5 cfu/g). Filamentous mould was not recovered in finish product. Out of 161 strains of microorganisms isolated, 81 isolates were yeasts and 80 isolates were lactic acid bacteria.

Table 14. Microbial load of kodo ko jaanr samples

Source	$\times 10^7$cfu/g fresh weight		
	Yeast	LAB	Total Viable Count
Rongli	1.1 (0.2-2.1)	0.03 (0.001-0.1)	1.1 (0.7-1.5)
Namchi	1.8 (1.7-1.9)	0.03 (0.02-0.04)	3.7 (1.5-5.8)
Aho	1.7 (1.0-2.5)	0.1 (0.05-0.2)	3.0 (1.5-4.5)
Kalimpong	1.0 (0.8-1.2)	0.2 (0.1-0.3)	1.4 (1.0-2.2)

Data represent the means of 10 samples from each source. Ranges are given in parentheses.

4.3.6.1. Characterisation and identification of yeasts

Representative strains of yeasts were selected on the basis of colony, cell morphology, vegetative reproduction and type of ascospore and were grouped into three genera (Table 15). These representative strains of yeasts were identified as *Pichia anomala* (E.C. Hansen) Kurtzman, *Saccharomyces cerevisiae* Meyen ex Hansen and *Candida glabrata* (Anderson) Meyer et Yarrow (Table 16). *Saccharomycopsis fibuligera* was not recovered in kodo ko jaanr samples. *Pichia anomala* and *Saccharomyces cerevisiae* were present in all samples showing 100 % prevalence whereas *Candida glabrata* showed only 40 % prevalence in forty samples of kodo ko jaanr analysed.

Table 6. Selection of representative strains of yeasts isolated from kodo ko jaanr samples[a]

Source	Number of strains isolated	Colony	Cell Shape	Mycelium	Ascospore	Grouped strains	Representative strains
Rongli	16	Ss	O-E	Pseudo	Hat-shaped	8	KR:YP2
		Ss	O-E	Pseudo	Globose	8	KR:YS1
Namchi	20	Ss	O-E	Pseudo	Hat-shaped	10	KN:YP3
		Ss	O-E	Pseudo	Globose	10	KN:YP4
Aho	25	Ss	O-E	Pseudo	Hat-shaped	8	KA:YP1
		Ss	O-E	Pseudo	Globose	10	KA:YS3
		Fs	O-E	–	–	7	KA:YC4
Kalimpong	20	Ss	O-E	Pseudo	Hat-shaped	10	KK:YP1
		Ss	O-E	Pseudo	Globose	6	KK:YS1
		Fs	O-E	–	–	4	KK:YC1

[a]Number of samples was 10 from each source. All isolates reproduced by multilateral budding.

Ss, smooth surface; Fs, fringed surface; O-E, Oval to ellipsoidal.

Table 16. Characteristics of representative strains of yeasts isolated from kodo ko jaanr

Parameter	KR:YP2	KN:YP3	KA:YS3	KA:YS1	KA:YC4
Cell width (μm)	1.1-3.2	1.3-3.2	1.6-3.2	1.6-4.0	0.8-2.5
Cell length (μm)	1.6-3.8	1.9-5.0	1.6-5.3	1.9-4.8	1.6-3.8
Nitrate reduction	+	+	–	–	–
Growth at 37^0C	+	+	+	+	+
Sugar fermentation:					
Glucose	+	+	+	+	+
Galactose	–	–	+	+	–
Lactose	–	–	–	–	–
Maltose	+	+	+	+	–
Raffinose	+	+	+	+	–
Sucrose	+	+	+	+	–
Starch	–	–	+	+	–
Trehalose	+	+	–	–	+
Sugar assimilation:					
Arabinose	+	$+_w$	–	–	–
Cellobiose	+	+	–	–	–
Galactose	+	+	+	+	–
Glycerol	–	–	–	–	$+_w$
Inositol	–	–	–	–	–
Lactose	–	–	–	–	–
Maltose	+	+	+	+	–
Melibiose	–	–	+	+	–
Mannitol	+	+	–	–	–
Rhamnose	–	–	–	–	–
Raffinose	+	+	+	+	–
Sucrose	+	+	+	+	–
Starch	+	+	+	+	–
Trehalose	+	+	+	+	+
Xylose	–	+	–	–	–
Identification	*Pichia*		*Saccharomyces*		*Candida*

4.3.6.2. Characterisation and identification of bacteria

Out of 80 strains of lactic acid bacteria, isolated from forty samples of kodo ko jaanr, 44 strains were coccii-shaped cells in tetrads and 36 strains were non-sporeforming rods (Table 17). Species of lactic acid bacteria were identified as *Pediococcus pentosaceus* Mees and *Lactobacillus bifermentans* Kandler, Schillinger and Weiss (Table 18). Prevalence of both of them was 100 % in finish products.

Table 17. Selection of representative strains of LAB isolated from kodo ko jaanr[a]

Source	Number of strains isolated	Cell Shape	Gas from glucose	NH_3 from arginine	Grouped strains	Representative strains
Rongli	18	Coccus	–	+	10	KR:C1
		Rod	+	–	8	KR:R2
Namchi	20	Coccus	–	+	12	KN:C2
		Rod	+	–	8	KN:R3
Aho	20	Coccus	–	+	10	KA:C1
		Rod	+	–	10	KA:R1
Kalimpong	22	Coccus	–	+	12	KK:C1
		Rod	+	–	10	KK:R1

[a]Number of samples was 10 from each source

[b]All isolates were Gram-positive, catalase-negative, non-sporeformers and non-motile

Table 18a. Phenotypic characters of representative strains of LAB isolated from kodo ko jaanr

Parameter	KR:C1	KN:C2	KA:C1	KK:C1	KR:R2	KN:R3	KA:R1	KK:R1
Cell shape	Ct	Ct	Ct	Ct	R	R	R	R
Cell diameter (μm)	0.2-0.5	0.2-0.6	0.2-0.5	0.4-0.7				
Cell width (μm)					0.2-0.3	0.2-0.4	0.2-0.3	0.2-0.3
Cell length (μm)					0.8-2.3	0.8-2.2	1.0-2.2	1.0-2.3
Anaerobic growth	+	+	+	+	+	+	+	+
Hydrolysis of:								
Casein	–	–	–	–	–	–	–	–
Gelatin	–	–	–	–	–	–	–	–
Arginine	+	+	+	+	–	–	–	–
Starch	–	–	–	–	–	–	–	–
Indole production	–	–	–	–	–	–	–	–
Nitrate reduction	–	–	–	–	–	–	–	–
Growth in NaCl:								
4.0 %	+	+	+	+	+	+	+	+
6.5 %	+	+	+	+	+	+	+	+
10.0 %	+	+	+	+	+	+	+	+
18.0 %	–	–	–	–	–	–	–	–
Growth in pH:								
4.2	+	+	+	+	+	+	+	+
7.5	+	+	+	+	+	+	+	+
8.5	+	+	+	+	+	+	+	+
Growth at:								
15° C	+	+	+	+	+	+	+	+
45° C	–	–	–	–	–	–	–	–

Ct, coccii-tetrad; R, rod in chain with 2-3 cells

Table 18b. Sugar fermentation of LAB strains using API 50 CHL system

Parameter	KR:C1	KN:C2	KA:C1	KK:C1	KR:R2	KN:R3	KA:R1	KK:R1
Glycerol	+	+	–	–	–	–	+	–
Erythritol	+	+	–	–	–	–	–	–
D-Arabinose	+	+	–	–	–	–	–	–
L-Arabinose	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	+
L-Xylose	–	–	–	–	–	–	–	$+_w$
Adonitol	+	+	–	–	–	–	+	–
β-Methyl-D-Xyloside	–	–	–	–	–	–	$+_w$	–
Galactose	+	+	+	+	+	$+_w$	–	$+_w$
D-Glucose	–	–	+	+	+	–	+	+
D-Fructose	–	–	+	+	+	+	+	+
D-Mannose	–	$+_w$	+	+	–	–	+	–
L-Sorbose	$+_w$	–	–	–	–	–	–	–
Rhamnose	+	+	–	–	–	–	+	–
Dulcitol	+	+	–	–	–	–	–	–
Inositol	–	–	–	–	–	–	–	–
Mannitol	–	–	–	–	–	–	–	$+_w$
Sorbitol	+	+	–	–	–	–	–	–
α-Methyl-D-Mannoside	+	+	–	–	–	$+_w$	+	–
α-Methyl-D-Glucoside	–	–	–	–	$+_w$	$+_w$	+	+
N-Acetyl-Glucosamine	+	+	+	+	$+_w$	–	+	+
Amygdalin	+	+	+	+	–	–	–	–
Arbutin	–	–	–	–	–	–	–	–
Esculin	–	–	–	–	–	–	–	–
Salicin	$+_w$	–	$+_w$	–	–	–	–	–
Cellobiose	+	+	+	+	–	–	–	–
Maltose	+	+	+	+	+	+	+	$+_w$

Parameter	KR:C1	KN:C2	KA:C1	KK:C1	KR:R2	KN:R3	KA:R1	KK:R1
Lactose	–	–	–	+	–	–	–	–
Melibiose	–	–	–	–	$+_w$	$+_w$	+	$+_w$
Sucrose	–	–	–	–	–	–	–	–
Trehalose	+	+	+	+	–	–	–	–
Inulin	–	–	–	–	–	–	–	–
Melezitose	–	–	–	–	–	–	–	–
Raffinose	–	–	–	–	–	–	–	–
Starch	–	–	–	–	–	–	–	–
Glycogen	–	–	–	–	–	–	–	–
Xylitol	–	–	–	–	–	–	–	–
Gentiobiose	+	+	+	+	–	–	–	–
D-Turanose	–	–	–	–	–	–	–	–
D-Lyxose	–	–	–	–	–	–	–	–
D-Tagatose	+	+	+	+	–	–	–	–
D-Fucose	–	–	–	–	–	–	–	–
L-Fucose	–	–	–	–	–	–	–	–
D-Arabitol	–	–	–	–	–	–	–	–
L-Arabitol	–	–	–	–	–	–	–	–
Gluconate	–	–	–	–	$+_w$	$+_w$	–	–
2-Keto-Gluconate	–	–	–	–	$+_w$	$+_w$	–	–
5-Keto-Gluconate	–	–	–	–	$+_w$	$+_w$	–	$+_w$
Identification	*Pediococcus*				*Lactobacillus*			

4.3.7. Proximate composition

Proximate composition of finger millet and kodo ko jaanr is presented in (Table 19). Average pH, acidity and alcohol content of the product was 4.1, 0.27 % and 4.8 %, respectively.

Table 19. Proximate composition of unfermented and fermented finger-millet

Parameter	Unfermented	Fermented (Kodo ko jaanr)			
	Cooked finger-millet	Rongli	Namchi	Aho	Kalimpong
pH	6.4 (6.3-6.5)	3.9 (3.7-4.1)	4.3 (3.8-4.4)	3.9 (3.5-4.3)	4.2 (3.8-4.5)
Moisture (%)	66.0 (64.0-72.0)	68.8 (64.5-72.8)	65.7 (62.8-78.2)	71.7 (62.4-78.2)	72.5 (67.2-79.9)
Acidity (%)	0.01 (0.01-0.01)	0.26 (0.23-0.31)	0.25 (0.21-0.29)	0.33 (0.25-0.50)	0.25 (0.20-0.34)
Alcohol (%)	0.1 (0.05-0.1)	2.4 (1.8-3.7)	7.1 (3.2-8.7)	5.2 (3.5-7.0)	4.5 (3.0-6.6)
Ash (% DM)	4.9 (4.5-5.5)	5.8 (4.8-7.1)	4.1 (3.5-5.3)	4.6 (4.2-6.0)	5.8 (4.8-6.0)
Fat (% DM)	2.4 (1.7-2.9)	2.0 (1.8-2.5)	2.0 (1.6-2.6)	1.8 (1.6-2.2)	2.1 (1.7-2.9)
Protein (% DM)	10.0 (9.5-11.0)	9.2 (8.2-10.5)	9.5 (8.5-10.8)	8.5 (8.2-10.5)	9.8 (8.3-11.0)
Crude fibre (% DM)	6.7 (5.8-7.0)	7.7 (6.8-8.7)	ND	ND	10.9 (10.6-11.3)
Carbohydrate (% DM)	82.7 (80.6-84.3)	83.0 (79.9-85.2)	84.4 (81.3-86.4)	85.1 (81.3-86.0)	82.3 (80.1-85.2)
Energy (Kcal/100g DM)	392.4 (375.7-407.3)	386.8 (368.6-405.3)	393.6 (373.6-412.2)	390.6 (372.4-405.8)	387.3 (368.9-410.9)

Data represent the means of 5 samples from each source. % DM, percentage on dry matter basis. Ranges are given in parentheses. ND, not determined.

Moisture content of the product was slightly higher in fermented product than unfermented cooked millet. No remarkable change was observed in ash, fat and protein contents of kodo ko jaanr over the substrate. Crude fibre content increased during fermentation in kodo ko jaanr. Calorie content of the unfermented millet and fermented product was almost same. Remarkable increase in mineral contents such as calcium, magnesium, manganese, iron, potassium and phosphorous was observed in jaanr (Table 20).

Table 20. Mineral contents of raw and fermented finger millets

Mineral	**mg/100 g dry matter**	
	Finger millet	**Kodo ko jaanr**
Calcium	206	281
Magnesium	76	118
Manganese	3.6	9.0
Copper	0.8	2.2
Iron	8.7	24
Zinc	1.0	1.2
Sodium	28	39
Potassium	252	398
Phosphorus	228	326

Data represent the means of 2 samples.

4.3.8. Successional studies during kodo ko jaanr fermentation

Kodo ko jaanr was prepared in the laboratory following the traditional method by using marcha, collected from Aho village, as mentioned in 3.3.6. Successional studies were carried at every 1 day interval within a range of 0-10 days.

4.3.8.1. Microbial changes

Table 21 shows the changes in microbial population in fermenting finger millet seeds during kodo ko jaanr fermentation. Mould population, which was originated from marcha, declined significantly ($P<0.05$) every day during fermentation and finally disappeared after 5 d (Fig 4). Population of yeasts increased significantly ($P<0.05$) from 10^5 cfu/g to 10^7 cfu/g within 2 d, and remained relatively constant at the same level till the end of fermentation. Subsequently, load of lactic acid bacteria increased significantly ($P<0.05$) from 10^6 cfu/g to 10^8 cfu/g in first day and decreased significantly ($P<0.05$) to a level of 10^3 cfu/g at the end. Total viable counts increased significantly ($P<0.05$) within first day and decreased in every interval of 1 d.

Table 21. Microbial changes during kodo ko jaanr fermentation

Fermentation time (days)	Log cfu/g			
	Mould	Yeast	LAB	Total Count
0	4.2 ± 0.29^{a}	5.2 ± 0.21^{e}	6.0 ± 0.16^{ef}	6.2 ± 0.05^{g}
1	3.1 ± 0.46^{b}	7.5 ± 0.08^{b}	8.2 ± 0.13^{a}	8.3 ± 0.16^{a}
2	2.4 ± 0.49^{c}	7.8 ± 0.13^{a}	7.9 ± 0.13^{b}	8.1 ± 0.08^{b}
3	1.8 ± 0.21^{d}	7.8 ± 0.13^{a}	7.9 ± 0.08^{b}	8.3 ± 0.08^{a}
4	< DL	7.8 ± 0.08^{a}	6.5 ± 0.21^{c}	7.9 ± 0.08^{c}
5	0	7.6 ± 0.08^{b}	6.3 ± 0.25^{d}	7.7 ± 0.08^{d}
6	0	7.6 ± 0.16^{b}	6.0 ± 0.16^{ef}	7.7 ± 0.08^{d}
7	0	7.3 ± 0.08^{bcd}	5.9 ± 0.17^{f}	7.3 ± 0.08^{e}
8	0	7.4 ± 0.13^{bc}	5.2 ± 0.08^{g}	7.4 ± 0.08^{e}
9	0	7.2 ± 0.08^{d}	4.7 ± 0.25^{h}	7.2 ± 0.08^{f}
10	0	7.3 ± 0.08^{bcd}	3.8 ± 0.33^{i}	7.3 ± 0.09^{e}

Data represent the means ± SD of three batches of fermentation. Data were transformed into logarithmic values. DL, detection limit (10 cfu/g).

Values bearing different superscripts in each column differ significantly ($P<0.05$).

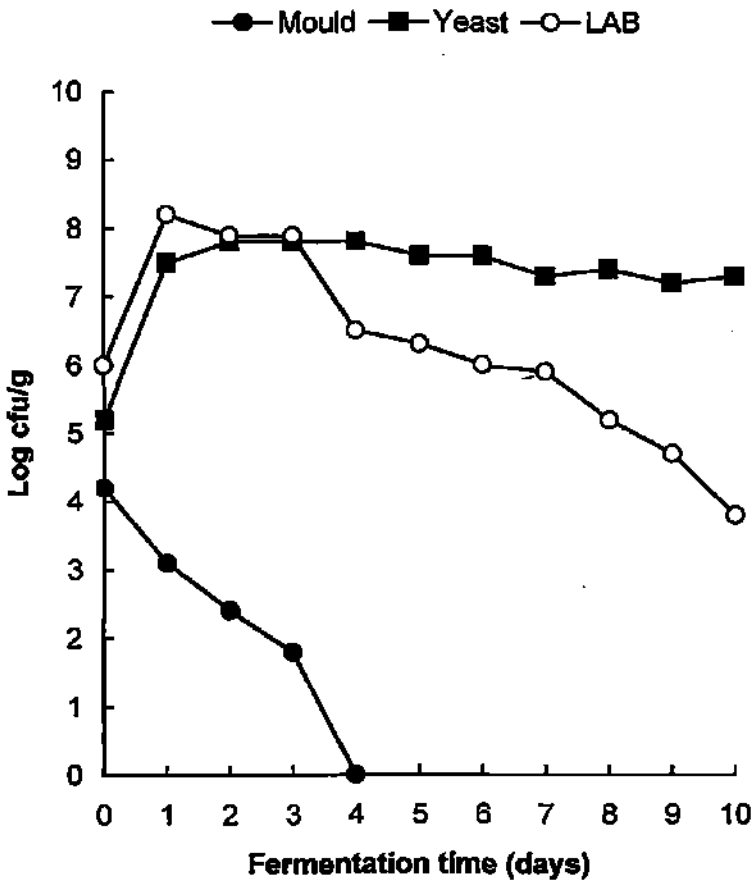


Fig 4. Changes in microbial load during kodo ko jaanr fermentation. Values are the means of three batches of fermentation. LAB, lactic acid bacteria.

4.3.8.2. Physico-chemical and enzymatic changes

Temperature of fermenting finger millet increased significantly ($P<0.05$) from 26° C to 30° C within 2 d and decreased to 28° C till the end during kodo ko jaanr fermentation (Table 22). The mean pH value decreased significantly ($P<0.05$) from 6.37 to 4.10 within 2 d of fermentation and after 2 d, decline in pH was non-significant. Titratable acidity increased significantly ($P<0.05$) from 0 d to 4 d, and remained the same till the end (Fig 5). Alcohol content increased significantly ($P<0.05$) from 0.1 % to 6.9 % within 6 d and slightly decreased to 6.5 % on 10 d.

Table 22. Physico-chemical changes during kodo ko jaanr fermentation

Fermentation time (days)	Temperature (°C)	pH	Acidity (%)	Alcohol (%)
0	26.0 ± 0.00^{f}	6.37 ± 0.01^{a}	0.01 ± 0.00^{e}	0.1 ± 0.11^{h}
1	28.8 ± 0.05^{e}	4.44 ± 0.01^{b}	0.08 ± 0.01^{d}	0.5 ± 0.08^{g}
2	30.0 ± 0.13^{a}	4.10 ± 0.01^{c}	0.14 ± 0.01^{c}	2.7 ± 0.08^{f}
3	29.5 ± 0.00^{b}	4.07 ± 0.02^{c}	0.18 ± 0.01^{b}	3.1 ± 0.08^{e}
4	29.0 ± 0.00^{c}	4.07 ± 0.01^{c}	0.24 ± 0.01^{a}	4.1 ± 0.08^{d}
5	29.0 ± 0.05^{c}	4.08 ± 0.01^{c}	0.23 ± 0.00^{a}	5.5 ± 0.08^{c}
6	28.8 ± 0.21^{c}	4.08 ± 0.01^{c}	0.23 ± 0.01^{a}	6.9 ± 0.21^{a}
7	28.3 ± 0.08^{d}	4.07 ± 0.01^{c}	0.22 ± 0.01^{a}	6.8 ± 0.13^{a}
8	28.0 ± 0.00^{e}	4.07 ± 0.02^{c}	0.23 ± 0.01^{a}	6.8 ± 0.08^{a}
9	28.0 ± 0.00^{e}	4.07 ± 0.01^{c}	0.22 ± 0.01^{a}	6.6 ± 0.08^{b}
10	28.0 ± 0.00^{e}	4.07 ± 0.01^{c}	0.23 ± 0.01^{a}	6.5 ± 0.08^{b}

Data represent the means ± SD of three batches of fermentation. Values bearing different superscripts in each column differ significantly ($P<0.05$).

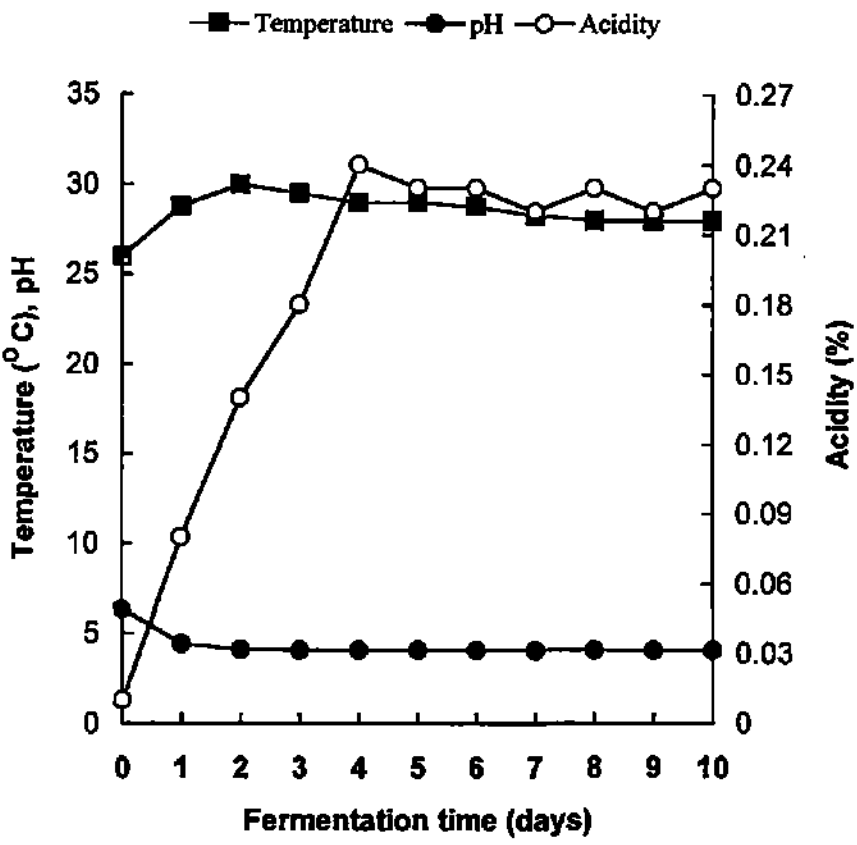


Fig 5. Changes in temperature, pH and acidity during kodo ko jaanr fermentation. Values are the means of three batches of fermentation.

Reducing sugar content was increased significantly ($P<0.05$) up to third day of fermentation and decreased significantly ($P<0.05$) everyday till the end (Table 23). Total sugar contents of fermenting finger millet decreased significantly ($P<0.05$) throughout the fermentation (Fig 6). Maximum activities of α–amylase and glucoamylase was observed on second day of fermentation and decreased significantly ($P<0.05$) till the end (Table 23 and Fig 7).

Table 23. Biochemical and enzymatic changes during kodo ko jaanr fermentation

Fermentation time (days)	Reducing sugar (%)	Total sugar (%)	α-amylase (U/g)	Glucoamylase (U/mg)
0	0.4 ± 0.16^{k}	85.9 ± 2.45^{a}	6.0 ± 0.16^{h}	33.2 ± 3.70^{f}
1	4.0 ± 0.08^{d}	72.9 ± 3.47^{b}	15.4 ± 0.50^{ef}	121.1 ± 2.11^{c}
2	4.6 ± 0.08^{c}	61.4 ± 1.18^{c}	36.0 ± 1.63^{a}	163.2 ± 4.91^{a}
3	7.0 ± 0.16^{a}	53.6 ± 0.57^{d}	27.1 ± 0.90^{b}	153.9 ± 6.97^{ab}
4	4.8 ± 0.16^{b}	44.3 ± 2.33^{e}	25.6 ± 1.14^{c}	147.8 ± 0.73^{b}
5	3.4 ± 0.08^{e}	40.0 ± 1.72^{f}	21.0 ± 1.30^{d}	147.0 ± 7.30^{b}
6	3.2 ± 0.16^{h}	38.2 ± 1.18^{f}	19.8 ± 1.03^{d}	145.7 ± 4.99^{b}
7	3.0 ± 0.08^{f}	34.2 ± 0.90^{g}	15.8 ± 0.82^{e}	146.5 ± 8.00^{b}
8	2.8 ± 0.08^{g}	31.6 ± 0.61^{gh}	14.3 ± 0.78^{f}	98.5 ± 9.68^{d}
9	1.8 ± 0.16^{i}	30.9 ± 0.57^{hi}	9.1 ± 0.82^{g}	85.1 ± 8.10^{e}
10	1.0 ± 0.08^{j}	28.7 ± 0.61^{i}	8.5 ± 0.41^{g}	34.1 ± 3.31^{f}

Data represent the means ± SD of three batches of fermentation.

Values bearing different superscripts in each column differ significantly ($P<0.05$).

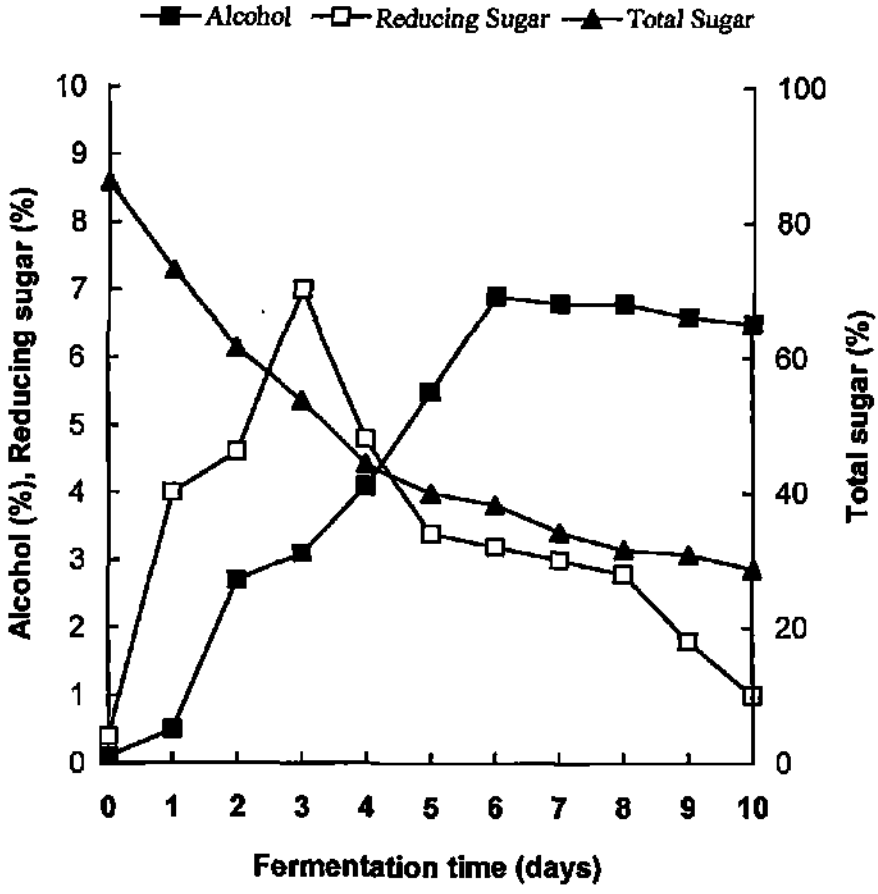


Fig 6. Changes in alcohol, reducing sugar and total sugar contents of finger millet during kodo ko jaanr fermentation. Values are the means of three batches of fermentation.

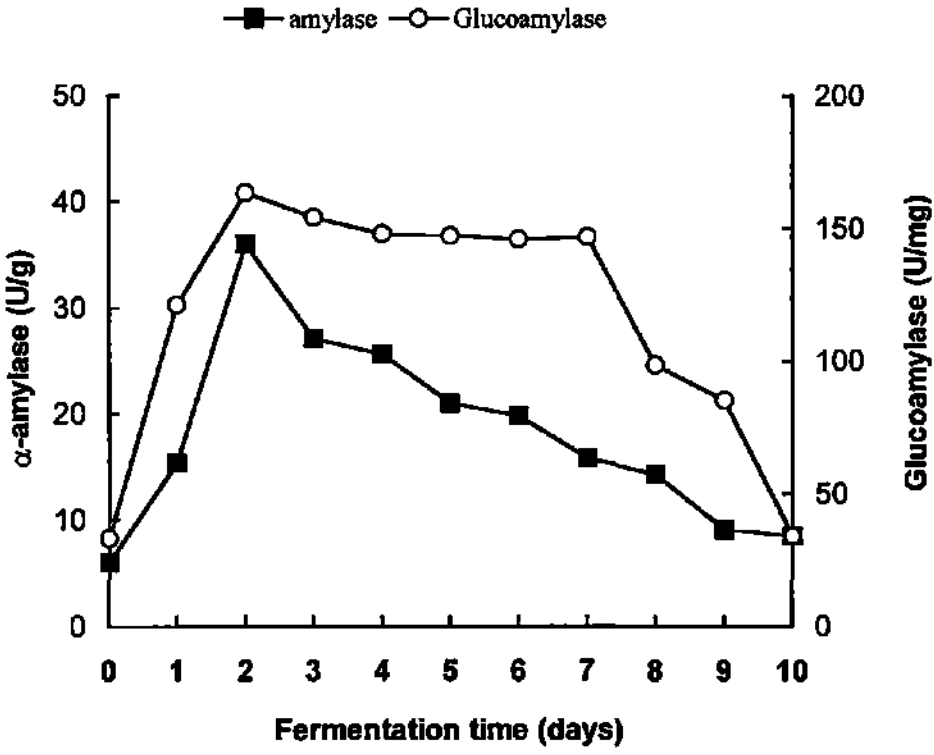


Fig 7. Changes in enzymatic activities in finger millet during kodo ko jaanr fermentation. Values are the means of three batches of fermentation.

4.3.9. Testing of isolates for producing kodo ko jaanr

Sterilised finger millet seeds were allowed to ferment with different combinations of *Rhizopus chinensis* MJ:Rh3 and *Saccharomycopsis fibuligera* MS:YD4, since both of them showed highest glucoamylase and ∝-amylase activities, respectively (Table 12) with other strains of mould and yeast: *Mucor* sp. (close to *M. hiemalis*) MJ:Mu1, *Saccharomyces cerevisiae* MJ:YS2, *Candida glabrata* MS:YC5, *Pichia anomola* MN:YP1, selected on the basis of high amylolytic activities; randomly selected LAB strains *Pediococcus pentosaceus* MA:B2 and *Lactobacillus bifermentans* MA:B5 for testing ability to produce kodo ko jaanr. Table 24 shows changes in pH, reducing sugar and alcohol production by each combination of strain during kodo ko jaanr fermentation. Jaanr prepared by a combination of *Rhizopus chinensis* MJ:Rh3 and *Saccharomycopsis fibuligera* MS:YD4 showed significantly ($P<0.05$) high reducing sugar contents during saccharification period of 2 d with low alcohol content of 1 % on 6 d. Cell suspension mixture of *Rhizopus chinensis* MJ:Rh3 and *Saccharomyces cerevisiae* MJ:YS2 produced jaanr with significantly ($P<0.05$) high reducing sugar and high alcohol content of 4.4 % in 6 d than jaanr samples fermented by other strains.

Table 25 shows the sensory evaluation of kodo ko jaanr produced by selected combination of strains. There was no significant ($P<0.05$) difference in aroma attribute of jaanr prepared by a cell suspension mixture of *Rhizopus chinensis* MJ:Rh3 with other strains, except jaanr prepared by a combination of *Saccharomycopsis fibuligera* MS:YD4 with *Saccharomyces cerevisiae* MJ:YS2, *Candida glabrata* MS:YC5, LAB strains and all

strains. There was significant ($P<0.05$) difference in taste score of jaanr prepared by cell mixture of *Rhizopus chinensis* MJ:Rh3 and *Saccharomyces cerevisiae* MJ:YS2 with that of other strains. However, significance ($P<0.05$) difference in texture and colour scores was observed in some jaanr samples. Jaanr product prepared by a combination of *Rhizopus chinensis* MJ:Rh3 and *Saccharomycopsis fibuligera* MS:YD4 had desirable sweet-sour taste but unpleasant odour due to low alcohol content. Hence based on sensory criteria, jaanr produced by these strains were unacceptable to consumers. Kodo ko jaanr prepared by a combination of *Rhizopus chinensis* MJ:Rh3 and *Saccharomyces cerevisiae* MJ:YS2 showed significantly ($P<0.05$) highest score in general acceptability. Kodo ko jaanr prepared by these strains had mild alcoholic-sweet flavour, significantly ($P<0.05$) acceptable to judges.

Table 24. Changes in pH, reducing sugar contents and alcohol production in fermented finger millet by selected strains

	pH		Reducing sugar (%)		Alcohol (%)	
Cooked millet (unfermented finger millet)	6.37 ± 0.01		0.4 ± 0.16		0.1 ± 0.06	
Strain	**2 days**	**6 days**	**2 days**	**6 days**	**2 days**	**6 days**
Rhizopus chinensis MJ:Rh3 **with**						
Mc	4.62 ± 0.02^{e}	4.82 ± 0.01^{e}	2.10 ± 0.19^{ef}	3.10 ± 0.24^{b}	0.25 ± 0.06^{f}	0.83 ± 0.06^{f}
Sc	4.31 ± 0.01^{f}	4.36 ± 0.01^{h}	4.21 ± 0.05^{c}	3.57 ± 0.05^{a}	2.50 ± 0.07^{a}	4.40± 0.13^{a}
Cg	4.32 ± 0.01^{f}	4.30 ± 0.00^{i}	3.95 ± 0.11^{c}	2.00 ± 0.19^{ef}	0.70 ± 0.06^{d}	1.80 ± 0.00^{d}
Pa	4.60 ± 0.00^{e}	4.70 ± 0.01^{f}	2.49 ± 0.08^{def}	3.13 ± 0.45^{b}	0.76 ± 0.00^{c}	2.20 ± 0.06^{c}
Lb + Pp	4.12 ± 0.01^{h}	4.16 ± 0.01^{j}	2.71 ± 0.46^{d}	2.89 ± 0.16^{bc}	1.00 ± 0.06^{b}	2.50 ± 0.06^{b}
Saccharomycopsis fibuligera MS: YD4 **with**						
Rc	4.20 ± 0.02^{g}	4.56 ± 0.02^{g}	6.28 ± 0.14^{a}	2.55 ± 0.05^{cd}	0.40 ± 0.07^{e}	1.00 ± 0.06^{e}
Mc	6.02 ± 0.01^{c}	6.10 ± 0.02^{bc}	4.94 ± 0.04^{b}	2.22 ± 0.05^{de}	0.15 ± 0.00^{g}	0.30 ± 0.00^{j}
Sc	6.08 ± 0.02^{b}	6.13 ± 0.01^{b}	4.74 ± 0.75^{bc}	2.63 ± 0.11^{c}	0.22 ± 0.06^{f}	0.50 ± 0.06^{hi}
Cg	5.95 ± 0.00^{d}	6.00 ± 0.04^{d}	3.07 ± 0.20^{d}	1.65 ± 0.01^{f}	0.15 ± 0.00^{g}	0.45 ± 0.00^{i}
Pa	6.10 ± 0.00^{b}	6.08 ± 0.00^{c}	2.61 ± 0.29^{de}	1.92 ± 0.11^{ef}	0.15 ± 0.06^{g}	0.68 ± 0.06^{g}
Lb + Pp	6.02 ± 0.01^{c}	6.12 ± 0.02^{bc}	2.29 ± 0.08^{def}	2.64 ± 0.04^{c}	0.20 ± 0.06^{g}	0.75 ± 0.00^{fg}
Strains*	6.43 ± 0.02^{a}	6.40 ± 0.00^{a}	1.91 ± 0.41^{f}	1.98 ± 0.02^{ef}	0.05 ± 0.06^{h}	0.15 ± 0.00^{k}

Data represent the means ± SD of three batches of fermentation. Values bearing different superscripts in each column differ significantly ($P<0.05$).

Mc, *Mucor* sp. (close to *M. hiemalis*) MJ:Mu1; **Rc,** *Rhizopus chinensis* MJ:Rh3

Sc, *Saccharomyces cerevisiae* MJ:YS2; **Cg,** *Candida glabrata* MS:YC5; **Pa,** *Pichia anomola* MN:YP1

Lb, *Lactobacillus bifermentans* MA:R5; **Pp,** *Pediococcus pentosaceus* MA:C1.

*Cell mixture of all above mentioned strains.

Table 25. Sensory evaluation of kodo ko jaanr produced by selected strains

Strain	Aroma	Taste	Texture	Colour	General acceptability
Rhizopus chinensis MJ:Rh3 with					
Mc	2.00 ± 0.93^{ab}	1.86 ± 0.52^{b}	2.14 ± 0.83^{b}	2.86 ± 0.83^{bcd}	2.29 ± 0.70^{bcd}
Sc	3.43 ± 0.50^{a}	2.79 ± 0.36^{a}	3.43 ± 0.90^{a}	4.29 ± 1.03^{a}	4.43 ± 0.73^{a}
Cg	2.43 ± 0.90^{ab}	1.57 ± 0.73^{b}	2.57 ± 0.73^{ab}	3.00 ± 0.93^{abc}	2.43 ± 0.50^{bc}
Pa	3.43 ± 0.90^{a}	1.86 ± 0.69^{b}	2.43 ± 0.50^{ab}	3.71 ± 0.88^{ab}	2.57 ± 0.50^{b}
Lb + Pp	2.29 ± 0.88^{ab}	1.79 ± 0.53^{c}	2.43 ± 1.18^{ab}	3.14 ± 0.99^{abc}	2.29 ± 0.88^{bcd}
Saccharomycopsis fibuligera MS: YD4 with					
Rc	1.86 ± 0.83^{ab}	1.29 ± 0.36^{b}	2.43 ± 0.50^{ab}	1.86 ± 0.64^{cde}	1.57 ± 0.50^{bcdf}
Mc	1.86 ± 0.83^{ab}	1.21 ± 0.36^{b}	2.29 ± 0.88^{ab}	1.86 ± 0.83^{cde}	1.43 ± 0.50^{cdef}
Sc	1.43 ± 0.73^{b}	1.14 ± 0.35^{b}	1.71 ± 0.88^{b}	1.29 ± 0.70^{e}	1.14 ± 0.35^{ef}
Cg	1.43 ± 0.73^{b}	1.21 ± 0.36^{b}	2.00 ± 0.76^{b}	1.29 ± 0.70^{e}	1.29 ± 0.70^{def}
Pa	2.29 ± 0.88^{ab}	1.21 ± 0.30^{b}	2.00 ± 1.07^{b}	1.43 ± 0.73^{e}	1.43 ± 0.73^{cdef}
Lb + Pp	1.57 ± 0.73^{b}	1.29 ± 0.36^{b}	2.29 ± 1.03^{ab}	1.57 ± 0.90^{de}	1.43 ± 0.73^{cdef}
Strains*	1.43 ± 0.73^{b}	1.00 ± 0.00^{b}	1.43 ± 0.73^{b}	1.14 ± 0.35^{e}	1.00 ± 0.00^{f}

Market kodo ko jaanr was used as control; score 1, bad; score 5, good.

Data represent the mean scores ± SD (n = 7). Values bearing different superscripts in each column differ significantly ($P<0.05$).

Mc, *Mucor* sp. (close to *M. hiemalis*) MJ:Mu1; **Rc,** *Rhizopus chinensis* MJ:Rh3

Sc, *Saccharomyces cerevisiae* MJ:YS2; **Cg,** *Candida glabrata* MS:YC5; **Pa,** *Pichia anomola* MN:YP1

Lb, *Lactobacillus bifermentans* MA:R5; **Pp,** *Pediococcus pentosaceus* MA:C1.

*Cell mixture of all above mentioned strains.

4.3.10. Consumers' Preference Trial

The consumers' preference trial showed that kodo ko jaanr prepared in the laboratory by cell suspension mixture of *Rhizopus chinensis* MJ:Rh3 and *Saccharomyces cerevisiae* MJ:YS2 as starter was more acceptable than the kodo ko jaanr prepared by conventional marcha. Market jaanr was liked extremely (score, 9) by 10 %, very much (score, 8) by 30 % and moderately (score, 7) by 60 %, the laboratory-made jaanr was liked extremely by 40 %, very much by 50 % and moderately by 10 % of the consumers.

4.4. BHAATI JAANR

Bhaati jaanr is a mild-alcoholic and juicy soft product with distinct sweet aroma, prepared from steamed glutinous rice (Plate 13). Both local and hybrid varieties of rice *(Oryza sativa* L.) are grown in low altitudes of these regions.

4.4.1. Synonym of bhaati jaanr

Bhaati jaanr is Nepali word for fermented rice beverage. Different ethnic people call it by their own dialect such as *tak thee* (Limboo), *kok umaak* (Rai), *kaiyan paa* (Gurung), *kaan chi* (Tamang), *kameshyaabu* (Sunwar), *chho haan* (Magar), *ja thon* (Newar), *dacchhang* (Sherpa), *laayakaa chhyaang* (Bhutia), and *jo chee* (Lepcha).

4.4.2. Traditional method of preparation

During traditional method of bhaati jaanr preparation, rice mainly glutinous, is cooked for about 15 min in an open cooker. Excess water is drained off and spread on a bamboo mat called mandro for cooling (~ 40° C). Powdered marcha (1 to 2 %) is sprinkled over cooked rice, mixed well and kept in a vessel or an earthen pot for 1-2 days at room temperature for saccharification. During saccharification sweet aroma is emitted out. After saccharification, the vessel is made airtight and fermented for 2-3 days in summer and 7-8 days in winter (Fig 8).

Plate 13. Rice and its fermented product bhaati jaanr

Plate 14. Maize and its fermented product makai ko jaanr

Plate 15. Wheat and its fermented product gahoon ko jaanr

Rice (glutinous)
↓
Cooked
↓
Dewatered, spread on a bamboo mat for cooling
↓
← added marcha (~ 2 %)
↓
Mixed
↓
Packed in earthen pot loosely, saccharified for 1-2 d
↓
Tightly closed the mouth of an earthen pot; fermented (25-30° C, 2-8 d)
↓
Bhaati jaanr

Fig 8. Flow sheet of bhaati jaanr preparation in South Sikkim

4.4.3. Mode of consumption

Bhaati jaanr is made into a thick paste by stirring the fermented mass with the help of a hand-driven wooden or bamboo-made stirrer. It is consumed directly. Sometimes, bhaati jaanr is stored in an earthenware crock for a week or more after desired fermentation is completed to make yellowish-white supernatant liquor called nigaar, collected at the bottom of the earthenware crock. Nigaar is drunk directly with or without addition of water. It is more alcoholic and slightly acidic in taste. It is a traditional diet for new mothers in villages who believe that it helps them to regain their strength.

Feeding frequency of bhaati jaanr is presented in Table 26. Only 5 % of people consume bhaati jaanr daily in rural areas of the Sikkim Himalayas. Per capita daily consumption of bhaati jaanr in the Darjeeling hills and Sikkim is 252.5 g and 323.5 g, respectively.

Table 26. Feeding frequency and consumption of bhaati jaanr

	The Darjeeling hills	Sikkim
	Feeding frequency (%)	
Daily	5	5
Weekly	20	25
Monthly	10	-
Occasional	20	10
Consumption *(g/capita/day)*	252.5 (0-893.0)	323.5 (0-1428.5)

Weekly means twice in a week. Occasional means every three months.

Values are the means of 100 households each in rural areas of the Darjeeling hills and Sikkim, respectively. Ranges are given in parentheses.

4.4.4. Similar product

Bhaati jaanr is similar to other fermented rice products of Asia such as *tapé ketan* (Indonesia), *lao-chao* (Cantonese in China), *tien-chiu-niang* (Mandarin in China), *yakju* (Korea) and *khao-maak* (Thailand).

4.4.5. Microorganisms

Twenty-four samples of bhaati jaanr were collected from different places of the Darjeeling hills and Sikkim and were analysed. Yeasts population was found higher than that of lactic acid bacteria (Table 27). Moulds were not recovered in any bhaati jaanr product analysed. Out of 127 strains of microorganisms isolated from bhaati jaanr samples, 69 isolates were yeasts and 58 isolates were lactic acid bacteria.

Table 27. Microbial load of bhaati jaanr

Source	$\times 10^7$ cfu/g fresh weight		
	Yeast	LAB	Total Viable Count
Rongli	5.0 (1.5-8.1)	0.8 (0.7-1.0)	5.6 (3.1-7.5)
Namchi	7.7 (6.8-8.7)	2.3 (2.0-2.4)	9.2 (7.5-11.5)
Aho	2.1 (1.6-2.6)	0.2 (0.07-0.3)	2.4 (1.4-4.1)
Kalimpong	0.2 (0.1-0.3)	0.01 (0.003-0.03)	0.2 (0.1-0.5)

Data represent the means of 6 samples from each source. Ranges are given in parentheses.

4.4.5.1. Characterisation and identification of yeasts

Representative strains of yeasts were selected on the basis of colony, cell morphology, vegetative reproduction and type of ascospores (Table 28). Only two types of yeasts were recovered from bhaati jaanr samples. Representative strains BR:YP1 and BN:YP1 were identified as *Pichia anomala* (E.C. Hansen) Kurtzman, and representative strains BA:YS1 and BK:YS2 as *Saccharomyces cerevisiae* Meyen ex Hansen (Table 29).

Table 28. Selection of representative strains of yeasts isolated from bhaati jaanr samples[a]

Source	Number of strains isolated	Colony	Cell shape	Mycelium	Ascospore	Grouped strains	Representative strains
Rongli	18	Ss	O-E	Pseudo	Hat-shaped	12	BR:YP1
		Ss	O-E	Pseudo	Globose	6	BR:YS1
Namchi	20	Ss	O-E	Pseudo	Hat-shaped	12	BN:YP1
		Ss	O-E	Pseudo	Globose	8	BN:YS2
Aho	15	Ss	O-E	Pseudo	Hat-shaped	8	BA:YP3
		Ss	O-E	Pseudo	Globose	7	BA:YS1
Kalimpong	16	Ss	O-E	Pseudo	Hat-shaped	8	BK:YP1
		Ss	O-E	Pseudo	Globose	8	BK:YS2

[a]Number of samples was 6 from each source. All isolates reproduced by multilateral budding.

Ss, smooth surface; O-E, Oval to ellipsoidal.

Table 29. Characteristics of representative strains of yeasts isolated from bhaati jaanr

Parameter	BR:YP1	BN:YP1	BA:YS1	BK:YS2
Cell width (μm)	1.1-3.1	1.3-3.0	1.4-3.0	1.5-3.8
Cell length (μm)	1.6-3.5	1.8-4.8	1.6-5.0	1.9-4.5
Nitrate reduction	+	+	–	–
Growth at 37°C	+	+	+	+
Sugar fermentation:				
Glucose	+	+	+	+
Galactose	–	–	+	+
Lactose	–	–	–	–
Maltose	+	+	+	+
Raffinose	+	+	+	+
Sucrose	+	+	+	+
Starch	–	–	+	+
Trehalose	+	+	–	–
Sugar assimilation:				
Arabinose	+	$+_w$	–	–
Cellobiose	+	+	–	–
Galactose	+	+	+	+
Glycerol	–	–	–	–
Inositol	–	–	–	–
Lactose	–	–	–	–
Maltose	+	+	+	+
Melibiose	–	–	+	+
Mannitol	+	+	–	–
Rhamnose	–	–	–	–
Raffinose	+	+	+	+
Sucrose	+	+	+	+
Starch	+	+	+	+
Trehalose	+	+	+	+
Xylose	–	+	–	–

Identification	*Pichia*	*Saccharomyces*

4.4.5.2. Characterisation and identification of bacteria

Out of 58 strains of lactic acid bacteria, isolated from bhaati jaanr samples, 31 strains were coccii-tetrads and 27 strains were non-sporeforming rods (Table 30). Species of lactic acid bacteria were identified as *Pediococcus pentosaceus* Mees and *Lactobacillus bifermentans* Kandler, Schillinger and Weiss (Table 31a & b).

Table 30. Selection of representative strains of LAB isolated from bhaati jaanr[a]

Source	Number of strains isolated	Cell shape	Gas from glucose	NH_3 from arginine	Grouped strains	Representative strains
Rongli	16	Coccus	–	+	8	BR:C1
		Rod	+	–	8	BR:R1
Namchi	12	Coccus	–	+	6	BN:C1
		Rod	+	–	6	BN:R2
Aho	16	Coccus	–	+	10	BA:C1
		Rod	+	–	6	BA:R2
Kalimpong	14	Coccus	–	+	7	BK:C1
		Rod	+	–	7	BK:R3

[a]Number of samples was 6 from each source

[b]All isolates were Gram-positive, catalase-negative, non-sporeformers and non-motile

Table 31a. Phenotypic characters of representative strains of LAB isolated from bhaati jaanr

Parameter	BR:C1	BN:C1	BA:C1	BK:C1	BR:R1	BN:R2	BA:R2	BK:R3
Cell shape	Ct	Ct	Ct	Ct	R	R	R	R
Cell diameter (μm)	0.2-0.7	0.2-0.7	0.2-0.5	0.4-0.7				
Cell width (μm)					0.2-0.3	0.2-0.3	0.2-0.4	0.2-0.3
Cell length (μm)					0.8-2.2	0.8-2.2	1.0-2.2	1.0-2.2
Anaerobic growth	+	+	+	+	+	+	+	+
Hydrolysis of:								
Casein	–	–	–	–	–	–	–	–
Gelatin	–	–	–	–	–	–	–	–
Arginin	+	+	+	+	–	–	–	–
Starch	–	–	–	–	–	–	–	–
Indole production	–	–	–	–	–	–	–	–
Nitrate reduction	–	–	–	–	–	–	–	–
Growth in NaCl:								
4.0 %	+	+	+	+	+	+	+	+
6.5 %	+	+	+	+	+	$+_w$	+	+
10.0 %	+	+	+	+	+	$+_w$	+	+
18.0 %	–	–	–	–	–	–	–	–
Growth in pH:								
4.2	+	+	+	+	+	+	+	+
7.5	+	+	+	+	+	+	+	+
8.5	+	+	+	+	+	+	+	+
Growth at:								
15° C	+	+	+	+	+	+	+	+
45° C	–	–	–	–	–	–	–	–

Ct, coccus, tetrad; R, rod in chain with 2-3 cells

Table 31b. Sugar fermentation of LAB strains using API 50 CHL system

Parameter	BR:C1	BN:C1	BA:C1	BK:C1	BR:R1	BN:R2	BA:R2	BK:R3
Glycerol	+	+	–	–	–	–	+	–
Erythritol	+	+	–	–	–	–	–	–
D-Arabinose	+	+	–	–	–	–	–	–
L-Arabinose	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	+
L-Xylose	–	–	–	–	–	–	–	$+_w$
Adonitol	+	+	–	–	–	–	+	–
β-Methyl-D-Xyloside	–	–	–	–	–	–	–	–
Galactose	+	+	+	+	+	$+_w$	–	+
D-Glucose	–	–	+	+	+	–	+	+
D-Fructose	–	–	+	+	+	+	+	+
D-Mannose	–	–	+	+	–	–	+	–
L-Sorbose	+	–	–	–	–	–	–	–
Rhamnose	+	+	–	–	–	–	+	–
Dulcitol	+	+	–	–	–	–	–	–
Inositol	–	–	–	–	–	–	–	–
Mannitol	–	–	–	–	–	–	–	$+_w$
Sorbitol	+	+	–	–	–	–	–	–
α-Methyl-D-Mannoside	+	+	–	–	–	–	+	–
α-Methyl-D-Glucoside	–	–	–	–	$+_w$	$+_w$	+	+
N-Acetyl-Glucosamine	+	+	+	+	$+_w$	$+_w$	+	+
Amygdalin	+	+	+	+	–	–	–	–
Arbutin	–	–	–	–	–	–	–	–
Esculin	–	–	–	–	–	–	–	–
Salicin	$+_w$	–	–	–	–	–	–	–
Cellobiose	+	+	+	+	–	–	–	–
Maltose	+	+	+	+	+	+	+	+

Parameter	BR:C1	BN:C1	BA:C1	BK:C1	BR:R1	BN:R2	BA:R2	BK:R3
Lactose	–	–	–	+	–	–	–	–
Melibiose	–	–	–	–	$+_w$	$+_w$	+	$+_w$
Sucrose	–	–	–	–	–	–	–	–
Trehalose	+	+	+	+	–	–	–	–
Inulin	–	–	–	–	–	–	–	–
Melezitose	–	–	–	–	–	–	–	–
Raffinose	–	–	–	–	–	–	–	–
Starch	–	–	–	–	–	–	–	–
Glycogen	–	–	–	–	–	–	–	–
Xylitol	–	–	–	–	–	–	–	–
Gentiobiose	+	+	+	+	–	–	–	–
D-Turanose	–	–	–	–	–	–	–	–
D-Lyxose	–	–	–	–	–	–	–	–
D-Tagatose	+	+	+	+	–	–	–	–
D-Fucose	–	–	–	–	–	–	–	–
L-Fucose	–	–	–	–	–	–	–	–
D-Arabitol	–	–	–	–	–	–	–	–
L-Arabitol	–	–	–	–	–	–	–	–
Gluconate	–	–	–	–	$+_w$	$+_w$	–	–
2-Keto-Gluconate	–	–	–	–	$+_w$	$+_w$	–	–
5-Keto-Gluconate	–	–	–	–	$+_w$	$+_w$	–	$+_w$
Identification	*Pediococcus*				*Lactobacillus*			

4.4.6. Proximate composition

Proximate composition of bhaati jaanr is presented in Table 32. Average pH of the product was 3.5, acidity and alcohol contents were 0.24 % and 5.9 %, respectively. Fat, protein and calorie contents remained same as the substrate. Considerable increase in calcium, manganese, iron, zinc,

sodium, potassium and phosphorous was observed in bhaati jaanr over the substrate (Table 33).

Table 32. Proximate composition of cooked rice and bhaati jaanr

Parameter	Unfermented	Fermented (Bhaati jaanr)			
	Cooked rice	Rongli	Namchi	Aho	Kalimpong
pH	6.01 (6.0-6.02)	3.5 (3.3-3.7)	3.4 (3.2-3.6)	3.3 (3.1-3.5)	3.7 (3.5-4.0)
Moisture (%)	67.2 (62.4-68.2)	82.8 (80.9-83.9)	85.2 (83.6-86.8)	82.4 (79.3-89)	83.0 (78.4-85.0)
Acidity (%)	0.01 (0.01-0.02)	0.20 (0.10-0.30)	0.25 (0.2-0.3)	0.25 (0.2-0.3)	0.27 (0.2-0.34)
Alcohol (%)	0.0 (0.0-0.0)	6.6 (5.3-7.4)	5.0 (4.0-6.0)	5.3 (4.0-8.3)	6.8 (6.6-6.9)
Ash (% DM)	0.6 (0.5-1.2)	1.7 (1.1-2.0)	2.0 (1.8-2.2)	0.8 (0.7-1.0)	2.1 (1.5-2.3)
Fat (% DM)	2.4 (1.9-2.9)	2.9 (1.9-3.2)	2.0 (1.5-2.5)	1.2 (1.0-2.0)	1.8 (1.2-2.6)
Protein (% DM)	9.5 (8.7-10.3)	9.4 (8.0-10.1)	9.8 (8.2-10.4)	9.3 (8.3-9.7)	9.5 (8.5-9.8)
Crude fibre (% DM)	0.6 (0.4-0.8)	1.5 (1.4-1.7)	ND	1.4 (1.2-1.7)	ND
Carbohydrate (% DM)	87.5 (85.6-88.9)	86.0 (84.7-89.0)	86.2 (84.9-88.5)	88.7 (87.2-90.0)	86.6 (85.3-88.8)
Energy (MJ/100g DM)	409.6 (394.3-422.9)	407.7 (387.9-425.2)	402.0 (385.9-418.1)	402.8 (391.0-416.8)	400.6 (386.0-417.8)

Data represent the means of 5 samples from each source. % DM, percentage on dry matter basis. Ranges are given in parentheses. ND, not determined.

Table 33. Mineral contents of raw and fermented rice

Mineral	mg/100 g dry matter	
	Rice	Bhaati jaanr
Calcium	2.5	12.8
Magnesium	22	50
Manganese	0.4	1.4
Copper	0.5	1.4
Iron	2.2	7.7
Zinc	0.6	2.7
Sodium	5.3	24.7
Potassium	57	146
Phosphorus	156	595

Data represent the means of 2 samples.

4.4.7. Successional studies during bhaati jaanr fermentation

Bhaati jaanr was prepared in the laboratory following the traditional method by using marcha, collected from Aho village, as mentioned in 3.3.6. Successional studies were carried at every 1 day interval within a range of 0-10 days.

4.4.7.1. Microbial changes

Loads of moulds decreased significantly ($P<0.05$) during fermentation and disappeared after the fifth day of fermentation (Table 34). Population of yeasts increased significantly ($P<0.05$) from 10^5 cfu/g to 10^8 cfu/g within 2 d, and decreased to a level of 10^5 cfu/g in 10 d (Fig 9). Exponential increase in load of lactic acid bacteria was significant ($P<0.05$) till second day of fermentation, and then declined slowly. However, the decrease was not significant till 6 d. Total viable count

increased significantly ($P<0.05$) from 0 d to 2 d, and decreased significantly ($P<0.05$) in every interval of 1 d.

Table 34. Microbial changes during bhaati jaanr fermentation

Fermentation time (days)	Log cfu/g			
	Mould	Yeast	LAB	Total Count
0	4.3 ± 0.13^{a}	5.9 ± 0.08^{h}	6.0 ± 0.13^{bc}	6.3 ± 0.08^{h}
1	3.5 ± 0.29^{b}	7.5 ± 0.13^{d}	6.4 ± 0.33^{ab}	7.5 ± 0.08^{d}
2	1.7 ± 0.08^{c}	8.0 ± 0.13^{a}	7.0 ± 0.08^{a}	8.1 ± 0.08^{a}
3	0.4 ± 0.26^{d}	7.9 ± 0.08^{b}	6.9 ± 0.08^{a}	8.0 ± 0.08^{b}
4	<DL	7.8 ± 0.08^{c}	6.6 ± 0.08^{a}	7.8 ± 0.08^{c}
5	0	7.5 ± 0.16^{d}	6.6 ± 0.08^{a}	7.5 ± 0.08^{d}
6	0	6.8 ± 0.08^{e}	6.6 ± 0.08^{a}	7.0 ± 0.08^{e}
7	0	6.5 ± 0.08^{f}	5.9 ± 0.13^{bc}	6.6 ± 0.13^{f}
8	0	6.4 ± 0.08^{g}	5.7 ± 0.13^{cd}	6.5 ± 0.16^{g}
9	0	5.8 ± 0.08^{i}	5.2 ± 0.13^{d}	6.3 ± 0.08^{h}
10	0	5.6 ± 0.08^{j}	5.2 ± 0.16^{d}	6.0 ± 0.08^{i}

Data represent the means ± SD of three batches of fermentation. Data were transformed into logarithmic values. DL, detection limit (10 cfu/g).

Values bearing different superscripts in each column differ significantly ($P<0.05$).

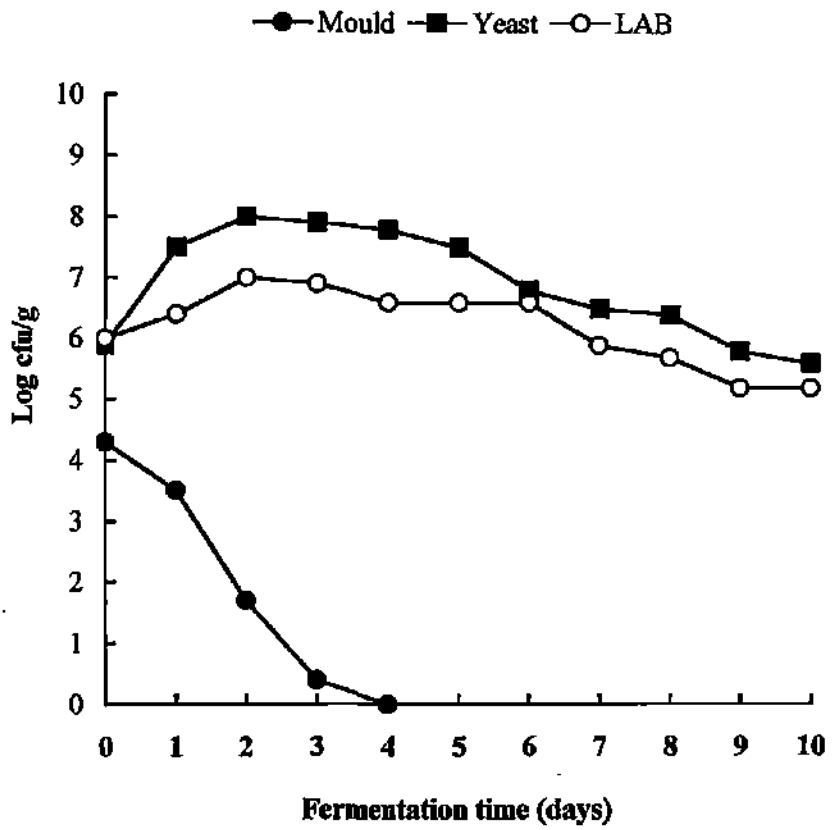


Fig 10. Changes in microbial load during bhaati jaanr fermentation. Values are the means of three batches of fermentation. LAB, lactic acid bacteria.

4.4.7.2. Physico-chemical and enzymatic changes

Temperature of fermenting rice remained relatively constant in between 28° C to 30° C throughout the fermentation after first day. During fermentation, pH decreased significantly ($P<0.05$) from 6.27 to 3.21 within 2 d and slightly increased to 3.96 at the end (Fig 10). Titratable acidity increased significantly ($P<0.05$) from 0.01 % to 0.20 % till 4 d and remained at a level of 0.17 % till end (Table 35). Alcohol content increased significantly ($P<0.05$) during fermentation (Fig 11).

Table 35. Physico-chemical changes during bhaati jaanr fermentation

Fermentation time (days)	Temperature (°C)	pH	Acidity (%)	Alcohol (%)
0	26.0 ± 0.00^{f}	6.27 ± 0.01^{a}	0.01 ± 0.00^{g}	0.1 ± 0.04^{e}
1	31.0 ± 0.00^{a}	3.36 ± 0.01^{i}	0.11 ± 0.01^{f}	0.4 ± 0.08^{e}
2	30.0 ± 0.00^{b}	3.21 ± 0.07^{j}	0.19 ± 0.01^{b}	2.8 ± 0.13^{d}
3	30.0 ± 0.00^{b}	3.37 ± 0.00^{i}	0.19 ± 0.02^{b}	3.6 ± 0.08^{d}
4	29.2 ± 0.13^{c}	3.45 ± 0.01^{h}	0.20 ± 0.00^{a}	6.8 ± 1.03^{c}
5	28.5 ± 0.00^{d}	3.51 ± 0.01^{g}	0.18 ± 0.01^{c}	7.3 ± 0.49^{bc}
6	28.5 ± 0.00^{d}	3.71 ± 0.01^{f}	0.18 ± 0.02^{c}	7.8 ± 0.29^{b}
7	28.5 ± 0.00^{d}	3.78 ± 0.01^{e}	0.18 ± 0.01^{c}	9.3 ± 0.29^{a}
8	28.3 ± 0.08^{e}	3.93 ± 0.01^{c}	0.18 ± 0.01^{c}	9.6 ± 0.29^{a}
9	28.3 ± 0.05^{e}	3.89 ± 0.01^{d}	0.18 ± 0.01^{c}	9.8 ± 0.13^{a}
10	28.2 ± 0.05^{e}	3.96 ± 0.01^{b}	0.17 ± 0.01^{d}	10.1 ± 0.29^{a}

Data represent the means ± SD of three batches of fermentation.

Values bearing different superscripts in each column differ significantly ($P<0.05$).

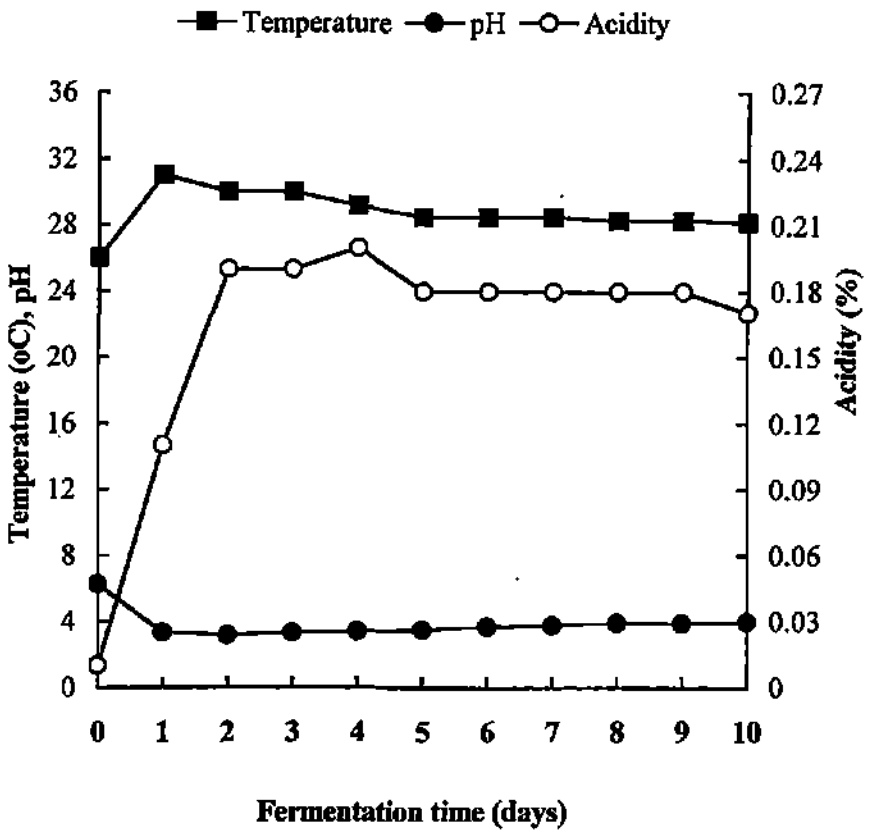


Fig. 11. Changes in temperature, pH and acidity during bhaati jaanr fermentation. Values are the means of three batches of fermentation.

Reducing sugar contents increased significantly ($P<0.05$) from 0.01 % to 12.6 % within 3 d, and declined till end (Table 36). Total sugar contents decreased significantly ($P<0.05$) throughout the fermentation. Maximum activities of α–amylase and glucoamylase was observed on third day of fermentation and decreased significantly ($P<0.05$) till the end (Table 36 and Fig 12).

Table 36. Biochemical and enzymatic changes during bhaati jaanr fermentation

Fermentation time (days)	Reducing sugar (%)	Total sugar (%)	α-amylase (U/g)	Glucoamylase (U/mg)
0	0.01 ± 0.00^{g}	64.1 ± 1.02^{a}	12.5 ± 1.23^{h}	61.7 ± 9.55^{e}
1	2.2 ± 0.41^{d}	61.6 ± 2.49^{a}	37.6 ± 0.65^{c}	181.1 ± 13.15^{c}
2	3.8 ± 0.21^{b}	49.1 ± 2.94^{b}	41.8 ± 0.82^{b}	227.9 ± 6.45^{b}
3	12.6 ± 0.13^{a}	39.3 ± 0.98^{c}	45.3 ± 0.25^{a}	510.9 ± 8.90^{a}
4	2.9 ± 0.21^{e}	30.9 ± 0.94^{d}	34.9 ± 0.74^{d}	115.5 ± 4.33^{d}
5	0.6 ± 0.08^{e}	27.7 ± 0.65^{e}	20.1 ± 0.08^{e}	41.3 ± 0.45^{e}
6	0.6 ± 0.05^{e}	27.6 ± 0.21^{e}	16.6 ± 0.49^{f}	44.4 ± 1.92^{e}
7	0.5 ± 0.05^{ef}	22.2 ± 0.94^{f}	15.5 ± 0.82^{g}	52.6 ± 1.18^{e}
8	0.4 ± 0.05^{ef}	18.1 ± 0.50^{g}	12.6 ± 0.33^{h}	40.3 ± 0.86^{e}
9	0.3 ± 0.08^{efg}	16.2 ± 0.05^{g}	8.8 ± 0.41^{i}	35.1 ± 3.39^{e}
10	0.2 ± 0.05^{fg}	13.4 ± 1.47^{h}	7.2 ± 0.16^{j}	33.5 ± 1.35^{e}

Data represent the means ± SD of three batches of fermentation.

Values bearing different superscripts in each column differ significantly ($P<0.05$).

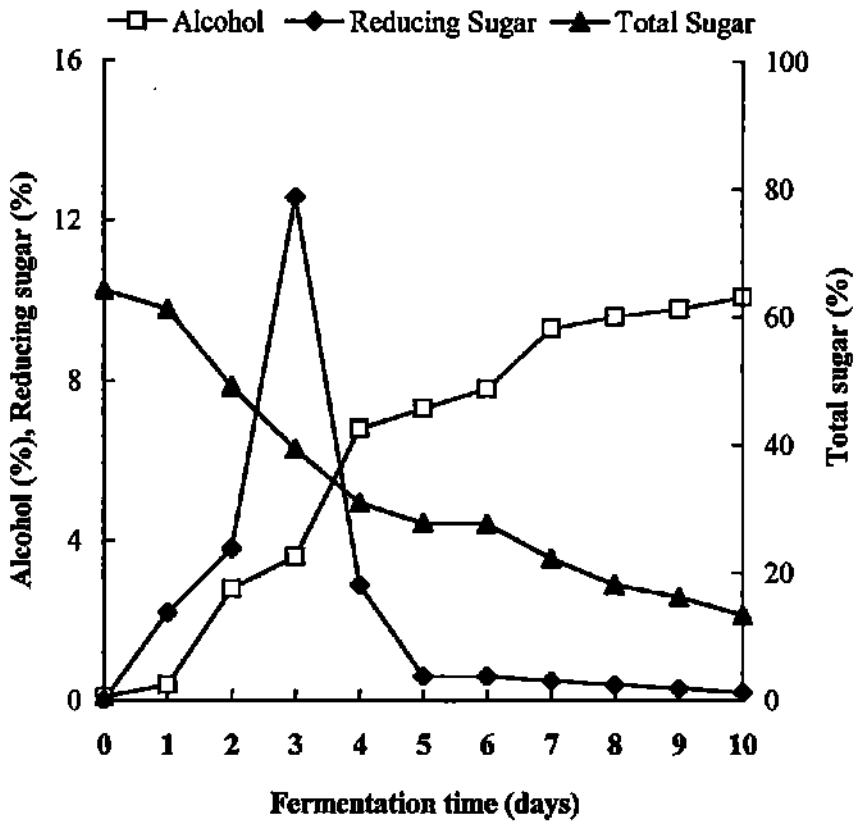


Fig. 12. Changes in alcohol, reducing sugar and total sugar contents of rice during bhaati jaanr fermentation. Values are the means of three batches of fermentation.

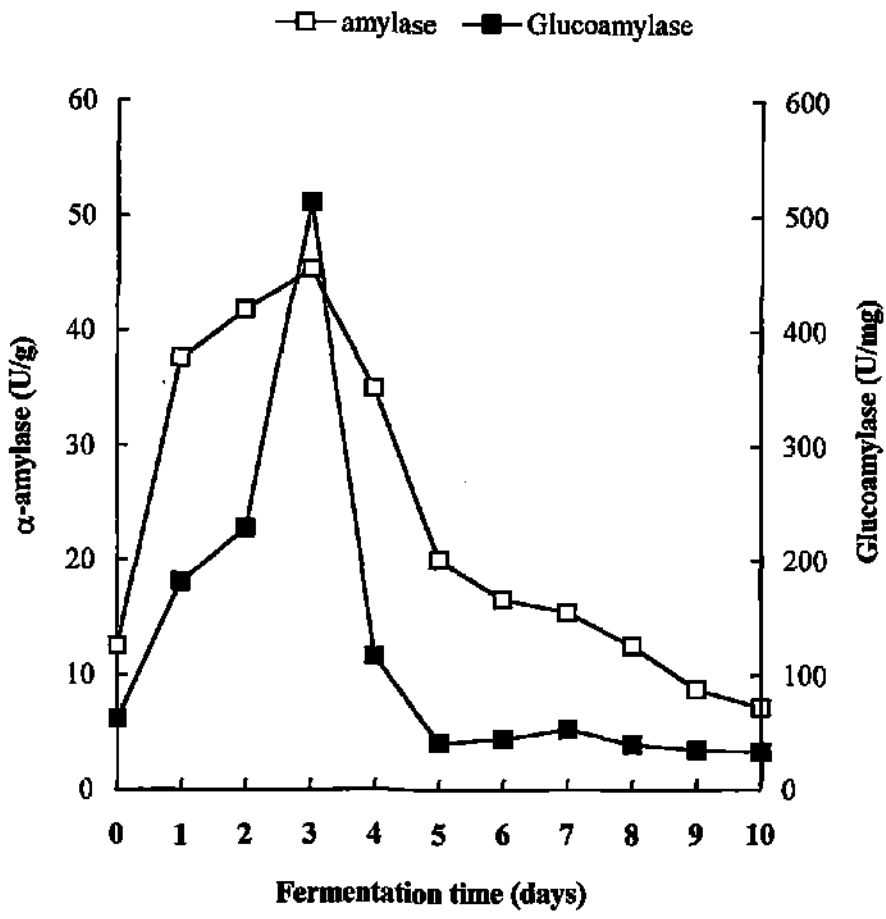


Fig. 13. Changes in enzymatic activities of rice during bhaati jaanr fermentation. Values are the means of three batches of fermentation.

4.5. MAKAI KO JAANR

Makai ko jaanr is a viscous, slightly bitter, mild-alcoholic beverage, fermented from maize (Plate 14). Preparation and consumption of makai ko jaanr are confined to few places of the Darjeeling hills and Sikkim.

4.5.1. Synonym of makai ko jaanr

Common name for the maize is makai in Nepali language. Different ethnic gropus call it such as *makai thee* by Limboo, *yobbhacha umaak* by Rai, *makhain paa* by Gurung, *maagnila jheen* by Tamang, *aakan shyaabu* by Sunwar, *makai haan* by Magar, *kahni thon* by Newar, *lichee chhyaang* by Sherpa, *kinya chhyaang* by Bhutia and *kanchung chee* by Lepcha.

4.5.2. Method of preparation

During traditional method of preparation of makai ko jaanr, dry seeds of maize *(Zea mays* L.) are grinded and dehuksed. Bigger grinded granules of maize called *chekhla* are selected for preparation of makai ko jaanr. Chekhla are washed, cooked to a thick porridge, cooled and inoculated with powdered marcha (1.0-2.0 %). Saccharification and fermentation method of makai ko jaanr are same as bhaati jaanr (Fig 13).

Dry maize seeds
↓
Grinded and dehuksed
↓
Bigger grinded granules of maize *(chekhla)* selected
↓
Washed and cooked
↓
Dewatered, spread on bamboo mat for cooling
↓
← added marcha (~2 %)
↓
Mixed
↓
Packed in earthen pot loosely, saccharified for 1-2 d
↓
Tightly closed the mouth of earthen pot; fermented (25-30° C, 2-8 d)
↓
Makai ko jaanr

Fig 15. Flow sheet of makai ko jaanr preparation at Algarah in Kalimpong

4.5.3. Mode of consumption

Fermented porridge is mashed, filtered and desirable amount of lukewarm water is added. Extract of makai ko jaanr is drunk directly. It is slightly bitter, mild alcoholic beverage. Feeding frequency of makai ko jaanr in rural areas of the Sikkim Himalayas is shown in Table 37.

Table 37. Feeding frequency and consumption of makai ko jaanr

	The Darjeeling hills	Sikkim
Feeding frequency (%)		
Daily	20	-
Weekly	10	15
Monthly	5	5
Occasional	10	15
Consumption *(g/capita/day)*	58.2 (0-285.7)	323.5 (0-476.2)

Weekly means twice in a week. Occasional means every three months.

Values are the means of 100 households each in rural areas of the Darjeeling hills and Sikkim, respectively. Ranges are given in parentheses.

4.5.4. Microorganisms

Load of yeasts was found 10 times higher than that of lactic acid bacteria in makai ko jaanr samples (Table 38). Moulds were not recovered in the final product.

Table 38. Microbial load of makai ko jaanr

Source	x 10^7cfu/g fresh weight		
	Yeast	LAB	Total Viable Count
Barnyak	2.5 (2.2-2.8)	0.4 (0.02-0.7)	7.4 (5.0-9.8)
Kalimpong	0.5 (0.3-0.7)	0.02 (0.01-0.03)	0.6 (0.5-0.7)

Data represent the means of 6 samples from each source. Ranges are given in parentheses.

Out of 100 strains of microorganisms isolated from twelve samples of makai ko jaanr, 54 isolates were yeasts and 46 isolates were lactic acid bacteria.

4.5.4.1. Characterisation and identification of yeasts

Table 39 shows selection of representative strains of yeasts, isolated from twelve samples of makai ko jaanr. Out of 54 strains of yeasts, 30 were oval with hat-shaped ascopres and 24 strains were oval with globose-shaped ascopores. The representative strains with hat-shaped ascospores MJB:YP1 and MJK:YP5 were identified as *Pichia anomala* (E.C. Hansen) Kurtzman; representative strains with globose-shaped ascospores MJB:YS4 and MJK:YS7 were identified *Saccharomyces cerevisiae* Meyen ex Hansen (Table 40).

Table 39. Selection of representative strains of yeasts isolated from makai ko jaanr samples[a]

Source	Number of strains isolated	Colony	Cell shape	Mycelium	Ascospore	Grouped strains	Representative strains
Barnyak	24	Ss	O-E	Pseudo	Hat-shaped	12	MJB:YP1
		Ss	O-E	Pseudo	Globose	12	MJB:YS4
Kalimpong	30	Ss	O-E	Pseudo	Hat-shaped	18	MJK:YP5
		Ss	O-E	Pseudo	Globose	12	MJK:YS7

[a]Number of samples was 6 from each source. All isolates reproduced by multilateral budding.

Ss, smooth surface; O-E, Oval to ellipsoidal.

Table 40. Characteristics of representative strains of yeasts isolated from makai ko jaanr

Parameter	MJB:YP1	MJK:YP5	MJB:YS4	MJK:YS7
Cell width (µm)	1.0-3.0	1.1-3.0	1.5-3.2	1.7-3.5
Cell length (µm)	1.6-4.6	1.6-4.4	1.6-5.1	1.9-4.9
Nitrate reduction	+	+	–	–
Growth at 37°C	+	+	+	+
Sugar fermentation:				
Glucose	+	+	+	+
Galactose	–	–	+	+
Lactose	–	–	–	–
Maltose	+	+	+	+
Raffinose	+	+	+	+
Sucrose	+	+	+	+
Starch	–	–	+	+
Trehalose	+	+	–	–
Sugar assimilation:				
Arabinose	+	+	–	–
Cellobiose	+	+	–	–
Galactose	+	+	+	+
Glycerol	–	–	–	–
Inositol	–	–	–	–
Lactose	–	–	–	–
Maltose	+	+	+	+
Melibiose	–	–	+	+
Mannitol	+	+	–	–
Rhamnose	–	–	–	–
Raffinose	+	+	+	+
Sucrose	+	+	+	+
Starch	+	+	+	+
Trehalose	+	+	+	+
Xylose	–	+	–	–
Identification	*Pichia*		*Saccharomyces*	

4.5.4.2. Characterisation and identification of bacteria

Representative strains of lactic acid bacteria were selected (Table 41). Out of 46 LAB strains isolated from makai ko jaanr, 28 strains were coccii-tetrads and 18 strains were non-sporeforming rods. Representative strains of coccii-tetrads MJB:C7 and MJK:C6 were identified as *Pediococcus pentosaceus* Mees and representative strains of rod-shaped isolates MJB:R4 and MJK:R5 were identified *Lactobacillus bifermentans* Kandler, Schillinger and Weiss (Table 42 a & b).

Table 41. Selection of representative strains of LAB isolated from makai ko jaanr[a]

Source	Number of strains isolated	Cell shape	Gas from glucose	NH_3 from arginine	Grouped strains	Representative strains
Barnyak	20	Coccus	–	+	12	MJB:C7
		Rod	+	–	8	MJB:R4
Kalimpong	26	Coccus	–	+	16	MJK:C6
		Rod	+	–	10	MJK:R5

[a]Number of samples was 6 from each source

[b]All isolates were Gram-positive, catalase-negative, non-sporeformers and non-motile

Table 42a. Phenotypic characteristics of representative strains of LAB isolated from makai ko jaanr

Parameter	MJB:C7	MJK:C6	MJB:R4	MJK:R5
Cell shape	Coccus, tetrads	Coccus, tetrads	Rod	Rod
Cell diameter (μm)	0.2-0.5	0.2-0.6		
Cell width (μm)			0.2-0.3	0.2-0.3
Cell length (μm)			1.0-2.2	1.0-2.3
Anaerobic growth	+	+	+	+
Hydrolysis of:				
Casein	–	–	–	–
Gelatin	–	–	–	–
Arginine	+	+	–	–
Starch	–	–	–	–
Indole production	–	–	–	–
Nitrate reduction	–	–	–	–
Growth in NaCl:				
4.0 %	+	+	+	+
6.5 %	+	+	+	+
10.0 %	+	+	+	+
18.0 %	–	–	–	–
Growth in pH:				
4.2	+	+	+	+
7.5	+	+	+	+
8.5	+	+	+	+
Growth at:				
15° C	+	+	+	+
45° C	–	–	–	–

Table 42 b. Sugar fermentation of LAB strains using API 50 CHL system

Parameter	MJB:C7	MJK:C6	MJB:R4	MJK:R5
Glycerol	+	+	–	+
Erythritol	+	+	–	–
D-Arabinose	+	+	–	–
L-Arabinose	+	+	+	+
Ribose	+	+	+	+
D-Xylose	+	+	+	+
L-Xylose	–	–	–	–
Adonitol	+	+	+	–
β-Methyl-D-Xyloside	–	–	–	–
Galactose	+	+	–	$+_w$
D-Glucose	–	–	+	+
D-Fructose	–	–	+	+
D-Mannose	–	$+_w$	+	–
L-Sorbose	$+_w$	–	–	–
Rhamnose	+	+	+	–
Dulcitol	+	+	–	–
Inositol	–	–	–	–
Mannitol	–	–	–	$+_w$
Sorbitol	+	+	–	–
α-Methyl-D-Mannoside	+	+	–	$+_w$
α-Methyl-D-Glucoside	–	–	+	+
N-Acetyl-Glucosamine	+	+	+	$+_w$
Amygdalin	+	+	–	–
Arbutin	–	–	–	–
Esculin	–	–	–	–
Salicin	$+_w$	–	–	–
Cellobiose	+	+	–	–
Maltose	+	+	+	$+_w$

Parameter	MJB:C7	MJK:C6	MJB:R4	MJK:R5
Lactose	–	–	–	–
Melibiose	–	–	+	+
Sucrose	–	–	–	–
Trehalose	+	+	–	–
Inulin	–	–	–	–
Melezitose	–	–	–	–
Raffinose	–	–	–	–
Starch	–	–	–	–
Glycogen	–	–	–	–
Xylitol	–	–	–	–
Gentiobiose	+	+	–	–
D-Turanose	–	–	–	–
D-Lyxose	–	–	–	–
D-Tagatose	+	+	–	–
D-Fucose	–	–	–	–
L-Fucose	–	–	–	–
D-Arabitol	–	–	–	–
L-Arabitol	–	–	–	–
Gluconate	–	–	–	–
2-Keto-Gluconate	–	–	–	–
5-Keto-Gluconate	–	–	$+_w$	$+_w$
Identification	*Pediococcus*		*Lactobacillus*	

4.5.5. Proximate composition

Proximate composition of makai ko jaanr is shown in Table 43. Mean average pH, acidity and alcohol content of the product was 3.3, 0.38 % and 2.5 %, respectively. Alcohol content was comparatively less in makai ko jaanr than that of other cereal-based jaanr products. Increase in crude fibre content was observed in makai ko jaanr. Calorie value

remained almost same in both substrate and the product. Remarkable increase in iron, potassium and phosphorous was observed in makai ko jaanr (Table 44).

Table 43. Proximate composition of cooked maize and makai ko jaanr

Parameter	Unfermented	Fermented (Makai ko jaanr)	
	Cooked maize	Barnyak	Kalimpong
PH	6.0 (5.9-6.2)	3.2 (3.1-3.5)	3.4 (3.1-3.8)
Moisture (%)	65.2 (63.0-68.2)	82.4 (79.2-84)	81.4 (79.0-83.1)
Acidity (%)	0.01 (0.01-0.01)	0.40 (0.34-0.62)	0.35 (0.31-0.56)
Alcohol (%)	0	2.0 (1.8-2.1)	3.0 (2.8-3.6)
Ash (% DM)	1.4 (1.2-2.2)	2.2 (1.5-2.7)	2.0 (1.5-2.5)
Fat (% DM)	3.7 (3.0-3.9)	3.0 (2.7-3.5)	3.2 (2.8-3.7)
Protein (% DM)	13.8 (11.0-14.5)	12.9 (10.5-14.0)	13.2 (10.8-14.3)
Crude fibre (% DM)	1.4 (1.0-2.8)	2.9 (2.0-3.8)	2.7 (2.3-3.2)
Carbohydrate (% DM)	81.1 (79.4-84.8)	81.9 (79.8-85.3)	81.6 (79.5-84.9)
Energy (MJ/100g DM)	412.9 (388.6-432.3)	406.2 (385.5-428.7)	408.0 (386.4-430.1)

Data represent the means of 5 samples from each source.

% DM, percentage on dry matter basis. Ranges are given in parentheses.

Table 44. Mineral contents of raw and fermented maize

Mineral	mg/100 g dry matter	
	Maize	Makai ko jaanr
Calcium	2.3	5.2
Magnesium	53	70
Manganese	0.3	0.5
Copper	0.4	0.9
Iron	5.5	17
Zinc	0.7	1.2
Sodium	12.3	21.5
Potassium	153	227
Phosphorus	342	538

Data represent the means of 2 samples.

4.5.6. Successional studies during makai ko jaanr fermentation

Makai ko jaanr was prepared in the laboratory following the traditional method by using marcha, collected from Aho village, as mentioned in 3.3.6. Successional studies were carried at every 1day interval within a range of 0-10 days.

4.5.6.1. Microbial changes

The load of moulds declined significantly ($P<0.05$) during makai ko jaanr fermentation and disappeared after the 4th day (Table 45). The load of yeasts increased significantly ($P<0.05$) from 0 day to 2 day and remained constant till 8th day, and decreased significantly ($P<0.05$) till an end of fermentation. Load of lactic acid bacteria increased significantly ($P<0.05$) from 0 day to 2 day and declined gradually till the end (Fig 14). Total viable counts increased significantly ($P<0.05$) from

10^6 to 10^7 cfu/g within second day and remained constant till 8 d, and then decreased significantly ($P<0.05$) to 10^6 cfu/g on 10 d.

Table 45. Microbial changes during makai ko jaanr fermentation

Fermentation time (days)	Log cfu/g			
	Mould	Yeast	LAB	Total Count
0	4.2 ± 0.08^{a}	6.9 ± 0.08^{d}	5.0 ± 0.17^{g}	6.7 ± 0.34^{de}
1	3.2 ± 0.05^{b}	7.6 ± 0.22^{b}	5.8 ± 0.29^{ef}	7.6 ± 0.08^{b}
2	1.5 ± 0.16^{c}	7.7 ± 0.08^{a}	7.1 ± 0.08^{ab}	7.8 ± 0.08^{ab}
3	< DL	7.8 ± 0.08^{a}	7.5 ± 0.08^{a}	7.9 ± 0.08^{a}
4	0	7.8 ± 0.08^{a}	6.9 ± 0.08^{bc}	7.8 ± 0.08^{ab}
5	0	7.8 ± 0.08^{a}	6.8 ± 0.08^{bc}	7.9 ± 0.08^{a}
6	0	7.8 ± 0.08^{a}	6.7 ± 0.08^{bcd}	7.9 ± 0.08^{a}
7	0	7.8 ± 0.08^{a}	6.6 ± 0.08^{cd}	7.8 ± 0.08^{ab}
8	0	7.7 ± 0.13^{a}	6.4 ± 0.08^{de}	7.8 ± 0.08^{ab}
9	0	7.2 ± 0.08^{c}	5.8 ± 0.08^{ef}	7.1 ± 0.08^{c}
10	0	6.5 ± 0.13^{e}	6.0 ± 0.54^{f}	6.6 ± 0.08^{e}

Data represent the means ± SD of three batches of fermentation. Data were transformed into logarithmic values. DL, detection limit (10 cfu/g).

Values bearing different superscripts in each column differ significantly ($P<0.05$).

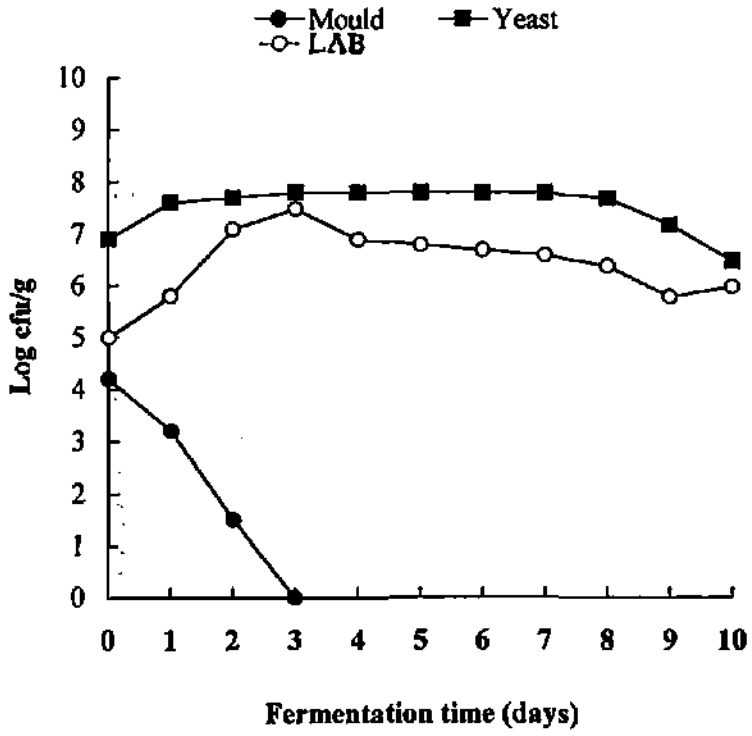


Fig 14. Changes in microbial load during makai ko jaanr fermentation. Values are the means of three batches of fermentation. LAB, lactic acid bacteria.

4.5.6.2. Physico-chemical and enzymatic changes

Temperature of fermenting maize remained relatively constant throughout the fermentation after third day (Table 46). During fermentation, pH dropped significantly ($P<0.05$) from 6.24 to 3.89 within 10 days. Acidity increased significantly ($P<0.05$) on each day till the sixth day, after which there was no significant change (Fig 15). The mean alcohol content increased significantly ($P<0.05$) during fermentation (Fig 16).

Table 46. Physico-chemical changes during makai ko jaanr fermentation

Fermentation time (days)	Temperature (°C)	pH	Acidity (%)	Alcohol (%)
0	24.0 ± 0.00^{f}	6.24 ± 0.00^{a}	0.01 ± 0.00^{g}	0^{j}
1	29.5 ± 0.41^{a}	3.14 ± 0.00^{j}	0.11 ± 0.01^{f}	0.5 ± 0.08^{i}
2	29.0 ± 0.41^{b}	3.18 ± 0.02^{i}	0.16 ± 0.01^{e}	2.9 ± 0.13^{h}
3	28.5 ± 0.00^{cd}	3.24 ± 0.01^{h}	0.17 ± 0.01^{d}	4.2 ± 0.08^{g}
4	28.3 ± 0.00^{de}	3.38 ± 0.01^{g}	0.22 ± 0.01^{a}	5.1 ± 0.08^{f}
5	28.2 ± 0.08^{de}	3.52 ± 0.01^{f}	0.21 ± 0.01^{b}	5.7 ± 0.25^{e}
6	28.0 ± 0.00^{e}	3.58 ± 0.01^{e}	0.18 ± 0.01^{c}	6.9 ± 0.08^{d}
7	28.0 ± 0.00^{e}	3.72 ± 0.01^{d}	0.17 ± 0.01^{d}	7.1 ± 0.13^{d}
8	28.0 ± 0.00^{e}	3.86 ± 0.01^{c}	0.17 ± 0.01^{d}	7.7 ± 0.21^{c}
9	28.0 ± 0.00^{e}	3.89 ± 0.01^{b}	0.17 ± 0.02^{d}	8.9 ± 0.13^{b}
10	28.0 ± 0.00^{e}	3.89 ± 0.02^{b}	0.17 ± 0.01^{d}	9.9 ± 0.21^{a}

Data represent the means ± SD of three batches of fermentation.

Values bearing different superscripts in each column differ significantly ($P<0.05$).

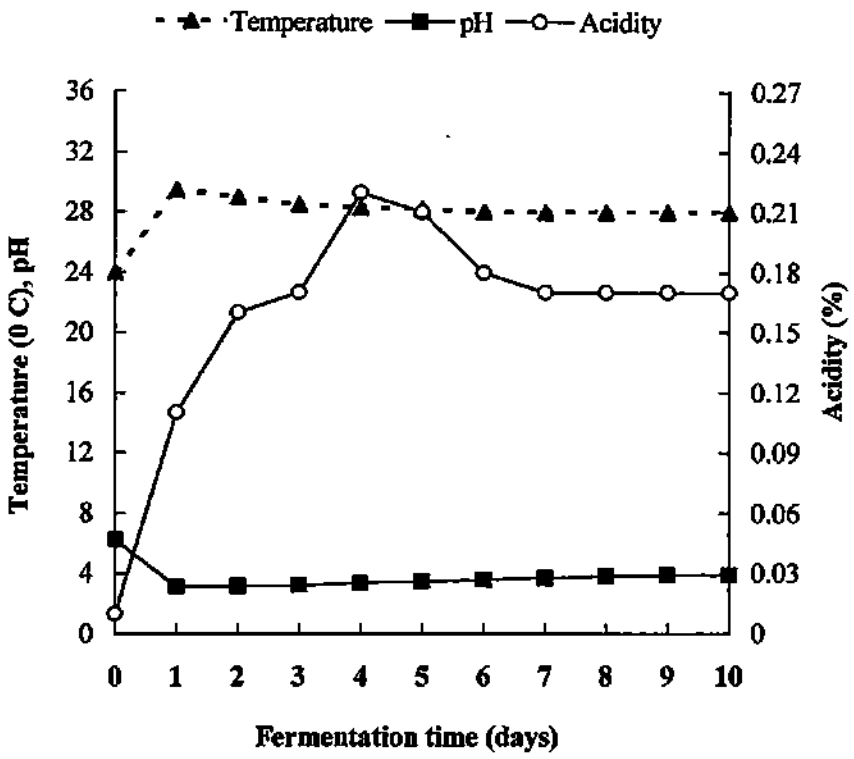


Fig 15. Changes in temperature, pH and acidity in fermenting maize during makai ko jaanr fermentation. Values are the means of three batches of fermentation.

Reducing sugar increased significantly ($P<0.05$) from 0 d to 1 d and then gradually reduced until 10 d (Table 16). Total sugar content decreased significantly ($P<0.05$) till 5 d, and the level decreased slowly towards the end of fermentation (Fig 17). Maximum ∝-amylase activities of fermenting millets was observed on 3 d and whereas that of glucoamylase activities was observed on 1 d (Table 47 and Fig 17).

Table 47. Biochemical and enzymatic changes during makai ko jaanr fermentation

Fermentation time (days)	Reducing sugar (%)	Total sugar (%)	∝-amylase (U/g)	Glucoamylase (U/mg)
0	0.01 ± 0.01^{i}	70.0 ± 6.61^{a}	18.2 ± 0.33^{e}	26.3 ± 1.88^{e}
1	4.1 ± 0.03^{a}	58.8 ± 4.41^{b}	34.9 ± 0.74^{d}	78.0 ± 9.10^{a}
2	3.9 ± 0.08^{a}	49.8 ± 4.33^{c}	47.7 ± 0.57^{b}	58.8 ± 4.74^{b}
3	1.5 ± 0.25^{bc}	38.4 ± 4.57^{d}	48.6 ± 1.10^{a}	58.3 ± 15.31^{b}
4	1.3 ± 0.08^{cd}	24.6 ± 4.90^{e}	47.2 ± 0.33^{b}	54.7 ± 5.47^{b}
5	1.2 ± 0.08^{de}	20.2 ± 2.29^{f}	45.9 ± 0.74^{c}	50.7 ± 7.69^{bc}
6	1.1 ± 0.08^{de}	18.8 ± 3.18^{fg}	18.1 ± 0.65^{e}	49.6 ± 0.49^{bc}
7	1.0 ± 0.25^{ef}	15.7 ± 2.53^{gh}	12.8 ± 0.65^{f}	42.0 ± 4.25^{cd}
8	0.8 ± 0.16^{f}	13.4 ± 2.21^{hi}	9.5 ± 0.41^{g}	37.5 ± 4.16^{de}
9	0.4 ± 0.08^{gh}	13.0 ± 2.61^{hi}	7.6 ± 0.25^{h}	35.0 ± 4.08^{de}
10	0.2 ± 0.08^{hi}	10.5 ± 2.29^{i}	7.0 ± 0.33^{h}	30.0 ± 1.63^{e}

Data represent the means ± SD of three batches of fermentation.

Values bearing different superscripts in each column differ significantly ($P<0.05$).

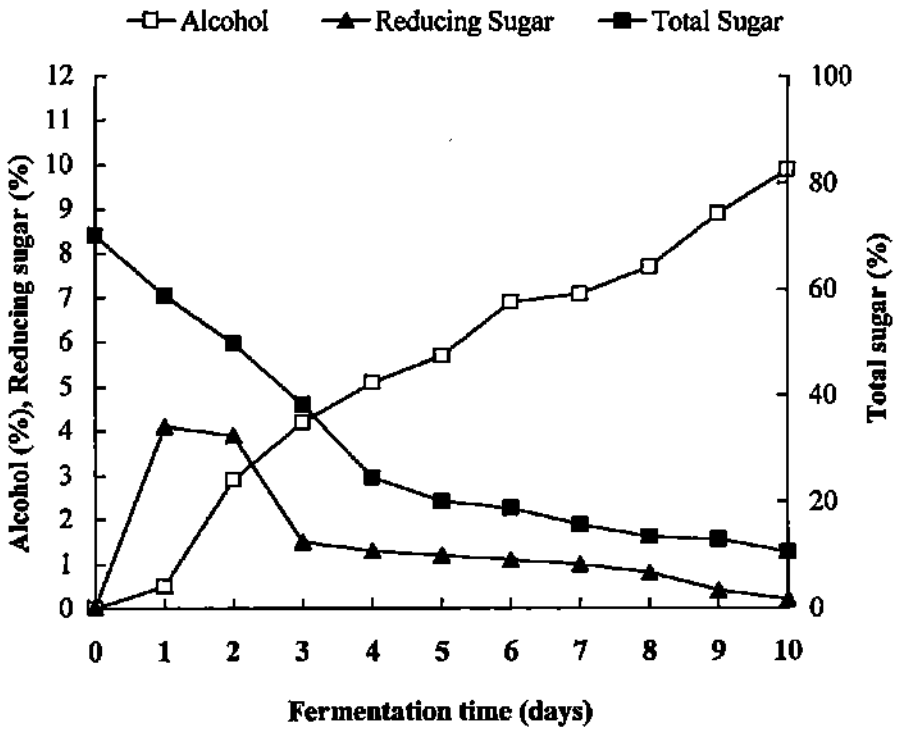


Fig 16. Changes in alcohol, reducing sugar and total sugar contents of fermenting maize during makai ko jaanr fermentation. Values are the means of three batches of fermentation.

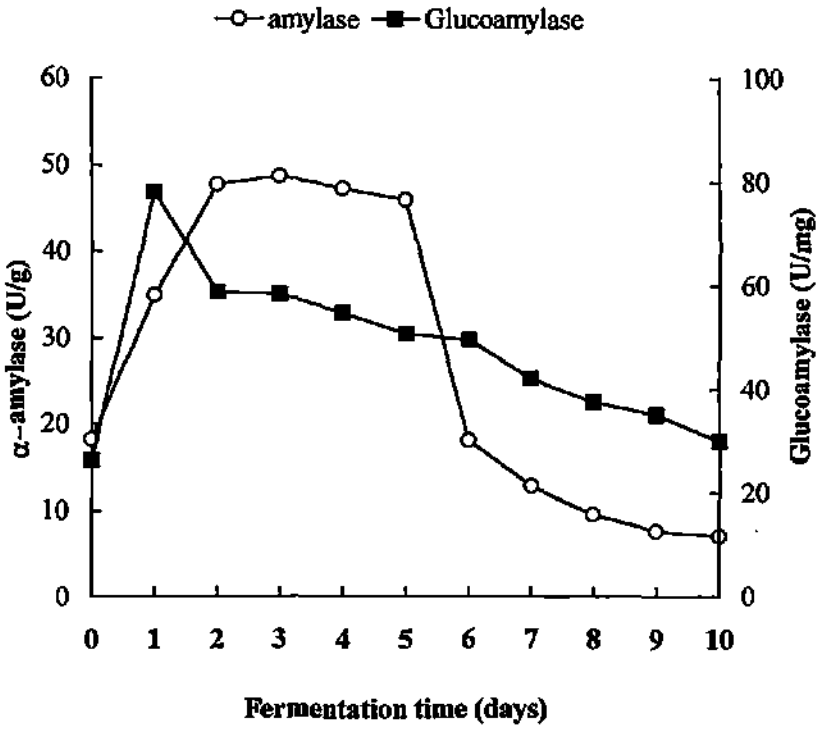


Fig 17. Changes in enzymatic activities of fermenting maize during makai ko jaanr fermentation. Values are the means of three batches of fermentation.

4.6. GAHOON KO JAANR

Gahoon ko jaanr is an alcoholic beverage, prepared from wheat (*Triticum aestivum* L.) (Plate 15). Method of preparation of gahoon ko jaanr is same as kodo ko jaanr (Fig 18). It is drunk directly by filtering the fermented grits. Sometimes, gahoon ko jaanr is mixed with kodo ko jaanr and filled up in toongbaa and consumed. Gahoon ko jaanr is mostly used for distillation to get raksi, clear distilled liquor.

Wheat
↓
Cleaned and Washed
↓
Cooked
↓
Excess water drained off
↓
Spread on bamboo mat for cooling (20 –25° C)
↓
← added marcha (~ 2 %)
↓
Mixed
↓
Packed in heaps and saccharified for 2-4 d
↓
Transferred into a closed earthen pot
↓
Fermented (25-30° C, 3-7 d)
↓
Gahoon ko jaanr

Fig 18. Flow sheet of gahoon ko jaanr preparation in East Sikkim

Feeding frequency of gahoon ko jaanr is shown in Table 48. Daily per capita consumption of gahoon ko jaanr is 13.5 g and 91.5 g in the Darjeeling hills and Sikkim, respectively.

Table 48. Feeding frequency and consumption of gahoon ko jaanr

	The Darjeeling hills	Sikkim
Feeding frequency (%)		
Daily	0	5
Weekly	15	10
Monthly	0	5
Occasional	0	5
Consumption *(g/capita/day)*	13.5 (0-142.3)	91.5 (0-357.1)

Weekly means twice in a week. Occasional means every three months.

Values are the means of 100 households each in rural areas of the Darjeeling hills and Sikkim, respectively. Ranges are given in parentheses.

4.6.1. Microorganisms

Ten samples of gahoon ko jaanr were collected from Aho village in East Sikkim and Algarah in Kalimpong, and were analysed for microbial load (Table 49). Yeasts population was 10 times higher than that of lactic acid bacteria. Moulds were not recovered in the final product.

Table 49. Microbial load of gahoon ko jaanr

Source	x 10^7cfu/g fresh weight		
	Yeast	LAB	Total Viable Count
Aho	4.8 (3.6-7.8)	0.4 (0.3-0.5)	5.6 (2.8-9.1)
Kalimpong	0.6 (0.2-1.0)	0.04 (0.03-0.05)	6.7 (5.0-10.0)

Data represent the means of 5 samples from each source. Ranges are given in parentheses.

4.6.1.1. Identification of yeasts and bacteria

Out of 47 strains of microorganisms isolated from ten samples of gahoon ko jaanr, 25 isolates were yeasts (Table 50) and 22 strains were lactic acid bacteria (Table 51). Representative strains of yeasts GJA:YP3 and GJK:YP2 were identified as *Pichia anomala* (E.C. Hansen) Kurtzman, and representative strains GJA:YS1 and GJK:YS3 were identified as *Saccharomyces cerevisiae* Meyen ex Hansen. Representative strains of lactic acid bacteria GJA:C1 and GJ:C2 were identified as *Pediococcus pentosaceus* Mees and GJA:R1 and GJK:R2 as *Lactobacillus bifermentans* Kandler, Schillinger and Weiss.

Table 50. Selection of representative strains of yeasts isolated from gahoon ko jaanr samples[a]

Source	Number of strains isolated	Colony	Cell shape	Mycelium	Ascospore	Grouped strains	Representative strains
Aho	15	Ss	O-E	Pseudo	Hat-shaped	9	GJA:YP3
		Ss	O-E	Pseudo	Globose	6	GJA:YS1
Kalimpong	10	Ss	O-E	Pseudo	Hat-shaped	5	GJK:YP2
		Ss	O-E	Pseudo	Globose	5	GJK:YS3

[a]Number of samples was 6 from each source. All isolates reproduced by multilateral budding.

Ss, smooth surface; O-E, Oval to ellipsoidal.

Table 51. Selection of representative strains of LAB isolated from gahoon ko jaanr[a]

Source	Number of strains isolated	Cell shape	Gas from glucose	NH_3 from arginine	Grouped strains	Representative strains
Aho	12	Coccus	–	+	6	GJA:C1
		Rod	+	–	6	GJA:R1
Kalimpong	10	Coccus	–	+	5	GJK:C2
		Rod	+	–	5	GJK:R2

[a]Number of samples was 6 from each source

[b]All isolates were Gram-positive, catalase-negative, non-sporeformers and non-motile

4.6.2. Proximate composition

Proximate composition of gahoon ko jaanr is presented in Table 52. The mean pH, acidity and alcohol content of the product was 3.9, 0.35 % and 3.1 %, respectively. Remarkable increase in crude fibre content was observed in gahoon ko jaanr. Calorie content remained same in the product. Magnesium, iron, sodium, potassium and phosphorous contents increased in finish product (Table 53).

Table 52. Proximate composition of cooked wheat and gahoon ko jaanr

Parameter	Unfermented	Fermented (Gahoon ko jaanr)	
	Cooked wheat	Aho	Kalimpong
pH	6.7 (6.6-6.7)	3.9 (3.4-4.0)	3.8 (3.6-4.0)
Moisture (%)	53.2 (52.0-55.0)	73.4 (70.0-75.5)	74.0 (67.0-81.0)
Acidity (%)	0.01 (0.01-0.01)	0.40 (0.34-0.62)	0.34 (0.25-0.42)
Alcohol (%)	0	2.6 (1.2-5.1)	3.5 (3.3-3.6)
Ash (% DM)	1.5 (1.3-1.8)	2.3 (1.5-2.8)	2.6 (1.8-2.9)
Fat (% DM)	0.6 (0.4-0.7)	0.6 (0.4-0.8)	0.5 (0.3-0.7)
Protein (% DM)	12.3 (11.8-12.7)	12.8 (11.0-13.0)	11.8 (11.0-12.0)
Crude fibre (% DM)	5.0 (3.3-6.0)	10.5 (9.2-11.9)	10.4 (9.5-11.0)
Carbohydrate (% DM)	85.6 (84.8-86.5)	84.3 (83.4-87.1)	85.1 (84.4-86.9)
Energy (MJ/100g DM)	397.0 (390.0-403.1)	393.8 (381.2-407.6)	392.1 (384.3-401.9)

Data represent the means of 5 samples from each source.

% DM, percentage on dry matter basis. Ranges are given in parentheses.

Table 53. Mineral contents of raw and fermented wheat

Mineral	mg/100 g dry matter	
	Wheat	Gahoon ko jaanr
Calcium	11.6	18.3
Magnesium	54	102
Manganese	1.7	2.9
Copper	0.8	1.0
Iron	4.7	13.6
Zinc	1.1	1.6
Sodium	13.1	26.7
Potassium	182	300
Phosphorous	392	763

Data represent the means of 2 samples.

4.7. RAKSI

Raksi is a clear distilled wine with characteristic aroma prepared from fermented cereal beverages such as kodo ko jaanr, bhaati jaanr, makai ko jaanr, gahoon ko jaanr, etc. Fermented masses of buckwheat, potato, canna, cassava roots are also distilled to get raksi.

4.7.1. Synonym of raksi

Raksi is a common term in Nepali meaning alcoholic drink. The Limboo calls it *sijongwaa aara*, Rai calls it *aarakha/hemma*, Gurung calls it *paa*, Tamang calls it *aaerak*, Sunwar calls it *rindho*, Newar calls it *aayala*, Magar calls it *dhise*, Sherpa calls it *aarak*, Bhutia calls it *aarak* and Lepcha calls it *aarok*.

4.7.2. Method of preparation

Fermented cereal beverages are distilled in a big cylindrical metallic vessel continuously for 2-3 h in an earthen-oven over firewood (Plate 16). At the top of the distilling vessel, cold water is kept in a metallic container used as condenser, water is replaced for 3-5 times after it gets boiled. Condensed raksi is collected in a small collecting metallic vessel called *poini*. Raksi prepared after replacing condensing water for 3 times is known as *theen pani raksi* which contains high alcohol and traditionally prepared for religious purposes. Raksi prepared after replacing the condensing water for 5 times is known as *panch pani raksi* which is a common alcoholic drink. Raksi is usually stored in bottle capped with piece of dry corncob (Plate 17).

Sometimes, petals of *Rhododendron* spp. are mixed during distillation to give distinct aroma in raksi. This type of raksi is

commonly prepared in Rimbik of Darjeeling and few places in West Sikkim.

4.7.3. Mode of consumption

Raksi is drunk directly without addition of water along with fried meat or side dish. Average daily per capita consumption of raksi is 91.5 ml and 66.9 ml in rural areas of the Darjeeling hills and Sikkim, respectively (Table 54).

The 100 ml of raksi costs about Rs.1.70 to Rs.2.00 per 100 ml in villages of the Sikkim Himalayas.

Table 46. Feeding frequency and consumption of raksi

	The Darjeeling hills	Sikkim
Feeding frequency (%)		
Daily	55	20
Weekly	15	20
Monthly	-	-
Occasional	15	20
Consumption *(ml/capita/day)*	91.5 (0-357.1)	66.9 (0-267.9)

Weekly means twice in a week. Occasional means every three months.

Values are the means of 100 households each in rural areas of the Darjeeling hills and Sikkim, respectively. Ranges are given in parentheses.

4.7.4. Equipment used

The traditional raksi distillation apparatus is made up of a metallic vessel (Plate 18). In main cylindrical metallic vessel measuring 40 cm × 30 cm × 25 cm, fermented grits (kodo ko jaanr or bhaati jaanr or gahoon ko jaanr) are steamed continuously for 2-3 h over firewood. Above main cylindrical vessel, a perforated container called *phunga* is placed. Inside *phunga*, a small metallic collector called *poini* is placed on iron-made tripod called *odhan* to collect distillate (raksi). Avove *phunga*, metallic vessel with cold water used as condenser is placed. Bottom of the condenser vessel is plastered by mud with the tip of *phunga* to prevent excess ventilation during distillation. This apparatus can distil 2-4 kg of jaanr to get 1-2 L of raksi after replacing condensing water 3 times.

4.7.5. Ethnical importance

Ethnical importance and essence of raksi in social activities by different ethnic communities was documented and noted in discussion chapter.

4.7.6. Proximate composition

Average pH, acidity and alcohol content of raksi, collected from different places of the Darjeeling hills and Sikkim, was 3.6, 0.06 % and 22.9 %, respectively (Table 55). Raksi distilled from bhaati jaanr mixed with few petals of *Rhododendron* showed highest alcohol content (~ 27 %) comparable to raksi prepared from other fermented cereals (Table 55).

Plate 16. *Raksi* distillation

Plate 17. *Raksi* in bottle capped with corncob

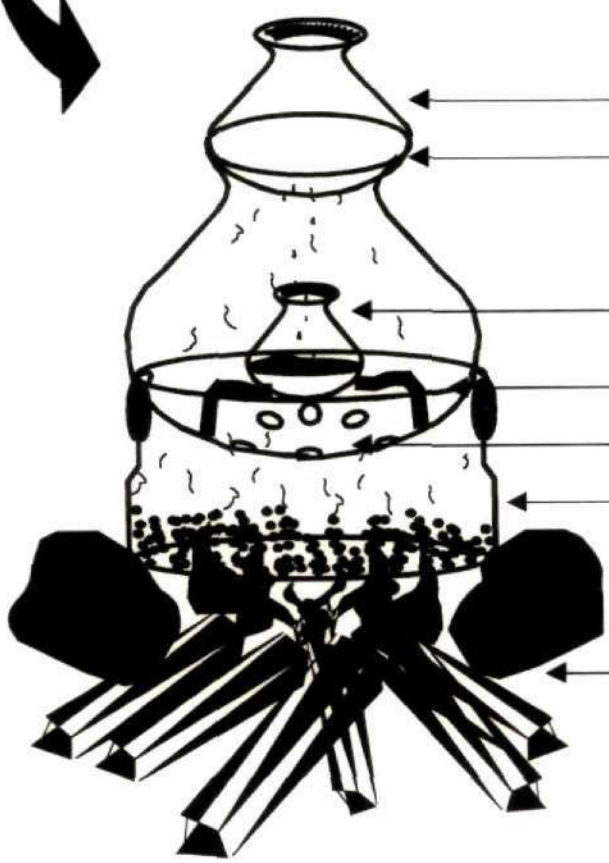


Plate 18. Internal diagrammatic view of *raksi* distillation apparatus

Table 55. Proximate composition of raksi

Parameter	RA	RRB	RR	RK	RN
pH	3.6 (3.2-3.8)	3.5 (3.3-3.7)	3.6 (3.3-3.8)	3.5 (3.4-3.6)	3.6 (3.5-3.7)
Acidity (%)	0.08 (0.03-0.1)	0.1 (0.1-0.1)	0.04 (0.03-0.04)	0.05 (0.04-0.06)	0.04 (0.04-0.05)
Alcohol (%)	22.6 (22.5-23.3)	24.0 (22.9-26.5)	22.8 (22.7-22.9)	22.7 (22.5-22.8)	22.5 (22.5-22.5)

RA: raksi (distilled from gahoon ko jaanr) collected from Aho

RRB: raksi (distilled from bhaati jaanr mixed with *Rododendron* petals) collected from Rimbik in Darjeeling

RR: raksi (distilled from bhaati jaanr) collected from Rongli

RK: raksi (distilled from makai ko jaanr) collected from Kalimpong

RN: raksi (distilled from kodo ko jaanr) collected from Namchi.

Data represent the means of 5 samples from each source. Ranges are given in parentheses.