4

Results

4.1. Survey

Following a moderate survey through a well-structured pretested proforma and by personal interviews, the distribution, indigenous methods of preparation, modes of consumption, shelf life and ethnic values of different legume-based traditional fermented foods used by the people of India were documented (Table 5).

4.1.1. Fermented legume products

4.1.1.1. Aakhuni

Aakhuni is a nonsalted soybean-fermented food traditionally prepared (Fig. 1) and consumed by the Sema tribe. It is also popular among the Aoo tribe who call it 'azukenshe'. The finished product

Table 5. Legume-based traditional fermented foods of India

Food	State/area of consumption	Substrate	Nature and mode of consumption
Legume			
Aakhuni	Nagaland	Soybean	Cooked beans with typical ammoniacal flavour, covered with sticky materials; made to thick curry; side dish
Amriti	West Bengal	Blackgram	Deep-fried, sugar syrup-dipped pretzel; confectionary or sweet dish
Bekang	Mizoram	Soybean	Cooked beans with typical ammoniacal flavour, covered with sticky materials; made to thick curry; side dish
Bethu	Manipur	Soybean	Cooked beans with typical ammoniacal flavour, covered with sticky materials; made to thick curry; side dish
Daler vada	West Bengal	Grass pea, lentil	Deep-fried spicy ball; snack
Daler nadu	West Bengal	Bengalgram, pigeon pea, grass pea	Sweet confectionary ball; snack
Hawaijar	Manipur	Soybean	Cooked beans with typical ammoniacal flavour, covered with sticky materials; made to thick curry; side dish
Kinema	Darjeeling hills of West Bengal, Sikkim	Soybean	Cooked beans with typical ammoniacal flavour, covered with sticky materials; made to thick curry; side dish
Masyaura	Darjeeling hills of West Bengal, Sikkim	Blackgram	Dried, hollow balls or cones; spicy condiment
Papad	All over India	Legume flours	Thin, circular, wafer-like product; deep-fried or roasted; snack or condiment
Ras vada	West Bengal	Green pea, mung dal	Sweet ball, with coconut fillings, immersed in sugar syrup; snack
Turumbai	Meghalaya, Arunachal Pradesh, Assam	Soybean	Cooked beans with typical ammoniacal flavour, covered with sticky materials; made to thick curry; side dish
Vada	Southern India	Legume beans	Deep-fried ball; breakfast food or snack with chutney, sambar or curd
Wadi	Northern and Eastern India	Blackgram	Dried, hollow balls or cones; spicy condiment
Cereal-legume mixture			
Adai	Southern India	Blackgram, Bengalgram, pigeon pea, rice	Confectionary pancake; savoury with sauces or chutney
Bhapa pitha	Orissa, West Bengal	Grass pea, rice	Soft and spongy steam-cooked cake; breakfast food or snack, with chutney
Chakuli	Orissa	Blackgram, rice	Fried pancake; snack, with sambar, sugar, jaggery and vegetable curry
Chhuchipatra pitha	Orissa	Blackgram, rice	Fried pancake stuffed with grated coconut, dahi-chhana (curd) and sugar; savoury
Chitou	Orissa	Bengalgram, rice	Fried pancake; snack, with sambar, sugar, jaggery and vegetable curry
Daler patisapta	West Bengal	Green pea, lentil, rice	Fried pancake stuffed with coconut and sugar; snack
Dhokla	Gujarat (now all over India)	Bengalgram, rice	Steamed-cooked soft cake; breakfast food or snack
Dosa	Southern (now all over India)	Blackgram, rice	Thin, fried, griddled pancake; breakfast food, along with chutney and sambar
Enduri pitha	Orissa	Blackgram, rice	Steam-cooked cake stuffed with coconut, dahi-chhana and sugar fillings; savoury
Idli	Southern (now all over) India	Blackgram, rice	Steam-cooked spongy cake; breakfast food or snack, along with chutney and sambar
Maunha pitha	Orissa	Blackgram, rice	Steam-cooked spongy cake supplemented with grated coconut, cashew nut, raisins, sugar or jaggery; breakfast food or snack
Poda pitha	Orissa	Blackgram/grass pea, rice	Oven-baked cake; breakfast food or snack

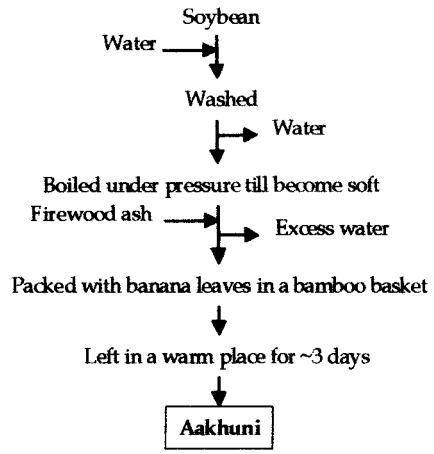

Fig. 1. Flow sheet for the preparation of aakhuni

resembles kinema in several organoleptic aspects including appearance, texture and flavour. Selling of aakhuni (sold by volume) packed with banana leaves and loosely tied by straw is a common scenario in the periodic markets ('haat').

4.1.1.2. Amriti

The method of preparation of amriti (Fig. 2 and 3) resembles that of jalebi, excepting that the refined wheat flour (maida) in jalebi is replaced by blackgram dal and the fermentation time is shorter. Amriti has shelf-life of 6-7 days and consumed during different festivals.

Fig. 2. Market sample of amriti

4.1.1.3. Bekang

The traditional method of preparation of bekang (Fig. 4) is similar to that of aakhuni, excepting the step of adding firewood ash. The finished product resembles kinema. Bekang is made to chutney or curry and served with rice.

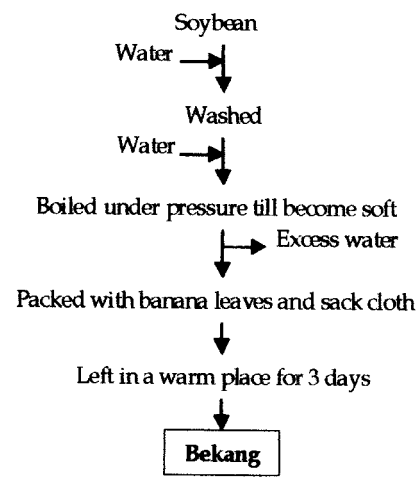

Fig. 4. Flow sheet for the preparation of bekang

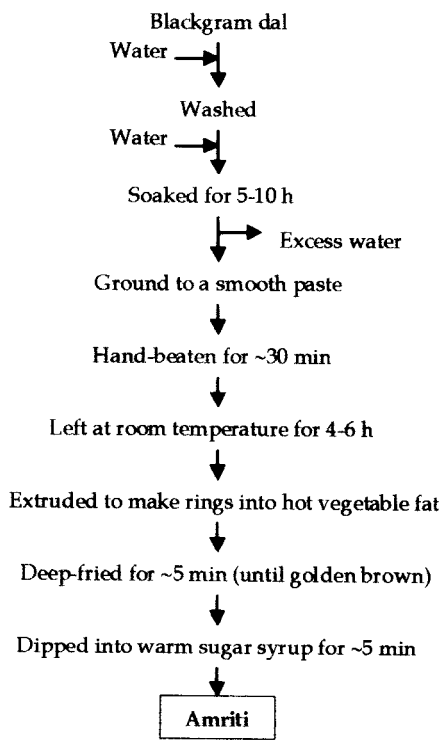

Fig. 3. Flow sheet for the preparation of amriti

4.1.1.4. Bethu

Bethu is traditionally prepared (Fig. 5) and consumed by the Kuki tirbe. Though the serving protocol of bethu varies among the consumers, it is generally used as curry to serve with rice.

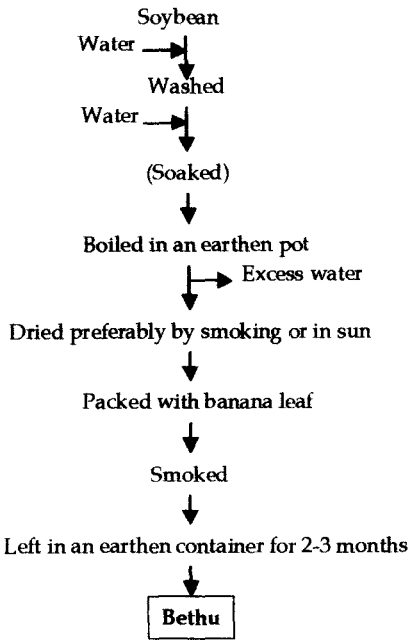

Fig. 5. Flow sheet for the preparation of bethu

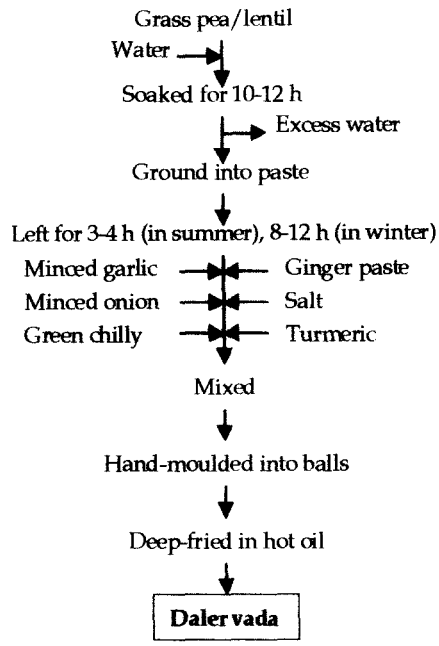

Fig. 6. Flow sheet for the preparation of daler vada

4.1.1.5. Daler vada

Traditionally, daler vada (literally, vada made up of legume) is prepared (Fig. 6) from varying proportions of grass pea and lentil. Sometimes a little amount of raw or parboiled rice flour may be added. Daler vada is served with chutney made up of ground nut, coconut and mustard seeds.

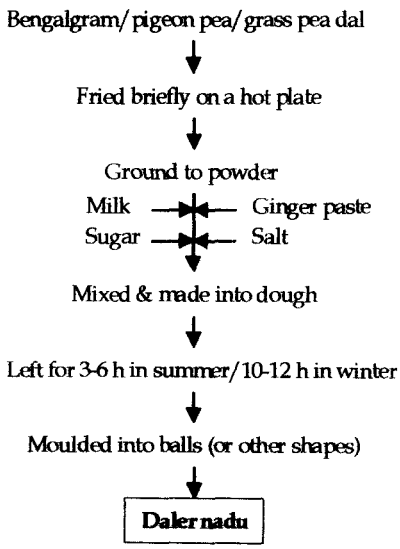

Fig. 7. Flow sheet for the preparation of daler nadu

4.1.1.6. Daler nadu

Traditionally, it is prepared (Fig. 7) in every house during festivals. These sweet balls are usually taken as snacks with puffed rice ('moori'). 'Chaitra sankranti' is the festival during which daler nadu is specially prepared.

4.1.1.7. Hawaijar

The traditional method of hawaijar preparation (Fig. 8) resembles that of kinema. In haats hawaijar is found to sell in small packets wrapped with banana leaves, loosely tied with straw.

4.1.1.8. Kinema

Kinema is used by the nonBrahmin Nepalis. In the traditional method of its preparation (Fig. 9), yellow seeded soybeans are preferred. The boiled soybean grits, containing teared hulls, are wrapped with fresh fern fronds or leaves of banana or *Leucosceptrum canum* Smith (sometimes by broad

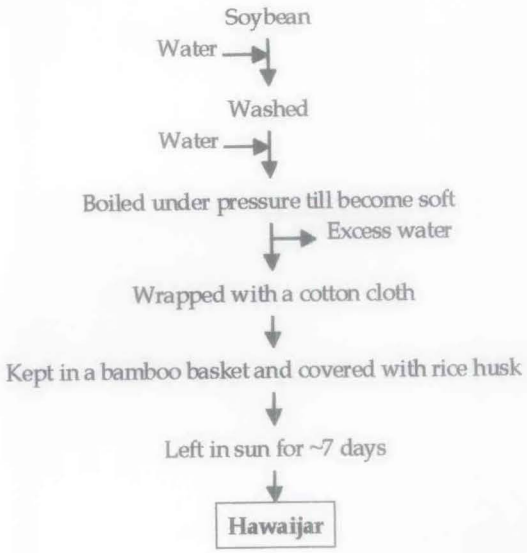

Fig. 8. Flow sheet for the preparation of hawaijar

Kinema has been used traditionally as an excellent substitute for animal protein sources. It is used to give a pleasant, nutlike flavour to curry. Fresh kinema (Fig. 10) keeps for 2-3 days during summer and a maximum of one week in winter. The shelf life of kinema is often lengthened to one month by drying in the sun or by keeping on earthen ovens in kitchens.

Fig. 10. Market sample of kinema

leaves of other plants such as *Macaranga pustulata* King, *Ficus hookeriana* Corner and *Bauhinia vahlii* Wt. and Arnott), covered with sackcloth and kept in a bamboo basket above an earthen oven in the kitchen to ferment for 1-3 days. The desired state of fermentation is indicated by the formation of a typical kinema flavour dominated by ammonia. Kinema shows long stringy threads when touched with fingers; the longer the thread better is the quality of kinema.

Fig. 9. Flow sheet for the preparation of kinema

4.1.1.9. Masyaura

Masyaura, consumed by certain castes of Nepalis, is dried, hollow, brittle, spongy friable ball of 5-10 cm in diameter and shares similarity with North Indian Punjabi wadi and South Indian sandige. It is prepared in cottage or home scale level. Traditionally, blackgram dal is the primary substrate for masyaura (Fig. 11), occasionally supplemented with starchy root/tuber of colocasia, dioscorea, radish or ash gourd, depending upon their availability. Masayura has shelf life of about one year.

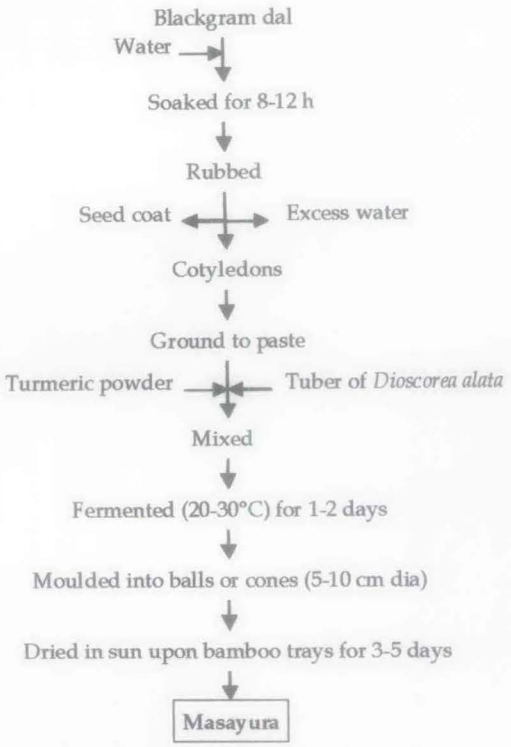

Fig. 11. Flow sheet for the preparation of masayura

However, partial replacement of blackgram with other legume and cereal flour is also being practiced in recent days. This is yet another option to reduce the cost of production. Moreover, a cereal-pulse combination offers a balanced blend from the standpoint of amino acid composition and improved nutritional value of the product.

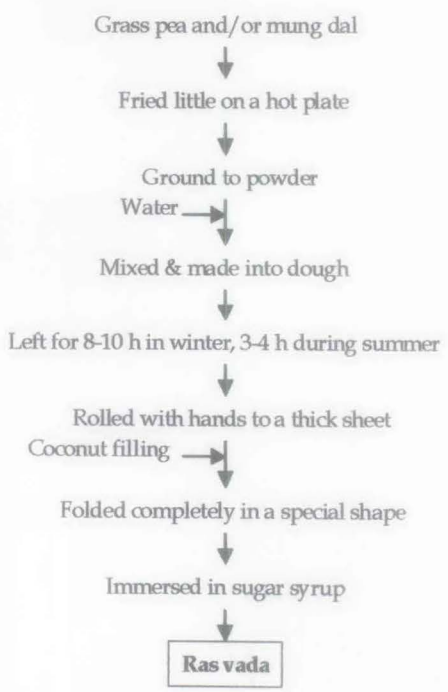

Fig. 14. Flow sheet for the preparation of ras vada

4.1.1.10. Papad

Papad (Fig. 12) is made from the legume-based dough often containing other farinaceous materials along with salt, spices and condiments (Fig. 13). Due to its mucilaginous texture, blackgram dal flour is the indispensable constituent in papad dough.

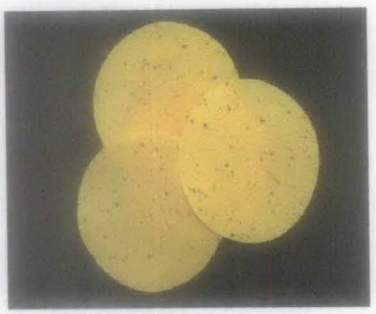

Fig. 12. Market sample of papad

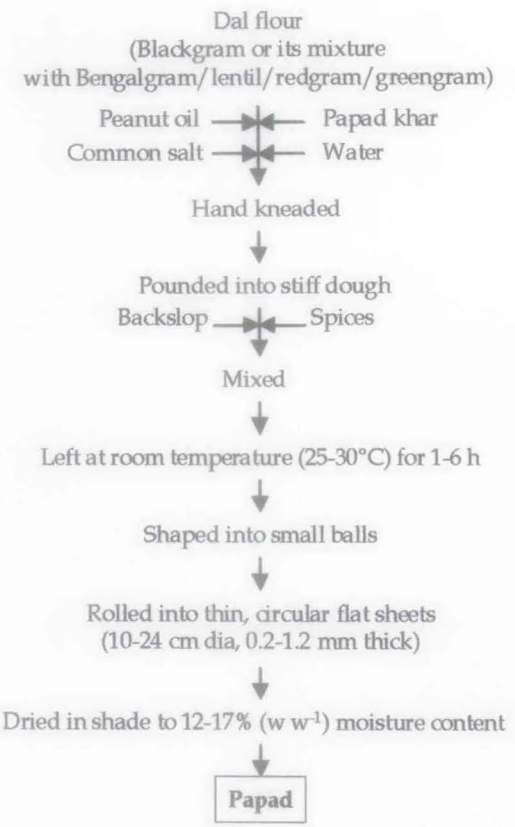

Fig. 13. Flow sheet for the preparation of papad

4.1.1.11. Ras vada

This confectionary is an attractive menu during special occasions, such as 'chaitra sankranti'. Traditionally, rasvada is made either from roasted grass pea or mung dal flour, or a combination of both (Fig. 14).

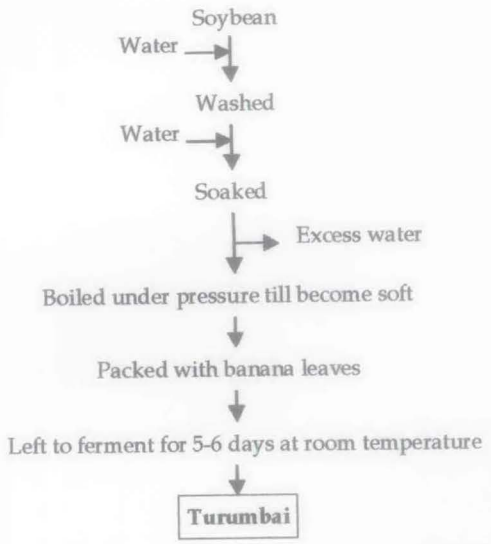

Fig. 15. Flow sheet for the preparation of turumbai

4.1.1.12. Turumbai

The traditional method of preparation (Fig. 15) of turumbai is similar to that of aakhuni, excepting that addition of firewood ash to the cooked beans is not practiced. The product resembles kinema in several organoleptic aspects.

4.1.1.13. Vada

The freshly prepared hot vada is relished as a breakfast item and is now popular among all the groups of people. Traditionally, this spicy fried ball is prepared from a variety of legumes (Fig. 16). Depending upon the kind of ingredients used different types of vada are being prepared throughout the country following the similar traditional method.

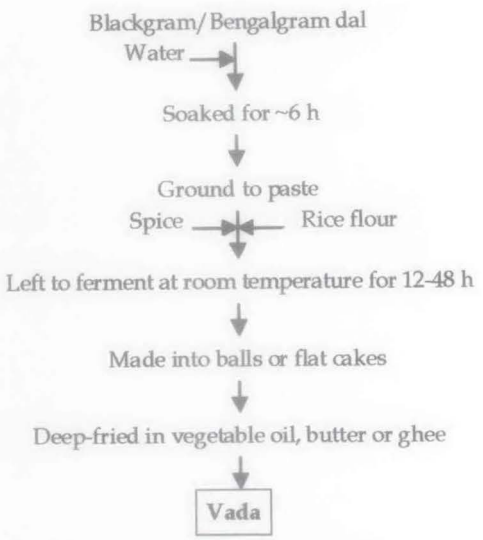

Fig. 16. Flow sheet for the preparation of vada

Fig. 17. Flow sheet for the preparation of wadi

4.1.1.14. Wadi

In India, 'wadi' has been called by different names. In Punjab and adjoining States of northern India, it is named as 'wadi' or 'Punjabi wadi'; in the States of Bihar and Jharkhand it is called 'adhauri', whereas in the States of West Bengal, Orissa and Assam the name 'bodi' or 'bori' is given for the same product.

Traditionally, wadi is prepared by soaking blackgram dal (Fig. 17). The dough is allowed to ferment for 1-3 days (for Punjabi wadi) or 8-10 h (for adhauri or bori) at room temperature (20-27°C), with or without spices, but generally with backslop added. In Punjabi wadi, spices, when added, include asafetida, caraway, cardamom, cloves, fenugreek, ginger and red pepper. In an alternative method, the dough is combined with shredded

waxgourd and whisked vigorously until it becomes light and fluffy due to incorporation of air. The fermented or whisked dough is hand-moulded into cones or balls (3-8 cm in diameter), deposited on bamboo or palm mats smeared with oil and sun-dried till the moisture content reaches 14-18% (w w^{-1}). Wadis look hollow with many air pockets in the interior and have a characteristic surface crust. Dried wadi (Fig. 18) is stored in an airtight container for future use.

Fig. 18. Market sample of wadi

4.1.2. Fermented cereal-legume mixed products

4.1.2.1. Adai

This pancake is popular among the children as well as adults. Traditionally, adai is prepared by mixing ground rice with blackgram, Bengalgram and pigeon pea dals (Fig. 19). It has a shelf life of about 2-3 days.

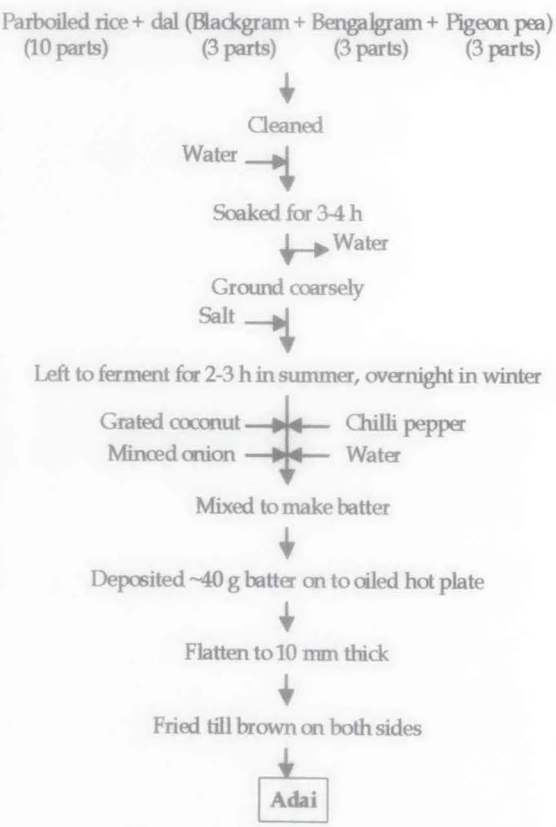

Fig. 19. Flow sheet for the preparation of adai

4.1.2.2. Bhapa pitha

This steam-cooked cake is popular in the rural pockets. Traditionally, bhapa pitha is prepared (Fig. 20) by mixing ground batter of rice and grass pea dal. A good quality bhapa pitha becomes soft and spongy like idli. The shelf life of bhapa pitha is one day, hence is usually consumed fresh.

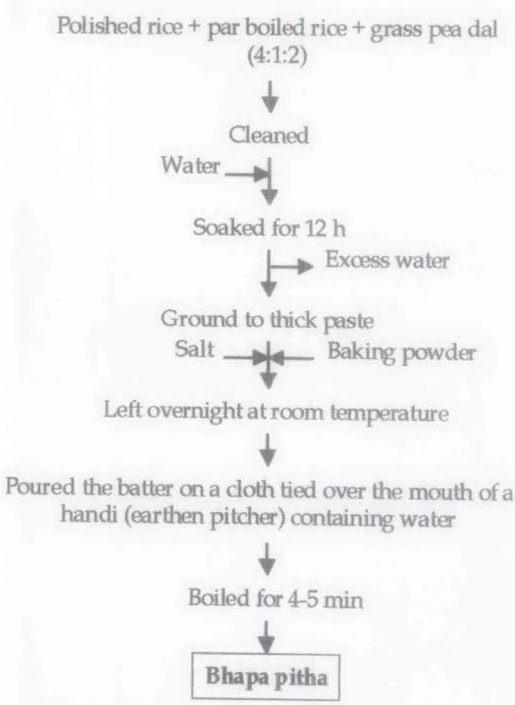

Fig. 20. Flow sheet for the preparation of bhapa pitha

4.1.2.3. Chakuli

Chakuli, which resembles dosa and consumed in the rural belt, is traditionally prepared from varying proportions of parboiled rice and blackgram dal (Fig. 21 and 22). A little amount of boiled rice may

Fig. 21. Flow sheet for the preparation of chakuli

Fig. 22. Preparation of chakuli: a, dewatering soaked rice through a perforated bowl; b, sun-drying briefly of soaked rice; c, grinding of rice; d, sieving pounded rice; e, grinding of blackgram; f, blending of rice powder with blackgram paste; g, leaving batter in a closed container to ferment; h, spreading fermented batter over a hot greased plate

be added, and blackgram may be substituted with juice of jackfruit (*Artocarpus heterophyllus* Lam.) or palmyra palm (*Borassus flabellifer* L.) fruit (in phal chakuli) during summer. Spices, like ginger, onion and black pepper powder are sometimes added at the time frying the fermented batter. Though the shelf life of chakuli (Fig. 23) is one day, it is consumed hot and fresh for optimum delicacy. Depending on the kind and ratio of ingredients used, different varieties of chakuli (such as phal chakuli (supplemented with fruit juice), mota chakuli (spicy) jau chakuli, and saru chakuli) are being prepared and consumed by the ethnic people during festivals.

Fig. 23. Chakuli, showing both the surfaces

4.1.2.4. Chhuchipatra pitha

The traditional method of chhuchipatra pitha preparation (Fig. 24) is similar to that of chakuli in respect of making and fermenting batter. The mixed fermented batter is flattened thin on a hot greased pan using a spatula. The filling is taken in the centre of the pancake which is then folded into a square shape and fried suitably (Fig. 25). It has shelf life of two days and is usually taken without any adjunct due to its sweet taste. Chhuchipatra pitha is prepared especially during 'bataosha' festival.

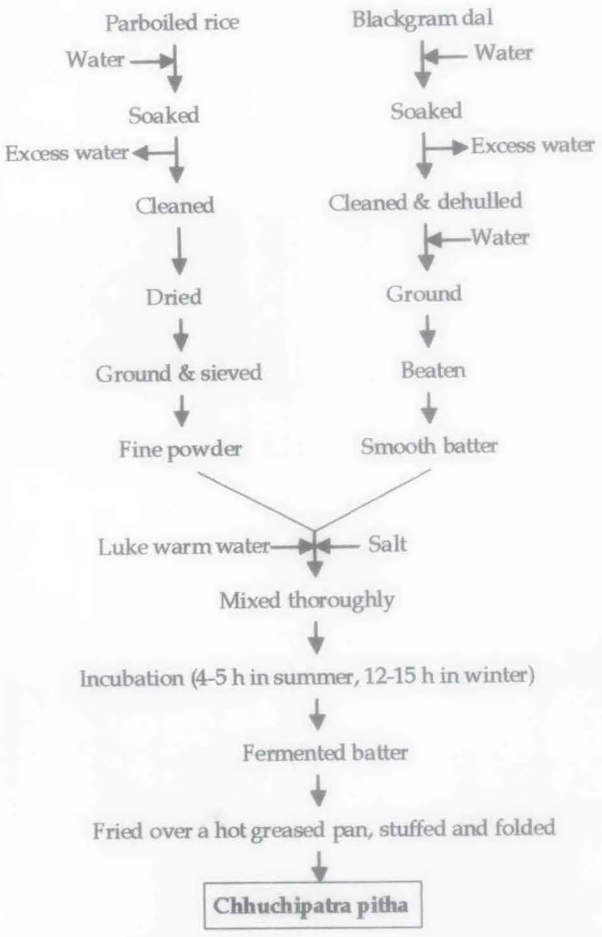

Fig. 24. Flow sheet for the preparation of chhuchipatra pitha

Fig. 25. Preparation of chuchipatra pitha: a, stuffing with coconut, b, pitha

4.1.2.5. Chitou

The traditional method of preparation of chitou (Fig. 26) is similar to that of chakuli in respect of making and fermenting batter. After fermentation, the batter is mixed with sugar and grated coconut and taken in a special earthen mould or deep bowl which is then covered with a lid. The junction is closed with a wet cloth and water is sprinkled intermittently. It is fried on a low heat. Although it has shelf life of one day, chitou is delicious when taken fresh and hot. Generally, it is taken with curry, sugar, curd or tea. Chitou is prepared in popular festivals, like 'makar sankranti' and 'chitou-amabasa'.

Fig. 26. Chitou

4.1.2.6. Daler patisapta

Daler patisapta is a popular confectionary, traditionally prepared (Fig. 27) in almost every house during festivals, especially during 'chaitra sankranti'. It has shelf-life of one day, and hence, these sweet pancake rolls are usually taken freshly as a snack.

4.1.2.7. Dhokla

Dhokla is a steamed product with appealing taste (mild sour), colour, flavour and spongy texture. It constitutes one of the Gujarati dishes known as 'farshana'. Traditionally, it is a part of the Gujarati meal (called, 'thali'), but very often is eaten a little part, as either a premeal appetizer or a break from

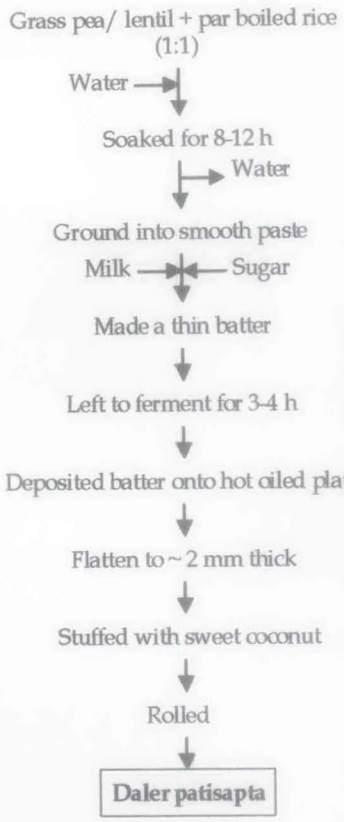

Fig. 27. Flow sheet for the preparation of daler patisapta

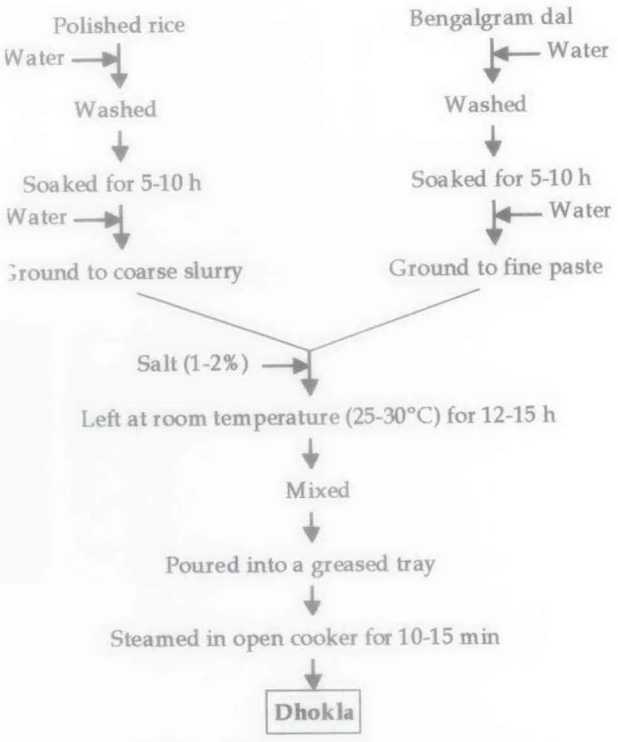

Fig. 28. Flow sheet for the preparation of dhokla

the meal. Because of its sensory attributes, nutritional quality and improved digestibility, dhokla is well-accepted by young children, adults and patients with digestive disorder, and can be ranked as one of the popular indigenous fermented foods of India.

Fig. 29. Dhokla

Traditionally, dhokla is prepared (Fig. 28) by overnight fermentation of a mixed batter of Bengalgram dal and rice at room temperature. However, both these ingredients can be substituted with suji, coarsely ground meal of wheat, maize or kodri for rice, and soybean, peas or moth beans for Bengalgram dal. The recipes usually made in Gujarati homes call for equal parts by volume of Bengalgram dal, rice, mung dal and blackgram dal. In the State of Maharashtra, dhokla is being prepared by fermenting a mixture of Bengalgram flour and curd for 16-18 h. Steamed dhokla is cut into diamond shape. A mixture of peanut or sesame oil with brown hot cracked seeds of mustard and sesame, chilli, pepper, ginger and curry leaves is poured over the steamed pieces of dhokla as seasonings (Fig. 29).

4.1.2.8. Dosa

Dosa (Fig. 30) is relished mainly as a breakfast food along with chutney and sambar. Due to its acceptable organoleptic properties, dosa is being prepared and consumed as a snack food throughout India. Traditionally, dosa is prepared using equal quantities of rice and blackgram dal (Fig. 31).

Fig. 30. Dosa

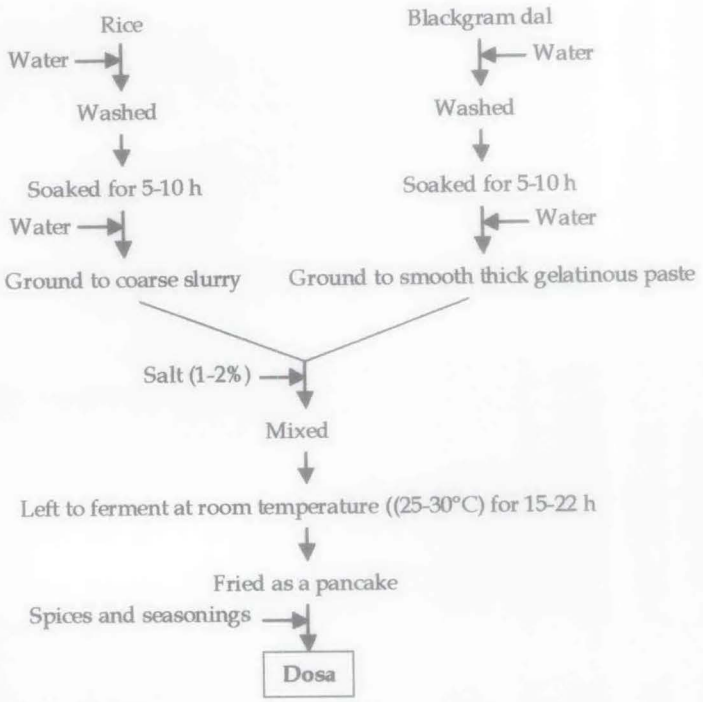

Fig. 31. Flow sheet for the preparation of dosa

4.1.2.9. Enduri pitha

Enduri pitha is a steamed, flavoured popular cereal-legume based fermented cake. The traditional preparation procedure (Fig. 32) of enduri pitha, a popular flavoured cake, is similar to that of chakuli in respect of making and fermenting batter. Its shelf life and mode of consumption are similar to those of chhuchipatra pitha. 'Prathama astami' is the festivity during which enduri pitha is specially prepared.

4.1.2.10. Idli

Idli (Fig. 33) belongs to an interesting group of cereal-legume based foods

Fig. 33. Idli

which are a major source of economical dietary energy and nutrients countrywide. It is relished mainly as a breakfast snack along with chutney and sambar. The ever-increasing popularity of idli is due to its appealing sour flavour, spongy texture, nutritional quality and easy digestibility. Traditionally, idli is made from naturally fermented batter prepared

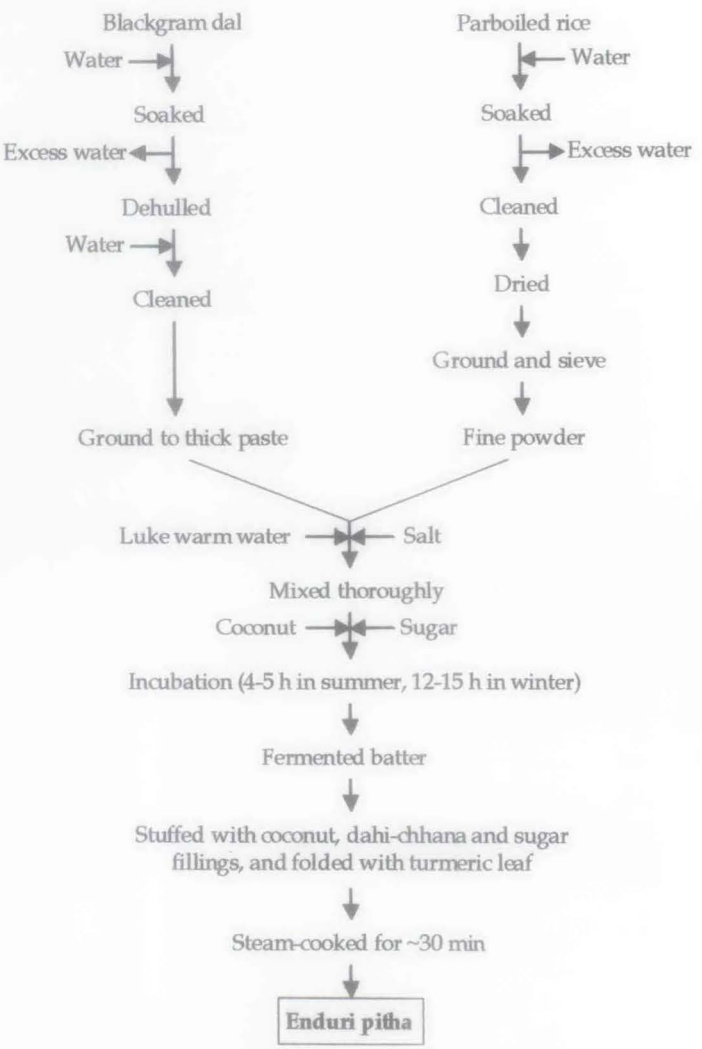

Fig. 32. Flow sheet for the preparation of enduri pitha

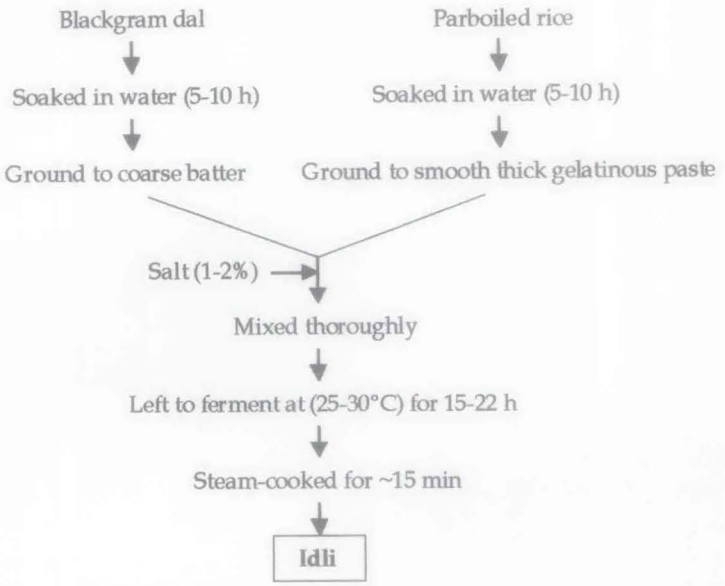

Fig. 34. Flow sheet for the preparation of idli

from a mixture of either parboiled or white polished rice with blackgram dal (Fig. 34). In general, idli is prepared on a household level, but in recent times it is widely prepared in catering establishments as well.

4.1.2.11. Maunha pitha

This delicious and nutritious cake (Fig. 35) is a popular fermented product. To prepare maunha pitha, parboiled rice powder and blackgram dal paste are mixed in the ratio of 3:1.

However, the traditional preparation procedure (Fig. 36 and 37) of maunha pitha is similar to that of chakuli in respect of making and fermenting batter. Sufficient water is taken in a handi, and a piece of cloth is tied over its mouth keeping a shallow cavity. When the water starts boiling, a thick

Fig. 33. Seved maunha pitha

batter is poured over the cloth. An empty handi is kept upside down over the previous one to capture the steam. The continuously generated steam cooks the material, and completion of cooking is checked by inserting a sharp object through the centre of the batter mass and observing if the batter has stuck to the surface. No adherence of batter to the object indicates completion of cooking even at the centre. A good quality munha pitha becomes spongy like idli and it is served by cutting into pieces. The shelf

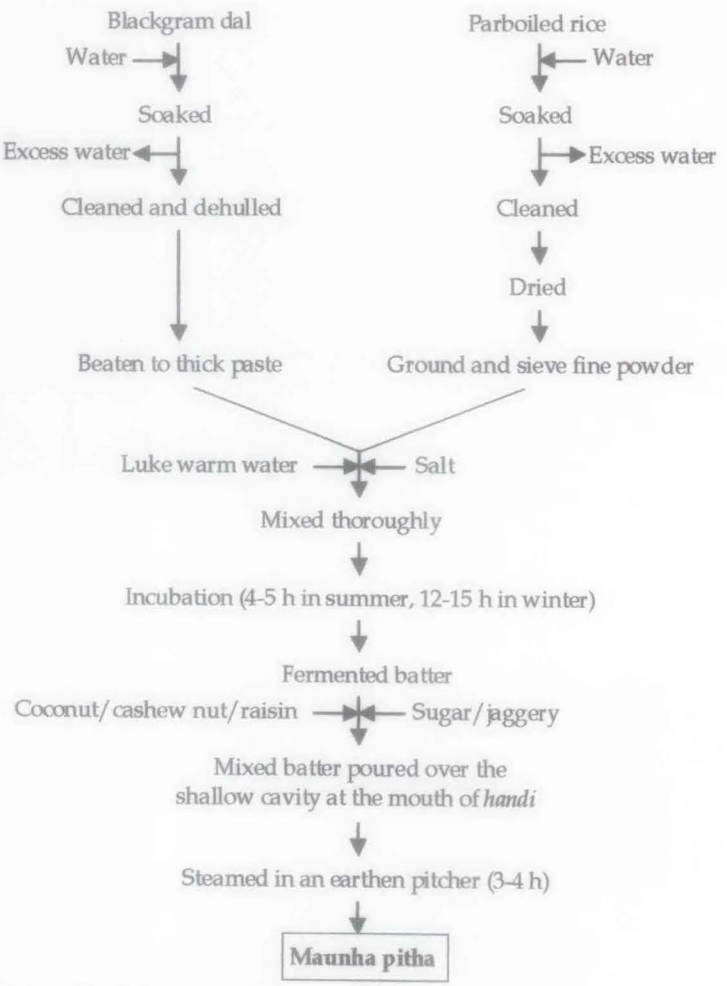

Fig. 36. Flow sheet for the preparation of maunha pitha

Fig. 37. Preparation of munha pitha: a, tying cloth around mouth of a handi; pouring fermented batter over the cloth; c, covering batter with another handi in an inverted position; d, pitha after steaming; e, pitha showing the lower (convex) surface

life of munha pitha is one day, and it is taken with sugar or curry. Munha pitha is prepared during different festivities.

4.1.2.12. Poda pitha

Poda pitha, a baked cake, is popular among the ethnic people belonging to the rural pockets. The traditional method of preparation (Fig. 38) of poda pitha is similar to that of chakuli in respect of making and fermenting batter. After preparation, the product is cut into pieces and served. Poda pitha has a shelf life of three days and is usually taken without a side dish. It is prepared during different festivals including 'bijoya dashami' and 'raja'.

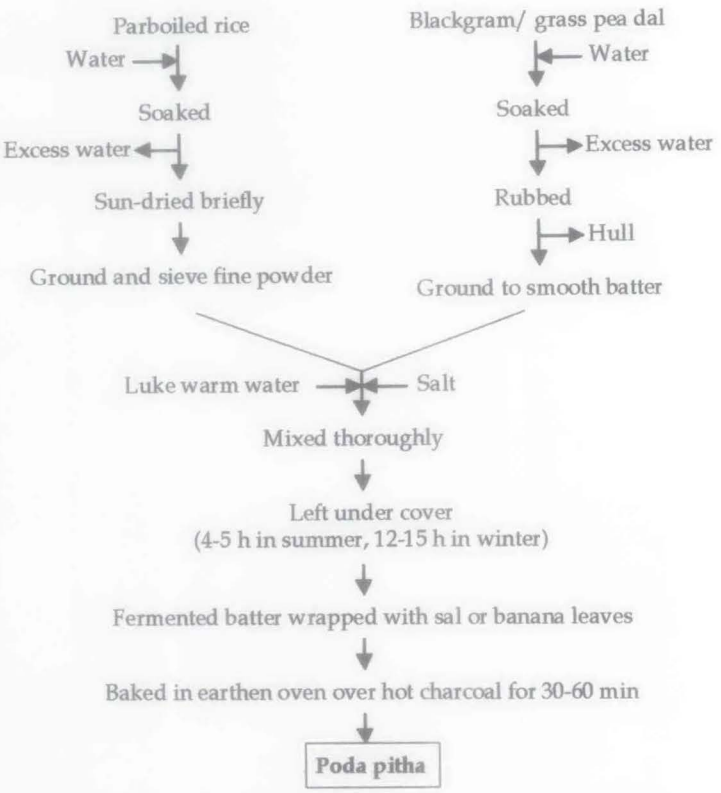

Fig. 38. Flow sheet for the preparation of poda pitha

4.2. Sampling

A total of 106 samples of five different kinds of most popular and commonly used legume-based traditional fermented foods were purchased from 75 different retail outlets and their sites of preparation scattered over five States in India (Table 6). The quality of the outlets, including sweetmeat parlours, restaurants, stationary and grocery shops and roadside cafés, represented a cross-section of the available standards. The collection included 10 samples of fermented dhokla batter, 12 samples each of fermented dosa and idli, 31 samples of papad and 41 samples of wadi. While dhokla, dosa and idli batters were freshly prepared, papad and wadi samples were packaged.

Table 6. Collection of different kinds of legume-based traditional foods from retail sources

Sl No.	Date of collection	Sample No	Kind of sample	Place of collection		Open/ Pkd[a]
				Town	State	
1	20.10.03	S1	Wadi	Shivmandir	West Bengal	Open
2	28.10.03	S2	Wadi	Haldibari	West Bengal	Pkd
3	10.11.03	S3	Wadi	Jalpaiguri	West Bengal	Pkd
4	17.11.03	S4	Wadi	Malda	West Bengal	Open
5	22.12.03	S5	Wadi	Sealdah	West Bengal	Open
6	29.12.03	S6	Wadi	Medinipur	West Bengal	Pkd
7	29.12.03	S7	Wadi	Medinipur	West Bengal	Open
8	25.01.04	S8	Wadi	Kalimpong	West Bengal	Open
9	09.02.04	S9	Wadi	Siliguri	West Bengal	Pkd
10	09.02.04	S10	Wadi	Bagdogra	West Bengal	Open
11	16.02.04	S11	Wadi	Mohitnagar	West Bengal	Pkd
12	16.02.04	S12	Wadi	Matigara	West Bengal	Open
13	08.03.04	S17	Wadi	Raiganj	West Bengal	Open
14	08.03.04	S18	Papad	Shivmandir	West Bengal	Pkd (B)
15	22.04.04	S23	Papad	Koch Bihar	West Bengal	Pkd (B)
16	25.05.04	S38	Wadi	Bokharo	Jharkhand	Open
17	25.05.04	339	Wadi	Madhubani	Bihar	Open
18	27.05.04	S40	Wadi	Muzaffarpur	Bihar	Open
19	27.05.04	S41	Wadi	Bhagalpur	Bihar	Open
20	29.05.04	S42	Wadi	Patna	Bihar	Open
21	31.05.04	S43	Wadi	Koch Bihar	West Bengal	Open
22	01.06.04	S44	Wadi	Mainaguri	West Bengal	Pkd
23	04.06.04	S45	Wadi	Garifa	West Bengal	Pkd
24	26.06.04	S46	Wadi	Aranghata	West Bengal	Open
25	26.06.04	S47	Wadi	Krishnanagar	West Bengal	Open
26	28.06.04	S48	Wadi	Shyambazar	West Bengal	Open
27	01.07.04	S49	Wadi	Bishnupur	West Bengal	Open
28	04.07.04	S50	Wadi	Uttarpara	West Bengal	Open
29	04.07.04	S51	Wadi	Uttarpara	West Bengal	Open
30	11.07.04	S52	Papad	Islampur	West Bengal	Pkd (B)
31	11.07.04	S53	Papad	Raiganj	West Bengal	Pkd (B)
32	16.07.04	S54	Papapd	Alipurduar	West Bengal	Pkd (B)
33	27.07.04	S56	Papad	Haldibari	West Bengal	Pkd (B)
34	27.07.04	S57	Papad	Haldibari	West Bengal	Pkd (B)
35	27.04.04	S58	Papad	Siliguri	West Bengal	Pkd (B)
36	09.08.04	S65	Wadi	Haora	West Bengal	Open
37	09.08.04	S66	Wadi	Haora	West Bengal	Open
38	10.08.04	S67	Wadi	Jadavpur	West Bengal	Pkd
39	10.08.04	S68	Wadi	Tollyganj	West Bengal	Pkd
40	10.08.04	S69	Papad	Bhadreswar	West Bengal	Pkd (B)
41	10.08.04	S70	Wadi	Srerampore	West Bengal	Open
42	10.08.04	S71	Wadi	Nungi	West Bengal	Open
43	10.08.04	S72	Papad	Titagarah	West Bengal	Open
44	10.08.04	S73	Papad	Nungi	West Bengal	Pkd (B)
45	10.08.04	S74	Papad	Nungi	West Bengal	Pkd (B)
46	11.08.04	S75	Papad	Haora	West Bengal	Pkd (B)
47	11.08.04	S76	Papad	Haora	West Bengal	Pkd (B)
48	11.08.04	S77	Papad	Naihati	West Bengal	Pkd (B)
49	11.08.04	S78	Papad	Titagarh	West Bengal	Pkd (B)
50	11.08.04	S79	Papad	C.R. Avenue	West Bengal	Pkd (B)
51	11.08.04	S80	Papad	Nadia	West Bengal	Pkd (B)
52	11.08.04	S81	Papad	Srerampore	West Bengal	Pkd (B)
53	11.08.04	S82	Papad	Santoshpur	West Bengal	Pkd (B)
54	11.08.04	S83	Papad	Titagarh	West Bengal	Pkd (B)
55	11.08.04	S84	Papad	Haora	West Bengal	Pkd (B)
56	11.08.04	S85	Papad	Naihati	West Bengal	Pkd (B)
57	08.11.04	S86	Papad	Chennai	Tamil Nadu	Pkd (B)
58	08.11.04	S87	Papad	Metupallayam	Tamil Nadu	Pkd (B)
59	15.12.04	S88	Wadi	Balasore	Orissa	Pkd
60	16.12.04	S89	Wadi	Mayurbhanj	Orissa	Open
61	18.12.04	S90	Wadi	Kharagpur	West Bengal	Pkd
62	18.12.04	S91	Wadi	Kharagpur	West Bengal	Open
63	18.12.04	S92	Papad	Kharagpur	West Bengal	Pkd (B)
64	18.12.04	S93	Papad	Kharagpur	West Bengal	Pkd (B)
65	31.01.05	S107	Wadi	Malda	West Bengal	Open
66	31.01.05	S108	Wadi	Malda	West Bengal	Open
67	31.01.05	S109	Papad	Malda	West Bengal	Pkd (B)

Sl No.	Date of collection	Sample No	Kind of sample	Place of collection		Open/ Pkd[a]
				Town	State	
68	31.01.05	S110	Papad	Malda	West Bengal	Pkd (B)
69	15.02.05	S111	Wadi	Farakka	West Bengal	Pkd
70	15.02.05	S112	Wadi	Farakka	West Bengal	Open
71	15.02.05	S113	Papad	Baharampur	West Bengal	Pkd (B)
72	15.02.05	S114	Papad	Baharampur	West Bengal	Pkd (B)
73	20.06.05	S118	Idli batter	Siliguri	West Bengal	Open
74	20.06.05	S119	Idli batter	Siliguri	West Bengal	Open
75	20.06.05	S120	Idli batter	Siliguri	West Bengal	Open
76	24.06.05	S122	Dosa batter	Bagdogra	West Bengal	Open
77	24.06.05	S123	Dosa batter	Bagdogra	West Bengal	Open
78	24.06.05	S124	Dosa batter	Siliguri	West Bengal	Open
79	27.06.05	S125	Idli batter	Siliguri	West Bengal	Open
80	27.06.05	S126	Idli batter	Bagdogra	West Bengal	Open
81	27.06.05	S127	Idli batter	Siliguri	West Bengal	Open
82	30.06.05	S128	Dosa batter	Shivmandir	West Bengal	Open
83	30.06.05	S129	Dosa batter	Shivmandir	West Bengal	Open
84	02.07.05	S130	Dosa batter	Kadamtala	West Bengal	Open
85	02.07.05	S131	Dosa batter	Kadamtala	West Bengal	Open
86	02.07.05	S132	Dosa batter	Kadamtala	West Bengal	Open
87	06.07.05	S133	Idli batter	Bagdogra	West Bengal	Open
88	06.07.05	S134	Idli batter	Bagdogra	West Bengal	Open
89	06.07.05	S135	Dosa batter	Siliguri	West Bengal	Open
90	10.07.05	S136	Idli batter	Jalpaiguri	West Bengal	Open
91	10.07.05	S137	Idli batter	Jalpaiguri	West Bengal	Open
92	10.07.05	S138	Dosa batter	Jalpaiguri	West Bengal	Open
93	16.07.05	S139	Dhokla batter	Sukuna	West Bengal	Open
94	16.07.05	S140	Dosa batter	Sukuna	West Bengal	Open
95	16.07.05	S141	Dosa batter	Sukuna	West Bengal	Open
96	16.07.05	S142	Dhokla batter	Siliguri	West Bengal	Open
97	16.07.05	S143	Dhokla batter	Siliguri	West Bengal	Open
98	20.07.05	S144	Dhokla batter	Siliguri	West Bengal	Open
99	20.07.05	S145	Dhokla batter	Siliguri	West Bengal	Open
100	24.07.05	S146	Dhokla batter	Kalimpong	West Bengal	Open
101	24.07.05	S147	Dhokla batter	Kalimpong	West Bengal	Open
102	24.07.05	S148	Idli batter	Kalimpong	West Bengal	Open
103	24.07.05	S149	Idli batter	Kalimpong	West Bengal	Open
104	25.07.05	S150	Dhokla batter	Rangpo	Sikkim	Open
105	25.07.05	S151	Dhokla batter	Melli	West Bengal	Open
106	30.07.05	S152	Dhokla batter	Sukuna	West Bengal	Open

[a]Pkd, packaged; B, branded.

4.3. Dhokla

4.3.1. Proximate composition

The proximate compositions of fermented batter and their substrates (white polished rice and Bengalgram dal) are presented in Table 7. The moisture content of fermented batters was approximately 5 times higher than that of the substrates. While the substrates had relatively neutral pH, the fermented batter was acidic. Titratable and free fatty acidity, and nonprotein and soluble nitrogen contents of the substrates increased significantly after fermentation. The ash, fat and carbohydrate contents of the fermented batter were in between those of the two substrates.

4.3.2. Microbial analysis

4.3.2.1. Isolation of microorganisms

A total of 375 strains of lactic acid bacteria (Table 8) and 245 strains of yeasts (Table 9) were isolated from 3 samples each of rice and Bengalgram dal, 10 samples of marketed batter and 18 samples of

Table 7. Proximate composition[a] of substrates and fermented batter of dhokla

Parameter	g (100 g)$^{-1}$ dry matter			
	Substrate		Fermented batter	
	Polished rice (n = 3)	Bengalgram dal (n = 3)	Marketed (n = 10)	Laboratory-made (n = 3)
pH	6.9a ± 0.07	7.0a ± 0.04	4.8b ± 0.07	4.7b ± 0.10
Moisture (g (100 g)$^{-1}$)	11.8c ± 0.20	14.2c ± 0.42	67.6a ± 0.82	60.5b ± 0.70
Ash	0.7c ± 0.03	7.5a ± 0.09	1.8b ± 0.15	2.0b ± 0.05
Titratable acid (as lactic acid)	0.1c ± 0.01	0.1c ± 0.01	1.5a ± 0.05	1.0b ± 0.05
Free fatty acid (as linoleic acid)	0.1b ± 0.01	0.1b ± 0.01	2.1a ± 0.06	2.2a ± 0.04
Total nitrogen	1.2b ± 0.04	3.8a ± 0.01	3.7a ± 0.12	3.9a ± 0.06
Protein nitrogen	1.1c ± 0.04	3.0a ± 0.02	2.5b ± 0.08	2.9a ± 0.05
Nonprotein nitrogen	0.1c ± 0.02	0.7b ± 0.02	1.3a ± 0.08	1.1a ± 0.10
Soluble nitrogen	0.3b ± 0.02	0.4b ± 0.02	1.0a ± 0.03	1.2a ± 0.02
Total protein (total N x 6.25[b])	7.4b ± 0.23	23.5a ± 0.04	23.4a ± 0.75	24.5a ± 0.04
Crude fat	0.4c ± 0.02	6.1a ± 0.35	4.4b ± 0.19	4.1b ± 0.10
Carbohydrate (by difference)	91.5a ± 0.27	62.9c ± 0.42	70.4b ± 0.92	69.4b ± 0.40
Energy (MJ (100 g)$^{-1}$ dry matter)	1.7a ± 0	1.7a ± 0.01	1.7a ± 0.01	1.7a ± 0

[a]Values are expressed as mean ± SEM. Means within a row sharing a common letter are not significantly different ($P < 0.05$).

[b]Protein factor is 5.95 for rice.

Table 8. Selection of representative strains of dominant lactic acid bacteria isolated from substrates and fermenting batter of dhokla

Source	Stage(s) of fermentation	Cell shape[a]	Gas from glucose	Growth in/at		Grouped strains[b]		No. of R[c] strains
				pH 4.8	45°C	Group	No.	
Substrate								
Bengalgram dal (n = 3)		Cp/c	+	-	-	Dk-LA	10	2
		Ct	-	+	+	Dk-LB	10	2
		Rod	+	ND	+	Dk-LC	10	2
White polished rice (n = 3)		Cp/c	+	-	-	Dk-LA	5	1
		Ct	-	+	+	Dk-LB	5	1
Mixed batter								
Marketed (n = 10)	Final	Cp/c	+	-	-	Dk-LA	35	7
		Ct	-	+	+	Dk-LB	20	4
		Rod	+	ND	+	Dk-LC	40	8
Laboratory-made (n = 18)	0 h - 15 h (3 h-interval)	Cp/c	+	-	-	Dk-LA	85	17
		Ct	-	+	+	Dk-LB	65	13
		Rod	+	ND	+	Dk-LC	90	18

[a]Cp/c, cocci in pair or short chain; Ct, cocci in tetrad.

[b]All the isolates were nonmotile, nonsporeforming, Gram positive and catalase negative.

[c]R, representative.

Table 9. Selection of representative strains of dominant yeasts isolated from substrates and fermenting batter of dhokla

Source	Stage(s) of fermentation	Colony[a]	Cell shape[b]	Pellicle formation	Grouped strains		No. of R[c] strains
					Group	No.	
Substrate							
Bengalgram dal (n = 3)		Cgs	G-O/E	-	Dk-YA	5	1
		Wbs	G-E	-	Dk-YE	10	2
Polished rice (n = 3)		Cgs	G-O/E	-	Dk-YA	5	1
Mixed batter							
Marketed (n = 10)	Final	Cgs	G-O/E	-	Dk-YA	25	5
		Wbs	G-E	-	Dk-YE	40	8
		Wsw	O-E	+	Dk-YC	5	1
Laboratory-made (n = 18)	0 h - 15 h (3 h-interval)	Cgs	G-O/E	-	Dk-YA	65	13
		Wbs	G-E	-	Dk-YE	85	17

[a]Cgs, cream colour with glistening and butyrous surface; Wsw, tannish white surface with wrinkled margin; Wbs, white, butyrous and smooth surface.

[b]G, globose; O, oval; E, ellipsoidal.

[c]R, representative.

laboratory-made fermenting and fermented batter. While all the lactic acid bacterial isolates were grouped into 3 cell morphotypes, the yeast isolates were grouped into three colony morphotypes. One representative strain, from each group of each positive sample, was selected randomly to ascertain their taxonomic status.

4.3.2.2. Taxonomical studies

4.3.2.2.1. Bacteria

On the basis of selected morphological, physiological and cultural characteristics of lactic acid bacteria shown in Table 8, all the representative strains of group Dk-LA belonged to the genus *Leuconostoc*, while those of the groups Dk-LB and Dk-LC belonged to the genera *Pediococcus* and *Lactobacillus*, respectively. The detailed morphological and physiological characteristics of all those representative strains are presented in Table 10. Following the criteria laid by Garvie (1986a, 1986b) as well as Intelligent Bacteria Identification System (IBIS) (Wijtzes *et al.*, 1997), the representative strains belonging

Table 10. Characteristics[a] of groups of representative strains of lactic acid bacteria isolated from substrates and fermenting batter of dhokla

Parameters	Dk-LA (n = 27)			Dk-LB (n = 20)			Dk-LC (n = 28)		
	+	d	-	+	d	-	+	d	-
Cell shape[b]	Cp/c			Ct			Rod		
Cell size (µm)	0.30-0.33			0.32-0.40			0.5-0.7 x 1.3-1.8		
Gas from glucose	100					100	100		
Growth in NaCl (g l^{-1})									
30	100			65	20	15	nd[c]		
65	100			100			nd		
80			100	100			nd		
Growth at pH									
4.2 (initial)	100			100			nd		
7.5 (initial)	100			100			nd		
Hydrolysis of									
arginine			100	100			100		
esculin	74	26		100					100
Growth at									
15°C	100			100					100
45°C			100	100			100		
Acid from									
L-arabinose	100			100			75	18	7
D-cellobiose	59	26	15	100			56	36	8
esculin	100			70		30			100
glycerol			100			100	nd		
D-lactose	48	52		40	35	25	83	17	
D-mannitol	100					100			100
D-mannose	100					100	100		
D-melibiose	67	26	7	50	30	20	100		
D-raffinose	78	19	3			100	64	25	11
L-rhamnose	nd			60	30	10			100
D-ribose	100			70	30		100		
salicin	71	22	7	100					100
sucrose	100				15	85			100
D-trehalose	100			100			64	25	11
D-xylose	67	22	11	65	20	15	72	14	14

[a]Data represent %strains showing + (positive), d (delayed) and - (negative) reactions. All the strains were Gram positive, nonmotile, nonsporeforming, catalase negative, capable of growing at 40°C and producing acid from D-fructose, D-galactose, D-glucose and D-maltose, however not capable of reducing nitrate, producing indole, hydrolyzing fat, gelatin and starch, and producing acid from sorbitol and starch.

[b]Cp/c, cocci in pair or chain; Ct, cocci in tetrad.

[c]nd, not determined.

to groups Dk-LA, Dk-LB and Dk-LC were tentatively identified as *Leuconostoc mesenteroides* (Tsenkovskii) van Tieghem 1879, *Pediococcus pentosaceus* Mees 1934 and *Lactobacillus fermentum* Beijerinck 1901, respectively.

4.3.2.2.2. Yeasts

The detailed morphological, cultural and physiological characteristics of the 48 representative strains of yeasts, comprising 3 different colony morphotypes, isolated from substrates and fermenting and fermented batter are shown in Table 11. Following the taxonomic keys of Barnett *et al.* (2000) and

Table 11. Characteristics[a] of groups of representative strains of yeasts isolated from fermenting batter of dhokla and its substrates

Parameters	Dk-YA (n = 20)			Dk-YC (n = 1)			Dk-YE (n = 27)		
	+	$+_w$	-	+	$+_w$	-	+	$+_w$	-
Colony morphology[b]	Cgs			Wsw			Wbs		
Cell shape[c]	(G-O)/E			O-E			O-E		
Cell width x length (µm)	3.6-5.2 x 4.4-7.9			2.8-5.7 x 5.3-8.5			3.0-5.1 x 3.5-6.6		
Ascus									
Asci evanescence	Persistent			Evanescent			Evanescent		
Ascospore[d]	(R-O)+Sm			Str/R			Hat		
Pellicle formation			100	100					100
Hydrolysis of fat			100	100					100
Fermentation of									
D-cellobiose			100			100	70	30	
D-galactose	65	25	10			100	81	19	
D-glucose	100				100		100		
D-maltose	55	30	15			100			100
D-melibiose	75	15	10			100			100
melezitose	60	15	25			100			100
D-raffinose	85	15				100			100
sucrose	75	20	5			100			100
D-trehalose	85		15			100			100
Assimilation of									
L-arabinose			100			100	100		
D-cellobiose			100			100	100		
citrate			100	100					100
m-erythritol			100			100	48	52	
D-galactose	65	15	20			100	100		
D-glucitol		25	75			100	100		
D-glucosamine			100	100					100
N-acetyl-glucosamine			100	100					100
glycerol	30		70	100			100		
D-maltose	70	20	10			100	56	18	26
D-mannitol		35	65			100	59	7	34
melezitose	45	30	25			100	74	26	
D-melibiose	60	30	10			100			100
D-raffinose	85		15			100			100
D-ribose			100			100	62	19	19
L-rhamnose			100			100	100		
sucrose	80		20			100	56	44	
D-trehalose	85		15			100	78		22
Vitamin-free medium			100	100					100
D-xylose			100			100	100		

[a]Data represent % stains showing + (positive), $+_w$ (weak positive) and - (negative) reactions. All the strains produced pseudomycelium, reproduced by multilateral budding and sexual reproduction, produced 1-4 ascospores per ascus, were capable of growing at 37°C and assimilating D-glucose, however not capable of reducing nitrate, forming starch, hydrolyzing gelatin, starch and urea, fermenting D-lactose and D-xylose, and assimilating *m*-inositol, D-lactose, nitrate and starch.

[b]Cgs, cream colour with glistening and butyrous surface; Wsw, tannish white surface with wrinkled margin; Wbs, white, butyrous and smooth surface.

[c]G, globose; O, oval; E, elongated.

[d]R, round, O, oval; Sm, smooth; Str, Saturn-shaped.

Kurtzman and Fell (2000), and using BioloMICSNet software (www.cbs.knaw.nl/yeast/BioloMICS.aspx), the taxonomic status of the representative strains of yeasts belonging to group Dk-YA, Dk-YC and Dk-YE were tentatively identified as *Saccharomyces cerevisiae* Meyen *ex* Hansen 1883, *Pichia membranifaciens* Hansen 1904 and *Pichia silvicola* (Wickerham) Kurtzman 1984, respectively.

4.3.2.3. Microbial loads in substrates and fermented batter

The average microbial load studied in 10 market samples of fermented batter, in 3 samples each of the two kinds of substrates (white polished rice and Bengalgram dal) and laboratory-made fermented dhokla batters are shown in Table 12. The total aerobic mesophilic bacterial (TAMB) cells and their spores (aMBS) were recovered from all the samples studied (Table 13). While *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* were recovered from both the substrates and the fermented batters, *Lactobacillus fermentum*, which dominated the fermented batter with an average load of $>10^8$ cfu g^{-1}, could not be recovered from raw rice. *Saccharomyces cerevisiae* was the only yeast isolated from both the two types of substrates and the fermented batter. *Pichia silvicola*, which dominated with an average count of $>10^6$ in batter, could be recovered from one of the two types of substrates. *Pichia membranifaciens* was recovered from marketed samples of fermented batter only.

Table 12. Dominant microbial load[a] in substrates and fermented batters of dhokla

Microbiota	Log cfu g^{-1} fresh weight			
	Substrate		Fermented batter	
	Polished rice (n = 3)	Bengalgram dal (n = 3)	Marketed (n = 10)	Laboratory-made (n = 3)
TAMB	4.4b ± 0.33	3.9b ± 0.14	9.7a ± 0.31	10.4a ± 0.25
aMBS	3.7c ± 0.35	3.3c ± 0.35	4.6a ± 0.31	4.0b ± 0.12
Yeasts[b]				
Saccharomyces cerevisiae	3.9c ± 0	3.5d ± 0.48	6.1a ± 0.33	5.6b ± 0.41
Pichia silvicola	<DL	4.0c ± 0.16	6.7b ± 0.35	7.1a ± 0.05
Pichia membranifaciens	<DL	<DL	5.8 ± 0	<DL
Lactic acid bacteria[c]				
Leuconostoc mesenteriodes	3.8b ± 0	4.3b ± 0.28	7.9a ± 0.44	7.5a ± 0.27
Pediococcus pentosaceus	3.3b ± 0	3.5b ± 0.17	6.6a ± 0.32	6.5a ± 0.20
Lactobacillus fermentum	<DL	3.7b ± 0.13	8.0a ± 0.32	8.4a ± 0.28

[a]Values are expressed as mean ± SEM. Means within a row sharing a common letter are not significantly different ($P < 0.05$).

[b]DL (detection limit), 2.0 log cfu g^{-1} fresh weight.

[c]DL, 1.0 log cfu g^{-1} fresh weight.

Table 13. Prevalence of dominant microorganisms in fermented batter of dhokla and its substrates

Microbiota	Positive sample (%)			
	Substrate		Fermented batter	
	Polished rice (n = 3)	Bengalgram dal (n = 3)	Marketed (n = 10)	Laboratory-made (n = 3)
TAMB	100	100	100	100
aMBS	100	100	100	100
Yeasts				
Saccharomyces cerevisiae	33	33	50	100
Pichia silvicola	0	67	80	100
Pichia membranifaciens	0	0	10	0
Lactic acid bacteria				
Leuconostoc mesenteroides	33	67	70	100
Pediococcus pentosaceus	33	67	30	100
Lactobacillus fermentum	0	67	80	100

4.3.3. Successional studies on batter during fermentation

4.3.3.1. Microbial changes

Table 14 shows the changes in microbial load of batter during fermentation under semicontrolled conditions. TAMB cells increased significantly at every 3 h-interval till 9 h of fermentation, while their spore count remained constant during the entire period of fermentation. *Saccharomyces cerevisiae* and *Pichia silvicola* were the dominant yeasts during fermentation. Their respective load of 3.7 and 3.9 log cfu g^{-1} fresh mixed batter increased to 5.6 and 7.1 log cfu g^{-1} after fermentation. *Lactobacillus fermentum* was the dominant bacterium encountered during fermentation; it increased significantly at every 3 h-interval. The load of *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* increased significantly during fermentation.

Table 14. Changes in microbial load[a] in mixed batter of dhokla during fermentation under semicontrolled conditions

Microbiota	Fermentation period (h)					
	0	3	6	9	12	15
TAMB	6.5d ± 0.13 (100)	7.5c ± 0.24 (100)	8.6b ± 0.25 (100)	10.0a ± 0.05 (100)	10.1a ± 0.10 (100)	10.4a ± 0.25 (100)
aMBS	3.9a ± 0.20 (100)	3.9a ± 0.57 (100)	4.0a ± 0.40 (100)	4.2a ± 0.17 (100)	3.8a ± 0.07 (100)	4.0a ± 0.12 (100)
Yeasts						
Saccharomyces cerevisiae	3.7d ± 0 (33)	4.3c ± 0 (33)	4.7b ± 0.21 (67)	4.8b ± 0.31 (100)	5.2ab ± 0.17 (100)	5.6a ± 0.41 (100)
Pichia silvicola	3.9e ± 0.11 (100)	4.5d ± 0.42 (100)	4.8cd ± 0.25 (100)	5.1c ± 0.23 (100)	6.6b ± 0.32 (100)	7.1a ± 0.05 (100)
Lactic acid bacteria						
Leuconostoc mesenteriodes	5.1d ± 0.16 (67)	5.2d ± 0.35 (100)	5.7c ± 0.27 (100)	6.3b ± 0.10 (100)	7.5a ± 0.38 (100)	7.5a ± 0.27 (100)
Pediococcus pentosaceus	4.9b ± 0 (33)	4.5b ± 0 (33)	4.9b ± 0.23 (67)	5.2b ± 0.42 (100)	6.4a ± 0.20 (100)	6.5a ± 0.20 (100)
Lactobacillus fermentum	4.5f ± 0.32 (100)	5.1e ± 0.22 (100)	6.2d ± 0.09 (100)	6.8c ± 0.18 (100)	7.7b ± 0.10 (100)	8.4a ± 0.28 (100)

[a]Values, expressed as mean ± SEM of log cfu g^{-1} fresh weight, were obtained from three different samples. Data within parentheses are expressed as %positive samples. Means within a row sharing a common letter are not significantly different ($P < 0.05$).

4.3.3.2. Changes in proximate composition

The changes in proximate composition of mixed batter during fermentation are shown in Table 15. The batter volume gained 1.8-fold increase during fermentation. The pH declined significantly from initial 6.3 to 4.7 with 10-fold increase in titratable acidity. Free fatty acidity of fermented batter registered 3.7-fold increase. While the nonprotein nitrogen content remained constant although, soluble nitrogen content increased significantly during batter fermentation. While the content of carbohydrate decreased significantly, there was no significant change in the contents of moisture, ash, crude fat and energy value during fermentation.

4.3.4. SDS-PAGE

Fig. 39 shows the electrophoretic pattern of whole-cell proteins of the substrates (white polished rice and Bengalgram dal) and mixed-batter of dhokla at different stages of fermentation. As could be seen from the protein fingerprinting, most of the resolved major protein subunits of the mixed batter revealed the combination of both the ingredients.

Table 15. Changes in proximate composition[a] of mixed batter of dhokla during fermentation under semicontrolled conditions

Parameter	Fermentation time (h)					
(g (100 g)$^{-1}$ dry matter)	0	3	6	9	12	15
pH	6.3a ± 0.05	6.1a ± 0.06	5.7b ± 0.11	5.2bc ± 0.03	5.0c ± 0.04	4.7d ± 0.10
Batter volume (ml)	100.0d ± 0.00	104.7d ± 0.67	128.0c ± 1.15	162.7b ± 0.67	178.7a ± 1.76	180.0a ± 2.31
Moisture (g (100 g)$^{-1}$)	60.7a ± 0.53	60.6a ± 0.53	60.8a ± 0.62	60.7a ± 0.74	60.8a ± 0.82	60.5a ± 0.70
Ash	2.2a ± 0.03	2.1a ± 0.03	2.1a ± 0.02	2.1a ± 0.04	2.1a ± 0.05	2.0a ± 0.05
Titratable acid (as lactic acid)	0.1c ± 0.01	0.2c ± 0.01	0.4b ± 0.01	0.6b ± 0.03	0.9a ± 0.02	1.0a ± 0.05
Free fatty acid (as linoleic acid)	0.6d ± 0.01	0.7d ± 0.01	1.1c ± 0.02	1.6b ± 0.03	2.0a ± 0.04	2.2a ± 0.04
Total nitrogen	3.4b ± 0.06	3.5b ± 0.05	3.5b± 0.02	3.8a ± 0.04	3.9a ± 0.05	3.9a ± 0.06
Protein nitrogen	2.4b ± 0.02	2.3b ± 0.04	2.2b ± 0.01	2.4b ± 0.03	2.6b ± 0.05	2.9a ± 0.05
Nonprotein nitrogen	1.1a ± 0.07	1.2a ± 0.02	1.3a ± 0.01	1.4a ± 0.02	1.3a ± 0.01	1.1a ± 0.10
Soluble nitrogen	0.4b± 0.02	0.6b ± 0.03	0.7b ± 0.02	1.0a ± 0.02	1.1a ± 0.01	1.2a ± 0.02
Total protein (total N x 6.25[b])	21.5b ± 0.38	21.7b ± 0.32	21.8b ± 0.14	23.8a ± 0.27	24.3a ± 0.30	24.5a ± 0.04
Crude fat	4.3a ± 0.17	4.2a ± 0.06	4.3a ± 0.10	4.2a ± 0.07	4.2a ± 0.07	4.1a ± 0.10
Carbohydrate (by difference)	72.1a ± 0.36	72.3a ± 0.21	71.8ab ± 0.17	69.9b ± 0.32	69.5b ± 0.32	69.4b ± 0.40
Energy (MJ (100 g)$^{-1}$ dry matter)	1.7a ± 0	1.7a ± 0	1.7a ± 0	1.7a ± 0	1.7a ± 0	1.7a ± 0

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a row sharing a common letter are not significantly different ($P < 0.05$).

[b]Protein factor is 5.95 for rice.

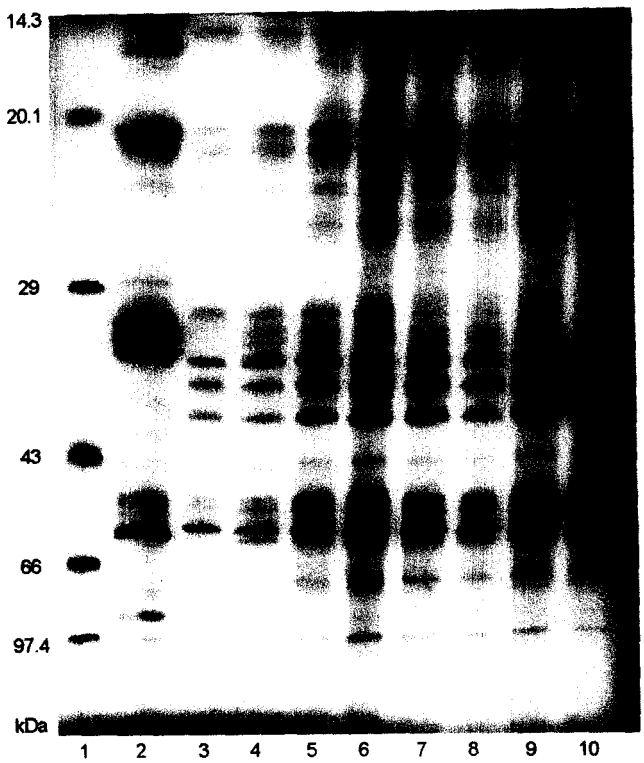

Fig. 39. SDS-PAGE profile of substrates and fermenting batter (a mixture of rice slurry and bengalgram dal paste, 1:4, 32°C for 18 h) of dhokla. Lanes: 1, protein molecular weight marker; 2, polished rice; 3, Bengalgram dal; 4, 0 h-old batter; 5, 3 h-old batter; 6, 6 h-old batter; 7, 9 h-old batter; 8, 12 h-old batter; 9, 15 h-old batter; 10, 18 h-old batter.

4.3.5. Evaluation of antioxidant properties

4.3.5.1. Extraction yield in methanol

The yield from fermented batter was significantly higher than that from either nonfermented batter or steamed product (Table 16).

4.3.5.2. Total phenol content

Table 16 shows the total phenol content of the methanolic extracts. After fermentation, the mean total phenol content of mixed batter increased by 154%. However, steaming of the fermented batter for 10-15 min during dhokla preparation caused a 130% decline in the total phenol content.

4.3.5.3. Reducing power

The mean reducing powers of the crude lyophilized extracts of samples in methanol are shown in Table 16. Extract from fermented batter registered the highest amount of reducing power, which was about double the value in nonfermented one. However, steaming of fermented batter for 10-15 min caused 96% decline in activity.

Table 16. Yield of methanolic extract, total phenol content and reducing power of dhokla at different stages of its preparation

Sample[a]	mg g^{-1} dry weight[b]		
	Yield	Total phenol content (gallic acid equivalent)	Reducing power (ascorbic acid equivalent)
Dk-0	74.80b ± 2.19	0.85c ± 0.04	4.65b ± 0.15
Dk-15	91.63a ± 3.71	2.16a ± 0.03	8.71a ± 0.22
Dk-SP	54.53c ± 1.04	0.94b ± 0.04	4.44b ± 0.11

[a]Dk-0, nonfermented dhokla batter; Dk-15, 15 h (fully)-fermented dhokla batter; Dk-SP, steamed product (dhokla).

[b]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a column sharing a common letter are not significantly different ($P < 0.05$).

4.3.5.4. Free radical-scavenging activity

Under different dosage (10-50 mg ml^{-1}) of methanolic extracts of nonfermented and fermented batter and steamed product, the scavenging effect for DPPH· increased correspondingly after 50 min of incubation (Table 17). During incubation, the DPPH·-scavenging activity of all the tested concentrations (10-50 mg ml^{-1}) increased significantly. IC_{50} of these samples, obtained by interpolation from linear

regression analysis, revealed that 161 mg ml^{-1}, 57 mg ml^{-1} and 76 mg ml^{-1} of the respective extracts could decrease the initial concentration of DPPH· by 50% when the reaction mixture was incubated for 50 min. While the relative scavenging activity of the batter was enhanced by 3.5-fold after fermentation, steam-cooking of the batter brought down the same to 1.6.

Table 17. DPPH·-scavenging activity[a] of methanolic extract of dhokla during different stages of its preparation

Sample[b]	Time (min)	%DPPH·-scavenging activity in extract (mg ml^{-1})					IC_{50} (mg ml^{-1} extract)[d]	RAE[e]
		10	20	30	40	50		
Dk-0							161.3 ± 2.19	1.00
	0	0.3c ± 0.10	0.6b ± 0.14	0.7b ± 0.04	1.0ab ± 0.16	1.4a ± 0.29		
	10	2.6e ± 0.29	4.5d ± 0.77	7.8c ± 1.06	9.5b ± 0.77	11.4a ± 0.93		
	20	3.0e ± 0.43	5.8d ± 0.59	9.2c ± 0.95	11.5b ± 0.70	14.0a ± 0.97		
	30	3.2e ± 0.46	6.6d ± 0.51	9.5c ± 0.83	12.0b ± 1.01	15.5a ± 0.88		
	40	3.5e ± 0.42	7.5d ± 0.66	9.9c ± 0.83	12.5b ± 0.75	16.0a ± 1.02		
	50	3.5e ± 0.45	7.7d ± 0.31	10.0c ± 0.39	12.5b ± 0.46	16.1a ± 1.01		
Dk-15							56.7 ± 0.67	3.50
	0	1.2d ± 0.14	1.3d ± 0.13	4.8c ± 0.61	7.2b ± 0.81	8.5a ± 0.48		
	10	6.9e ± 0.53	8.9d ± 0.51	16.8c ± 0.87	20.7b ± 0.96	22.1a ± 1.16		
	20	12.5e ± 1.13	15.6d ± 0.41	27.3c ± 0.80	32.9b ± 1.00	36.6a ± 0.96		
	30	13.8e ± 0.94	22.5d ± 0.42	31.1c ± 1.06	35.4b ± 0.98	39.5a ± 0.98		
	40	13.9e ± 0.99	23.0d ± 0.44	31.7c ± 0.85	38.0b ± 0.84	41.9a ± 1.10		
	50	14.1e ± 0.78	23.2d ± 0.60	32.0 c± 0.75	38.0b ± 0.92	42.0a ± 1.10		
Dk-SP							76.3 ± 1.20	1.55
	0	1.2e ± 0.25	2.4d ± 0.58	4.0c ± 0.87	4.3b ± 0.41	4.8a ± 0.46		
	10	4.6e ± 0.58	8.4d ± 0.87	11.2c ± 0.84	14.9b ± 0.67	17.4a ± 0.74		
	20	5.5e ± 0.87	11.4d ± 0.99	16.9c ± 0.99	23.6b ± 0.99	29.5a ± 0.98		
	30	5.7e ± 0.58	12.2d ± 0.88	20.9c ± 0.99	28.0b ± 1.06	30.6a ± 0.79		
	40	6.0e ± 0.47	12.5d ± 0.91	21.3c ± 0.69	28.6b ± 1.12	31.2a ± 0.98		
	50	6.1e ± 0.57	12.5d ± 0.92	21.5c ± 0.71	28.9b ± 0.92	31.2a ± 0.98		

[a]Values, expressed as mean ± SEM, were obtained from three independent samples. Means within a row sharing a common letter are not significantly different ($P < 0.05$).

[b]Dk-0, nonfermented dhokla batter; Dk-15, 15 h (fully)-fermented dhokla batter; Dk-SP, steamed product (dhokla).

[c]Incubation time of reaction mixture.

[d]IC_{50} (the efficient concentration of antioxidant decreasing initial concentration of DPPH· by 50%) was obtained by interpolation from linear regression analysis.

[e]Relative antioxidant effect was obtained by dividing extraction rate with IC_{50} value and compared with that o nonfermented batter which was assigned as 1.0.

4.3.5.5. Metal-chelating activity

The chelation power of methanolic extracts of batters and product examined against Fe^{2+} is shown in Table 18. At 10 mg ml^{-1}, the dhokla extract had a significant (51%) Fe^{2+}-chelating capacity which was demonstrated by the decrease in purple colour formed due to ferrozine-Fe^{2+} complex formation. At the same dosage level, the respective activities of the methanolic extracts of nonfermented and fermented

Table 18. Fe^{2+}-chelating activity[a] of methanolic extracts of dhokla during different stages of its preparation

Sample[b]	%Fe^{2+}-chelating activity in extract (mg ml^{-1})					IC_{50} (mg ml^{-1} extract)[c]	RAE[d]
	10	20	30	40	50		
Dk-0	24.7c ± 1.36	48.1c ± 2.49	66.5b ± 3.12	68.0b ± 3.44	69.0b ± 3.62	21.83a ± 1.09	1.00b
Dk-15	34.2b ± 2.31	61.4a ± 3.48	79.4a ± 3.29	79.9a ± 2.74	80.6a ± 3.11	15.83b ± 0.88	1.69a
Dk-SP	51.0a ± 3.48	56.1b ± 2.15	58.9c ± 2.87	60.3c ± 3.34	60.3c ± 3.44	9.83c ± 0.60	1.63a

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a column sharing a common letter are not significantly different ($P < 0.05$).

[b]Dk-0, nonfermented dhokla batter; Dk-15, 15 h (fully)-fermented dhokla batter; Dk-SP, steamed product (dhokla).

[c]IC_{50} (the efficient concentration of antioxidant decreasing initial concentration by 50%) was obtained by interpolation from linear regression analysis.

[d]Relative antioxidant effect was obtained by dividing extraction rate with IC_{50} value and compared with that of non-fermented batter which was assigned as 1.0.

batters were 25% and 34%. Though their chelating abilities varied significantly at 10 mg ml^{-1}, those increased up to a certain extent with the increase in dosage level, and then leveled off despite further increase in concentration. At 30 mg ml^{-1}, the extracts of nonfermented and fermented batters and steamed product showed 67, 79 and 59%, respectively, of Fe^{2+}-chelating ability. Further increase in the dosages, however, could not increase the percentage of chelation ability significantly. The mean half-inhibition concentration (IC_{50}) of the extracts was obtained by interpolation from linear regression analysis. The steamed product showed an IC_{50}-value of 9.8 mg ml^{-1} methanol, while 21.8 mg ml^{-1} and 15.8 mg ml^{-1} methanolic extracts of nonfermented and fermented batters, respectively, could chelate the initial Fe^{2+} concentration by 50%. However, a higher relative Fe^{2+}-chelating ability was observed with the fermented batter extract than the steamed product extract, when their initial yield of crude extract in methanol was taken in account. The relative Fe^{2+}-chelating ability increased by about 69% after fermentation, which revealed that the Fe^{2+}-chelating ability of dhokla batter enhanced after fermentation. Steaming the fermented batter for 10-15 min, however, showed a significant decline in the relative Fe^{2+}-chelating ability.

4.3.5.6. Lipid peroxidation inhibitory activity

Table 19 showed the *in vitro* assay of lipid peroxidation inhibitory activity of the methanolic extracts of nonfermented and fermented batters and steamed product, at different concentrations (10-50 mg ml^{-1} methanol) on the peroxidation of linoleic acid emulsion system. After 24 h of incubation at 37°C, methanolic extracts of fermented batter showed the highest antioxidant activity, followed by steamed product and nonfermented batter. A significant increase in percentage inhibition of linoleic acid peroxidation with the increase in concentration of the extract was evident at all the tested concentrations (10-50 mg ml^{-1}) of the extracts. At 50 mg ml^{-1}, the extract of nonfermented batter exhibited a total antioxidant activity with 24.2% inhibition of linoleic acid peroxidation at 24 h, while the fermented batter and steamed product extracts inhibited 41.6% and 31.8%, respectively, of linoleic acid peroxidationat similar concentration and incubation period. However, the mean peroxidation inhibition of the extracts was found to decline with time and reached 19%, 30% and 28%, respectively, after 72 h. In comparison, the extract of fermented batter exhibited effective antioxidant activity than the steamed product and nonfermented batter at tested concentrations, revealing its ability to deter lipid peroxidation.

Table 19. Lipid peroxidation inhibitory activity[a] of methanolic extracts of dhokla at different stages of its preparation

Sample[b]	Time[c] (h)	%inhibition by lyophilized extract (mg ml^{-1} methanol)				
		10	20	30	40	50
Dk-0	24	6.24d ± 0.45	12.46c ± 0.82	18.53b ± 1.00	23.53a ± 1.08	24.23a ± 0.89
	48	5.90d ± 0.58	10.25c ± 1.20	15.83b ± 0.78	20.53a ± 0.63	21.49a ± 0.66
	72	5.50d ± 0.74	9.06c ± 1.16	15.28b ± 0.83	16.83ab ± 0.85	18.53a ± 0.94
Dk-15	24	12.57e ± 0.90	24.72d ± 0.96	33.77c ± 1.08	40.55b ± 0.79	41.62a ± 1.10
	48	8.21d ± 0.80	17.54c ± 1.03	23.67b ± 0.82	29.59a ± 0.66	31.54a ± 1.09
	72	8.29d ± 0.88	16.20c ± 0.80	21.63b ± 0.89	28.70a ± 0.97	29.79a ± 0.86
Dk-SP	24	8.38e ± 0.79	16.51d ± 0.90	25.48c ± 0.94	29.67b ± 0.77	31.84a ± 0.52
	48	6.86e ± 1.04	13.30d ± 1.00	18.81c ± 0.98	25.43b ± 0.79	27.43a ± 0.82
	72	6.85e ± 0.81	12.90d ± 1.05	19.86c ± 0.81	26.93b ± 0.83	28.44a ± 1.07

[a]Values, expressed as mean ± SEM, were obtained from three independent samples. Means within a row sharing a common letter are not significantly different ($P < 0.05$).

[b]Dk-0, nonfermented dhokla batter; Dk-15, 15 h (fully)-fermented dhokla batter; Dk-SP, steamed product (dhokla).

[c]Incubation time of reaction mixture.

4.3.6. Correlation and regression

Correlation coefficients between every two antioxidative parameters tested for nonfermented and fermented batters and steamed product are shown in Table 20. The data exhibited all the five antioxidant parameters exhibited significant positive correlation among the every two parameters. In batters, the highest correlation was observed between metal-chelating activity and reducing power (r = 0.995), and lipid peroxidation inhibitory activity and reducing power (r = 0.989), respectively, while in dhokla it was total phenol content with lipid peroxidation inhibitory activity (r = 0.977), being closely followed by radical-scavenging activity and total phenol content (r = 0.944).

Table 20. Coefficient of correlations[a] among various antioxidative parameters[b] of nonfermented dhokla batter (Dk-0), 15 h (fully)-fermented dhokla batter (Dk-15) and steamed product or dhokla (Dk-SP) (13 d.f.)

Parameter	Sample	TPC	RP	RSA	MCA	TAA
TPC	Dk-0	1.000				
	Dk-15	1.000				
	Dk-SP	1.000				
RP	Dk-0	0.869	1.000			
	Dk-15	0.906	1.000			
	Dk-SP	0.880	1.000			
RSA	Dk-0	0.978	0.914	1.000		
	Dk-15	0.947	0.953	1.000		
	Dk-SP	0.944	0.865	1.000		
MCA	Dk-0	0.858	0.995	0.903	1.000	
	Dk-15	0.843	0.942	0.900	1.000	
	Dk-SP	0.653	0.793	0.686	1.000	
LPIA	Dk-0	0.909	0.920	0.937	0.926	1.000
	Dk-15	0.936	0.989	0.965	0.918	1.000
	Dk-SP	0.977	0.901	0.936	0.679	1.000

[a]Significant at P <0.01 (two-tailed).
[b]TPC, total phenol content; RP, reducing power; RSA, radical-scavenging activity; MCA, metal-chelating activity; LPIA, lipid peroxidation inhibitory activity (after 72 h).

The regression equations and coefficients of correlations between different antioxidant parameters and total phenol content of the extracts are presented in Table 21. All the tested samples showed that the total phenol contents were positively correlated (P <0.01) with DPPH·-scavenging activity, reducing power, metal-chelating ability and lipid peroxidation inhibitory activity. Relevant regression equations exhibited that the antiradical activity is greatly influenced by the total phenol content which accounted for 96%, 90% and 89% DPPH·-scavenging activities in nonfermented and fermented batters and dhokla, respectively (Table 21: eq. 1, 5 and 9). Similarly, the respective correlations between total phenol content and reducing power were 76%, 82% and 77% (Table 21: eq. 2, 6 and 10). Total phenol content

Table 21. Regression equations[a] for antioxidant parameters[b] as related to total phenol content (TPC) of nonfermented dhokla batter (Dk-0), 15 h (fully)-fermented dhokla batter (Dk-15) and steamed product or dhokla (Dk-SP)

Sample	Equation		R^2	TPC accounted for (%)
Dk-0	1. RSA	$y = -340.260TPC^2 + 273.920TPC - 23.091$	0.998	95.65
	2. RP	$y = -4.453TPC^2 + 2.668TPC - 0.194$	0.988	75.52
	3. MCA	$y = -1670.100TPC^2 + 961.140TPC - 66.334$	0.969	73.62
	4. LPIA	$y = -308.500TPC^2 + 199.970TPC - 14.623$	0.996	82.63
Dk-15	5. RSA	$y = -51.918TPC^2 + 95.479TPC - 3.143$	0.997	89.68
	6. RP	$y = -0.812TPC^2 + 1.197TPC + 0.115$	1.000	82.10
	7. MCA	$y = -246.140TPC^2 + 315.220TPC - 17.407$	0.988	71.10
	8. LPIA	$y = -47.229TPC^2 + 84.581TPC - 6.749$	0.987	87.61
Dk-SP	9. RSA	$y = 4.818TPC^2 + 29.705TPC - 0.733$	0.971	89.11
	10. RP	$y = -3.401TPC^2 + 2.666TPC - 0.204$	0.958	77.44
	11. MCA	$y = -115.860TPC^2 + 98.414TPC - 39.599$	0.977	42.64
	12. LPIA	$y = -64.152TPC^2 + 104.680TPC - 6.928$	0.997	95.45

[a]Significant at P <0.01 (two-tailed).
[b]RSA, radical-scavenging activity; RP, reducing power; MCA, metal-chelating activity; LPIA, lipid peroxidation inhibitory activity (after 72 h).

could also explain 74%, 71% and 43% respective metal-chelating ability (Table 21: eq. 3, 7 and 11), and 83%, 88% and 95% respective lipid peroxidation inhibitory activity (Table 21: eq. 4, 8 and 12). On the whole, total phenol content of the extracts exhibited a high positive correlation ($P < 0.01$) with all the antioxidant parameters tested.

4.4. Dosa

4.4.1. Proximate composition

Table 22 shows the proximate composition of market sample of dosa batter. The average moisture content of the acidic batter was 67.8 g 100 g^{-1}. The mean ash and crude fat contents of the batter were 1.1 g and 1.5 g 100 g^{-1} dry weight, respectively. Free fatty acid content in the dosa batter was 1.8%. Total nitrogen, protein nitrogen, nonprotein nitrogen and soluble nitrogen contents of dosa batter were 3.0, 1.8, 1.2 and 1.0 g (100 g)$^{-1}$ dry matter, respectively.

Table 22. Proximate composition[a] of substrates and fermented batter of dosa

Parameter	g (100 g)$^{-1}$ dry matter		
	Substrate		Marketed batter (n = 12)
	Parboiled rice (n = 3)	Blackgram dal (n = 3)	
pH	6.8a ± 0.05	7.0a ± 0.05	4.5b ± 0.04
Moisture (g (100 g)$^{-1}$)	9.4c ± 0.15	11.0b ± 0.09	67.8a ± 0.75
Ash	0.6c ± 0.05	6.3a ± 0.09	1.1b ± 0.03
Titratable acid (as lactic acid)	0.1b ± 0.01	0.1b ± 0.01	0.8a ± 0.04
Free fatty acid (as linoleic acid)	0.7b ± 0.01	0.7b ± 0.01	1.8a ± 0.04
Total nitrogen	0.9c ± 0.03	4.1a ± 0.03	3.0b ± 0.11
Protein nitrogen	0.9c ± 0.03	3.6a ± 0.03	1.8b ± 0.08
Nonprotein nitrogen	0.1c ± 0.01	0.4b ± 0.03	1.2a ± 0.05
Soluble nitrogen	0.2c ± 0.02	0.5b ± 0.02	1.0a ± 0.02
Total protein (total N x 6.25[b])	5.5c ± 0.20	25.4a ± 0.21	18.6b ± 0.67
Crude fat	0.2b ± 0.04	1.5a ± 0.10	1.5a ± 0.02
Carbohydrate (by difference)	93.7a ± 0.20	66.8c ± 0.30	78.8b ± 0.69
Energy value (MJ (100 g)$^{-1}$ dm)	1.7a ± 0	1.6a ± 0	1.7a ± 0

[a]Values are expressed as mean ± SEM. Means within a row sharing a common letter are not significantly different ($P < 0.05$)..
[b]Protein factor is 5.95 for rice.

4.4.2. Microbial analysis

4.4.2.1. Isolation of microorganisms

A total of 90 strains of lactic acid bacteria (Table 23) and 85 strains of yeasts (Table 24) were isolated from 12 samples. While all the lactic bacterial isolates were grouped into two cell morphotypes, the

Table 23. Selection of representative strains of dominant lactic acid bacteria isolated from marketed samples of fermented batter of dosa

Parameter	Character	
Cell shape	Cocci in pair/short chain	Cocci in tetrad
Gas from glucose	+	-
Growth in/at		
pH 4.8	-	+
45°C	-	+
Grouped strains[a]		
Group	Do-LA	Do-LB
Total number of strain in group	60	30
No. of representative strains	12	6

[a]All the strains were nonmotile, nonsporeforming, Gram positive and catalase negative.

Table 24. Selection of representative strains of dominant yeasts isolated from marketed samples of fermented batter of dosa

Parameter	Character		
Colony[a]	Cgs	Tds	Pgs
Cell shape[b]	G-O/E	O-E	G-E
Pellicle formation	-	+	-
Grouped strains			
Group	Do-YA	Do-YB	Do-YD
Total number strains in group	40	40	5
No. of representative strains	8	8	1

[a]Cgs, cream colour with glistening and butyrous surface; Tds, tarnish white colour with dull and flat surface; Pgs, pink colour with glistening and butyrous surface.
[b]G, globose; O, oval; E, ellipsoidal.

yeast isolates were grouped into three colony morphotypes. One representative strain, from each group of each positive sample, was selected randomly to ascertain its taxonomic status.

4.4.2.2 Taxonomical studies

4.4.2.2.1. Bacteria

On the basis of selected morphological, physiological and cultural characteristics of lactic acid bacteria shown in Table 23 all the representative strains of the group Do-LA belonged to the genus *Leuconostoc*, while that of the group Do-LB belonged to the genus *Pediococcus*. The detailed morphological and physiological characteristics of all those representative strains are presented in Table 25. Following the criteria laid by Garvie (1986a, 1986b) as well as IBIS (Wijtzes *et al.*, 1997), the representative strains of group Do-LA and Do-LA were tentatively identified as *Leuconostoc mesenteroides* (Tsenkovskii) van Tieghem 1879 and *Pediococcus pentosaceus* Mees 1934, respectively.

Table 25. Characteristics[a] of groups of representative strains of lactic acid bacteria isolated from marketed samples of fermented batter of dosa

Parameters	Do-LA (n = 12)			Do-LB (n = 6)		
	+	d	-	+	d	-
Cell shape	Cocci in pair or chain			Cocci in tetrad		
Cell diameter (µm)	0.30 - 0.70			0.30 - 0.65		
Gas from glucose	100					100
Growth in NaCl (g l^{-1})						
30	100			67	33	
65	67	33		100		
80			100	100		
Growth at pH 4.8			100	100		
Hydrolysis of						
arginine			100	100		
esculin	58	42		83	17	
Growth at 45°C			100	100		
Acid from						
D-cellobiose	42	50	8	100		
esculin	83	17		75	25	
D-lactose	78	22		33	50	17
D-mannitol	100					100
D-mannose	100					100
D-melibiose	50	42	8	76	33	
D-raffinose	75	25				100
D-ribose	100			65	35	
salicin	67	33		100		
sucrose	100			50		50
D-xylose	58	42		67	17	16

[a]Data represent %strains showing + (positive), d (delayed) and - (negative) reactions. All the strains were Gram positive, nonmotile, nonsporeforming, catalase negative, capable of growing at pH 6.5 and 15-40°C, and producing acid from L-arabinose, D-fructose, D-galactose, D-glucose, D-maltose and D-trehalose, however not capable of reducing nitrate, producing indole, hydrolyzing fat, gelatin and starch, and producing acid from glycerol, sorbitol and starch.

4.4.2.2.2. Yeasts

The detailed morphological, cultural and physiological characteristics of the 17 representative strains of yeasts, comprising three different colony morphotypes, isolated from the samples of marketed dosa batter are shown in Table 26. Following the taxonomic keys of Barnett *et al.* (2000) and Kurtzman and Fell (2000), and using BioloMICSNet software (http://www.cbs.knaw.nl/yeast/BioloMICS.aspx), the representative strains of yeasts belonging to group Do-YA, Do-YB and Do-YD were tentatively identified as *Saccharomyces cerevisiae* Mayen *ex* Hansen 1883, *Issatchenkia orientalis* Kudryavtsev 1960 and *Rhodotorula minuta* (Saito) Harrison 1928, respectively.

Table 26. Characteristics[a] of groups of representative strains of yeasts isolated from marketed samples of fermented batter of dosa

Parameter	Do-YA (n = 8)			Do-YB (n = 8)			Do-YD (n = 1)		
	+	$+_w$	-	+	$+_w$	-	+	$+_w$	-
Colony morphology[b]	Cgs			Tds			Pgs		
Cell shape[c]	(G-O) / E			O-E			G-E		
Cell width x length (µm)	3.4-4.7 x 4.0-8.8			2.7-6.5 x 3.1-7.1			3.8-3.7 x 3.8-4.8		
Mycelium[d]	Psd			Psd					100
Vegetative reproduction	100			100					100
Budding	Multilateral			Multilateral					
Sexual reproduction	100			100					100
Ascus	100			100					100
Asci evanescence	Persistent			Persistent					100
Ascospore[e]	(R-O)+Sm			O+Sm					100
Ascospore/ ascus	1-4			1-2					100
Pellicle formation			100	100					100
Hydrolysis of									
fat	48	52		100					100
urea			100			100	100		
Fermentation of									
D-galactose	52	27	21			100			100
D-glucose	100			90	10				100
D-maltose	61	15	24			100			100
D-melibiose	64		36			100			100
melezitose	52	27	21			100			100
D-raffinose	100					100			100
sucrose	100					100			100
Assimilation of									
L-arabinose			100			100		100	
D-cellobiose			100			100	100		
citrate			100	100					100
D-galactose	43	21	36		30	70	100		
D-glucitol			100			100		100	
D-glucosamine			100	100					100
N-acetyl-glucosamine			100	95	5		100		
glycerol			100	100			100		
D-lactose			100			100		100	
D-maltose	100				15	85			100
melezitose	62	25	13			100			100
D-melibiose	50	25	25			100			100
D-raffinose	100					100			100
D-ribose			100			100	100		
sucrose	100					100	100		
D-trehalose	100					100		100	
vitamin-free medium			100	100					100
D-xylose			100			100		100	

[a]Data represent %strains showing + (positive), $+_w$ (weak positive) and - (negative) reactions. All the strains were capable of growing at 37°C and assimilating D-glucose, however not capable of reducing nitrate, forming starch, hydrolyzing gelatin and starch, fermenting D-cellobiose, D-lactose, D-trehalose and D-xylose, and assimilating *m*-erythritol, *m*-inositol, D-mannitol, nitrate, L-rhamnose and starch.

[b]Cgs, Cream colour with glistening and butyrous surface; Tds, tannish white colour with dull and flat surface; Wsw, tannish white surface with wrinkled margin; Pgs, pink colour with glistening and butyrous surface.

[c]G, globose; O, oval; E, elongated.

[d]Psd, pseudomycelium/pseudohyphae.

[e]R, round, O, oval; Sm, smooth; Str, Saturn-shaped.

4.4.2.3. Microbial load in fermented batter

The average microbial load and their percentage occurrence in 12 market samples dosa batter are shown in Table 27. TAMB cells and their spores were recovered from all the samples studied. While *Leuconostoc mesenteroides* and *Pediococcisus pentosaceus* were the two dominant bacteria occurring in 100% and 50% of dosa batter, respectivey, yeast microbiota comprises *Saccharomyces cerevisiae*, followed by *Issatchenkia orientalis* and *Rhodotorula minuta*. Each of the former two members of yeast was

encountered in 67% of the samples, while *R. minuta* was isolated from only 8% of the samples studied.

Table 27. Dominant microbial load in the marketed samples (n = 12) of fermented batter of dosa

Microbiota	Log cfu g^{-1} fresh wt (mean ± SEM)	%occurrence
TAMB	9.8 ± 0.15	100
aMBS	4.1 ± 0.14	100
Yeasts		
Saccharomyces cerevisiae	5.8 ± 0.57	67
Issatchenkia orientalis	4.9 ± 0.61	67
Rhodotorula minuta	4.1 ± 0.45	8
Lactic acid bacteria		
Leuconostoc mesenteroides	9.4 ± 0.17	100
Pediococcus pentosaceus	7.5 ± 0.25	50

4.5. Idli

4.5.1. Proximate composition

The proximate composition of substrates (parboiled rice and blackgram dal) and fermented idli batter from different sources are presented in Table 28. The moisture content of fermented idli batter was six times higher than that of the substrates. Blackgram dal showed a significantly higher ash content than the fermented idli batter and parboiled rice. The fermented batter was distinctly acidic in contrast to the neutral pH of the substrates. The titratable and free fatty acidity of the ingredients increased significantly during fermentation. While the mean protein content of blackgram dal was higher than that of parboiled rice and the fermented batter, the crude fat content of rice was less than that of blackgram dal and fermented batter.

Table 28. Proximate composition[a] of substrates and fermented batter of idli

Parameter	g (100 g)$^{-1}$ dry matter			
	Substrate		Fermented batter	
	Parboiled rice (n = 3)	Blackgram dal (n = 3)	Marketed (n = 12)	Laboratory-made (n = 3)
pH	6.8a ± 0.05	7.0a ± 0.05	4.7b ± 0.08	4.3b ± 0.04
Moisture (g (100 g)$^{-1}$)	9.4c ± 0.15	11.0c ± 0.09	57.0b ± 0.72	61.1a ± 1.46
Ash	0.6c ± 0.05	6.3a ± 0.09	1.2b ± 0.05	1.5b ± 0.02
Titratable acid (as lactic acid)	0.1c ± 0.01	0.1c ± 0.01	0.7b ± 0.03	1.8a ± 0.03
Free fatty acid (as linoleic acid)	0.7b ± 0.01	0.7b ± 0.01	1.6a ± 0.04	1.6a ± 0.01
Total nitrogen	0.9c ± 0.03	4.1a ± 0.03	2.5b ± 0.07	2.7b ± 0.12
Protein nitrogen	0.9c ± 0.03	3.6a ± 0.03	1.9b ± 0.06	1.4b ± 0.03
Nonprotein nitrogen	0.1c ± 0.01	0.4bc ± 0.03	0.6b ± 0.04	1.3a ± 0.13
Soluble nitrogen	0.2c ± 0.02	0.5bc ± 0.02	0.5bc ± 0.03	0.7a ± 0.03
Total protein (total N x 6.25[b])	5.5d ± 0.20	25.4a ± 0.21	15.4c ± 0.45	16.7b ± 0.75
Crude fat (ether extract)	0.2c ± 0.04	1.5a ± 0.10	1.5a ± 0.02	1.1b ± 0.02
Carbohydrate (by difference)	93.7a ± 0.20	66.8c ± 0.30	81.9b ± 0.49	80.7b ± 0.79
Energy (MJ (100 g)$^{-1}$ dry matter)	1.7a ± 0	1.6a ± 0	1.7a ± 0	1.7a ± 0

[a]Values are expressed as mean ± SEM. Means within a row sharing a common letter are not significantly different ($P < 0.05$).

[b]Protein factor is 5.95 for rice.

4.5.2. Microbial analysis

4.5.2.1. Isolation of microorganisms

A total of 295 strains of lactic acid bacteria (Table 29) and 365 strains of yeasts (Table 30) were isolated from three samples of raw blackgram dal, 12 samples of marketed batter and 21 samples of laboratory-made fermenting and fermented batter. However, the occurrence of lactic acid bacteria and yeasts were below the detection limit in parboiled rice. While all the lactic acid bacterial isolates were grouped into two cell morphotypes, the yeast isolates were clustered into four colony morphotypes. A total of 30 strains of mould were isolated from 0 h and 3 h-old batch of idli batter fermenting under laboratory conditions (Table 31). One representative strain, from each group of each positive sample, was selected randomly to ascertain its taxonomic status.

Table 29. Selection of representative strains of dominant lactic acid bacteria isolated from substrates and fermenting batter of idli

Source	Stage(s) of fermentation	Cell shape[a]	Gas from glucose	Growth in/at		Grouped strains[b]		No. of R[c] strains
				pH 4.8	45°C	Group	No.	
Substrate								
Parboiled rice (n = 3)							0	
Blackgram dal (n = 3)		Cp/c	+	-	-	Id-LA	15	3
Mixed batter								
Marketed (n = 12)	Final	Cp/c	+	-	-	Id-LA	60	12
		Ct	-	+	+	Id-LB	35	7
Laboratory-made	0 h-18 h	Cp/c	+	-	-	Id-LA	105	21
(n = 21)	(3 h-interval)	Ct	-	+	+	Id-LB	80	16

[a]Cp/c, cocci in pair or short chain; Ct, cocci in tetrad.
[b]All the isolates were nonmotile, nonsporeforming, Gram positive and catalase negative.
[c]R, representative.

Table 30. Selection of representative strains of dominant yeasts isolated from substrates and fermenting batter of idli

Source	Stage(s) of fermentation	Colony[a]	Cell shape[b]	Pellicle formation	Grouped strains		No. of R[c] strains
					Group	No.	
Substrates							
Parboiled rice (n = 3)						0	
Blackgram dal (n = 3)		Cgs	G-O/E	-	Id-YA	10	2
Mixed batter							
Marketed (n = 12)	Final	Cgs	G-O/E	-	Id-YA	50	10
		Tds	O-E	+	Id-YB	30	6
		Wsw	O-E	+	Id-YC	5	1
		Pgs	G-E	-	Id-YD	10	2
Laboratory-made	0 h-18 h	Cgs	G-O/E	-	Id-YA	105	21
(n = 21)	(3 h-interval)	Tds	O-E	+	Id-YB	70	14
		Wsw	O-E	+	Id-YC	85	17

[a]Cgs, Cream colour with glistening and butyrous surface; Tds, tannish white colour with dull and flat surface; Wsw, tannish white surface with wrinkled margin; Pgs, pink colour with glistening and butyrous surface.
[b]G, globose; O, oval; E, ellipsoidal.
[c]R, representative.

Table 31. Selection of representative strains of moulds isolated from fermenting batter of idli

Source	Number of		
	strains isolated[a]	grouped strains	representative strains
Nonfermented batter (n = 3)	15	15	3
3 h-fermenting batter (n = 3)	15	15	3

[a]All the isolates had mycelia without septation, rhizoid and stolon; sporangiophores branched and arose directly from the substrate mycelia; sporangiospores, ellipsoidal to globose; columellae, ellipsoidal to pyriform; chlamydospores, numerous and barrel-shaped.

4.5.2.2. Taxonomical studies

4.5.2.2.1. Bacteria

On the basis of selected morphological, physiological and cultural characteristics of lactic acid bacteria shown in Table 29, all the representative strains of the group Id-LA belonged to the genus *Leuconostoc*, while those of the group Id-LB belonged to the genus *Pediococcus*. The detailed morphological and physiological characteristics of all those representative strains are shown in Table 32. Following the criteria laid by Garvie (1986a, 1986b) as well as IBIS (Wijtzes *et al.*, 1997), the representative strains belonging to group Id-LA and Id-LB were tentatively identified as *Leuconostoc mesenteroides* (Tsenkovskii) van Tieghem 1879 and *Pediococcus pentosaceus* Mees 1934, respectively.

Table 32. Characteristics[a] of groups of representative strains of lactic acid bacteria isolated from substrates and fermenting batter of idli

Parameters	Id-LA (n = 36)			Id-LB (n = 23)		
	+	d	-	+	d	-
Cell shape	Cocci in pair or chain			Cocci in tetrad		
Cell diameter (µm)	0.30 - 0.70			0.30 - 0.65		
Gas from glucose	100					100
Growth in NaCl (g l^{-1})						
30	100			61	39	
65	69	31		100		
80			100	100		
Growth at pH 4.8			100	100		
Hydrolysis of						
arginine			100	100		
esculin	56	44		87	13	
Growth at 45°C			100	100		
Acid from						
D-cellobiose	47	53		100		
esculin	86	14		74	26	
D-lactose	78	22		39	52	9
D-mannitol	100					100
D-mannose	100					100
D-melibiose	56	44		30	48	22
D-raffinose	75	25				100
D-ribose	100			65	35	
salicin	61	39		100		
sucrose	100			43		57
D-xylose	58	42		65	13	22

[a]Data represent %strains showing + (positive), d (delayed) and - (negative) reactions. All the strains were Gram positive, nonmotile, nonsporeforming, catalase negative, capable of growing at 15-40°C and producing acid from L-arabinose, D-fructose, D-galactose, D-glucose, D-maltose and D-trehalose, however not capable of reducing nitrate, producing indole, hydrolyzing fat, gelatin and starch, and producing acid from glycerol, sorbitol and starch.

4.5.2.2.2. Yeasts

Table 33 shows the detailed morphological, cultural and physiological characteristics of the 73 representative strains of yeast, comprising four different colony morphotypes, isolated from the substrates and fermenting and fermented batters. Following the taxonomic keys of Barnett *et al.* (2000) and Kurtzman and Fell (2000), and using BioloMICSNet software (http://www.cbs.knaw.nl/yeast/BioloMICS.aspx), the representative strains of yeasts belonging to group Id-YA, Id-YB, Id-YC and Id-YD were tentatively identified as *Saccharomyces cerevisiae* Mayen *ex* Hansen 1883, *Issatchenkia orientalis* Kudryavtsev 1960, *Pichia membranifaciens* Hansen 1904, and *Rhodotorula minuta* (Saito) Harrison 1928, respectively.

4.5.2.2.3. Moulds

The detailed morphological characteristics of the six representative strains of mould, isolated from samples of fermenting batter at 0 h and after 3 h, are shown in Table 34. Following the taxonomic keys of Hesseltine (1983) and Samson and van Reenen-Hoekstra (1988) all the representative strains of the mould were identified as *Mucor racemosus* Fres.

4.5.2.3. Microbial load in substrates and fermented batter

The average microbial load studied in 12 market samples of fermented batter, three samples each of two kinds of substrates (blackgram dal and parboiled rice) and laboratory-made fermented idli batters

Table 33. Characteristics[a] of groups of representative strains of yeasts isolated from fermenting batter of idli and its substrates

Parameters	Id-YA (n = 33)			Id-YB (n = 20)			Id-YC (n = 18)			Id-YD (n = 2)		
	+	$+_w$	-	+	$+_w$	-	+	$+_w$	-	+	$+_w$	-
Colony morphology[b]	Cgs			Tds			Wsw			Pgs		
Cell shape[c]	(G-O)/E			O-E			O-E			G-E		
Cell width x length (µm)	3.4-4.7 x 4.0-8.8			2.7-6.5 x 3.1-7.1			2.7-5.3 x 3.4-7.3			3.3-4.2 x 3.9-5.9		
Mycelium[d]	Psd			Psd			Psd					100
Vegetative reproduction	100			100			100					100
Budding	Multilateral			Multilateral			Multilateral					
Sexual reproduction	100			100			100					100
Ascus	100			100			100					100
Asci evanescence	Persistent			Persistent			Evanescent					
Ascospore[e]	(R-O)+Sm			O+Sm			Str/R					
Ascospore/ascus	1-4			1-2			1-4					
Pellicle formation			100	100			100					100
Hydrolysis of												
fat	48	52		100					100			100
urea			100			100			100	100		
Fermentation of												
D-galactose	52	27	21			100			100			100
D-glucose	100			90	10		83	17				100
D-maltose	61	15	24			100			100			100
D-melibiose	64		36			100			100			100
melezitose	52	27	21			100			100			100
D-raffinose	100					100			100			100
sucrose	100					100			100			100
Assimilation of												
L-arabinose			100			100			100	50	50	
D-cellobiose			100			100			100	50		50
citrate			100	100			100					100
D-galactose	43	21	36		30	70			100	50	50	
D-glucitol			100			100			100	50		50
D-glucosamine			100	100					100			100
N-acetyl-glucosamine			100	95	5		100			100		
glycerol			100	100			100			100		
D-lactose			100			100			100	50	50	
D-maltose	100				15	85			100			100
melezitose	52	15	33			100			100			100
D-melibiose	40	33	27			100			100			100
D-raffinose	100					100		28	72			100
D-ribose			100			100			100	50	50	
sucrose	100					100			100	100		
D-trehalose	100					100		17	83	50	50	
vitamin-free medium			100	100			100					100
D-xylose			100			100	100			50	50	

[a]Data represent %strains showing + (positive), $+_w$ (weak positive) and - (negative) reactions. All the strains were capable of growing at 37°C and assimilating of D-glucose, however not capable of reducing nitrate, forming starch, hydrolyzing gelatin and starch, fermenting D-cellobiose, D-lactose, D-trehalose and D-xylose, and assimilating *m*-erythritol, *m*-inositol, D-mannitol, nitrate, L-rhamnose and starch.

[b]Cgs, cream colour with glistening and butyrous surface; Tds, tannish white colour with dull and flat surface; Wsw, tannish white surface with wrinkled margin; Pgs, pink colour with glistening and butyrous surface.

[c]G, globose; O, oval; E, elongated.

[d]Psd, pseudomycelium/pseudohyphae.

[e]R, round, O, oval; Sm, smooth; Str, Saturn-shaped.

are shown in Table 35. TAMB cells and their spores (aMBS) were recovered from all the samples studied (Table 36). While *Leuconostoc mesenteroides* was dominant in fermented batters and raw blackgram dal, *Pediococcus pentosaceus* was recovered from the fermented batters only. The average load of *Saccharomyces cerevisiae* in the blackgram dal was $>10^3$ cfu g^{-1}. However, the occurrence of lactic acid bacteria and yeasts in parboiled rice was below the detection limits. While the average load of yeast in fermented batter ranges between 10^3 and 10^6 cfu g^{-1}, all the four morphotypes of representative strains were present in market samples. *S. cerevisiae* was most dominant followed by *Issatchenkia*

orientalis and *Pichia membranifaciens*. *Rhodotorula minuta*, with an average load of $>10^4$, was recovered from 17% of the market samples.

Table 34. Characteristics of representative strains of moulds isolated from nonfermented and fermenting batter of idli

Character	Representative strains[a]					
	LId-M0$_A$	LId-M0$_B$	LId-M0c	LId-M3$_A$	LId-M3$_B$	LId-M3c
Sporangiospore						
Length (μm)	4.5-6.4	4.6-6.3	4.4-6.4	4.6-6.7	4.5-6.5	4.0-6.7
Breadth (μm)	4.4-5.5	4.3-5.2	4.0-4.8	4.5-5.3	4.5-5.5	4.0-5.5
Columella						
Length (μm)	40-47	42-46	38-44	35-45	42-48	40-45
Breadth (μm)	40-45	35-45	40-45	38-45	40-48	30-45
Chlamydospore						
Length (μm)	15-18	14-19	16-19	15-22	17-21	15-20
Breadth (μm)	12-15	12-15	11-14	13-16	13-16	10-15

[a]All the isolates had aseptate mycelium, rhizoid, stolon, branched sporangiophores which arose directly from the substrate mycelium, ellipsoidal to globose sporangiospores, ellipsoidal to pyriform columellae, and numerous, barrel-shaped chlamydospores, however were not capable of growing at 37°C.

Table 35. Dominant microbial load[a] in substrates and fermented batter of idli

Microbiota	Log cfu g^{-1} fresh weight			
	Substrate		Fermented batter	
	Parboiled rice (n = 3)	Blackgram dal (n = 3)	Marketed (n = 12)	Laboratory-made (n = 3)
TAMB	4.7c ± 0.14	5.3c ± 0.08	9.8b ± 0.26	11.0a ± 0.35
aMBS	3.2a ± 0.08	3.2a ± 0.15	3.1a ± 0.49	3.1a ± 0.13
Lactic acid bacteria[b]				
Leuconostsoc mesenteroides	<DL	4.8c ± 0.09	9.4b ± 0.16	11.8a ± 0.31
Pediococcus pentosaceus	<DL	<DL	8.7b ± 0.71	9.8a ± 0.14
Yeasts[c]				
Saccharomyces cerevisiae	<DL	3.6b ± 0.10	6.0a ± 0.37	5.9a ± 0.12
Issatchenkia orientalis	<DL	<DL	4.9b ± 0.41	6.2a ± 0.83
Pichia membranifaciens	<DL	<DL	5.6a ± 0.38	3.3b ± 0.08
Rhodotorula minuta	<DL	<DL	4.9 ± 0.45	<DL

[c]Values are expressed as mean ± SEM. Means within a row sharing a common letter are not significantly different ($P < 0.05$).
[b]DL (detection limit), 1.0 log cfu g^{-1} fresh weight.
[c]DL, 2.0 log cfu g^{-1} fresh weight.

Table 36. Prevalence of dominant microorganisms in fermented batter of idli and its substrates

Microbiota	Positive sample (%)			
	Substrate		Fermented batter	
	Parboiled rice (n = 3)	Blackgram dal (n = 3)	Marketed (n = 12)	Laboratory-made (n = 3)
TAMB	100	100	100	100
aMBS	100	100	100	100
Lactic acid bacteria				
Leuconostsoc mesenteroides	0	100	100	100
Pediococcus pentosaceus	0	0	58	100
Yeasts				
Saccharomyces cerevisiae	0	67	83	100
Issatchenkia orientalis	0	0	50	100
Pichia membranifaciens	0	0	8	100
Rhodotorula minuta	0	0	17	0

4.5.3. Optimization of traditional process parameters

Table 37 shows the average sensory scores for optimizing proportions of substrates (parboiled rice: blackgram dal) during idli batter fermentation. All the treatments differed significantly among themselves with respect to flavour, body texture, colour and total score. The treatment B (2:1) had the highest score compared to the treatment A (1:1); treatment C (3:1) and treatment D (4:1). Hence, the proportion of substrates for the preparation of idli was optimized at fermenting mixture of two parts of parboiled rice and one part of blackgram dal.

Table 37. Average sensory scores[a] of idli for optimizing ingredients' proportions of mixed batter for fermentation (30°C, 18 h)

Attributes	Proportion of rice and blackgram dal (w w^{-1})			
	A (1:1)	B (2:1)	C (3:1)	D (4:1)
Taste (35)	21.7b ± 0.5	31.8a ± 0.5	31.1a ± 1.0	30.7a ± 1.2
Texture (30)	21.0b ± 0.3	25.8a ± 0.8	25.8a ± 0.8	21.2b ± 0.5
Flavour (30)	21.4b ± 0.4	25.9a ± 0.5	24.8a ± 0.5	23.1ab ± 0.4
Colour (5)	4.0a ± 0.1	4.2a ± 0	4.3a ± 0.1	4.1a ± 0.1
Total score (100)	68.1c ± 0.3	87.7a ± 1.3	85.6a ± 0.8	79.1b ± 1.2

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a row sharing a common letter are not significantly different (P <0.05).

Table 38 reveals that all the treatments considered for optimizing fermentation period of batter during idli preparation differed significantly among themselves with respect to every sensory attribute. The treatment C (fermentation period of 18 h) had the highest score compared to the treatment A (12 h), treatment B (15 h) and treatment D (21 h). Hence, 18 h is optimum time for the fermentation of mixed idli batter during idli preparation.

Table 38. Average sensory scores[a] of idli for optimizing fermentation (30°C) period of mixed batter[b]

Attribute	Fermentation period (h)			
	A (12)	B (15)	C (18)	D (21)
Taste (35)	22.6c ± 0.8	27.7b ± 0.7	31.8a ± 0.5	31.5a ± 0.6
Texture (30)	23.0b ± 0.9	24.0ab ± 0.8	25.8a ± 0.8	25.6a ± 0.4
Flavour (30)	22.6c ± 0.4	24.8b ± 0.8	25.9a ± 0.5	23.4b ± 0.4
Colour (5)	4.2a ± 0.1	4.1a ± 0.1	4.2a ± 0	4.1a ± 0.1
Total score (100)	72.4c ± 1.3	80.6b ± 1.1	87.7a ± 1.3	84.6ab ± 0.6

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a row sharing a common letter are not significantly different (P <0.05).

[b]Proportions of ingredients: parboiled rice and blackgram dal, 2:1 (w w^{-1}).

All the treatments considered for optimizing temperature of incubation (fermentation) during idli batter fermentation differed significantly from each other in respect of flavour, texture and total score (Table 39). The treatment B (temperature of 30°C during idli batter fermentation) had the highest total score compared to the treatment A (25°C) and the treatment C (35°C). Hence, 30°C was the optimum temperature for the fermentation of idli batter during idli preparation.

Table 39. Average sensory scores[a] of idli for optimizing fermentation (18 h) temperature of mixed batter[b]

Attribute	Fermentation temperature (°C)		
	A (25)	B (30)	C (35)
Taste (35)	26.4b ± 0.5	31.6a ± 1.0	22.3c ± 0.6
Texture (30)	24.0a ± 0.3	25.4a ± 0.4	24.8a ± 0.4
Flavour (30)	23.3ab ± 0.2	25.8a ± 0.5	20.9b ± 0.9
Colour (5)	3.9b ± 0.7	4.3a ± 0.1	3.9b ± 0.1
Total score (100)	77.6b ± 0.6	87.1a ± 1.4	71.9c ± 0.9

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a row sharing a common letter are not significantly different (P <0.05).

[b]Proportions of ingredients: parboiled rice and blackgram dal, 2:1 (w w^{-1}).

4.5.4. Successional studies on batter during fermentation

4.5.4.1. Microbial changes

Table 40 shows quantitative changes in the count of dominant microorganisms during idli batter fermentation under optimized conditions. TAMB cells increased significantly from 10^6 to 10^{11} cfu g

Table 40. Changes in microbial load[a] in mixed batter of idli during fermentation under optimized conditions

Microbiota	Fermentation period (h)						
	0	3	6	9	12	15	18
TAMB	6.6f ± 0.09	7.3e ± 0.34	8.4d ± 0.14	9.3c ± 0.07	10.4b ± 0.05	11.0a ± 0.28	11.0a ± 0.35
	(100)	(100)	(100)	(100)	(100)	(100)	(100)
aMBS	4.2a ± 0.12	3.1b ± 0.09	2.8b ± 0.42	3.2b ± 0.07	3.3b ± 0.10	3.2b ± 0.13	3.1b ± 0.13
	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Lactic acid bacteria[b]							
Leuconostsoc mesenteroides	4.9f ± 0.35	5.4e ± 0.25	7.2d ± 0.05	8.1c ± 0.06	10.7b ± 0.12	11.8a ± 0.12	11.8a ± 0.31
	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Pediococcus pentosaceus	<DL	4.5f ± 1.51f	5.9e ± 1.95	6.9d ± 0.07	7.9c ± 0.13	9.1b ± 0.10	9.8a ± 0.14
	(0)	(67)	(67)	(100)	(100)	(100)	(100)
Yeasts[c]							
Saccharomyces cerevisiae	4.3c ± 0.16	4.8b ± 0.14	4.8b ± 0.06	5.2ab ± 0.22	5.8a ± 0.22	5.9a ± 0.13	5.9a ± 0.12
	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Issatchenkia orientalis	<DL	<DL	4.7c ± 0.15	4.8c ± 0.11	5.5b ± 0.34	6.1a ± 0.25	6.2a ± 0.23
	(0)	(0)	(67)	(100)	(100)	(100)	(100)
Pichia membranifaciens	<DL	4.1c ± 0.15	5.2b ± 0.34	6.0a ± 0.06	6.2a ± 0.12	3.6de ± 0.09	3.3e ± 0.08
	(0)	(67)	(100)	(100)	(100)	(100)	(100)
Mould[c]							
Mucor racemosus	4.4a ± 0.12	3.3b ± 0.11	<DL	<DL	<DL	<DL	<DL
	(100)	(100)	(0)	(0)	(0)	(0)	(0)

[a]Values, expressed as mean ± SEM of log cfu g^{-1} fresh weight, were obtained from three different samples. Data within parentheses are expressed as %positive samples. Means within a row sharing a common letter are not significantly different ($P < 0.05$).

[b]DL (detection limit), 1.0 log cfu g^{-1} fresh weight.

[c]DL, 2.0 log cfu g^{-1} fresh weight.

fresh weight, while the count of their spores decreased by one log-cycle after fermentation. Since idli fermentation is an acidic one, *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* were the most predominant microorganisms encountered throughout fermentation. The number of cells of *L. mesenteroides* increased significantly at every 3 h-interval till 15 h of fermentation. The presence of *Pediococcus pentosaceus* and yeasts was variable. *P. pentosaceus*, although not found at the onset of fermentation, was detected after 3 h and increased significantly after every 3 h-interval till the end. *S. cerevisiae*, which occurred in raw dal, was present at the initial stage and increased during fermentation. *Issatchenkia orientalis*, which was detected only after 6 h of fermentation, increased during fermentation. *Pichia membranifaciens*, which appeared after 3 h, increased at the midway of fermentation, but decreased at the end. Unlike market samples, no *Rhodotorula minuta* was recovered during entire process of fermentation. *Mucor racemosus*, the only mould involved in idli batter fermentation, was found at the onset of fermentation, however its cell number declined after 3 h and eventually perished after 6 h onwards of fermentation.

4.5.4.2. Changes in proximate composition

The proximate compositional changes in idli batter during fermentation are presented in Table 41. Fermentation caused a 3-fold increase in titratable acidity with a decrease in pH. During initial 3 h of fermentation, no appreciable change was observed in the batter volume. However, from 3 h onward the batter volume increased significantly at every 3 h interval till 12 h of fermentation, during which the batter gained double the original volume. Further change in the batter volume, beyond 12 h, was not significant. Total protein content showed mild fluctuations during intermediate stages of fermentation, but eventually remained the same. While protein nitrogen content decreased, nonprotein nitrogen increased during fermentation. No significant change in the contents of moisture, carbohydrate, crude fat, total protein, free fatty acid, ash, soluble nitrogen and energy value was observed during idli batter fermentation.

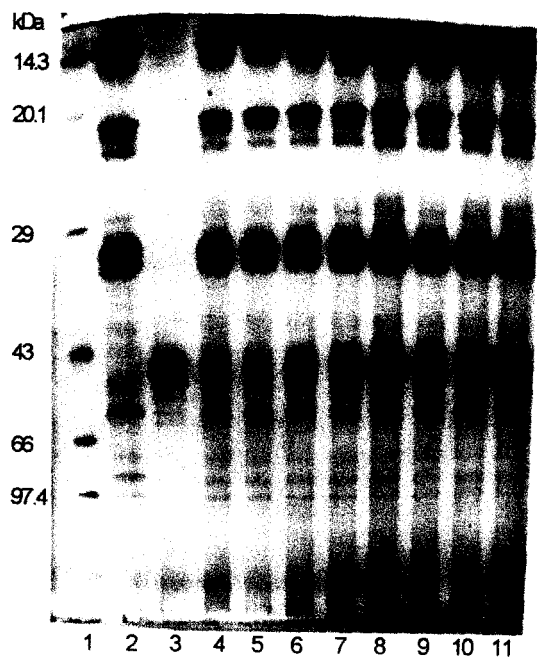

Fig. 40. SDS-PAGE profile of raw ingredients and fermenting batter (a mixture of rice slurry and blackgram dal paste, 2:1; 30°C) of idli. Lanes: 1, protein molecular weight marker; 2, parboiled rice; 3, blackgram dal; 4, 0 h-old batter; 5, 3 h-old batter; 6, 6 h-old batter; 7, 9 h-old batter; 8, 12 h-old batter; 9, 15 h-old batter; 10, 18 h-old batter; 11, 21 h-old batter.

4.5.5. SDS-PAGE

Fig. 40 showed the electrophoretic pattern of whole-cell proteins of the substrates (parboiled rice and blackgram dal) and mixed-batter of idli at different stages of fermentation. As could be seen from the electrophoretic pattern, the rice protein resolved in as much as 10 major bands with molecular weights ranging between 14.3 to 97.4 kDa. Blackgram dal had one major bands corresponding to molecular weight 45 kDa and six minor but significant protein fractions corresponding to molecular weight ranging between 45 to 55 kDa. The protein fingerprint of the mixed batter revealed the combination of both rice and blackgram dal protein subunits.

Table 41. Changes in proximate composition[a] of mixed batter of idli during fermentation under optimized conditions

Parameter	Fermentation time (h)						
(g (100 g)$^{-1}$ dry matter)	0	3	6	9	12	15	18
pH	5.9a ± 0.06	5.8a ± 0.02	5.6a ± 0.03	5.1b ± 0.04	4.7c ± 0.02	4.4d ± 0.04	4.3d ± 0.04
Batter volume (ml)	60.0d ± 0	60.0d ± 0	80.0c ± 1.73	104.0b ± 2.08	120.7a ± 1.77	121.7a ± 0.88	120.7a ± 0.67
Moisture (g (100 g)$^{-1}$)	62.7a ± 0.27	62.7a ± 0.14	62.2a ± 1.35	61.8a ± 0.88	61.3a ± 1.09	61.1a ± 0.94	61.1a ± 1.46
Ash	1.5a ± 0.03	1.5a ± 0.03	1.5a ± 0.02	1.5a ± 0.07	1.5a ± 0.04	1.5a ± 0.02	1.5a ± 0.02
Titratable acid (as lactic acid)	0.6c ± 0.03	1.1b ± 0.02	1.2b ± 0.03	1.1b ± 0.03	1.5a± 0.06	1.7a ± 0.07	1.8a ± 0.03
Free fatty acid (as linoleic acid)	1.3a ± 0.02	1.4a ± 0.07	1.4a ± 0.01	1.4a ± 0.05	1.6a ± 0 .14	1.6a ± 0.04	1.6a ± 0.01
Total nitrogen	2.6a ± 0.03	2.8a ± 0.03	2.6a ± 0.03	2.5a ± 0.07	2.3a ± 0.07	2.5a ± 0.06	2.7a ± 0.12
Protein nitrogen	2.2a ± 0	2.2a ± 0.03	1.8b ± 0.07	1.5bc ± 0.03	1.1c ± 0.03	1.3bc ± 0.10	1.4bc ± 0.03
Non-protein nitrogen	0.4c ± 0	0.6bc ± 0.03	0.8b ± 0.03	1.0ab ± 0.03	1.2a ± 0.07	1.2a ± 0.06	1.3a ± 0.13
Soluble nitrogen	0.4a ± 0.0a	0.4a ± 0.02	0.5a ± 0.03	0.5a ± 0.03	0.6a ± 0.03	0.6a ± 0.03	0.7a ± 0.03
Total protein (total N x 6.25[b])	16.5a ± 0.21	17.3a ± 0.21	16.0a± 0.21	15.4ab ± 0.42	14.2b ± 0.42	15.6ab ± 0.36	16.7a ± 0.75
Crude fat (ether extract)	1.1a ± 0.02	1.2a ± 0.02	1.0a ± 0.04	1.1a ± 0.04	1.1a ± 0.03	1.1a ± 0.02	1.1a ± 0.02
Carbohydrate (by difference)	80.9a ± 0.27	80.0a ± 0.25a	81.5a ± 0.19	82.0a ± 0.56	83.2a ± 0.48	81.8a ± 0.38	80.7a ± 0.79
Energy (MJ (100 g)$^{-1}$ dry matter)	1.7a ± 0	1.7a ± 0.02	1.7a ± 0	1.7a ± 0.01	1.7a ± 0.05	1.7a ± 0	1.7a ± 0

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a row sharing a common letter are not significantly different ($P < 0.05$).

[b]Protein factor is 5.95 for rice.

4.5.6. Evaluation of antioxidant properties

4.5.6.1. Extraction yield in methanol

As shown in Table 42 the yield of the crude lyophilized extracts in methanol from the fermented batter was significantly higher than that from either nonfermented batter or steamed product. Fermentation resulted in 1.6-fold increase in the extract yield.

Table 42. Yield of methanolic extract, total phenol content and reducing power of idli at different stages of its preparation[a]

Sample[b]	mg g^{-1} dry weight		
	Yield	Total phenol content (gallic acid equivalent)	Reducing power (ascorbic acid equivalent)
Id-0	32.73b ± 4.77	0.79c ± 0.01	0.60b ± 0.03
Id-18	52.66a ± 2.17	2.33a ± 0.01	1.35a ± 0.05
Id-SP	31.18b ± 1.89	1.05b ± 0.01	0.59b ± 0.02

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a column sharing a common letter are not significantly different ($P < 0.05$).

[b]Id-0, nonfermented idli batter; Id-18, 18 h (fully)-fermented idli batter; Id-SP, steamed product (idli).

4.5.6.2. Total phenol content

Table 42 shows the total phenol content of the methanolic extracts. The mean total phenolic content of the mixed batter increased by 135% after fermentation. However, steam-cooking of the fermented batter for 10-15 min during idli preparation significantly reduced the total phenol content.

4.5.6.3. Reducing power

The mean reducing power of the crude lyophilized extracts of samples in methanol is shown in Table 42. Crude extract of fermented batter registered the highest amount of reducing power, which was about 125% higher than that of nonfermented batter. However, steam-cooking of fermented batter for 10-15 min resulted in about 2.3-fold decrease in the reducing activity which eventually leveled off with that of nonfermented batter.

4.5.6.4. Free radical-scavenging activity

Table 43 shows the dose-time-response of DPPH·-scavenging activity of extracts at different dose levels of nonfermented and fermented batter and steamed product, incubated for 50 min. The mean DPPH·-scavenging activity of all the tested concentrations (10-50 mg ml^{-1}) increased significantly during incubation. The IC_{50} of these samples, obtained by interpolation from linear regression analysis, revealed that 128 mg ml^{-1}, 79 mg ml^{-1} and 118 mg ml^{-1} of the respective extracts could decrease the initial concentration of DPPH· by 50% when the reaction mixture was incubated for 50 min (Table 43). While the relative scavenging activity of the idli batter was enhanced by 2.6-fold after fermentation, steam-cooking of fermented batter reduced the relative radical-scavenging activity to that of the nonfermented batter (Table 43).

4.5.6.5. Metal-chelating activity

The data generated through *in vitro* assay of chelating ability of extract of batters and products examined against Fe^{2+} are shown in Table 44. The Fe^{2+}-chelating activity of all the extracts increased up to a

Table 43. DPPH•-scavenging activity[a] of methanolic extract of idli during different stages of its preparation

Sample[b]	Time[c] (min)	%DPPH•-scavenging activity in extract (mg ml^{-1})					IC_{50} (mg ml^{-1} extract)[d]	RAE[e]
		10	20	30	40	50		
Id-0							128.3 ± 15.06	1.00
	0	2.2d ± 0.37	2.7c ± 0.44	3.6b ± 0.71	4.1a ± 0.49	4.3a ± 0.73		
	10	5.4e ± 0.65	7.8d ± 0.49	10.1c ± 1.12	13.8b ± 1.01	16.8a ± 1.62		
	20	6.5e ± 0.60	8.9d ± 0.41	12.8c ± 1.35	16.6b ± 1.60	18.0a ± 1.66		
	30	7.2e ± 0.63	10.0d ± 0.85	13.2c ± 1.49	17.6b ± 1.75	19.7a ± 1.80		
	40	7.2e ± 0.62	10.1d ± 0.84	13.2c ± 1.48	17.7b ± 1.73	19.9a ± 1.83		
	50	7.2e ± 0.62	10.1d ± 0.83	13.2c ± 1.47	17.7b ± 1.72	19.9a ± 1.82		
Id-18							79.0 ± 3.00	2.60
	0	3.9e ± 0.21	4.5d ± 0.24	6.5c ± 0.95	7.6b ± 0.20	9.0a ± 0.36		
	10	7.6e ± 0.53	13.2d ± 1.23	18.1c ± 1.06	21.4b ± 0.64	26.9a ± 0.45		
	20	8.6e ± 1.07	14.5d ± 1.54	19.8c ± 0.98	23.9b ± 0.40	29.4a ± 0.28		
	30	9.4e ± 1.28	15.4d ± 1.48	21.0c ± 0.91	25.4b ± 0.99	31.2a ± 1.54		
	40	9.4e ± 1.28	15.5d ± 1.51	21.1c ± 0.88	25.6b ± 0.90	31.8a ± 1.59		
	50	9.4e ± 1.28	15.5d ± 1.52	21.1c ± 0.88	25.7b ± 1.00	31.8a ± 1.58		
Id-SP							117.7 ± 3.28	1.03
	0	0.9e ± 0.06	1.7d ± 0.22	2.4c ± 0.23	3.8b ± 0.22	4.1a ± 0.19		
	10	3.6e ± 0.21	5.5d ± 0.13	7.5c ± 0.29	10.5b ± 0.38	14.1a ± 0.39		
	20	4.3e ± 0.34	8.1d ± 0.30	11.7c ± 0.66	15.5b ± 0.56	19.1a ± 0.24		
	30	4.8e ± 0.16	8.8d ± 0.33	14.1c ± 0.71	16.4b ± 0.51	20.2a ± 0.22		
	40	4.8e ± 0.49	9.3d ± 0.38	14.4c ± 0.81	16.7b ± 0.57	20.8a ± 0.37		
	50	4.8e ± 0.75	9.3d ± 0.62	14.5c ± 0.80	16.8b ± 0.72	20.9a ± 0.51		

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a row sharing a common letter are not significantly different ($P < 0.05$).

[b]Id-0, nonfermented idli batter; Id-18, 18 h (fully)-fermented idli batter; Id-SP, steamed product (idli).

[c]Incubation time of reaction mixture.

[d]IC_{50} (the efficient concentration of antioxidant decreasing initial concentration of DPPH• by 50%) was obtained by interpolation from linear regression analysis.

[e]Relative antioxidant effect was obtained by dividing extraction rate with IC_{50} value and compared with that of nonfermented batter which was assigned as 1.0.

certain extent with the increase in dosage level (20 mg ml^{-1} for steamed product and 30 mg ml^{-1} for batters), and then leveled off despite further increase in concentration. The respective mean IC_{50} for Fe^{2+}-chelating activity of extracts obtained by interpolation from linear regression analysis revealed that fermented batter of idli has efficient Fe^{2+}-chelating ability superior to the nonfermented batter and steamed product. A higher relative Fe^{2+}-chelating ability was observed in fermented batter than the steamed product, when their initial yields in methanol were taken in account. However, steam-cooking of the fermented batter reduced the relative Fe^{2+}-chelating ability of the extract by 84%.

Table 44. Fe^{2+}-chelating activity[a] of methanolic extracts of idli during different stages of its preparation

Sample[b]	%Fe^{2+}-chelating activity in extract (mg ml^{-1})					IC_{50} (mg ml^{-1} extract)[c]	RAE[d]
	10	20	30	40	50		
Id-0	19.56c ± 1.68	30.48c ± 2.31	37.88b ± 2.54	38.29c ± 1.48	38.41c ± 1.52	55.67a ± 0.88	1.00c
Id-18	23.91b ± 1.31	39.01b ± 1.65	47.61a ± 1.38	48.71a ± 2.11	49.30a ± 2.07	40.33c ± 0.33	2.22a
Id-SP	26.61a ± 1.91	44.27a ± 2.13	45.78a ± 1.63	46.15b ± 1.68	46.17b ± 2.21	43.33b ± 1.01	1.21b

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a column sharing a common letter are not significantly different ($P < 0.05$).

[b]Id-0, nonfermented idli batter; Id-18, 18 h (fully)-fermented idli batter; Id-SP, steamed product (idli).

[c]IC_{50} (the efficient concentration of antioxidant decreasing initial concentration by 50%) was obtained by interpolation from linear regression analysis.

[d]Relative antioxidant effect was obtained by dividing extraction rate with IC_{50} value and compared with that of nonfermented batter which was assigned as 1.0.

4.5.6.6. Lipid peroxidation inhibitory activity

The results of the *in vitro* assay of lipid peroxidation inhibitory activity (LPIA) of the extracts of nonfermented and fermented batters and steamed product of idli at different concentrations (10-50 mg ml^{-1} methanol) on the peroxidation of linoleic acid emulsion system is shown in Table 45. A significant

increase in LPIA with the increase in concentration of the extract was evident at all the tested concentrations of the extracts. At 50 mg ml^{-1}, the extract of unfermented batter exhibited 21% of LPIA when the reaction mixture was kept for 24 h, while the extracts of fermented batter and steamed product showed better but relatively similar percentage of LPIA at same concentrations and time (Table 45). However, the total antioxidant activity of the extracts declined significantly with time. At all the tested concentrations the extracts of fermented batter and steamed product showed better LPIA than the unfermented batter, when the reaction mixtures were incubated for 24, 48 and 72 h at 37°C, revealing their ability to deter lipid peroxidation.

Table 45. Lipid peroxidation inhibitory activity[a] of methanolic extracts of idli at different stages of its preparation

Sample[b]	Time[c] (h)	% inhibition by lyophilized extract (mg ml^{-1} methanol)				
		10	20	30	40	50
Id-0	24	3.72e ± 0.56	8.50d ± 1.00	15.36c ± 0.76	18.59b ± 1.11	20.83a ± 0.90
	48	3.67e± 0.55	6.93d ± 0.99	12.30c ± 0.81	16.40b ± 1.19	18.66a ± 0.92
	72	3.60e ± 0.42	5.67d ± 0.69	8.60c ± 1.09	13.92b ± 0.90	15.27a ± 0.98
Id-18	24	8.14e ± 1.00	18.97d ± 1.32	27.65c ± 1.41	32.58b ± 1.01	36.64a ± 0.98
	48	6.89e ± 0.59	13.33d ± 0.79	18.82c ± 1.02	25.44b ± 0.89	27.90a ± 1.26
	72	5.58e ± 0.94	11.43d ± 1.16	16.09c ± 0.81	23.51b ± 1.15	26.37a ± 0.98
Id-SP	24	5.75d ± 0.93	17.19c ± 0.92	26.53b ± 1.06	34.44a ± 1.30	35.73a ± 1.03
	48	5.31e ± 0.85	12.77d ± 1.07	19.57c ± 0.57	23.95b ± 1.09	26.20a ± 0.82
	72	4.84e ± 0.57	12.40d ± 0.97	18.18c ± 1.05	21.67b ± 1.29	24.87a ± 0.99

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a row sharing a common letter are not significantly different (P <0.05).

[b]Id-0, nonfermented idli batter; Id-18, 18 h (fully)-fermented idli batter; and Id-SP, steamed product (idli).

[c]Incubation time of reaction mixture.

4.5.6.7. Correlation and regression

Correlation coefficients between every two antioxidative parameters tested for nonfermented and fermented batters and steamed products are shown in Table 46. The data exhibited that all the five antioxidant parameters exhibited a significant positive correlation among the every two parameters. In batters, the highest correlation was observed between metal-chelating activity and lipid peroxidation inhibitory activity (r = 0.923), and metal chelating activity and reducing power (r = 0.986), respectively, while in idli it was radical-scavenging activity with lipid peroxidation inhibitory activity (r = 0.968), being closely followed by metal-chelating activity and total phenol content (r = 0.951).

Table 46. Coefficient of correlations[a] among various antioxidative parameters of nonfermented idli batter (Id-0), 18 h (fully)-fermented idli batter (Id-18) and steamed product or idli (Id-SP) (13 d. f.)

Parameters[b]	Sample	TPC	RP	RSA	MCA	TAA
TPC	Id-0	1.000				
	Id-18	1.000				
	Id-SP	1.000				
RP	Id-0	0.705	1.000			
	Id-18	0.897	1.000			
	Id-SP	0.768	1.000			
RSA	Id-0	0.863	0.758	1.000		
	Id-18	0.959	0.878	1.000		
	Id-SP	0.951	0.860	1.000		
MCA	Id-0	0.707	0.906	0.828	1.000	
	Id-18	0.908	0.986	0.879	1.000	
	Id-SP	0.641	0.816	0.789	1.000	
LPIA	Id-0	0.790	0.916	0.835	0.923	1.000
	Id-18	0.953	0.884	0.935	0.883	1.000
	Id-SP	0.916	0.820	0.968	0.838	1.000

[a]Significant at P <0.01 (two-tailed).

[b]TPC, total phenol content; RP, reducing power; RSA, radical-scavenging activity; MCA, metal-chelating activity; LPIA, lipid peroxidation inhibitory activity (after 72 h).

The regression equations and coefficients of correlations between different antioxidant

parameters and total phenol content of the extracts are presented in Table 47. All the tested samples showed that the total phenol content was positively correlated with DPPH·-scavenging activity, reducing power, metal-chelating ability and lipid peroxidation inhibitory activity. Relevant regression

Table 47. Regression equations[a] for antioxidant parameters[b] as related to total phenol content (TPC) of nonfermented idli batter (Id-0), 18 h (fully)-fermented idli batter (Id-18) and steamed product or idli (Id-SP)

Product	Equation		R^2	TPC accounted for (%)
Id-0	1. RSA	$y = -12.346TPC^2 + 31.220TPC + 0.382$	0.979	61.62
	2. RP	$y = -0.284TPC^2 + 0.625TPC - 0.056$	0.866	49.70
	3. MCA	$y = -30.861TPC^2 + 63.839TPC - 0.074$	0.962	49.98
	4. LPIA	$y = -18.681TPC^2 + 41.197TPC - 2.917$	0.989	62.41
Id-18	5. RSA	$y = 2.550TPC^2 + 5.150TPC + 8.301$	0.966	91.97
	6. RP	$y = -0.114TPC^2 + 0.405TPC + 0.121$	0.957	80.46
	7. MCA	$y = -12.311TPC^2 + 42.888TPC + 2.308$	0.965	82.45
	8. LPIA	$y = 2.521TPC^2 + 5.221TPC + 4.391$	0.964	90.82
Id-SP	9. RSA	$y = -5899TPC^2 + 21.658TPC - 0.802$	0.951	90.44
	10. RP	$y = -0.246TPC^2 + 0.643TPC + 0.020$	0.969	89.98
	11. MCA	$y = -24.406TPC^2 + 58.906TPC + 4.879$	0.712	41.09
	12. LPIA	$y = -11.188TPC^2 + 35.097TPC - 3.748$	0.950	83.91

[a]Significant at $P < 0.01$ (two-tailed).

[b]RSA, radical-scavenging activity; RP, reducing power; MCA, metal-chelating activity; LPIA, lipid peroxidation inhibitory activity (after 72 h).

equations exhibited that the total phenol content accounted for 62%, 92% and 90 % DPPH·-scavenging activities in nonfermented and fermented batters and idli, respectively (Table 47: eq. 1, 5 and 9). Similarly, the respective correlations between total phenol and reducing power were 50%, 81% and 90% (Table 47: eq. 2, 6 and 10). Total phenol content could also explain 50%, 80% and 41% metal-chelating ability (Table 47: eq. 3, 7 and 11), and 62%, 91% and 84% lipid peroxidation inhibitory activity (Table 47: eq. 4, 8 and 12). On the whole, total phenol content of the extracts exhibited a high positive correlation with all the antioxidant parameters tested.

4.6. Kinema

4.6.1. Evaluation of antioxidant properties

4.6.1.1. Extraction yield in methanol

The yields of crude lyophilized extracts of cooked nonfermented (CNF) soybean and kinema in methanol are shown in Table 48. Kinema registered 1.7-fold higher yield of crude extract than that of CNF soybean.

Table 48. Yield of methanolic extract, total phenol content and reducing power of cooked nonfermented (CNF) soybean and kinema

Sample	mg g^{-1} dry weight[a]		
	Yield	Total phenol content (gallic acid equivalent)	Reducing power (ascorbic acid equivalent)
CNF soybean	90.50b ± 8.32	3.26b ± 0.14	3.80b ± 0.27
Kinema	153.83a ± 5.45	7.95a ± 0.61	9.74a ± 0.14

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a column sharing a common letter are not significantly different ($P < 0.05$).

4.6.1.2. Total phenol content

As shown in Table 48, the mean total phenol content of kinema was about 144% higher than that of CNF soybean.

4.6.1.3. Reducing power

The reducing powers of the crude lyophilized extracts of CNF soybean and kinema are shown in Table 48. The extract of kinema registered about 2.6-fold reducing power than the CNF soybean. A significant increase in the reducing activity of kinema extracts revealed the influence of fermentation on the enhancement of reducing activity of soybean.

4.6.1.4. Free radical-scavenging activity

Table 49 shows the dose-time-response for the antiradical activities of different dosages (10-50 mg ml^{-1}) of the crude extracts of CNF soybean and kinema, incubated for 40 min. The mean DPPH$^{\cdot}$-scavenging activity of the extracts increased significantly ($P < 0.05$) with the increase in concentration up to 40 min of reaction, and leveled off with further increase in time. At all the tested dosages, the extracts of kinema was better DPPH$^{\cdot}$-scavenger than that of CNF soybean. The IC_{50} of CNF soybean and kinema obtained by interpolation from linear regression analysis revealed that 50 mg ml^{-1} and 41 mg ml^{-1} of the respective extracts could scavenge the initial DPPH$^{\cdot}$ concentration by 50% when the reaction mixture was incubated for 40 min. The relative DPPH$^{\cdot}$-scavenging activity of CNF soybean increased by 2.1-fold after fermentation.

Table 49. DPPH$^{\cdot}$-scavenging activity[a] of methanolic extract of cooked nonfermented (CNF) soybean and kinema

Sample	Time[b] (min)	%DPPH$^{\cdot}$-scavenging activity in extract (mg ml^{-1})					IC_{50} (mg ml^{-1} extract)[c]	RAE[d]
		10	20	30	40	50		
CNF soybean							50.0 ± 1.19	1.00
	0	2.1e ± 0.52	5.5d ± 0.53	7.4c ± 0.11	10.0b ± 1.01	12.0a ± 1.38		
	10	5.6e ± 0.30	15.7d ± 1.58	21.5c ± 1.91	29.8b ± 1.38	35.7a ± 1.54		
	20	8.6e ± 0.24	19.2d ± 1.16	26.8c ± 0.76	34.5b ±1.44	38.9a ± 1.50		
	30	9.7e ± 0.05	22.0d ± 1.03	31.5c ± 1.02	38.9b ± 1.83	44.5a ± 1.56		
	40	10.2e ± 0.65	22.6d ± 1.08	33.0c ± 0.47	41.3b ± 1.22	47.4a ± 1.14		
	50	10.4e ± 0.66	22.8d ± 1.62	33.4c ± 3.05	41.5b ± 1.98	47.5a ± 1.66		
	60	10.5e ± 0.62	23.0d ± 1.53	33.7c ± 3.07	41.8b ± 1.96	47.5a ± 1.70		
Kinema							40.8 ± 0.74	2.10
	0	3.1e ± 0.53	7.3d ± 0.85	9.5c ± 0.35	12.5b ± 0.41	16.6a ± 0.49		
	10	8.4e ± 0.83	21.0d ± 0.74	28.4c ± 0.89	34.5b ± 1.31	40.0a ± 0.28		
	20	11.3e ± 0.43	25.9d ± 1.45	33.5c ± 0.49	42.5b ± 0.25	50.7a ± 0.96		
	30	12.7e ± 0.31	29.3d ± 1.11	37.4c ± 1.83	46.4b ± 1.36	54.6a ± 1.32		
	40	13.4e ± 0.41	31.1d ± 1.73	38.9c ± 1.31	47.9b ± 1.67	59.2a ± 1.28		
	50	13.5e ± 0.44	31.4d ± 1.79	39.4c ± 0.94	48.3b ± 1.82	60.1a ± 1.10		
	60	13.5e ± 0.43	31.5d ± 1.77	39.4c ± 0.94	48.4b ± 1.81	60.1a ± 1.10		

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a row sharing a common letter are not significantly different ($P < 0.05$).

[b]Incubation time of reaction mixture.

[c]IC_{50} (the efficient concentration of antioxidant decreasing initial concentration of DPPH$^{\cdot}$ by 50%) was obtained by interpolation from linear regression analysis.

[d]Relative antioxidant effect was obtained by dividing extraction rate with IC_{50} value and compared with that of CNF soybean which was assigned as 1.0.

4.6.1.5. Metal-chelating activity

The *in vitro* assay of chelating ability of the extracts of CNF soybean and kinema examined against Fe^{2+} are shown in Table 50. The chelating ability of the extracts increased with the increase in concentration. At all the tested concentrations (10-50 mg ml^{-1}), the extract of kinema had a superior metal-chelating ability, which was demonstrated by the decrease in purple colour formed due to ferrozine-Fe^{2+} complex formation, to that of the CNF soybean. Also illustrated in the Table 50 is the mean IC_{50} of the extracts obtained by interpolation from linear regression analysis. The ability of kinema extract to chelate the initial Fe^{2+} concentration by 50% was 2-fold than that of CNF soybean. Kinema exhibited higher relative Fe^{2+}-chelating ability than the CNF soybean, when their extract yields were taken in account.

Table 50. Fe^{2+}-chelating activity[a] of methanolic extract of cooked non-fermented (CNF) soybean and kinema

Sample	%Fe^{2+}-chelating activity in extract (mg ml^{-1})					IC_{50} (mg ml^{-1} extract)[b]	RAE[c]
	10	20	30	40	50		
CNF soybean	22.33b ± 0.47	38.62b ± 1.09	55.77b ± 0.98	64.22b ± 0.51	68.45b ± 0.31	31.02b ± 0.41	1.00
Kinema	63.57a ± 1.96	70.86a ± 0.93	74.34a ± 0.79	78.00a ± 0.69	82.35a ± 0.24	16.05a ± 0.53	3.27

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a column sharing a common letter are not significantly different ($P < 0.05$).

[b]IC_{50} (the efficient concentration of antioxidant decreasing initial concentration by 50%) was obtained by interpolation from linear regression analysis.

[c]Relative antioxidant effect was obtained by dividing extraction rate with IC_{50} value and compared with that of CNF soybean which was assigned as 1.0.

4.6.1.6. Lipid peroxidation inhibitory activity

Table 51 showed the *in vitro* assay of lipid peroxidation inhibitory activity (LPIA) of the extracts CNF soybean and kinema on the peroxidation of linoleic acid emulsion system. At all the tested concentrations (10-50 mg ml^{-1}), kinema extracts exhibited better LPIA than that of CNF soybean, when the reaction mixtures were incubated for 24 at 37°C. At 50 mg ml^{-1}, the extracts of kinema and CNF soybean exhibited 44% and 36% of LPIA, respectively. Though LPIA of extracts increased significantly with the increase in the dosage levels, the values declined significantly with the time of incubation. After 48 h, the extracts of both CNF soybean and kinema, at a concentration of 50 mg ml^{-1}, exhibited only 29% of LPIA.

Table 51. Lipid peroxidation inhibitory activity[a] of methanolic extracts of cooked nonfermented (CNF) soybean and kinema

Sample	Time[c] (h)	%inhibition by lyophilized extract (mg ml^{-1} methanol)				
		10	20	30	40	50
CNF soybean	24	6.33e ± 0.36	14.36d ± 1.19	18.53c ± 1.18	30.68b ± 1.7	35.59a ± 0.53
	48	4.09e ± 0.38	10.79d ± 1.01	15.25c ± 1.03	25.29b ± 1.17	29.37a ± 0.48
	72	3.47e ± 0.43	8.41d ± 1.14	13.46c ± 0.70	21.88b ± 1.36	26.02a ± 0.96
Kinema	24	9.34e ± 1.12	20.54d ± 2.37	32.19c ± 2.99	39.13b ± 1.50	44.26a ± 1.79
	48	6.70e ± 0.71	12.30d ± 1.84	20.48c ± 2.15	25.67b ± 1.48	29.37a ± 1.93
	72	4.02e ± 0.48	7.76d ± 1.49	13.90c ± 1.52	20.41b ± 2.20	26.16a ± 0.70

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a row sharing a common letter are not significantly different ($P < 0.05$).

[c]Incubation time of reaction mixture.

4.6.1.7. Correlation and regression

Table 52 shows correlation coefficients between every two antioxidative parameters tested for CNF soybean and kinema. All the five antioxidant parameters exhibited significant positive correlation

among the every two parameters. In CNF soybean, the highest correlation was observed between radical-scavenging activity and Fe^{2+}-chelating activity (r = 0.988), while in kinema it was radical-scavenging activity with lipid peroxidation inhibitory activity (r = 0.959), being closely followed by Fe^{2+}-chelating activity (r = 0.953).

Table 52. Coefficient of correlations[a] among various antioxidative parameters of cooked non-fermented (CNF) soybean and kinema (13 d.f.)

Parameter[b]	Sample	LPIA	RSA	MCA	RP	TPC
LPIA	CNF soybean	1.000				
	Kinema	1.000				
RSA	CNF soybean	0.958	1.000			
	Kinema	0.979	1.000			
MCA	CNF soybean	0.947	0.988	1.000		
	Kinema	0.926	0.960	1.000		
RP	CNF soybean	0.968	0.970	0.961	1.000	
	Kinema	0.915	0.953	0.949	1.000	
TPC	CNF soybean	0.815	0.822	0.763	0.823	1.000
	Kinema	0.910	0.959	0.953	0.940	1.000

[a]Significant at P <0.01 (two tailed).

[b]LPIA, lipid peroxidation inhibitory activity; RSA, radical-scavenging activity; MCA, metal-chelating activity; RP, reducing power; TPC total phenol content.

The regression equations and coefficients of correlations between different antioxidant parameters and total phenol content of the extracts are presented in Table 53. All the tested samples showed that the total phenol content was positively correlated with DPPH·-scavenging, reducing, Fe^{2+}-chelating and lipid peroxidation inhibitory activities. Relevant regression equations exhibited that the total phenol content accounted for 68% and 92% DPPH·-scavenging activities in CNF soybean and kinema, respectively (Table 53: eq. 1 and 5). Similarly, 68% and 88% correlations between total phenol content and reducing power were found in CNF soybean and kinema, respectively (Table 53: eq. 2 and 6). Total phenol content could also explain 58% and 91% Fe^{2+}-chelating ability (Table 53: eq. 3 and 7), and 66% and 83% lipid peroxodation inhibitory activity of the CNF soybean and kinema extracts, respectively (Table 53: eq. 4 and 8). On the whole, total phenol content of the extracts exhibited a high positive correlation with all the antioxidant parameters used.

Table 53. Regression equations[a] for antioxidant parameters[b] as related to total phenol content of cooked nonfermented (CNF) soybean and kinema

Equation	R^2	R^2 (adjusted)	TPC accounted for (%)
CNF soybean			
1. RSA = -27.862TPC^2 + 85.320TPC - 14.356	0.958	0.951	68
2. RP = -0.069TPC^2 + 0.219TPC + 0.006	0.915	0.901	68
3. MCA = -42.975TPC^2 + 126.153TPC - 13.184	0.941	0.931	58
4. LPIA = -24.862TPC^2 + 76.094TPC - 17.802	0.963	0.957	66
Kinema			
5. RSA = -52.397TPC^2 + 154.680TPC - 51.310	0.946	0.937	92
6. RP = -0.177TPC^2 + 0.431TPC - 0.086	0.951	0.943	88
7. MCA = -30.079TPC^2 + 79.980TPC^2 + 30.621	0.951	0.942	91
8. LPIA = -66.306TPC^2 + 166.420TPC - 58.340	0.882	0.862	83

[a]Significant at P< 0.01 (two tailed).

[b]RSA, radical-scavenging activity; TPC, total phenol content; RP, reducing power; MCA, metal-chelating activity; LPIA, lipid peroxidation inhibitory activity.

4.7. Papad

4.7.1. Proximate composition

The proximate compositions of substrates (blackgram and mung dal flours) and papad from different sources are presented in Table 54. The moisture content of papad was about 3 times higher than the moisture contents of the substrates. The ash content of papad was >3 times higher than that of its

substrates. The pH, titratable and free fatty acidity, soluble nitrogen and crude fat contents of the papads were higher than those of the substrates. The carbohydrate contents of the substrates were higher than those of the products.

Table 54. Proximate composition[a] of papad and its substrates

Parameter	g (100 g)$^{-1}$ dry matter			
	Substrate (legume flour)		Papad	
	Blackgram dal (n = 3)	Mung dal (n = 3)	Market (n = 31)	Laboratory-made (n = 3)
pH	6.98bc ± 0.17	6.34c ± 0.14	8.72a ± 0.42	7.08b ± 0.01
%Diametric expansion[b]	ND	ND	25.90a ± 2.13	27.60a ± 0.36
Moisture (g (100g)$^{-1}$ dm)	5.39c ± 0.38	5.93c ± 0.11	18.00a ± 0.87	14.44b ± 0.37
Ash	3.39b ± 0.08	3.23b ± 0.06	11.20a ± 0.33	9.87a ± 0.21
Titratable acid (as lactic acid)	0.11b ± 0.02	0.13b ± 0.01	0.67a ± 0.08	0.58a ± 0.03
Free fatty acid (as linoleic acid)	0.21b ± 0.03	0.21b ± 0.03	1.63a ± 0.06	1.33a ± 0.04
Total nitrogen	4.04a ± 0.04	3.07b ± 0.06	2.06c ± 0.05	3.88ab ± 0.03
Protein nitrogen	3.37a ± 0.06	2.21b ± 0.07	1.39c ± 0.05	2.41b ± 0.02
Nonprotein nitrogen	0.68b ± 0.04	0.86b ± 0.03	0.67b ± 0.01	1.47a ± 0.02
Soluble nitrogen	0.85c ± 0.28	0.71c ± 0.04	2.13a ± 0.21	1.32b ± 0.04
Total protein (total N x 6.25)	25.29a ± 0.23	19.17b ± 0.39	13.94c ± 0.74	24.27a ± 0.20
Crude fat	1.44c ± 0.04	1.52c ± 0.03	3.92b ± 0.12	4.73a ± 0.08
Carbohydrate (by difference)	69.87b ± 0.25	76.08a ± 0.31	53.94d ± 0.34	61.13c ± 0.10
Energy value (MJ (100 g)$^{-1}$ dm)	1.65a ± 0	1.65a ± 0	1.28a ± 0	1.61a ± 0

[a]Values are expressed as mean ± SEM. Means within a row sharing a common letter are not significantly different (P <0.05).
[b]After deep-fat frying

4.7.2. Microbial analysis

4.7.2.1. Isolation of microorganisms

A total of 80 strains of lactic acid bacteria (Table 55) and 75 strains of yeasts (Table 56) were isolated from 3 samples each of raw blackgram and mung dal flours, and 18 samples of laboratory-made

Table 55. Selection of representative strains of dominant lactic acid bacteria isolated from substrates and fermenting dough of papad

Source	Stage(s) of fermentation	Cell shape[a]	Gas from glucose	Growth in/at		Total No. of strains[b]	No. of representative strains
				pH 4.8	45°C		
Substrate (dal flour)							
Blackgram (n = 3)		Ct	-	+	+	15	3
Mung (n = 3)		Ct	-	+	+	10	2
Papad/dough							
Marketed (n = 31)	Final					0	
Laboratory-made (n = 18)	0 h - 8 h (2 h-interval)	Ct	-	+	+	55	11

[a]Ct, cocci in tetrad.
[b]All the isolates were nonmotile, nonsporeforming, Gram positive and catalase negative.

Table 56. Selection of representative strains of dominant yeasts isolated from substrates and fermenting dough of papad

Source	Stage(s) of fermentation	Colony[a]	Cell shape[b]	Pellicle formation	Total No. of strains	No. of representative strains
Substrate (dal flour)						
Blackgram (n = 3)		Cgs	G-O/E	-	10	2
Mung (n = 3)		Cgs	G-O/E	-	10	2
Papad/dough						
Marketed (n = 31)	Final				0	
Laboratory-made (n = 18)	0 h - 8 h (2 h-interval)	Cgs	G-O/E	-	55	11

[a]Cgs, cream colour with glistening and butyrous surface.
[b]G, globose; O, oval; E, ellipsoidal.

fermenting papad dough and drying papads. While all the isolates of lactic acid bacteria were grouped into a single cell morphotye, yeast isolates were grouped into a single colony morphotype. One representative strain, from each group of each positive sample, was selected randomly to ascertain their taxonomic status.

4.7.2.2. Taxonomical studies

4.7.2.2.1. Bacteria

On the basis of selected morphological, physiological and cultural characteristics of lactic acid bacteria shown in Table 55, all the 15 representative strains belonged to the genus *Pediococcus*. The detailed morphological and physiological characteristics of all the representative strains of lactic acid bacteria are presented in Table 57. Following the criteria laid by Garvie (1986a, 1986b) as wellas IBIS (Wijtzes *et al.*, 1997), all the 15 representative strains of lactic acid bacteria were tentatively identified as *Pediococcus pentosaceus* Mees 1934.

Table 57. Characteristics[a] of representative strains of lactic acid bacteria isolated from papad, and its substrates and fermenting dough

Parameter	Representative strains (n = 15)		
	+	d	-
Growth in 30 g NaCl l^{-1}	60	27	13
Acid from			
D-lactose	60	27	13
D-melibiose	54	33	13
L-rhamnose	47	33	20
D-ribose	60	33	7
sucrose		20	80
D-xylose	67	27	6

[a]Data represent %strains showing + (positive), d (delayed) and - (negative) reactions. All the strains were Gram positive, nonmotile, nonsporeforming, catalase negative cocci (0.35-0.6 µm in diameter), capable of growing at 80 g NaCl l^{-1}, pH 4.2 and 7.5, 15-45°C, hydrolyzing arginine and esculin, and producing acid from L-arabinose, D-cellobiose, esculin, D-fructose, D-galactose, D-glucose, D-maltose, D-mannose, salicin and D-trehalose, however not capable of growing at 50°C, reducing nitrate, producing indole, hydrolyzing fat, gelatin and starch, and producing acid from glycerol, D-mannitol, D-raffinose, sorbitol and starch.

Table 58. Characteristics[a] of representative strains of yeasts isolated from papad and its substrates and fermenting dough

Parameter	Representative strains (n = 15)		
	+	$+_w$	-
Hydrolysis of fat	67	33	
Fermentation of			
D-galactose	47	33	20
D-maltose	33	54	13
D-melibiose	60	40	
melezitose	73	14	13
D-raffinose	73	20	7
sucrose	67	20	13
D-trehalose	27	53	20
Assimilation of			
D-galactose	60	33	7
D-glucitol	14	53	33
glycerol	60	20	20
D-maltose	53	14	33
D-mannitol	47	33	20
melezitose	80	13	17
D-melibiose	73	20	7
D-raffinose	87		13
sucrose	80	13	7
D-trehalose	60	27	13

[a]Data represent %strains showing + (positive), $+_w$ (weak positive) and - (negative) reactions. All the strains were globose to oval or elongated (3.4-5.8 x 5.5-8.7 µm), produced cream colour with glistening and butyrous surface, pseudomycelium, reproduced by multilateral budding and sexual reproduction, produced round to oval and smooth persistent 1-4 ascospores per ascus, were capable of growing at 37°C, fermenting glucose, and assimilating D-glucose, however not capable of producing pellicle, reducing nitrate, forming starch, hydrolyzing gelatin, starch and urea, fermenting D-cellobiose, D-lactose and D-xylose, and assimilating L-arabinose, D-cellobiose, citrate, m-erythritol , D-glucosamine, N-acetyly glucosamine, *m*-inositol, D-lactose, nitrate, D-ribose, L-rhamnose, starch, vitamin-free medium and D-xylose.

4.7.2.2.2. Yeasts

The detailed morphological, cultural and physiological characteristics of the 15 representative strains of yeasts, comprising a single colony morphotype, isolated from substrates and laboratory-made fermenting dough and drying papads are shown in Table 58. Following the taxonomic keys of Barnett *et al.* (2000) and Kurtzman and Fell (2000), and using BioloMICSNet software (http://www.cbs.knaw.nl/yeast/BioloMICS.aspx), all the representative strains of yeasts were tentatively identified as *Saccharomyces cerevisiae* Mayen *ex* Hansen 1883.

4.7.2.3. Microbial load in substrates and product

The average microbial load studied in 31 market samples of papad, 3 samples each of two kinds of substrates (blackgram dal and mung dal flours) and laboratory-made papad are shown in Table 59.

Table 59. Dominant microbial load in papad and its substrate from different sources

Microbiota	Log cfu g^{-1} fresh weight (mean ± SEM)			
	Substrate (dal flour)		Papad	
	Blackgram (n = 3)	Mung (n = 3)	Marketed (n = 31)	Laboratory-made (n = 3)
TAMB	3.85b ± 0.14	3.95b ± 0.42	4.98a ± 0.17	4.67a ± 0.25
aMBS	3.17c ± 0.05	3.39bc ± 0.26	4.36a ± 0.17	4.01a ± 0.16
Saccharomyces cerevisiae[a]	3.59b ± 0.29	3.97a ± 0.19	<DL	<DL
Pediococcus pentosaceus[b]	4.94a ± 0.09	3.46b ± 0.24	<DL	<DL

[a]DL (detection limit), 2.0 log cfu g^{-1} fresh weight. Means within a row sharing a common letter are not significantly different (P <0.05).
[b]DL, 1.0 log cfu g^{-1} fresh weight.

TAMB cells were recovered from all the samples studied. The average loads of TAMB cells and their spore (aMBS) count in the papads were higher than those of substrates. While *Pediococcus pentosaceus* was found below the detection limit (2.0 log cfu g^{-1} fresh weight) in the papads, they occurred in 100% and 67% of blackgram and mung dal flours, respectively (Table 60). Similarly, *Saccharomyces cerevisiae*, the only yeast present in the substrates, was not encountered in the finished product.

Table 60. Prevelance of dominant microorganisms in papad and its substrates from different sources

Microbiota	Positive sample (%)			
	Substrate (dal flour)		Papad	
	Blackgram (n = 3)	Mung (n = 3)	Marketed (n = 31)	Laboratory-made (n = 3)
TAMB	100	100	100	100
aMBS	67	100	94	100
Saccharomyces cerevisiae	67	67	0	0
Pediococcus pentosaceus	100	67	0	0

4.7.3. Optimization of traditional process parameters

Table 61 shows the average sensory scores for optimizing proportions of substrates (blackgram dal flour and mung dal flour) during the preparation of papad. All the treatments differed significantly

Table 61. Average sensory scores[a] for optimizing proportions of ingredients during papad preparation[b]

Treatment	Flour blend (BF:MF)	Attribute					
		Dough		Papad			
		Handfeel	Rolling	Appearance	Texture	Taste	Total score
A	1:0	19.7bc ± 0.44	20.3c ± 0.33	9.8d ± 0.17	13.5b ± 0.29	10.0c ± 0.58	73.3c ± 1.17
B	0:1	16.1c ± 0.55	14.8e ± 0.17	13.0b ± 0.50	11.0c ± 0.29	6.8d ± 0.22	61.8d ± 0.90
C	1:1	19.9b ± 0.82	20.3c ± 0.52	11.9c ± 0.51	13.1b ± 0.36	10.8bc ± 0.25	75.9c ± 1.23
D	2:1	20.3b ± 0.73	19.6cd ± 0.30	12.1bc ± 0.58	13.1b ± 0.36	11.7b ± 0.51	76.8c ± 0.66
E	3:1	20.2b ± 0.60	18.5d ± 0.29	11.1cd ± 0.22	12.8b ± 0.25	11.5b ± .029	74.0c ± 1.13
F	1:2	23.3a ± 0.14	23.5a ± 0.29	14.0a ± 0.29	18.2a ± 0.33	13.8a ± 0.14	92.7a ± 0.46
G	1:3	21.0b ± 0.29	22.0b ± 0.29	13.2b ± 0.44	13.3b ± 0.17	13.5a ± 0.29	83.7b ± 0.50

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a column sharing a common letter are not significantly different (P <0.05).
[b]Made by using 15 g papad khar, 70 g common salt and 500 ml water kg^{-1} flour blend (BF, blackgram dal flour; MF, mung dal flour) which was then cabinet-dried at 70 ± 5% relative humidity and 30 ± 1°C for 8 h, to final moisture content of 14-16%.

among themselves with respect to handfeel and rolling of dough, appearance, texture, taste of the product and total score. The treatment F, 1:2, had the highest score compared to the treatment A, blackgram dal flour alone; treatment B, mung dal flour alone; treatment C, 1:1; treatment D, 2:1; treatment E, 3 :1; and treatment G, 1:3. Hence, proportion of ingredients for the preparation of papad was optimized by fermenting mixture of one part of blackgram dal flour and two parts of mung dal flour.

Table 62 reveals that all the treatments considered for optimizing amount of papad khar added to the flour mixture during the preparation of papad, differed significantly among themselves with respect to every sensory attribute and total score. The treatment B, 15 g papad khar kg^{-1} legume flour blend, had the highest score compared to the treatment A, 10 g; treatment C, 20 g; and treatment D, 25 g. Hence addition of 15 g papad khar kg^{-1} legume flour blend is optimum for the preparation of papad.

Table 62. Average sensory scores[a] for optimizing amount of papad khar to be added during papad preparation[b]

Treatment	Papad khar (g kg^{-1}flour blend)	Attribute					
		Dough		Papad			
		Handfeel	Rolling	Appearance	Texture	Taste	Total score
A	10	20.5c ± 0.52	20.7c ± 0.44	10.3d ± 0.38	14.8d ± 0.22	11.6c ± 0.35	77.9d ± 0.59
B	15	23.3a ± 0.20	23.9a ± 0.69	13.6a ± 0.32	18.0a ± 0.38	13.4a ± 0.32	92.1a ± 0.70
C	20	22.3b ± 0.56	21.8b ± 0.46	12.3b ± 0.30	17.3b ± 0.14	12.3b ± 0.25	86.0b ± 0.35
D	25	20.9c ± 0.30	20.2c ± 0.33	11.6c ± 0.44	16.1c ± 0.22	12.2b ± 0.86	80.9c ± 0.97

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a column sharing a common letter are not significantly different ($P < 0.05$).

[b]Made by using 70 g common salt and 500 ml water kg^{-1} flour blend (blackgram dal flour : mung dal flour, 1:2) which was then cabinet-dried at 70 ± 5% relative humidity and 30 ± 1°C for 8 h, to final moisture content of 14-16%.

All the treatments considered for optimizing amount of water added during papad preparation differed significantly from each other in respect of dough hand feel, rolling properties, papad texture, taste and total score (Table 63). The treatment D, 500 ml water kg^{-1} legume flour blend during papad preparation, had the highest total score compared to the treatment A, B, C and E with 350, 400, 450, and 550 ml water kg^{-1} legume flour blend, respectively.

Table 63. Average sensory scores[a] for optimizing amount of luke warm water to be added during papad preparation[b]

Treatment	Water (ml kg^{-1} flour blend)	Attribute					
		Dough		Papad			
		Handfeel	Rolling	Appearance	Texture	Taste	Total score
A	350	15.1d ± 0.46	12.8e ± 0.52	7.9c ± 0.30	11.2d ± 0.44	6.0e ± 0.29	52.9d ± 0.46
B	400	19.6c ± 0.47	17.9c ± 0.22	10.3b ± 0.60	13.3c ± 0.44	8.4d ± 0.36	69.6c ± 1.11
C	450	22.1b ± 0.22	20.0b ± 0.76	12.1a ± 0.22	15.3b ± 0.38	11.2c ± 0.17	80.6b ± 0.55
D	500	24.1a ± 0.22	22.9a ± 0.30	12.8a ± 0.43	18.0a ± 0.29	13.3a ± 0.30	91.1a ± 0.88
E	550	21.3bc ± 0.38	16.2d ± 0.44	12.0a ± 0.29	15.7b ± 0.17	12.8b ± 0.52	77.8bc ± 1.02

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a column sharing a common letter are not significantly different ($P < 0.05$).

[b]Made by using 15 g papad khar and 70 g common salt kg^{-1} flour blend (black gram flour: mung dal flour, 1:2) which was then cabinet-dried at 70 ± 5% relative humidity and 30 ± 1°C for 8 h, to final moisture content of 14-16%.

Table 64 shows the average sensory scores for optimizing amount of common added during preparation of papad. All the treatments differed significantly among themselves with respect to every sensory attribute. The treatment C, 70 g common salt kg^{-1} legume flour blend, had the highest sensory score compared to the treatment A, 40 g; treatment B, 60 g; treatment D, 80 g; treatment E, 90 g; and treatment F, 100 g common salt kg^{-1} legume flour blend. Hence, amount of the common salt added during papad preparation was optimized at 70 g kg^{-1} legume flour blend.

Table 64. Average sensory scores[a] for optimizing amount of common salt to be added during papad preparation[b]

Treatment	Salt (g kg^{-1} flour blend)	Attribute					
		Dough		Papad			
		Handfeel	Rolling	Appearance	Texture	Taste	Total score
A	40	19.2c ± 0.36	19.3b ± 0.22	11.7b ± 0.22	13.6c ± 0.36	6.8f ± 0.15	70.5d ± 0.50
B	60	21.3ab ± 0.14	19.8ab ± 0.65	12.7a ± 0.08	14.1b ± 0.22	11.6cd ± 0.17	79.4bc ± 1.10
C	70	21.8a ± 0.38	20.4a ± 0.51	13.0a ± 0.29	15.0a ± 0.38	14.6a ± 0.51	84.8a ± 1.80
D	80	20.5b ± 0.38	20.2a ± 0.33	12.8a ± 0.14	14.3b ± 0.25	13.5b ± 0.25	81.2b ± 0.46
E	90	20.8b ± 0.29	19.9ab ± 0.36	12.3ab ± 0.25	14.2b ± 0.46	12.0c ± 0.14	79.1bc ± 0.22
F	100	20.4b ± 0.51	19.6b ± 0.30	12.3ab ± 0.22	14.2b ± 0.22	8.7e ± 0.30	75.2c ± 0.82

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a column sharing a common letter are not significantly different ($P < 0.05$).

[b]Made by using 15 g papad khar and 500 ml water kg^{-1} flour blend (blackgram dal flour: mung dal flour, 1:2) and cabinet-drying at 70 ± 5% relative humidity and 30 ± 1°C for 8 h to final moisture content of 14-16%.

4.7.4. Successional studies on dough during fermentation and drying

4.7.4.1. Microbial changes

Table 65 shows the quantitative changes in microbiota during preparation of papad under optimized conditions. TAMB cells and their spore count prevailed throughout the preparation process. *Saccharomyces cerervisiae*, the only yeast recovered from the ingredients, occurred relatively at constant load before perished after 6 h of drying. The initial load of the *Pediococcus pentosaceus*, the only lactic acid bacteria encountered in the ingredients, was increased by a log cycle during 3 h fermentation of the dough. Their count, however, gradually declined to below detection limit (2.0 log cfu g^{-1} fresh weight) beyond 6 h of drying of papad sheets.

4.7.4.2. Changes in proximate composition

The changes in proximate composition of the papad dough fermented for 3 h and subsequent drying of papad sheets are presented in Table 66. The data exhibited no significant change in the proximate composition of the papad dough during initial 3 h of fermentation. However, during the subsequent drying of papad sheets the mean moisture content of the papad decreased significantly by 3-fold. The pronounced effect of decline in moisture content of papad was evident from the significant increase in percentage diametric expansion of papad sheets after every 2 h-interval till 6 h of drying when the raw papad sheets attained the mean moisture content of 14.4 g (100 g)$^{-1}$ dry weight. The other parameters were relatively constant, and changes in proximate compositions, if any, were insignificant during the fermentation and subsequent drying of papad under optimized conditions.

4.8. Wadi

4.8.1. Proximate composition

The proximate composition of substrate (blackgram dal) and fermented wadi from different sources are presented in Table 67. The mean moisture content of the wadi was higher than that of the substrate. While the substrate had neutral pH, wadi was acidic. While titratable and fatty acidity contents of the wadi were higher than that of blackgram dal, the ash content of the substrate 2.6 times higher than of wadi. Protein nitrogen of the substrate decreased, while the nonprotein nitrogen and soluble nitrogen contents increased by 2.5-3.5 times, and 2.6-2.8 times respectively, after fermentation. Mean carbohydrate and fat contents of wadi was higher than that of blackgram dal.

Table 65. Changes in microbial load[a] in papad dough during fermentation and subsequent drying under semicontrolled conditions

Microbiota	Fermentation period (h)		Drying period (h)				
	0	3	0	2	4	6	8
TAMB[b]	4.06d ± 0.06 (100)	4.60c ± 0.16 (100)	4.60c ± 0.16 (100)	4.91b ± 0.17 (100)	5.01a ± 0.10 (100)	4.63c ± 0.30 (100)	4.67c ± 0.25 (100)
aMBS[c]	4.04c ± 0.10 (100)	4.14b ± 0.11 (100)	4.14b ± 0.11 (100)	4.25a ± 0.24 (100)	4.05c ± 0.15 (100)	4.04c± 0.22 (100)	4.01c ± 0.16 (100)
Yeast[c]							
Saccharomyces cerevisiae	4.48b ± 0.11 (67)	4.60a ± 0.15 (100)	4.60a ± 0.15 (100)	4.68a ± 0.25 (100)	4.40b ± 0.18 (100)	<DL	<DL
Lactic acid bacteria[b]							
Pediococcus pentosaceus	4.82b ± 0.31 (100)	5.25a ± .26 (100)	5.25a ± .26 (100)	4.33c ± 0.25 (100)	3.75d ± 0.14 (67)	<DL	<DL

[a]Values, expressed as mean ± SEM of log cfu g^{-1} fresh weight, were obtained from three different samples. Data within parentheses are expressed as % positive samples. Means within a row sharing a common letter are not significantly different ($P < 0.05$).

[b]DL (detection limit), 1.0 log cfu g^{-1} fresh weight.

[c]DL, 2.0 log cfu g^{-1} fresh weight

Table 66. Changes in proximate composition[a] of papad dough during fermentation and subsequent drying under optimized conditions

Parameter	Fermentation period (h)		Drying period (h)				
(g (100 g)$^{-1}$ dry matter)	0	3	0	2	4	6	8
pH	7.04a ± 0.04	7.08a ± 0.07	7.08a ± 0.07	6.95a ± 0.03	7.02a ± 0.07	7.08a ± 0.01	6.95a ± 0.01
% diametric expansion after frying	ND[b]	ND	6.6d ± 1.58	14.27c ± 0.88	24.6b ± 0.40	27.6a ± 0.36	28.0a ± 0.71
Moisture (g (100 g)$^{-1}$)	39.79a ± 0.89	39.76a ± 0.91	39.76a ± 0.91	23.67b ± 0.63	18.38c ± 0.29	14.44d ± 0.37	13.79d ± 0.51
Ash	9.91a ± 0.15	9.9a ± 0.18	9.9a ± 0.18	9.92a ± 0.15	9.95a ± 0.11	9.87a ± 0.21	9.97a ± 0.13
Titratable acid (as lactic acid)	0.56a ± 0.03	0.59a ± 0.02	0.59a ± 0.02	0.52a ± 0.03	0.55a ± 0.06	0.58a ± 0.03	0.55a ± 0.05
Free fatty acid (as linoleic acid)	1.28ab ± 0.03	1.23b ± 0.05	1.23b ± 0.05	1.30a ± 0.07	1.31a ± 0.03	1.33a ± 0.04	1.34a ± 0.02
Total nitrogen	3.86a ± 0.11	3.82a ± 0.07	3.82a ± 0.07	3.89a ± 0.07	3.89a ± 0.02	3.88a ± 0.03	3.77a ± 0.04
Protein nitrogen	2.46a ±0.07	2.47a ± 0.11	2.47a ± 0.11	2.41a ± 0.10	2.38b ± 0.10	2.41a ± 0.02	2.37b ± 0.02
Nonprotein nitrogen	1.40ab ± 0.07	1.35b ± 0.05	1.35b ± 0.05	1.49a ± 0.09	1.51a ± 0.14	1.47a ± 0.02	1.40ab ± 0.03
Soluble nitrogen	1.17b ± 0.01	1.28a ± 0.02	1.28a ± 0.02	1.28a ± 0.04	1.29a ± 0.03	1.32a ± 0.04	1.31a ± 0.03
Total protein (total N x 6.25)	24.13a ± 0.68	23.88a ± 0.42	23.88a ± 0.42	24.31a ± 0.43	24.31a ± 0.40	24.25a± 0.20	23.56a ± 0.26
Crude fat (ether extract)	4.77ab ± 0.09	4.69c ± 0.08	4.69c ± 0.08	4.79ab ± 0.24	4.83a ± 0.19	4.73b ± 0.08	4.93a ± 0.15
Carbohydrate (by difference)	61.18a ± 0.77	61.55a ± 0.46	61.55a ± 0.46	60.97a ± 0.47	61.34a ± 0.53	61.13a ± 0.10	61.59a ± 0.27
Energy (MJ (100 g)$^{-1}$ dry matter)	1.61a ± 0.00	1.60a ± 0.00	1.60a ± 0.00	1.61a ± 0.01	1.61a ± 0.01	1.61a ± 0.00	1.61a ± 0.00

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a row sharing a common letter are not significantly different ($P < 0.05$).

[b]ND, not determined.

Table 67. Proximate composition[a] of wadi and its substrate

Parameter	g (100 g)$^{-1}$ dry matter		
	Substrate	Wadi	
	(blackgram dal) (n = 3)	Marketed (n = 41)	Laboratory-made (n = 3)
pH	7.0a ± 0.04	5.6b ± 0.04	5.4b ± 0.05
Moisture (g (100 g)$^{-1}$)	11.1b ± 0.10	14.7a ± 0.37	14.5a ± 0.40
Ash	6.4a ± 0.07	2.5b ± 0.05	2.6b ± 0.10
Titratable acid (as lactic acid)	0.1c ± 0.01	1.0b ± 0.03	1.6a ± 0.04
Free fatty acid (as linoleic acid)	0.7b ± 0.01	2.5a ± 0.06	2.7a ± 0.04
Total nitrogen	4.1a ± 0.02	3.4b ± 0.08	4.1a ± 0.07
Protein nitrogen	3.7a ± 0.03	2.4b ± 0.08	2.7b ± 0.05
Nonprotein nitrogen	0.4b ± 0.05	1.0a ± 0.05	1.4a ± 0.09
Soluble nitrogen	0.5b ± 0.01	2.3a ± 0.09	2.4a ± 0.04
Total protein (total N x 6.25)	25.5a ± 0.11	21.0b ± 0.51	25.6a ± 0.44
Crude fat	0.3b ± 0.02	1.9a ± 0.03	2.2a ± 0.03
Carbohydrate (by difference)	67.8c ± 0.16	74.6a ± 0.50	69.6b ± 0.43
Energy (MJ (100 g)$^{-1}$ dm)	1.7a ± 0.01	1.7a ± 0.01	1.7a ± 0.01

[a]Values are expressed as mean ± SEM. Means within a row sharing a common letter are not significantly different (P <0.05).

4.8.2. Microbial analysis

4.8.2.1. Isolation of microorganisms

A total of 555 strains of lactic acid bacteria (Table 68) and 635 strains of yeasts (Table 69) were isolated from 3 samples of raw blackgram dal, 41 samples of market wadi, and 21 samples of laboratory-made

Table 68. Selection of representative strains of dominant lactic acid bacteria isolated from substrate and fermenting batter of wadi

Source	Stage(s) of fermentation	Cell shape[a]	Gas from glucose	Growth in/at		Grouped strains[b]		No. of R[c] strains
				pH 4.8	45°C	Group	No.	
Substrate								
Blackgram dal		Cp/c	+	-	-	W-LA	15	3
(n = 3)		Ct	-	+	+	W-LB	10	2
Wadi/batter								
Marketed	Final	Cp/c	+	-	-	W-LA	135	27
(n = 41)		Ct	-	+	+	W-LB	185	37
Laboratory-made	0 h-60 h	Cp/c	+	-	-	W-LA	105	21
(n = 21)	(12 h-interval)	Ct	-	+	+	W-LB	105	21

[a]Cp/c, cocci in pair or short chain; Ct, cocci in tetrad.
[b]All the isolates were nonmotile, nonsporeforming, Gram positive and catalase negative.
[c]R, representative.

samples of fermenting and fermented wadi. While all the lactic acid bacterial isolates were grouped into 2 cell morphotypes, the yeast isolates were grouped into 4 colony morphotypes. A total of 30 strains of mould were isolated from 6 market samples of wadi (Table 70). Since all the mould isolates exhibited identical morphological characters, they were clustered under a single group. One representative strain, from each group of each positive sample, was selected randomly to ascertain their taxonomic status.

4.8.2.2. Taxonomical studies

4.8.2.2.1. Bacteria

On the basis of selected morphological, physiological and cultural characteristics of lactic acid bacteria shown in Table 68, all the representative strains of group W-LA belonged to the genus *Leuconostoc*,

Table 69. Selection of representative strains of dominant yeasts isolated from substrate and fermenting batter of wadi

Source	Stage(s) of fermentation	No. of samples	Colony[a]	Cell shape[b]	Pellicle formation	Grouped strains		No. of R[d] strains
						Group	No.	
Substrate								
Blackgram dal (n = 3)		3	Cgs	G-O/E	-	W-YA	15	3
Wadi/batter								
Marketed (n = 41)	Final	41	Cgs	G-O/E	-	W-YA	50	10
			Tds	O-E	+	W-YB	110	22
			Wsw	O-E	+	W-YC	180	36
			Pgs	G-E	-	W-YD	25	5
Laboratory-made (n = 21)	0 h - 60 h (12 h-interval)	21	Cgs	G-O/E	-	W-YA	105	21
			Tds	O-E	+	W-YB	80	16
			Wsw	O-E	+	W-YC	70	14

[a]Cgs, cream colour with glistening and butyrous surface; Tds, tannish white colour with dull and flat surface; Wsw, tannish white surface with wrinkled margin; Pgs, pink colour with glistening and butyrous surface.
[b]G, globose; O, oval; E, ellipsoidal.
[d]R, representative.

while those of the group W-LB belonged to the genus *Pediococcus*. The detailed morphological and physiological characteristics of all those representative strains are presented in Table 71. Following the criteria laid by Garvie (1986a, 1986b) and IBIS (Wijtzes *et al.*, 1997), the representative strains belonging to groups W-LA and W-LB were tentatively identified as *Leuconostoc mesenteroides* (Tsenkovskii) van Tieghem 1879 and *Pediococcus pentosaceus* Mees 1934, respectively.

4.8.2.2.2. Yeasts

The detailed morphological, cultural and physiological characteristics of the 127 representative strains of yeasts, comprising of 4 different colony morpho-types, isolated from substrates market samples of wadi and laboratory-made fermenting and fermented wadi are shown in Table 72.

Table 70. Selection of representative strains of moulds isolated from wadi[a]

Source	Number of		
	Strains isolated[a]	Grouped strains	Representative strains
Raw ingredients (n = 3)	0	0	0
Marketed wadi (n = 41)	30	30	6
Laboratory-made wadi (n = 21)	0	0	0

[a]All the isolates had mycelia without septation, rhizoid and stolon; sporangiophores, branched and arose directly from the substrate mycelium; sporangiospores, ellipsoidal to globose; columellae, ellipsoidal to pyriform; chlamydospores, numerous and barrel-shaped.

Table 71. Characteristics[a] of groups of representative strains of lactic acid bacteria isolated from wadi, and its substrates and fermenting batter

Parameters	W-LA (n = 51)			W-LB (n = 60)		
	+	d	-	+	d	-
Cell shape	Cocci in pair or chain			Cocci in tetrad		
Cell diameter (µm)	0.30-0.65			0.33-0.68		
Gas from glucose	100					100
Growth in NaCl (g l^{-1})						
30	100			56	32	12
65	71	25	4	100		
80			100	100		
Growth at pH 4.8			100	100		
Hydrolysis of						
arginine			100	100		
esculin	71	25	4	100		
gelatin			100	100		
Growth at 45°C			100	100		
Acid from						
D-cellobiose	65	29	6	100		
esculin	70	22	8	100		
D-lactose	55	45		52	33	15
D-mnnitol	100					100
D-mannose	100					100
D-melibiose	69	25	6	71	22	7
D-raffinose	72	28				100
D-ribose	100			68	32	
salicin	63	25	12	100		
sucrose	100				23	77
D-xylose	67	19	14	67	25	8

[a]Data represent %strains showing + (positive), d (delayed) and - (negative) reactions. All the strains were Gram positive, nonmotile, nonsporeforming, catalase negative, capable of growing at 15-40°C and producing acid from L-arabinose, D-fructose, D-galactose, D-glucose, D-maltose and D-trehalose, however not capable of reducing nitrate, producing indole, hydrolyzing fat and starch, and producing acid from glycerol, sorbitol and starch.

Table 72. Characteristics[a] of groups of representative strains of yeasts isolated from wadi and its substrates and fermenting batter

Parameter	W-YA (n = 34)			W-YB (n = 38)			W-YC (n = 50)			W-YD (n = 5)		
	+	$+_w$	-	+	$+_w$	-	+	$+_w$	-	+	$+_w$	-
Colony morphology	Cgs			Tds			Wsw			Pgs		
Cell shape	(G-O)/E			O-E			O-E			G-E		
Cell width x length (µm)	3.2-5.7 x 4.8-7.5			2.7-6.4 x 3.7-7.3			2.2-5.6 x 3.1-7.4			2.3-4.3 x 2.9-5.7		
Mycelium[d]	Psd			Psd			Psd					100
Vegetative reproduction	100			100			100					100
Budding	Multilateral			Multilateral			Multilateral					100
Sexual reproduction	100			100			100					100
Ascus	100			100			100					100
Asci evanescence	Persistent			Persistent			Evanescent					100
Ascospore[e]	(R-O)+Sm			O+Sm			Str/R					100
Ascospore/ascus	1-4			1-2			1-4					100
Pellicle formation			100	100			100					100
Hydrolysis of												
fat	65	35		100			78	22		40	60	
urea			100			100			100	80	20	
Fermentation of												
D-galactose	59	12	29			100			100			100
D-glucose	100			100			82	18	0			100
D-maltose	53	29	18			100			100			100
D-melibiose	54	23	23			100			100			100
melezitose	62	29	9			100			100			100
D-raffinose	70	15	15			100			100			100
sucrose	62	26	12			100			100			100
D-trehalose	18	32	50			100			100			100
Assimilation of												
L-arabinose			100			100			100	60	20	20
D-cellobiose			100			100			100	80		20
citrate			100	100			100			80	20	
D-galactose	70	18	12		24	76			100	40	40	20
D-glucitol		56	44			100			100	20	60	20
D-glucosamine			100	100					100			100
N-acetyl-glucosamine			100	100			100			100		
glycerol	56	6	38	76	24		58	30	12	80	20	
D-lactose			100			100			100	60	20	20
D-maltose	62	20	18			100			100			100
D-mannitol	15	56	29			100			100	20		80
melezitose	50	18	32			100			100	40	40	20
D-melibiose	76	18	16			100			100			100
D-raffinose	73	9	18			100			100	20		80
D-ribose			100	55	24	21			100	40	40	20
sucrose	68		32			100			100	100		
D-trehalose	70	9	21			100			100	80		20
vitamin-free medium			100	68	19	13	78	22	0			100
D-xylose			100			100	70	22	8	60	40	

[a]Data represent %strains showing + (positive), $+_w$ (weak positive) and - (negative) reactions. All the strains were capable of growing at 37°C and assimilating D-glucose, however not capable of reducing nitrate, forming starch, hydrolyzing gelatin and starch, fermenting D-cellobiose, D-lactose and D-xylose, and assimilating *m*-erythritol, *m*-inositol, nitrate, L-rhamnose and starch.

[b]Cgs, cream colour with glistening and butyrous surface; Tds, tannish white colour with dull and flat surface; Wsw, tannish white surface with wrinkled margin; Pgs, pink colour with glistening and butyrous surface.

[c]G, globose; O, oval; E, elongated.

[d]Psd, pseudomyceliuml/pseudohyphae.

[e]R, round, O, oval; Sm, smooth; Str, Saturn-shaped.

Following the taxonomic keys of Barnett *et al.* (2000) and Kurtzman and Fell (2000), and using BioloMICSNet software (http://www.cbs.knaw.nl/yeast/BioloMICS.aspx) the taxonomic status of the representative strains belonging to group W-YA, W-YB, W-YC and W-YD were tentatively identified as *Saccharomyces cerevisiae* Mayen *ex* Hansen 1883, *Issatchenkia orientalis* Kudryavtsev 1960, *Pichia membranifaciens Hansen* 1904, and *Rhodotorula minuta* (Saito) Harrison 1928, respectively.

4.8.2.2.3. Moulds

Table 73 shows the detail morphological characteristics of 6 representative strains of mould, recovered from the market samples of wadi. The mycelia were aseptate, and without rhizoids and stolons. The sporangiophores with ellipsoidal to globose sporangiospores were branched and arose directly from the substrate mycelium, while the chlamydospores were numerous and barrel-shaped. Following the taxonomic keys of Hesseltine (1983) and Samson and van Reenen-Hoekstra (1988), all the representative strains of the mould were tentatively identified as *Mucor racemosus* Fres.

Table 73. Characteristics of representative strains of moulds isolated from market wadi

Character	Representative strains[a]					
	W-M1	W-M2	W-M3	W-M4	W-M5	W-M6
Sporangiospore						
Length (µm)	4.0-6.2	3.8-6.8	4.8-6.6	4.1-6.6	4.6-5.7	4.0-6.9
Breadth (µm)	4.5-5.3	4.8-5.6	3.8-4.3	3.6.-4.7	4.1-5.0	3.5-5.3
Columella						
Length (µm)	35-40	46-50	43-48	44-50	40-48	44-47
Breadth (µm)	35-40	35-44	38-45	38-40	35-43	30-41
Chlamydospore						
Length (µm)	22-25	18-22	20-24	17-25	17-22	20-24
Breadth (µm)	14-17	15-20	16-20	14-18	14-16	13-17

[a]All the isolates had aseptate mycelium, rhizoid, stolon, branched sporangiophores which arose directly from the substrate mycelium, ellipsoidal to globose sporangiospores, ellipsoidal to pyriform columellae, and numerous, barrel-shaped chlamydospores, however were not capable of growing at 37°C.

4.8.2.3. Microbial loads in substrate and product

The average microbial load studied in 41 market samples of wadi, 3 samples each of blackgram dal and laboratory-made wadi are shown in Table 74. TAMB cells and their spores (aMBS) were recovered form all the samples studied (Table 74). While the average load of TAMB cells in wadi was 3 log cycles

Table 74. Dominant microbial load in substrates and fermented batter of wadi

Microbiota	Log cfu g^{-1} fresh weight[a]		
	Substrate	Wadi	
	(blackgram dal) (n = 3)	Marketed (n = 41)	Laboratory-made (n = 3)
TAMB	5.2c ± 0.11	8.0b ± 0.91	9.2a ± 0.18
aMBS	3.5b ± 0.34	4.1a ± 0.18	3.8ab ± 0.17
Yeasts			
Saccharomyces cerevisiae	3.4c ± 0.43	4.4b ± 0.84	5.2a ± 0.27
Issatchenkia orientalis	<DL[b]	5.2a ± 1.31	5.2a ± 0.21
Pichia membranifaciens	<DL	5.1a ± 1.14	4.5b ± 0.87
Rhodotorula minuta	<DL	4.0 ± 0.60	<DL
Mould			
Mucor racemosus	<DL	4.1 ± 0.50	<DL
Lactic acid bacteria			
Leuconostoc mesenteroides	4.3c ± 0.45	7.9b ± 1.28	9.3a ± 0.31
Pediococcus pentosaceus	3.1b± 0.08	8.1a ± 1.30	8.6a ± 0.28

[a]Values are expressed as mean ± SEM. Means within a row sharing a common letter are not significantly different (P <0.05).
[b]DL (detection limit), 2.0 log cfu g^{-1} fresh weight.

more than that of substrate, their spore count was between 3.5 to 3.9 log cfu g^{-1} fresh weight, in the samples studied. Similarly, the mean load of *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* in substrate was increased by 3.5 log cycles during fermentation. In laboratory-made wadi, the average load of *Saccharomyces cerevisiae*, the only yeast found in substrate, was highest followed by *Issatchenkia orientalis* and *Pichia membranifaciens*. Market wadi was dominated by *I. orientalis*, followed by *P. membranifaciens*, *S. cerevisiae*, and *Rhodotorula minuta*. *Mucor racemosus*, the only mold recovered had the average load of 4.1 log cfu g^{-1} fresh weight sample. However, both *R. minuta* and *M. racemosus*, encountered in 12% and 15% of market samples, respectively, were recovered neither from the raw blackgram dal nor from the laboratory-made wadi (Table 75).

Table 75. Prevalence of dominant microorganisms in wadi and its substrate from different sources

Microbiota	Positive sample (%)		
	Substrate	Wadi	
	(blackgram dal) (n = 3)	Marketed (n = 41)	Laboratory-made (n = 3)
TAMB	100	100	100
aMBS	100	93	100
Yeasts			
Saccharomyces cerevisiae	100	24	100
Issatchenkia orientalis	0	54	100
Pichia membranifaciens	0	88	67
Rhodotorula minuta	0	12	0
Mould			
Mucor racemosus	0	15	0
Lactic acid bacteria			
Leuconostoc mesenteroides	100	66	100
Pediococcus pentosaceus	67	90	100

4.8.3. Successional studies on batter during fermentation and drying

4.8.3.1. Microbial changes

Table 76 shows the quantitative changes in microbial load of wadi during fermentation and subsequent drying. The load of lactic acid bacteria, yeast and TAMB cells increased significantly during 10 h-fermentation, followed by 24 h of drying when the population size increased by 4-5 log cycles. The average load of *Leuconostoc mesenteroides* increased significantly, and it formed the dominant component of the wadi microbiota. However, the final 12 h-drying (from 48 h to 60 h) showed a sharp declined in their count by 2-log cycles.

Similarly the average count of *Pediococcus pentosaceus*, after initial increased by about 4 log cycles, declined gradually after 24 h of drying. *Saccharomyces cerevisiae*, the most predominant yeast encountered during the wadi preparation, increased significantly during 10 h-fermentation and at every 12 h-interval of drying till 24 h, followed by the steady fall in the population. The decrease was significant after every 12 h-interval. *Issatchenkia orientalis*, which was detected only after 10 h fermentation of wadi dough, increased significantly by 3.5-log cycle during initial 24 h drying, eventually declined to the final load of $>10^5$ cfu g^{-1} fresh weight at the end. The decrease was significant after every 12 h-interval. *Pichia membranifaciens*, which was not recovered during the onset of process, appeared after 12 h of drying and remained constant before marginal decrease at the end.

4.8.3.2. Changes in proximate composition

The proximate compositional changes of wadi batter during fermentation and drying are shown in Table 77. During the initial 10 h-fermentation, though the moisture content of the batter remained unchanged, the volume increased by 1.4-fold. However, the subsequent drying (sun-drying for 8 h followed by 16 h shade-drying at room temperature (28-30°C) daily) for a total of 60 h, reduced the initial moisture content of wadi by 4.3 times. The pH declined during the initial 10 h from 6.2 to 5.8, and then at every 12 h-interval till 36 h of drying to reach 4.9. The titratable and free fatty acidity of the batter increased significantly during fermentation and drying. The protein nitrogen declined during the initial fermentation, but gradually increased during the subsequent drying period. While the content of soluble nitrogen increased by 3.4 times during wadi preparation, there was no significant change in the contents of nonprotein nitrogen, crude fat and energy value during wadi preparation.

Table 76. Changes in microbial load[a] in wadi batter during fermentation and subsequent drying under semicontrolled conditions

Microbiota	Fermentation period (h)		Drying period (h)					
	0	10	0	12	24	36	48	60
TAMB	6.2f ± 0.20	8.7e ± 0.25	8.7e ± 0.25	9.9c ± 0.50	11.2ab ± 0.31	11.4a ± 0.22	10.9b ± 0.22	9.2d ± 0.18
	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
aMBS	3.1b ± 0.03	4.2a ± 0.12	4.2a ± 0.12	4.0a ± 0.43	3.9a ± 0.20	4.0a ± 0.08	4.0a ± 0.10	3.8a ± 0.17
	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Yeasts								
Saccharomyces cerevisiae	4.3e ± 0.31	7.1c ± 0.27	7.1c ± 0.27	8.1b ± 0.11	8.8a ± 0.30	7.0c ± 0.08	5.2 ± 0.25	5.2d ± 0.27
	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Issatchenkia orientalis	<DL[b]	3.9d ± 0.64	3.9d ± 0.64	6.3b ± 0.24	7.5a ± 0.32	6.4b ± 0.31	5.4c ± 0.21	5.2c ± 0.21
		(33)	(33)	(100)	(100)	(100)	(100)	(100)
Pichia membranifaciens	<DL	<DL	<DL	5.0b ± 0.43	5.5a ± 0.23	5.5a ± 0.16	5.4a ± 0.42	4.5c ± 0.87
				(100)	(100)	(100)	(100)	(67)
Lactic acid bacteria								
Leuconostoc mesenteroides	5.6c ± 0.24	9.1b ± 0.06	9.1b ± 0.06	11.2a ± 0.33	11.4a ± 0.33	11.7a ± 0.3	11.3a ± 0.28	9.3b ± 0.31
	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Pediococcus pentosaceus	5.8d ± 0.08	8.6c ± 0.27	8.6c ± 0.27	10.1a ±0 .18	10.1a ± 0.09	9.9b ± 0.05	9.4bc ± 0.25	8.6c ± 0.28
	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)

[a]Values, expressed as mean ± SEM of log cfu g^{-1} fresh weight, were obtained from three different samples. Data within parentheses are expressed as % positive samples. Means within a row sharing a common letter are not significantly different ($P < 0.05$).

[b]DL (detection limit), 2.0 log cfu g^{-1} fresh weight.

Table 77. Changes in proximate composition[a] of wadi dough during fermentation and subsequent drying under semi-controlled conditions

Parameter	Fermentation period (h)		Drying period (h)					
(g (100 g)$^{-1}$ dry matter)	0	10	0	12	24	36	48	60
pH	6.2a ± 0.11	5.8b ± 0.10	5.8b ± 0.10	5.6b ± 0.05	5.3c ± 0.06	4.9d ± 0.04	5.0d ± 0.06	5.0d ± 0.05
Moisture (g (100 g)$^{-1}$)	61.9a ± 1.03	61.9a ± 0.96	61.9a ± 0.96	53.8b ± 0.82	47.1c ± 0.70	33.6d ± 0.38	26.2e ±1 .05	14.5f ± 0.40
Volume (ml)	250.0b ± 0	346.7a ± 1.67	ND[b]	ND	ND	ND	ND	ND
Ash	3.4a ± 0.10	3.2a ± 0.23	3.2a ± 0.23	2.9b ± 0.17	2.7b ± 0.12	2.7b ± 0.11	2.7b ± 0.13	2.6b ± 0.10
Total nitrogen	3.8b ± 0.08	3.3b ± 0.20	3.3b ± 0.20	3.5b ± 0.08	3.6ab ± 0.04	3.9a ± 0.02	4.0a ± 0.08	4.1a ± 0.07
Protein nitrogen	2.6a ± 0.14	2.1b ± 0.19	2.1b ± 0.19	2.3ab ± 0.4	2.5a ± 0.05	2.6a ± 0.12	2.7a ± 0.04	2.7a ± 0.05
Nonprotein nitrogen	1.2a ± 0.04	1.a2 ± 0.04	1.2a ± 0.04	1.2a ± 0.10	1.2a ± 0.09	1.3a ± 0.12	1.3a ± 0.08	1.4a ± 0.09
Soluble nitrogen	0.7d ± 0.06	1.2c ± 0.02	1.2c ± 0.02	1.8b ± 0.04	1.9b ± 0.04	2.2a ± 0.02	2.3a ± 0.04	2.4a ± 0.04
Total protein (total N x 6.25)	23.8ab ± 0.50	20.8d ± 1.25	20.8d ± 1.25	21.7c ± 0.48	23.0b ± 0.20	24.2a ± 0.14	25.2a ± 0.47	25.6a ± 0.44
Titratable acid (as lactic acid)	0.6c ± 0.05	1.1b ± 0.05	1.1b ± 0.05	1.2b ± 0.02	1.3ab ± 0.04	1.4a ± 0.04	1.5a ± 0.05	1.6a ± 0.04
Free fatty acid acid (as linoleic acid)	1.5c ± 0.04	2.0b ± 0.02	2.0b ± 0.02	2.2b ± 0.02	2.3ab ± 0.04	2.5a ± 0.02	2.6a ± 0.05	2.7a ± 0.04
Crude fat	2.2a ± 0.04	2.1a ± 0.11	2.1a ± 0.11	2.2a ± 0.14	2.2a ± 0.06	2.2a ± 0.03	2.2a ± 0.01	2.2a ± 0.03
Carbohydrate(by difference)	70.1c ± 0.39c	73.9a ± 1.25	73.9a ± 1.25	73.2ab ± 0.69	72.1b ± 0.15	71.0bc ± 0.23	69.0d ± 0.51	69.6c ± 0.43
Energy (MJ (100 g)$^{-1}$ dry matter)	1.7a ± 0.01	1.7a ± 0. 01	1.7a ± 0. 01	1.7a ± 0.00	1.7a ± 0.00	1.7a ± 0.00	1.7a ± 0.01	1.7a ± 0.01

[a]Values, expressed as mean ± SEM, were obtained from three different samples. Means within a row sharing a common letter are not significantly different ($P < 0.05$).

[b]ND, not determined.