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Microbiological and physico-chemical changes during fermentation of kodo ko jaanr, a traditional alcoholic beverage of the Darjeeling hills and Sikkim

S. Thapa and J. P. Tamang*
Food Microbiology Laboratory, Department of Botany, Sikkim Government College, Gangtok 737102, Sikkim

Kodo ko jaanr is a traditional fermented finger millet beverage, commonly consumed in the Darjeeling hills and Sikkim. In this paper, microbial and physico-chemical changes during fermentation of kodo ko jaanr were investigated. Population of filamentous moulds declined significantly (P<0.05) every d and finally disappeared after fifth d. Load of yeasts increased significantly (P<0.05) from 10^5 cfu g^{-1} to 10^7 cfu g^{-1} within second d. Lactic acid bacteria increased significantly (P<0.05) from 10^6 cfu g^{-1} to 10^8 cfu g^{-1} in first d and decreased significantly (P<0.05) to 10^3 cfu g^{-1} at the end. The pH decreased and acidity increased during fermentation. Alcohol content increased significantly (P<0.05) from 0.1 % to 6.9 % within sixth d. Reducing sugar content increased significantly (P<0.05) till third d and decreased, followed by decrease in total sugar content. Maximum activities of saccharification and liquefaction of millets were observed on second d of fermentation. It was revealed that *Saccharomycopsis fibuligera* and *Rhizopus* spp. play the major role in saccharification process of finger millet in kodo ko jaanr fermentation. A combination of mould and yeast strains, isolated from kodo ko jaanr during natural fermentation, were tested for production of the same product under lab-condition. Kodo ko jaanr prepared by a mixture of *Rhizopus chinensis* MJ:R3 and *Saccharomyces cerevisiae* MJ:YS2 had scored highest in general acceptability.

Keywords: Kodo ko jaanr, finger millet, yeasts, lactic acid bacteria

Fermented beverages are prepared by the action of microorganisms, either spontaneously or by adding starter cultures(s), which modify the substrates biochemically and organoleptically, and are thus generally palatable, safe and nutritious[1]. Fermentation yielding alcohol generally offers low-cost ways of preserving food for majority of people who cannot afford canned or frozen foods or beverages[2]. Kodo ko jaanr is a popular mild alcoholic beverage prepared from dry seeds of finger millet [*Eleusine coracana* (L) Gaertn.] in the Eastern Himalayan regions of the Darjeeling hills and Sikkim in India, Nepal and Bhutan[3]. Ethnic people of the Sikkim Himalayas have been consuming kodo ko jaanr or chhang for centuries[4, 5]. Product characterisation of market samples of kodo ko jaanr including microbial composition and proximate composition was studied[6]. The present paper deals with the study of microbial and physico-chemical changes during kodo ko jaanr fermentation.

Materials and Methods

Laboratory preparation of kodo ko jaanr: Kodo ko jaanr was prepared in the laboratory following the traditional method[6]. Dry seeds of finger millet {*Eleusine coracana* (L.) Gaertn.), local variety 'mudke kodo', were purchased from Gangtok market of Sikkim in India. Finger millet seeds were cleaned, washed and cooked for 30 min in an open cooker, and excess water was drained off. Marcha, a dry round to flattened, ball-like mixed dough starter[7], obtained from Gangtok market was powdered aseptically, and about 2 % of it was added to cooked substrates. About 100 g of inoculated seeds were kept in sterile 250 ml-bottles with loosely covered lids, and fermented at 28° C for 2 days for saccharification. After 2 days, lids were tightly capped to make anaerobic condition and kept for 8 days at 28° C for further fermentation. Samplings were collected at every 1 d interval until 10th d of fermentation for microbial, physico-chemical and enzymatic analysis.

Microbial analysis: A 10 g of sample was mixed in 90 ml of 0.85 % (w/v) sterile physiological saline and homogenized in a stomacher lab-blender (400, Seward, UK) for 1 min and serially diluted in the same diluent. Moulds and yeasts were isolated on potato dextrose agar (M096, HiMedia) and yeast-malt (YM) agar

*e-mail: jyoti_tamang@hotmail.com
Tel: 91 3592 231053

(M424, HiMedia), respectively supplemented with 10 IU ml^{-1} benzylpenicillin and 12 mg ml^{-1} streptomycin sulphate and incubated aerobically at 28° C for 48-72 h. Lactic acid bacteria (LAB) were isolated on MRS agar (M641, HiMedia) plates supplemented with 1 % $CaCO_3$ and incubated under anaerobic condition in an Anaerobic Gas-Pack system (LE002, HiMedia) and incubated at 30° C for 48-72 h. Total viable counts were determined using plate count agar (MO91A, HiMedia) and incubated at 30° C for 48 h. Colonies were selected randomly from the plates. Purity of the isolates was checked by streaking again on fresh agar plates of the isolation medium, followed by microscope examination. Microbiological data were transformed into logarithms of the numbers of cfu g^1.

Characterisation and identification: Characterisation and identification of filamentous moulds was carried out following the keys of Schipper[8,9] and Hesseltine[10]. Characterisation and identification of yeasts were carried out following the methods of Kreger-van Rij[11] and Kurtzman and Fell[12]. Phenotypic characterization of LAB was performed following the methods of Schillinger and Lücke[13] and were identified following the taxonomic keys of Wood and Holzapfel[14].

Physico-chemical analysis: A 10 g of sample was blended with 20 ml of carbon-dioxide free-distilled water in a homogeniser for 1 min and the pH of the slurry was determined directly using a digital pH meter (Type 361, Systronics) calibrated with standard buffer solutions (Merck). The temperature (^oC) change of the fermenting substrates during beverage fermentation was recorded directly by a thermometer. Titratable acidity, expressed as percent lactic acid, was determined by titrating 10 ml of sample using 0.1 (N) sodium hydroxide. Alcohol content of sample was determined by dichromate oxidation method[15]. Reducing sugar content of sample was determined by modified colorimetric method[16] using glucose as standard solution[17]. Total sugar was determined by determining reducing sugar in hydrolysed sample with HCl[15].

Amylolytic activity: Surface-dried plates of starch agar[18] were streaked with 24 h-old cultures, incubated at 28^o C for yeasts and moulds, and 30^o C for LAB for 72 h, respectively. After incubation, the plates were flooded with iodine solution for 15-30 min and examined the clear zone underneath (after the colony was scrapped off) for amylolytic activity.

Preparation of supernatant (enzyme solution): A 10 g sample was homogenised and centrifuged at 17,000 rpm for 10 min and the supernatant was filtered. Separately, cultures were grown on broth medium (1.0 % soluble starch, 1.0 % yeast extract, 1.0 % peptone, and 0.3 % NaCl, pH 7.0) in shaking incubator at 28^o C for 48 h and were immediately centrifuged at 17,000 rpm for 10 min. The supernatant (enzyme solution) was diluted to an appropriate concentration for estimation of liquefying and saccharifying activities.

Liquefying (μ-amylase) activity assay: The blue value method of Fuwa[19] as modified by Kawaguchi et al.[20] was followed for a-amylase activity. UV-VIS Spectrophotometer (Specord 200, Analytik Jena, Germany) was used at 660 nm for absorbance of solution. One unit of a-amylase activity (liquefying activity) was defined as the amount of a-amylase which produced 10% fall in the intensity of blue colour at the above condition.

Saccharifying (glucoamylase) activity assay: Saccharifying (glucoamylase) activity was determined according to modified method of Ueda and Saha[21]. The glucose was determined by calorimetric method[16]. One unit of glucoamylase activity (saccharifying activity) was defined as the amount of enzyme, which liberated 1 mg glucose in 1 min under the above condition. Unit of activity was expressed as mg glucose released per ml per 10 min.

Preparation of inocula for kodo ko jaanr production: Kodo ko jaanr was prepared in the laboratory using selected strains of moulds and yeasts, isolated from conventionally prepared kodo ko jaanr samples. The suspension of mould cultures was made by adding 5 ml of sterile distilled water to each 4-day-old slant culture on potato dextrose agar (M096, HiMedia) and mycelia and sporangia were scraped off the agar with an sterile inoculating wire. Yeasts inocula were prepared by introducing 5 ml each of sterile distilled water onto 48 h-old slant culture on YM agar (M424, HiMedia). Tubes were agitated for 30 sec in a cyclomixer (Remi). Number of cells in the suspension was determined using a Neubauer's

counting chamber and a phase contrast microscope (Olympus CH3-BH-PC, Japan). Cell suspensions of selected strains were prepared (10^5 to 10^6 cells ml^{-1}) and 2 ml of each mixture was inoculated to 100 g sterilised (121° C for 15 min, cooled to ~ 40° C) seeds of finger millet. Inoculated seeds were saccharified in sterile 250 ml-bottles, which were loosely capped, incubated at 28° C for 2 days. After saccharification, lids were tightly capped and further fermented at same temperature for 6 days. The rationale behind selecting the inoculum size was based on the observation on conventional method of adding ~ 2 % of marcha in boiled seeds of finger millets during kodo ko jaanr preparation. The products were evaluated physico-chemically and organoleptically using the method as described.

*Sensory evaluation***:** Sensory properties of product were evaluated in terms of aroma, taste, texture, colour and general acceptability. Kodo ko jaanr samples produced by selected strains were organoleptically evaluated by a panel of 7 judges with score rate of 1, bad and 5, good considering market jaanr as control with scoring rate of 3, moderate.

Consumers' Preference Trial: Market samples of kodo ko jaanr as well as kodo ko jaanr prepared in the laboratory by using a mixture of selected strains were served to 50 consumers representing different ethnic groups of people of Sikkim who were familiar with kodo ko jaanr. The 9-point scale used in this study ranged from 'dislike extremely' (score, 1) to 'like extremely' (score, 9).

Statistical analysis: Data were analysed by determining standard deviation (SD), standard error of measurement (SEM) and analysis of variance (ANOVA) [22].

Results and Discussion

Successional studies during kodo ko jaanr fermentation were carried daily from 0-10 d (Table 1). Population of filamentous moulds, which were originated from marcha, declined significantly ($P<0.05$) every d during fermentation and finally disappeared on fifth d. Filamentous moulds isolated during fermentation were identified, based on the taxonomical keys[8,9,10] (data not shown) as *Mucor circinelloides*, *Rhizopus chinensis* and *Rhizopus stolonifer*. Load of yeasts increased significantly ($P<0.05$) from 10^5 cfu g^{-1} to 10^7 cfu g^{-1} within second d, indicating their roles in amylase production during fermentation. Isolated yeasts were from fermenting finger millets were characterised based on the identification keys of Kreger-van Rij[11] and Kurtzman and Fell[12] (data

Table 1. Changes in microbial load during kodo ko jaanr fermentation.

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

Data represent the means ± SD of three batches of fermentation. Data were transformed into logarithmic values.

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

not shown), and were identified as *Saccharomycopsis fibuligera, Pichia anomala, Saccharomyces cerevisiae* and *Candida glabrata*. Subsequently, load of LAB increased significantly ($P<0.05$) from 10^6 cfu g^{-1} to 10^8 cfu g^{-1} in the first d and decreased significantly ($P<0.05$) to a level of 10^3 cfu g^{-1} at the end. LAB strains were isolated and identified as *Pediococcus pentosaceus* and *Lactobacillus bifermentans*, based on phenotypic characters described by Schillinger and Lücke[13], Wood and Holzapfel[14]. Total viable counts increased significantly ($P<0.05$) within first d and gradually decreased every d during fermentation.

Temperature of fermenting finger millet increased significantly ($P<0.05$) from 26° C to 30° C within second d due to exponential growth of mixed population of microorganisms, and then remained constant around 28° C (Table 2). The pH decreased significantly ($P<0.05$) from 6.37 to 4.10 within second d of fermentation and after second d, decline in pH was non-significant. Titratable acidity increased significantly ($P<0.05$) till the fourth d, and remained the same till the end (Table 2). The cause of increase in acidity and consequent drop in pH during fermentation of cereal was likely due to utilization of free sugars of the substrate by yeasts and LAB[23, 24], since all the strains were able to ferment glucose. Alcohol content increased significantly ($P<0.05$) from 0.1 % to 6.9 % within sixth d and slightly decreased to 6.5 % at the end (Table 2). The result showed that reducing sugar content increased significantly ($P<0.05$) till third d followed by decrease in total sugar content (Table 3). This is due to maximum break down of starch of substrates to reducing sugars by amylolytic enzymes, produced by moulds and yeasts during fermentation[25]. Maximum activities of saccharification (glucoamylase) and liquefaction (μ-amylase) of finger millets were observed on the second d of fermentation (Table 3).

Preliminary screenings of amylolytic activities of all 110 isolates (27 mould isolates, 53 yeasts isolates and 30 LAB isolates), isolated from fermenting finger millets during succesional studies were tested in starch agar plates. On the basis of amylolytic activity (strains showing >2.0 mm hydrolysis zone in agar plate), 4 strains of *Rhizopus* spp., 2 strains of *Mucor* spp., 5 strains of *Saccharomycopsis fibuligera*, 4 strains of *Pichia anomala*, 4 strains of *Saccharomyces cerevisae* and 3 strains of *Candida glabrata* were selected for liquefying and saccharifying activities (Table 4). None of the LAB strains showed amylolytic activity; hence, they were not selected for amylolytic enzyme assay. Saccharifying activities were mostly shown by *Rhizopus* spp. and *Saccharomycopsis*

Table 2. Physico-chemical changes during kodo ko jaanr fermentation.

Fermentation time (days)	Temperature (°C)	pH	Acidity (%)	Alcohol (%)
0	26.0 ± 0.00^{f}	6.4 ± 0.01^{a}	0.01 ± 0.00^{e}	0.1 ± 0.11^{h}
1	28.8 ± 0.05^{c}	4.4 ± 0.01^{b}	0.08 ± 0.01^{d}	0.5 ± 0.08^{g}
2	30.0 ± 0.13^{a}	4.1 ± 0.01^{c}	0.14 ± 0.01^{c}	2.7 ± 0.08^{f}
3	29.5 ± 0.00^{b}	4.1 ± 0.02^{c}	0.18 ± 0.01^{b}	3.1 ± 0.08^{e}
4	29.0 ± 0.00^{c}	4.1 ± 0.01^{c}	0.24 ± 0.01^{a}	4.1 ± 0.08^{d}
5	29.0 ± 0.05^{c}	4.1 ± 0.01^{c}	0.23 ± 0.00^{a}	5.5 ± 0.08^{c}
6	28.8 ± 0.21^{c}	4.1 ± 0.01^{c}	0.23 ± 0.01^{a}	6.9 ± 0.21^{a}
7	28.3 ± 0.08^{d}	4.1 ± 0.01^{c}	0.22 ± 0.01^{a}	6.8 ± 0.13^{a}
8	28.0 ± 0.00^{e}	4.1 ± 0.02^{c}	0.23 ± 0.01^{a}	6.8 ± 0.08^{a}
9	28.0 ± 0.00^{e}	4.1 ± 0.01^{c}	0.22 ± 0.01^{a}	6.6 ± 0.08^{b}
10	28.0 ± 0.00^{e}	4.1 ± 0.01^{c}	0.23 ± 0.01^{a}	6.5 ± 0.08^{b}

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

Table 3. Biochemical and enzymatic changes during kodo ko jaanr fermentation.

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

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

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

Table 4. Amylolytic activities of functional microorganisms isolated during fermentation of kodo ko jaanr.

Group	Strain	Liquefying activity (U ml-^{1})	Saccharifying activity (U ml-^{1})
Mould	*Rhizopus stolonifer* KJ:R1	5.3	71.3
	Rhizopus stolonifer KJ:R2	2.8	59.3
	Rhizopus chinensis MJ:R3	5.8	96.3
	Rhizopus chinensis MK:R5	0.7	87.4
	Mucor circinelloides MS:M1	1.8	16.8
	Mucor circinelloides MS:M7	2.6	24.0
Yeasts	*Saccharomycopsis fibuligera* KJ:S2	4.2	47.0
	Saccharomycopsis fibuligera KJ:S4	4.5	43.1
	Saccharomycopsis fibuligera KJ:S5	6.8	80.1
	Saccharomycopsis fibuligera KJ:S7	5.6	63.3
	Saccharomycopsis fibuligera KJ:S9	4.8	62.8
	Pichia anomala MA:YP2	4.4	45.7
	Pichia anomala MA:YP3	4.3	23.1
	Pichia anomala MA:YP5	2.0	37.0
	Pichia anomala MA:YP8	2.2	24.5
	Saccharomyces cerevisiae MJ:YS1	4.1	18.8
	Saccharomyces cerevisiae MJ:YS2	5.6	29.5
	Saccharomyces cerevisiae MJ:YS3	4.6	24.5
	Saccharomyces cerevisiae MJ:YS5	1.2	9.1
	Candida glabrata MS:YC2	1.2	19.4
	Candida glabrata MS:YC4	1.6	27.1
	Candida glabrata MS:YC5	1.7	35.8

Data represent the means of 3 sets of experiment.

fibuligera whereas liquefying activities were shown by *Saccharomycopsis fibuligera* and *Saccharomyces cerevisiae* (Table 4). Fermented rice, sweet-sour paste of Indonesia, *Saccharomycopsis fibuligera* produced mainly a-amylase and *Rhizopus* sp. produced glucoamylase[26]. *Saccharomycopsis fibuligera* played the main roles in amylase production whereas *Rhizopus* seemed to supplement the saccharification[27,28,29,30,31]. *Rhizopus* is known to produce good amount of glucomaylase[32,33]. The result indicated that *Saccharomycopsis fibuligera* and *Rhizopus* spp play the major role in saccharification process of kodo ko jaanr fermentation, breaking starch of substrates into glucose for ethanol production. *Mucor* spp., *Pichia anomala* and *Candida glabrata, Saccharomyces cerevisiae* may supplement the saccharification.

For testing ability of selected strains to produce kodo ko jaanr, *Rhizopus chinensis* MJ:R3 and *Saccharomycopsis fibuligera* KJ:S5 were selected on the basis of highest saccharifying and liquefying activities, respectively (Table 4). One strain of mould *Mucor circinnelloides* MS:M7, three strains of yeasts *Saccharomyces cerevisiae* MJ:YS2, *Candida glabrata* MS:YC5 and *Pichia anomola* MA:YP2 were also selected on the basis of high amylolytic activities among the same genera (Table 4); two LAB strains *Lactobacillus bifermentans* MA:R5 and *Pediococcus pentosaceus* MA were selected randomly. These strains were inoculated with the sterilised finger millet seeds to produce kodo ko jaanr. Kodo ko jaanr prepared by a combination of *Rhizopus chinensis* MJ:R3 and *Saccharomycopsis fibuligera* KJ:S5 showed significantly (P<0.05) high reducing sugar contents during saccharification period of 2 d with low alcohol content of 1 % on 6 d (Table 5). Cell suspension mixture of *Rhizopus chinensis* MJ:R3 and *Saccharomyces cerevisiae* KJ:S5 produced jaanr with significantly (P<0.05) high reducing sugar and

Table 5. Changes in pH, reducing sugar and alcohol in fermented finger millets by selected strains.

	pH		Reducing sugar (%)		Alcohol (%)	
Cooked millet (non-inoculated)	6.37 ± 0.01		0.4 ± 0.16		0.1 ± 0.06	
Strain	2 days	6 days	2 days	6 days	2 days	6 days
Rhizopus chinensis MJ:R3 with						
Mucor circinnelloides MS:M7	4.6 ± 0.02^{e}	4.8 ± 0.01^{e}	2.10 ± 0.19^{ef}	3.10 ± 0.24^{b}	0.25 ± 0.06^{f}	0.83 ± 0.06^{f}
Saccharomyces cerevisiae MJ:YS2	4.3 ± 0.01^{f}	4.4 ± 0.01^{h}	4.21 ± 0.05^{c}	3.57 ± 0.05^{a}	2.50 ± 0.07^{a}	4.40± 0.13^{a}
Candida glabrata MS:YC5	4.3 ± 0.01^{f}	4.3 ± 0.00^{i}	3.95 ± 0.11^{c}	2.00 ± 0.19^{ef}	0.70 ± 0.06^{d}	1.80 ± 0.00^{d}
Pichia anomola MA:YP2	4.6 ± 0.00^{e}	4.7 ± 0.01^{f}	2.49 ± 0.08^{def}	3.13 ± 0.45^{b}	0.76 ± 0.00^{c}	2.20 ± 0.06^{c}
Lb. bifermentans MA:R5 + *P. pentosaceus* MA:C1	4.1 ± 0.01^{h}	4.2 ± 0.01^{j}	2.71 ± 0.46^{d}	2.89 ± 0.16^{bc}	1.00 ± 0.06^{b}	2.50 ± 0.06^{b}
Saccharomycopsis fibuligera KJ:S5 with						
Rhizopus chinensis MJ:R3	4.2 ± 0.02^{g}	4.6 ± 0.02^{g}	6.28 ± 0.14^{a}	2.55 ± 0.05^{cd}	0.40 ± 0.07^{e}	1.00 ± 0.06^{e}
Mucorc circinnelloides MS:M7	6.0 ± 0.01^{c}	6.1 ± 0.02^{bc}	4.94 ± 0.04^{b}	2.22 ± 0.05^{de}	0.15 ± 0.00^{g}	0.30 ± 0.00^{j}
Saccharomyces cerevisiae MJ:YS2	6.1 ± 0.02^{b}	6.1 ± 0.01^{b}	4.74 ± 0.75^{bc}	2.63 ± 0.11^{c}	0.22 ± 0.06^{f}	0.50 ± 0.06^{hi}
Candida glabrata MS:YC5	5.9 ± 0.00^{d}	6.0 ± 0.04^{d}	3.07 ± 0.20^{d}	1.65 ± 0.01^{f}	0.15 ± 0.00^{g}	0.45 ± 0.00^{i}
Pichia anomola MA:YP2	6.1 ± 0.00^{b}	6.1 ± 0.00^{c}	2.61 ± 0.29^{de}	1.92 ± 0.11^{ef}	0.15 ± 0.06^{g}	0.68 ± 0.06^{g}
Lb. bifermentans MA:R5 + *P. pentosaceus* MA:C1	6.0 ± 0.01^{c}	6.1 ± 0.02^{bc}	2.29 ± 0.08^{def}	2.64 ± 0.04^{c}	0.20 ± 0.06^{g}	0.75 ± 0.00^{fg}
All strains*	6.4 ± 0.02^{a}	6.4 ± 0.00^{a}	1.91 ± 0.41^{f}	1.98 ± 0.02^{ef}	0.05 ± 0.06^{h}	0.15 ± 0.00^{k}

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

*Cell mixture of all above mentioned strains.

Table 6. Sensory evaluation of kodo ko jaanr produced by selected strains.

Strain	Aroma	Taste	Texture	Colour	General acceptability
Rhizopus chinensis MJ:R3 with					
Mucor circinnelloides MS:M7	2.00 ± 0.93^{ab}	1.86 ± 0.52^{b}	2.14 ± 0.83^{b}	2.86 ± 0.83^{bcd}	2.29 ± 0.70^{bcd}
Saccharomyces cerevisiae MJ:YS2	3.43 ± 0.50^{a}	2.79 ± 0.36^{a}	3.43 ± 0.90^{a}	4.29 ± 1.03^{a}	4.43 ± 0.73^{a}
Candida glabrata MS:YC5	2.43 ± 0.90^{ab}	1.57 ± 0.73^{b}	2.57 ± 0.73^{ab}	3.00 ± 0.93^{abc}	2.43 ± 0.50^{bc}
Pichia anomola MA:YP2	3.43 ± 0.90^{a}	1.86 ± 0.69^{b}	2.43 ± 0.50^{ab}	3.71 ± 0.88^{ab}	2.57 ± 0.50^{b}
Lb. bifermentans MA:R5 + *P. pentosaceus* MA:C1	2.29 ± 0.88^{ab}	1.79 ± 0.53^{c}	2.43 ± 1.18^{ab}	3.14 ± 0.99^{abc}	2.29 ± 0.88^{bcd}
Saccharomycopsis fibuligera KJ:S5 with					
Rhizopus chinensis MJ:R3	1.86 ± 0.83^{ab}	1.29 ± 0.36^{b}	2.43 ± 0.50^{ab}	1.86 ± 0.64^{cde}	1.57 ± 0.50^{bcdf}
Mucor circinnelloides MS:M7	1.86 ± 0.83^{ab}	1.21 ± 0.36^{b}	2.29 ± 0.88^{ab}	1.86 ± 0.83^{cde}	1.43 ± 0.50^{cdef}
Saccharomyces cerevisiae MJ:YS2	1.43 ± 0.73^{b}	1.14 ± 0.35^{b}	1.71 ± 0.88^{b}	1.29 ± 0.70^{e}	1.14 ± 0.35^{ef}
Candida glabrata MS:YC5	1.43 ± 0.73^{b}	1.21 ± 0.36^{b}	2.00 ± 0.76^{b}	1.29 ± 0.70^{e}	1.29 ± 0.70^{def}
Pichia anomola MA:YP2	2.29 ± 0.88^{ab}	1.21 ± 0.30^{b}	2.00 ± 1.07^{b}	1.43 ± 0.73^{e}	1.43 ± 0.73^{cdef}
Lb. bifermentans MA:R5 + *P. pentosaceus* MA:C1	1.57 ± 0.73^{b}	1.29 ± 0.36^{b}	2.29 ± 1.03^{ab}	1.57 ± 0.90^{de}	1.43 ± 0.73^{cdef}
All strains*	1.43 ± 0.73^{b}	1.00 ± 0.00^{b}	1.43 ± 0.73^{b}	1.14 ± 0.35^{e}	1.00 ± 0.00^{f}

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

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

*Cell mixture of all above mentioned strains.

high alcohol content of 4.4 % in 6 d than jaanr samples fermented by other strains (Table 5).

Table 6 shows the sensory evaluation of kodo ko jaanr produced by selected combination of strains. There was no significant ($P<0.05$) difference in aroma attribute of jaanr prepared by a cell suspension mixture of *Rhizopus chinensis* MJ:R3 with other strains, except jaanr prepared by a combination of *Saccharomycopsis fibuligera* KJ:S5 with *Saccharomyces cerevisiae* MJ:YS2, *Candida glabrata* MS:YC5, and all strains. There was a significant ($P<0.05$) difference in taste score of jaanr prepared by cell mixture of *Rhizopus chinensis* MJ:R3 and *Saccharomyces cerevisiae* MJ:YS2 with that of other strains. However, significance ($P<0.05$) difference in texture and colour scores was observed in some jaanr samples. Jaanr product prepared by a combination of *Rhizopus chinensis* MJ:R3 and *Saccharomycopsis fibuligera* KJ:S5 had desirable sweet-sour taste but unpleasant odour due to low alcohol content. Hence based on sensory criteria, jaanr produced by these strains were unacceptable to consumers. Kodo ko jaanr prepared by a combination of *Rhizopus chinensis* MJ:R3 and *Saccharomyces cerevisiae* MJ:YS2 showed significantly ($P<0.05$) highest score in general acceptability. Kodo ko jaanr prepared by these strains had mild alcoholic-sweet flavour, significantly ($P<0.05$) acceptable to consumers. *Saccharomyces cerevisiae* posseses strong tendency to ferment sugars into alcohol[34]. Whereas jaanr prepared by a combination of *Rhizopus chinensis* MJ:R3 and *Saccharomycopsis fibuligera* KJ:S5 had sweet-sour taste but due to low-alcohol content, the product had unpleasant odour, which could not be considered as good quality jaanr. *Saccharomycopsis fibuligera* is reported to produce high biomass during fermentation of cassava starch, which leads to low ethanol yield[35].

The consumers' preference trial showed that kodo ko jaanr prepared by a mixture of *Rhizopus chinensis*

MJ:R3 and *Saccharomyces cerevisiae* MJ:YS2 as starter was more acceptable than the kodo ko jaanr prepared by conventional marcha. Market jaanr was liked extremely (score, 9) by 10 %, very much (score, 8) by 30 % and moderately (score, 7) by 60 %; the laboratory-made jaanr was liked extremely by 40 %, very much by 50 % and moderately by 10 % of the consumers (data not shown). The results of the present studies demonstrate that to make a good quality jaanr, a mixture of a selected strain of mould *(Rhizopus*) and one amylolytic yeast (*Saccharomyces cerevisiae*) may be used as a mixed pure starter. Similar observation of using a mixture of mould and yeast in production of Vietnamese rice wine was reported[36]. Kodo ko jaanr prepared by a mixed pure culture may have more advantages over the jaanr prepared by using marcha due to better quality in product, maintaining consistency and maximum utilization of substrates.

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