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Summary

The present study reveals that some of the indigenous legume-based fermented products, particularly made from the legume (such as akuni, bekang, hawaijar, kinema, masuyra and turumbai), are still confined to the area of their preparation, while others know no geographical barriers. Like many other states in India, the people of Orissa have a tradition of relishing a variety of cakes, locally called pitha, specially prepared during various festivals and rituals. These products include chakuli, chhuchipatra pitha, enduri pitha, maunha pitha, poda pitha and chitou, which are unknown to the scientific community.

Dhokla constitutes one of the categories of the traditional Gujarati dishes. Dhokla batter had higher moisture than its substrates. In the fermented batter titratable and free fatty acid values increased, but the pH value decreased. The respective contents of crude fat and carbohydrate in Bengalgram dal and polished rice were higher than those of fermented batter. All the samples showed the occurrence of total aerobic mesophilic bacteria (TAMB) and aerobic mesophilic bacterial spores (aMBS). A total of 375 strains of lactic acid bacteria (135 strains of *Leuconostoc mesenteroides*, 100 strains of *Lactobacillus*

fermentum and 140 strains of *Pediococcus pentosaceus*) and 240 strains of yeast (100 strains of *Saccharomyces cerevisiae* 135 strains of *Pichia silvicola* and 5 strains of *Pichia membranifaciens*) were isolated from three samples each of white polished rice and Bengalgram dal, 10 market samples of fermented batter, and 21 samples of laboratory-made fermenting and fermented batters of dhokla. *L. mesenteroides*, *P. pentosaceus* and *S. cerevisiae* were recovered from all the samples studied. *L. fermentum* and *P. silvicola* were found in raw dal and mixed batter only, but not in rice. *P. membranifaciens* was encountered in 10% of fermented batter of marketed samples only.

Succession of microbiota during dhokla batter fermentation under semicontrolled conditions (Bengalgram dal and rice in 4:1, fermentation at 32°C for 15 h, steam-cooking for 10-15 min) revealed that *L. fermentum* was the dominant bacterium increasing significantly at every 3 h-interval, followed by *L. mesenteroides* and *P. pentosaceus*, resulting in a decreased pH (4.7). Although *S. cerevisiae* and *P. silvicola* were present althrough, the latter dominated the fermentation. The contents of titratable acid, free fatty acid, soluble nitrogen and also batter volume increased significantly after fermentation.

The antioxidant activities of methanolic extracts of nonfermented and fermented batters of dhokla and steam-cooked product were evaluated by four *in vitro* methods. The yield of lyophilized crude extract of the fermented batter was higher than that of nonfermented batter and steamed product. While fermentation enhanced phenolic content of the batter by about 2.5-fold, steam-cooking of fermented batter for 10-15 min significantly reduced the content. At all the tested concentrations (10-50 mg ml^{-1}), the methanolic extract of fermented batter showed a better antiradical activity than that of corresponding nonfermented batter and steamed product, which increased in a time-dose-response manner. The DPPH·-scavenging ability of the extracts increased significantly up to 30 min of reaction and then leveled off despite further increase in time. Fermented batter had the better IC_{50} and higher relative DPPH·-scavenging activity than those of steamed product and nonfermented batter. Fermentation also caused a 1.9-fold increase in the reducing power of dhokla batter which eventually reduced by 96% during steam-cooking. Though the Fe^{2+}-chelating activity of the crude extract of steam-cooked dhokla batter was superior to the corresponding batters at 10 mg ml^{-1} concentration, fermented batter showed better chelating activities at higher dose levels. The chelating effects of the extracts increased up to a certain extent with the increase in dosage level, and then leveled off despite further increase in concentration. IC_{50} of the methanolic extracts of the samples indicates that steamed product was an efficient Fe^{2+}-chelating agent, followed by fermented and nonfermented batters. While fermentation significantly enhanced the relative Fe^{2+}-chelating ability of the batter, steam-cooking of the batter reduced the same by 69%. The extracts of fermented batter also exhibited a better LPIA than its corresponding nonfermented batter and steamed product at all the tested concentrations. However, when kept for a longer period of incubation, the LPIA of the extracts was found to decline significantly. The total phenol contents of nonfermented and fermented batters and steamed product exhibited a positive correlation with respective values of antiradical activity, reducing power, metal-chelating activity and LPIA. Relevant regression equations showed that the total phenol contents of nonfermented batter, fermented batter and dhokla could explain 96, 90 and 89% DPPH·-scavenging activities, 76, 82 and 77% reducing power, 74, 71 and 43% metal-chelating ability, and 83, 88 and 95% LPIA, respectively. The data also revealed a significant correlation between any two of these five parameters, indicating while total phenol content as the dependent variable, all other parameters as the dependent variables.

Dosa, a thin, fairly crisp, fried and highly seasoned griddled pancake-like food is indigenous to southern India. The fermented batter was acidic (pH 4.5) and had moisture content of 67.8%, and per 100 g dry weight basis 1.1 g ash, 1.5 g crude fat, 3 g total nitrogen, 1.8 g protein nitrogen, 1.2 g nonprotein nitrogen and 1 g soluble nitrogen. The titratable and free fatty acid contents of fermented

batter were 0.8% and 1.8%, respectively. The toal protein and carbohydrate contents of fermented batter indicate dosa as a nutritionally rich fermented product. A total of 90 strains of lactic acid bacteria (60 of *L. mesenteroides* and 30 of *P. pentosaceus*) and 85 strains of yeasts (40 each of *S. cerevisiae* and *Issatchenkia orientalis* and 5 of *Rhodotorula minuta*) were isolated from 12 market-samples of fermented batter of dosa. While the dominant lactic acid bacteria were identified as *L. mesenteroides* and *P. pentosaceus*, the dominant yeast was *S. cerevisiae*. *R. minuta* was encountered in only 8% of the samples. While *L. mesenteroides* was recovered from all the samples studied, the prevalence of each of *S. cerevisiae* and *I. orientalis* was in 67% of the samples.

Idli,is highly popular and widely consumed as a snack food in India. The moisture content of fermented idli batter was six-fold higher than that of the substrates. The acidic batter registered a significantly higher titratable and free fatty acid contents. The process parameters for the preparation of idli, with respect to ingredient proportions and fermentation time-temperature, were optimized under semicontrolled conditions. The proportion of parboiled rice and blackgram dal as 2:1 and fermentation period of 18 h at 30°C were found optimum with respect to all sensory scores. A total of 295 strains of lactic acid bacteria (180 of *L. mesenteroides* and 115 of *P. pentosaceus*), 365 strains of yeasts (165 of *S. cerevisiae*, 100 of *I. orientalis*, 90 of *P. membranifaciens* and 10 of *R. minuta*) and 30 strains of *Mucor racemosus* (mould) were isolated from three samples of raw blackgram dal, 12 marketed samples of fermented batter and 21 samples of batters fermenting under optimized conditions.

Heterofermentative *L. mesenteroides* was recovered from all the samples studied, excepting raw rice, confirming the source and role of the organism in idli batter fermentation. *P. pentosaceus*, although appeared only after 3 h of the start of fermentation, dominated thereafter. Like *L. mesenteroides*, *S. cerevisiae* was recorded in raw blackgram dal and in 100% samples of the batter fermenting althrough. *I. orientalis* and *P. membranifaciens*, hitherto unreported from fermenting idli batter, were although not present at the start, dominated the later stages of fermentation. During the fermentation, pH of the batter dropped from 5.9 to 4.3 along with a three-fold increase in titratable acidity and a two-fold increase in batter volume. *M. racemosus* was present at the onset of fermentation, but declined after 3 h and disappeared after 6 h. Among the isolates, the last one happened to be the only organism capable of hydrolyzing starch. So its importance in idli batter fermentation by hydrolyzing rice starch to simple sugars conducive for the growth of lactic acid bacteria and yeasts is indicated.

The fermented batter of idli showed a higher yield of lyophilized crude extract than its corresponding steamed product and nonfermented batter when extracted in methanol. Fermentation also caused 135% increase in total phenolic content of idli batter. However, steam-cooking for 10-15 min significantly reduced the total phenolic content. At all the tested concentrations, the methanolic extract of fermented batter exhibited a better free radical-scavenging activity than the corresponding nonfermented batter and steamed product, which increased in a time-dose-response manner. The scavenging ability of the extracts increased nearly up to 30 min of reaction and then leveled off despite further increase in time. IC_{50} and the relative DPPH-scavenging activity of the extracts of fermented batter were superior to the corresponding steamed product and nonfermented batter. The methanolic extract of fermented batter of idli showed 125% more reducing power than that of nonfermented batter. Steam-cooking for 10-15 min resulted in about 2.3-fold decrease in the reducing activity of the same. While the extract of idli showed a higher metal-chelating activity at lower dose (10 mg ml^{-1}), the chelating activity of fermented batter was better than that of corresponding nonfermented and steamed product at higher dose (30 mg ml^{-1}). Chelating effects of the extracts increased up to a certain extent with the increase in dosage level, and leveled off despite further increase in concentration. Methanolic extracts of fermented batter of idli showed a better IC_{50} closely followed by the steamed product. While

the fermentation enhanced the relative chelating activity of the batter by 2.2-fold, steam-cooking of the fermented batter for 10-15 min reduced the same by 46%. Extracts of fermented batter also exhibited a better LPIA than those of nonfermented batter and steamed product at all the tested concentrations. However, the LPIA of all the samples declined with time after 72 h.

Total phenol contents of the nonfermented and fermented batters and steamed product exhibited a positive correlation with antiradical activity, reducing power, metal-chelating activity and LPIA. Relevant regression equations showed that the total phenol contents of nonfermented and fermented batters and idli could explain 61%, 92% and 90% antiradical activity, 50%, 80% and 90% reducing power, 50%, 82% and 41% metal-chelating ability and 62%, 91% and 84% LPIA, respectively. The data also revealed a significant correlation between any two of these five parameters, indicating total phenol content as the dependent variable, while all other parameters as the dependent variables.

The antioxidant activities of methanolic extract of kinema, fermented using *Bacillus subtilis*, and cooked nonfermented (CNF) soybean were evaluated by four *in vitro* methods. The average yields of lyophilized methanolic extracts of CNF soybean and kinema were 91 and 154 mg g^{-1} (dry weight basis), respectively. The total phenol content of kinema was 144% higher than that of CNF soybean. At all the tested concentrations, kinema extract was found to be a better free radical-scavenger, which increased in a time and dose-dependent manner than the corresponding CNF soybean extract. The scavenging activity increased nearly up to 40 min of reaction and then leveled off with further increase in time. IC_5 of CNF soybean and kinema was better than that of CNF soybean. Fermentation caused a two-fold enhancement of the relative DPPH·-scavenging activity of CNF soybean. The reducing power of kinema was 147% higher than that of CNF soybean, which reflects the influence of fermentation on the enhancement of reducing power of CNF soybean. At 10 mg ml^{-1}, the methanolic extract of kinema exhibited 64% of metal-chelation which was much higher than the activity shown by CNF soybean (22%). The chelating ability increased with the increase in concentration. The methanolic extracts of kinema was two-fold efficient than that of CNF soybean for the chelation of the initial Fe^{2+} concentration by 50%. A higher relative Fe^{2+}-chelating ability was observed in kinema than the CNF soybean. The extracts of kinema exhibited a better LPIA than the CNF soybean. The inhibition of both of them was found to decline with time.

The total phenol contents of kinema and CNF soybean were positively correlated with the respective values of free radical-scavenging activity, reducing power, metal-chelating activity and LPIA. Whereas the total phenol content in CNF soybean accounted for 68% of each of free radical-scavenging activity and reducing power, 58% of Fe^{2+}-chelating activity and 66% of LPIA, that in kinema could reflect 83-92% of all the antioxidant parameters studied. The data also revealed a significant correlation ($P < 0.01$) between any two of these five parameters, indicating while total phenol content as the independent variable, all other parameters as the dependent variables. Thus, kinema may be exploited as a functional food to alleviate oxidative stress.

Papad constitutes an important food adjunct, manufactured and extensively consumed in India. While pH, titratable and free fatty acidity, and the contents of moisture, ash soluble nitrogen and crude fat of papad were significantly higher than those of its substrates, the carbohydrate contents of the substrates were higher than those of the products. Traditional process parameters for the preparation of papad were optimized with respect to ingredients' proportions, and amount of papad khar, common salt and water to be added to the flour blend. The proportion of blackgram and mung dal flours as 1:2 and the addition of 15 g, 70 g and 500 ml kg^{-1} legume flour blend of papad khar, common salt and water, respectively, per kg flour blend were found optimum with respect to the rolling property and handfeel of the papad dough as well as to all sensory scores when the mixed

dough fermented for 3 h at room temperature (28± 2°C) was rolled into thin sheets and dried under semicontrolled conditions (30± 1 °C and 70 ± 5 % relative humidity) for 8 h.

A total of 80 strains of *P. pentosaceus* and 75 strains of *S. cerevisiae* were isolated from 3 samples each of raw blackgram and mung dal flours, and 18 samples of laboratory-made fermenting papad dough and drying papads. Though both *P. pentosaceus* and *S. cerevisiae* were isolated from the substrates, they were not recovered from the papads. While TAMB cells and their spore counts prevailed throughout the preparation process, S. *cerervisiae* and *P. pentosaceus*, after initial increase in their load exhibited gradual decrease and eventually went below the detection limit after 6 h of drying. The papad sheets showed sharp decline in their moisture content during drying. The pronounced effect of decline in moisture content of papad was evident from the significant increase in percentage diametric expansion of papad sheets.

Wadi, a savoury prepared traditionally from bengalgram dal by fermentation following natural inoculation and sun-drying, is popularly consumed in many places in India. The similar product has different synonyms in different parts of country (Punjabi wadi in the States of northern India, bori in West Bengal and Orissa, adhauri or wadi in Bihar and Jharkhand, and masyuara in Darjeeling hills of West Bengal and Sikkim. Wadi had the higher moisture content than that of the substrate. The contents of moisture, nonprotein nitrogen, soluble nitrogen, crude fat and carbohydrate were higher in the wadi than those of substrates. The fermentation of blackgram dal to wadi caused a decrease in pH. The titratable and free fatty acid, ash, protein-nitrogen and crude fat contents of wadi were higher than those of the blackgram dal.

A total of 555 strains of lactic acid bacteria (255 of *L. mesenteroides* and 300 of *P. pentosaceus*) and 635 strains of yeasts (170 of *S. cerevisiae*, 190 of *I. orientalis*, 250 of *P. membranifaciens* and 25 of *R. minuta*) were isolated from three samples of raw blackgram dal, 41 samples of market-fermented wadi, and 21 samples of laboratory-made fermenting and fermented wadi. A total of 30 strains of *M. racemosus* were also recovered from the market samples.

Wadi was prepared under semicontrolled conditions which mimiced the traditional method of preparation. The blackgram dal batter, after fermentation at 32°C for 10 h, was hand-moulded to small cones (3-5 cm diameter) and deposited on a greased bamboo mat and sun-dried (29-33°C) for 8 h followed by 16 h shade-drying at room temperature (28-30°C) daily for three successive days. *L. mesenteroides*, *P. pentosaceus* and *S. cerevisiae* comprised the major components of the wadi microbiota. Their prevalence in the laboratory-made wadi (100%) was significantly higher than that of market samples and the substrates. After a significant increase during fermentation and initial drying for 24 h, their loads gradually declined towards the end. The growth kinetics of the TAMB cells was also similar to that of dominant microbiota. While *I. orientalis* was detected only after 10 h-fermentation of wadi dough, *P. membranifaciens* appeared after 12 h of drying. Though they dominated the later stages of the drying, their occurrence in the market samples was 54% and 88%, respectively. *R. minuta* and *M. racemosus*, though isolated from 12 and 15% of the marketed wadi, respectively, were neither encountered during the preparation of wadi under semicontrolled conditions, nor recovered from the ingredients. The batter volume of the wadi increased by 1.4-fold after fermentation. Subsequent drying of wadi reduced the initial moisture content by 4.3 times. The overall pH declined from 6.2 to 4.9. Fermentation also resulted in the significant increase in titratable and free fatty acidity and soluble nitrogen contents of the substrate. The protein nitrogen declined during the fermentation, but gradually increased during the subsequent drying period.