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Summary

Legume-based traditional fermented foods that impart variety, nutrition and delicacy to the diet, may become an important carrier of various micro-organisms implicating possible health hazards for consumers. This project was undertaken with the objective of evaluating microbiological quality of legume-based traditional fermented foods marketed in West Bengal, India and understanding some behavioural patterns of the pathogenic isolates from those. A total of 105 samples of six different kinds of foods collected from 83 retail outlets scattered over 16 districts of the State of West Bengal was investigated to determine their microbiological quality status. Majority of the samples of each of the six kinds foods, except dosa, had a high count (> 10^4 cfu g^{-1}) of these bacteria, indicating a lapse in good hygiene practices followed in preparing these foods. Every kind of foods was contaminated with *Bacillus cereus*, Enterobacteriaceae and coliforms. *Staphylococcus aureus* was present in only one sample of dhokla (4×10^4 cfu g^{-1}). Faecal coliforms were present in dhokla, idli, papad and wadi. One sample each of idli (3.8×10^3 cfu g^{-1}) and wadi (3.2×10^4 cfu g^{-1}) were found contaminated with *Escherichia coli*. *Salmonella* was present in 11.4% (12 of 105) of the total samples analysed. It was present in idli, papad

and wadi. *Clostridium perfringens* and *Shigella* could not be detected in any of the samples. *B. cereus*, Enterobacteriaceae, coliforms and faecal coliforms were present in 20, 46, 28, and 11%, respectively, of the samples analysed. among the critical control points (CCPs) are raw materials, water, beating or mixing batter or dough, utensils, drying environment (in case of papad and dough), post-preparative storage conditions, and dish cloth used at the time of serving.

Strains of foodborne bacterial pathogens that are resistant to a variety of antimicrobial agents have become a major health concern. The extent of prevalence of antimicrobial resistance among the food isolates were determined. The tested strains of *B. cereus*, Enterobacteriaceae and *Salmonella* were found resistant to at least nine, four and five antimicrobials, respectively. Most of the antibiotics against which these isolates showed resistance inhibit synthesis of prokaryotic cell wall, however were sensitive to those inhibiting protein and nucleic acid syntheses. This situation invites a challenge to tackle the problem of antibiotic resistance in foods

The *D*-values for spores help to understand the hazardous potential of these organisms which can survive the cooking processes. In glucose-supplemented brain heart infusion broth, the mean $D_{100°C}$-values for 12 different isolates *B. cereus* spores ranged from 3.0-9.2 min, suggesting that time-temperature exposure at an appropriate level during cooking may destroy heat-sensitive spores, but not the heat-resistant ones. The data can be used as an aid to predict the time required at 100 °C to achieve a certain number of log-cycle reductions of this pathogen.

Activity of the enzymes, like protease, lipase and amylase indicates spoilage potentiality of the producing organisms. So, it was found that 50% of the tested strains of *B. cereus* present in fermented foods had the potentiality of causing food spoilage too.

All the representative isolates, except *B. cereus*, grew optimally at a pH range of 6.11-7.26. The The minimum inhibitory concentrations (MICs) of sodium chloride were 65-110 mg ml^{-1} and of benzoic acid for *B. cereus*, *S. aureus*, *E. coli* and *Salmonella* were 400-650 µg ml^{-1}. Similarly, the MICs of sorbic acid for all the tested isolates were 500-800 µg ml^{-1}. Most (80%) of the tested 10 strains were resistant to 300 µg nisin ml^{-1} nutrient agar. The effects of combination of pH, sodium chloride, benzoic acid and nisin on the growth of *B. cereus* 37-B1, and of pH, sodium chloride and benzoic acid on *Salmonella* 1-S4 were investigated in order to understand the scientific basis for an efficient application of hurdle technology in the preservation of food which can be contaminated by these micro-organisms. The best combination found for the cessation of growth of *B. cereus* was 20 mg sodium chloride ml^{-1}, 300 µg benzoic acid and 25 µg nisin ml^{-1} at pH 5.6. The logical combination for the cessation of the growth of *Salmonella* was 50 mg ml^{-1} sodium chloride and 600 µg ml^{-1} benzoic acid at pH 5.4. The results will be the basis for an efficient application of hurdle technology in preserving the legume-based fermented foods.

Whole-cell protein fingerprinting (WCPF) analysis of 48 strains of *B. cereus* and 33 strains of *Salmonella* revealed a diversity of these organisms and a relative preference of the WCPF subclusters to particular types of legume-based fermented foods.

Challenge study by intentional inoculation of a batter or dough with pathogenic bacteria revealed that fermentation alone could not completely inhibit the growth of the inoculated strains of *B. cereus*, *S. aureus* and *E. coli* from the fermenting batter of dhokla and idli, and dough of wadi. Steaming of the fully fermented batter for 15 min according to the traditional culinary process of making dhokla and idli, completely inhibited *S. aureus* and *E. coli*. However, a low level of *B. cereus* survived the steaming process, indicating that although cooking inactivates most contaminating micro-organisms, heat-resistant bacterial endospores may survive. None of the inoculated pathogenic bacteria could survive in wadi after 36 h of sun- and air-drying of fermented dough. Good hygienic and manufacturing practices are advised to ensure best quality products.