

Abstract

Legumes are a nutritionally rich and inexpensive source of dietary protein in many parts of the world, especially in developing countries like India where majority of the population is vegetarian. The diversity of the population of India has given rise to a large number of traditional legume-based foods. Most of these foods are fermented and hence have numerous health benefits. The microbiological safety of these foods is an important issue as the various steps involved in their preparation and distribution provide ample scope for contamination with pathogenic and spoilage microorganisms. Foodborne illnesses are a health problem all over the world especially in developing and under-developed countries. Thus, processing technologies that ensure food safety, quality and shelf-life are required to be adopted both at the household and industrial levels.

A total of 72 samples of eight different kinds of legume-based traditional foods were collected from different market sources of India. Besides the market samples, three kinds of legume-based foods namely sattoo, idli and wadi were collected during the processing stages from different sites of production. Moisture content, pH, a_w and microbial load were determined for all the samples.

Among the market samples analyzed, dhokla, idli and kinema were the intermediate-moisture foods (moisture content, 61-64 g/100 g), while the other foods

contained less moisture (8-29 g/100 g). Sattoo had the lowest moisture content. Papad and kinema were alkaline, while all other foods were acidic. Dhokla, dosa, idli and kinema displayed the highest a_w (0.949-0.997), while the other products like besan, sattoo, papad and wadi displayed a lower a_w -value (0.603-0.703). Kinema and wadi contained total aerobic mesophilic bacteria (TAMB) cells at a level of $>10^8$ cfu/g while the other food samples contained 10^5 - 10^6 cfu/g. *Bacillus cereus* s.l. occurred at a potentially hazardous level ($\geq 10^4$ cfu/g) in kinema and sattoo. Only one sample of wadi was found to be contaminated with *Staphylococcus aureus* with a load of 10^4 cfu/g. Enterobacteriaceae was absent in dosa and idli. One sample each of dhokla, kinema and wadi were found to be contaminated with *Escherichia coli*.

TAMB were present at all the processing stages of sattoo, idli and wadi. *Bacillus cereus* s.l. counts during idli production decreased ($p < 0.05$) after fermentation and cooking, but were not eliminated even after the cooking stage. It was present below the hazardous level ($>10^4$ cfu/g) in both idli and sattoo, while it was absent in wadi. *Staphylococcus aureus* was absent in all the three foods studied. Fermentation during idli production eliminated *S. aureus*. Enterobacteriaceae occurred in all the processing stages of sattoo while, in case of idli production it occurred at the start of fermentation at a level of 10^5 cfu/g, but were not detected after cooking. Although, drying reduced ($p < 0.05$) the Enterobacteriaceae counts by 8%, they were not eliminated from wadi.

Raffinose family oligosaccharides (RFOs) are important antinutritional factors present in blackgram seeds. In the present study, response surface methodology (RSM) was used to optimize the soaking conditions of blackgram in order to reduce these flatogenic sugars. A central composite rotatable design (CCRD) was used to study the effect of four separate soaking parameters, viz. bean-water ratio (1:3-10, w/v) and soaking temperature (15-35°C), time (0-24 h) and pH (4-8) on the total RFO content of the beans. Thirty-one experimental runs were carried out in triplicate to obtain the optimum soaking conditions at bean-water ratio of 1:10 and soaking temperature, time and pH being 16°C, 21 h and 6.0, respectively, in which the total RFO content predicted was 1.97 g/kg (dry wt basis) and the experimentally obtained value was 2.02 g/kg (96.42% decrease over raw seeds). Lack of significant difference ($p < 0.05$) between the experimental and predicted values validates the accuracy of the model.

The mechanization of the drying phase of wadi was optimized using RSM. The effect of three independent variables, viz. RH (20-65%), drying time (24-120 h) and sample diameter (15-35 mm) on the overall acceptability (OAA) based on its sensory attributes and the a_w were studied using a CCRD. Twenty experimental runs in triplicate were conducted to obtain the optimal conditions as 42.5% RH, 72 h time and a sample diameter of 25 mm. Although all the three factors had an influence on the OAA, the study indicated that the drying time and size of wadi had a greater influence. As per the optimization, the predicted maximum OAA of wadi was 16.74 with a_w of 0.56. The experimental values for OAA and a_w were 16.83 and 0.56, respectively which showed no significant difference ($p < 0.05$) from the predicted values. The conditions, thus optimized, were found to produce wadi of satisfactory sensory qualities and a_w -values.

A microbial challenge study was performed in order to understand the survivability and growth of inoculated (10^4 cfu/g) foodborne pathogens such as *E. coli*, *B. cereus* s.l. and *S. aureus* during the production of idli and wadi. During idli production, *Bacillus cereus* s.l. could survive through the entire fermentation process and also the cooking

stage on account of its ability to form heat-resistant spores. However, both *E. coli* and *S. aureus* were absent in the final steamed product. None of the pathogens survived the drying process during the production of wadi and were not recovered on storing the wadi at 25°C for 14 days.

A total of 130 isolates of LAB were obtained during the production of idli and wadi and these were screened for antibacterial activity. Among these, 17 isolates (13%) showed antagonistic activity against the bacterial pathogens, *B. cereus* s.l., *S. aureus* and *E. coli*. Out of 17, only 2 isolates of *Lactococcus lactis* showed antibacterial activity due to bacteriocin production.

Kinema is a traditional non-salted, alkaline food produced by *Bacillus*-fermentation of soybeans. The present study aimed to understand whether NaCl addition prior to fermentation and moisture reduction after the fermentation had any influence on the safety and keeping quality of kinema. The pH, a_w and proteolytic activity of kinema decreased with an increase in the concentration of NaCl up to 4 g/100 g where there was no significant change in cell count of the fermenting microorganism (*Bacillus subtilis*) and sensory score of the product. This added salt level in the product did not impair the taste of cooked kinema, but eliminated *Bacillus cereus*, an important foodborne bacterial pathogen. The population of *Escherichia coli* was reduced by 1 log cycle in the presence of NaCl but eliminated when the water activity (a_w) of kinema was reduced to 0.5 after fermentation. Salt had no effect on its shelf-life.

Moisture sorption characteristics of kinema and wadi were investigated at temperature of 15-45°C and a_w of 0.11-0.97 and their sorption isotherms were of sigmoid type. The Caurie model was used to determine the various properties of sorbed water which decreased with an increase in temperature. The optimum a_w for storage of kinema was 0.5 while the monolayer moisture content, which is the safe lower limit of moisture levels for foods ranged from 6.0 to 8.3 g/100 g. The thermodynamic functions in kinema decreased with increasing moisture content. The optimum storage temperature and a_w for wadi were 15-25°C and <0.7.