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Determination of microbiological status of chanachur, a traditional spicy ready-to-eat snack and its quality improvement by gamma radiation

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ABSTRACT

In this study microbiological status of chanachur, a ready-to-eat traditional snack was determined and its quality was improved by applying gamma radiation. To assess the microbial load in chanachur samples four types (non-branded coded as S_1 and branded coded as S_2, S_3 and S_4) were collected and Total Viable Bacterial Count (TVBC), Total Coliform Count (TCC), Total Fungal Count (TFC) were determined by spread plate method using Nutrient Agar (NA), MacConkey Agar and Potato Dextrose Agar (PDA), respectively. TVBC, TCC and TFC values for four types of chanachur samples were ranged from 1.18×10^5 to 2.1×10^4 CFU/g, 1.2×10^4 to 3.0×10^3 CFU/g and 1.5×10^4 to 2.0×10^3 CFU/g respectively which were beyond the satisfactory or acceptable level. To improve the quality of chanachur samples, a series of doses of gamma radiation viz. 2.0, 5.0, 10.0 and 15.0 kGy were applied. Irradiation with 2.0, 5.0 and 10.0 kGy, microbial load was decreased by 1 to 3 log but not completely eliminated. After applying the radiation dose of 15.0 kGy, all kinds of viable microorganisms including spores were eliminated rendering the chanachur samples sterile and safe for consumption. Bacteria associated with the chanachur samples were also identified based on cultural, morphological and different biochemical characteristics.

KEYWORDS

Chanachur;
Microbial load;
Contamination;
Coliform;
Gamma-radiation.

INTRODUCTION

Street vended foods are ready-to-eat foods prepared and sold by vendors especially in streets, markets and other similar public places. There are many different kinds of traditional chanachur in Indian subcontinental region and consumed mostly as one of the favorite street food. It is also known as Namkeen. This product has a combination of maximum 12 items. The most common components are fried noodles made from pulse flour (bason); flour chips of different sizes and shapes; fried and puffed dhals; cereals and peanuts. Spices and condiments are used as to flavour the mixture and to get spicy, sour and salty taste. The combination of ingredients varies considerably depending on the price and the market[1]. Chanachur is available in almost all grocery shops, even in the most remote villages of Bangladesh. Vendors often are found selling

"chanachur" in public places like stations, bus terminals or in front of schools. It is taken by all aged people from lower to upper level. It is frequently taken when people wait in traffic jam, in bus, train or launch journey, when friends gather together and gossip and for entertaining the guests etc. Mostly street-vended open sale chanachur are served with some chopped chilli, onions and tomatoes and a little salt. Sometimes it is consumed with puffed rice (muri) and with other snack foods by children and working people.

Chanachur consumption is increasing in rural areas. Producers are coming up with more and more new ideas on chanachur: from new packaging to new varieties. Entrepreneurs are taking interest. This product is becoming one of the important food processing businesses in Bangladesh. Not only it is economically important but also providing a means of earning for the groups of low income people in Bangladesh. Practically all the food we prepare, purchase or grow, be it bakery food, fruit, vegetable, meat, cereal, meat, home made drinks and juices or dairy products harbor a variety of microorganisms. This is not surprising when one considers that bacteria and fungi are ubiquitous and are especially plentiful in soil and around us (air) and could easily contaminate foods. From the microbial perspective, food can be viewed as a fertile ecosystem in which these organisms uptake for their nutrients[2]. It is estimated that between 24 and 81 million cases of food borne diarrhea diseases occur each year in the United States, costing between $5 million and $17 billion in medical care and loss of productivity[3]. Food suppliers or sellers bring the public into intimate contact with a variety of microorganisms that are present in the various food production and processing environment from around the world[4].

Our study snack food chanachur of branded and non-branded is contaminated with a huge load of microorganisms which is not in satisfactory level[5]. This contamination mainly occurs during processing, mixing, packaging and storage of foods. Foods may be contaminated by physical contaminants (e.g., pieces of glass, wood, metal, plastic, film, human hair and fingernails, plasters, jewelery, small personal belongings, pests, paper etc. chemical contaminants (e.g., residues of cleaning and disinfection chemicals, machinery lubricants, synthetic preservatives, food additives, pesticides etc) and microbiological contaminants (e.g., bacteria, yeasts, mould, mycotoxins, viruses etc.)[6]. Food with microbiological contaminants can transmit a wide range of diseases in a condition termed food infection, where the food serves as a vehicle for the transfer of the pathogen to the consumer, in whom the pathogen grow and causes disease[7]. Another condition that might arise is food intoxication, where the pathogens grow in the food and produce toxins that can then affect the consumer of the food[8]. Improper handling of food including improper use of preparation and storage temperatures, cross contamination and poor personal hygiene etc are responsible for most cases of food-borne diseases and intoxication. When food handlers do not practice proper personal hygiene or correct food preparation, they may become vehicles for microorganisms, through their hands, mouth, skin among others[9].

A high number of foods sold in our communities are contaminated to a large extent with pathogenic micro-organisms. Foods sold near polluted environments are prone to contamination by pathogenic microorganisms. The numbers of deaths due to pathogens that cause acute gastroenteritis in the United States is 1,381 of which 67% are attributed to food-borne transmission[10]. In developing countries such as Bangladesh, there are serious concerns about sanitation of ready-to-eat foods where the incidence of food borne diseases is very high due to maintenance of improper hygienic practice both in food production and serving areas. Moreover, most of the consumer are not aware of this food quality and related risk though this category of food pose serious health risk. Considering this points this study was conducted to evaluate the microbiological quality of this ready-to- eat food for providing valuable data on the incidence of such pathogenic microorganisms with a view to increasing public awareness as well as for necessary action on the part of food sellers, consumers and authorities. For quality improvement, this food items was irradiated with a series of doses of gamma radiation and satisfactory quality was obtained at the dose 15.0 kGy that eliminated all kinds of viable microorganisms including spores rendering the chanachur samples safe for consumption.

MATERIALS AND METHODS

Sample collection and preparation

One street-vended non-branded and three branded

Regular Paper

chanachur samples were collected from the street food vendors and nearby grocery shop of Balivadra Bazar, Atomic Energy Research Establishment, Savar, Dhaka. The street-vended, non-branded chanachur sample was coded as S_1 and the three branded were coded as S_2, S_3 and S_4. All samples were processed within 4 hours of collection. From each sample 10g of chanachur was aseptically weighed and put in a sterile polybag. Ninety (90) ml of sterile saline water was added in the polybag and then sample is macerated with stomacher Lab-Blender 80 [Model BA6020]. From the macerated sample 1ml aliquot was transferred in the test tube containing 9 ml of sterile saline water and mixed uniformly with a vortex mixture giving a 1:10 dilution. By repeating the process a serial dilution was made up to $1:10^5$.

Estimation of bacteria load

For estimation of Total Viable Bacterial Count (TVBC) and Total Coliform Count (TCC), Nutrient Agar (NA) and MacConkey Agar were used. Potato Dextrose Agar (PDA) was used to determine Total Fungal Count (TFC). Duplicate plates of Nutrient Agar, MacConkey Agar, and Potato Dextrose Agar were inoculated with 0.1 ml of sample from different dilutions using spread plate technique. All plates except PDA plate were incubated at a temperature of 37°C for 24 h before colony enumeration and isolation. PDA plates were incubated at a temperature of 30°C for 72h. After incubation counting of different microorganisms was performed using Gallenkamp colony counter and different microbial counts were expressed as Colony Forming Unit per gram (CFU/g) sample.

Isolation and identification

After cultivation/ growth on media (NA), discrete colonies were picked up, sub-cultured by streak plate method on the respective media. By repeated sub-culturing, pure culture of the picked up discrete colonies were obtained which are used for identification purpose. Identification of different isolates was carried out as per standard method[11] on the basis of different cultural, morphological and biochemical characteristics. Cultural characteristic include the colonial morphology with respect to growth vigor, size, shape, margin, elevation, pigmentation, opacity and viscosity etc[12]. Gram staining was carried out to study Gram reaction pattern and morphological characteristics e.g., size, shapes, arrangement of the cells of different isolates. Different biochemical tests e.g., catalase, oxidase, glucose fermentation, oxidative/ fermentative test, spore stain etc. were done to determine different biochemical characteristics of the isolates for identification. Overall cultural, morphological and biochemical results were compared with the standard features of different bacterial genus and as per maximum similarity isolates were identified.

Treatment with gamma-radiation

For microbiological quality improvement, chanachur samples were subject to irradiation at a series of doses viz. 2, 5, 10 and 15 kilo Gray (kGy) with a cobalt-60 gamma irradiator at the doze rate of 1.5kGy/h. After radiation each of irradiated samples were again tested for microbiological parameters as mentioned above to observe the effect of different doses of radiation.

RESULTS

Microbial load in non-irradiated (control) chanachur samples

Microbiological quality assessment of chanachur samples were carried out both qualitatively and quantitatively. Total Viable Bacterial Count (TVBC), Total Coliform Count (TCC) and Total Fungal Count (TFC) of the chanachur samples were determined and expressed in colony forming unit per gram (CFU/g).

In non-branded sample (S_1) TVBC was very high and that was 1.08×10^5 CFU/g. TCC and TFC were also high in this non-branded sample and that were 1.2×10^4 CFU/g and 1.5×10^4 CFU/g respectively (TABLE 1).

TABLE 1 : Microbiological load of non-irradiated chanachur samples.

Chanachur sample	TVBC (CFU/g)	TCC (CFU/g)	TFC (CFU/g)
*S_1	$1.08\times10^5 \pm 1.4\times10^3$	$1.2\times10^4 \pm 1.4\times10^3$	$1.5\times10^4 \pm 2.8\times10^3$
S_2	$1.18\times10^5 \pm 11.3\times10^3$	$3.0\times10^3 \pm 2.8\times10^3$	$7.0\times10^3 \pm 1.4\times10^3$
S_3	$2.1\times10^4 \pm 5.6\times10^3$	$8.0\times10^3 \pm 2.8\times10^3$	$4.0\times10^3 \pm 2.1\times10^3$
S_4	$4.1\times10^4 \pm 7.7\times10^3$	$7.0\times10^3 \pm 4.2\times10^3$	$2.0\times10^3 \pm 7.0\times10^2$

Asterisk mark (*) indicates street-vended non-branded sample.

Among the branded samples, maximum TVBC was found in S_2 sample and that was 1.18×10^5 CFU/

Regular Paper

g which was unsatisfactory and the minimum TVBC was found in S_3 sample and that was 2.1×10^4 CFU/g (TABLE 1). Maximum TCC (8.0×10^3 CFU/g) was found in S_3 sample and the minimum TCC (3.0×10^3 CFU/g) was found in S_2 sample. Maximum and minimum TFC of branded samples were found in S_2 and S4 sample respectively that were 7.0×10^3 CFU/g and 2.0×10^3 CFU/g.

Among the four chanachur samples though TVBC was higher in the branded sample than non-branded sample, TCC and TFC were higher in non-branded sample than the branded chanachur sample. As per microbiological specification of this food, none of the sample was found with satisfactory quality.

Microorganisms associated with chanachur samples

Microorganisms associated with chanachur samples were isolated and identified as per standard method on the basis of different cultural, morphological and biochemical characteristics. From, non-branded chanachur eleven different types of bacterial strains were isolated (TABLE 2). All of them were gram-positive except two isolates i.e., *Cardiobacterim and Enterobacteria*. Most of the isolates were oxidase negative, motility negative and glucose positive, anaerobic growth positive.

TABLE 2 : Bacterial isolates associated with branded and non-branded chanachur samples

Bacterial Isolates	Gram reaction	Chanachur samples
Bacillus	Positive bacilli	S_1
Clostridium	Positive bacilli	S_1
Cardiobacterim	Negative bacilli	S_1
Enterobacteria	Negative bacilli	S_1, S_2 and S_4
Corynebacterium	Positive bacilli	S_1, S_2, S_3 and S_4
Erysipelothrix	Positive bacilli	S_1
Staphylococcus	Positive cocci	S_1
Streptococcus	Positive cocci	S_1
Aerococcus	Positive cocci	S_1
Micrococcus	Positive cocci	S_1
Listeria	Positive bacilli	S_1
Mycobacterium	Positive bacilli	S_2

From three different branded chanachur only three types of bacterial strains were isolated and identified as *Enterobacteria, Corynebacterium* and *Mycobacterium* (TABLE 2). All of them were bacilli and two were gram-positive and one was gram-negative. All of them were catalase positive and oxidase negative.

Frequency of occurrence of the various isolates

Total twenty seven representative colonies were isolated and identified from the inoculated plates of four types of chanachur samples. Among the twenty seven colonies, *Bacillus, Clostridium, Streptococcus, Aerococcus* were two in number (Figrure 1). These represented 7% of the isolated colonies. *Cardiobacterim., Erysipelothrix, Micrococcus* and *Mycobacterium* were one in number and represented 4% of the isolated microorganisms.

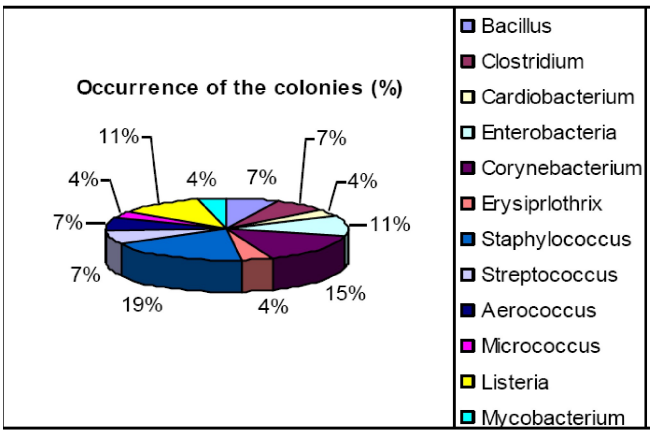

Figure 1 : Occurrence of colonies of various bacterial isolates (%) in the chanachur samples.

Enterobacteria and *Listeria* were three in the number of colonies and represented 11% of the isolated microbes. *Corynebacterium* was four among the twenty seven colonies and represented 15% of the isolated microorganisms. The highest number was *Staphylococcus*; this was five in number and represented 19% of the isolated microorganisms.

Quality Improvement of chanachur Samples by gamma radiation

As all the chanachur samples contains high amount of coliform indictors along with other harmful bacteria, it is essential to improve the quality of this food item before it is consumed. To improve the quality, chanachur samples were irradiated with different doses (2.0, 5.0, 10.0 and 15.0 kGy) of radiation. Result showed that upon irradiation of the food sample microbial counts

Regular Paper

were decreased gradually as the radiation doses were increased.

At 2.0 and 5.0 kGy doses of radiation treatment the survival level of the microorganisms were reduced by 1 to 3 log of TVBC and TCC. Maximum TVBC and TCC were found in S_1 sample and that were 6.0×10^3 and 2.5×10^3 CFU/g respectively at 2.0 kGy (TABLE 3). TFC became nil in all samples after radiation treatment with 2.0 kGy.

TABLE 3 : Microbiological load in chanachur samples irradiated with dose of 2.0 kGy.

Chanachur sample	TVBC (CFU/g)	TCC (CFU/g)	TFC (CFU/g)
S_1	$6.0\times10^3 \pm 1.4\times10^3$	$2.5\times10^3 \pm 0.0$	Nil
S_2	$5.5\times10^3 \pm 7.0\times10^2$	Nil	Nil
S_3	$3.0\times10^3 \pm 0.0$	$1.0\times10^3 \pm 0.0$	Nil
S_4	$1.5\times10^3 \pm 0.0$	Nil	Nil

At the dose of 5.0 kGy, maximum TVBC and TCC were found in the sample S_1 and that were 4.0×10^3 and 1.0×10^3 CFU/g respectively (TABLE 4). TCC except in S1 and TFC in all the samples irradiated with the same dose became nil.

TABLE 4 : Microbiological load in chanachur samples irradiated with dose of 5.0 kGy.

Chanachur sample	TVBC (CFU/g)	TCC (CFU/g)	TFC (CFU/g)
S_1	$4.0\times10^3 \pm 2.8\times10^3$	$1.0\times10^3 \pm 0.0$	Nil
S_2	$3.0\times10^3 \pm 1.4\times10^3$	Nil	Nil
S_3	$2.0\times10^3 \pm 0.0$	Nil	Nil
S_4	$1.0\times10^3 \pm 0.0$	Nil	Nil

The irradiation dose of 10 kGy completely eliminated coliform and fungi though some bacteria survived in the sample S_1 and S_2 (TABLE 5). At the dose of 15 kGy, all kinds of viable microorganisms including spores were eliminated rendering the chanachur samples sterile and safe for consumption.

Radio-resistant microorganisms in chanachur samples

To observe the radio-resistant microorganisms in the irradiated food samples, several representative colonies were picked up from plates inoculated with samples irradiated with 5.0 and 10.0 kGy. Then survived isolates were identified as *Aerococcus, Staphylococcus, Listeria and Corynebacterium* (TABLE 6).

TABLE 5 : Microbiological load in chanachur samples irradiated with dose of 10.0 kGy.

Chanachur sample	TVBC (CFU/g)	TCC (CFU/g)	TFC (CFU/g)
S_1	$1.0\times10^3 \pm 0.0$	Nil	Nil
S_2	$5.0\times10^2 \pm 7.0\times10^2$	Nil	Nil
S_3	Nil	Nil	Nil
S_4	Nil	Nil	Nil

TABLE 6 : Microorganisms associated with non-branded and branded chanachur samples survived at 5.0 and 10.0 kGy dose level.

Bacterial Isolates	Gram reaction	Chanachur samples
Aerococcus	Positive cocci	S_1
Staphylococcus	Positive cocci	S_1, S_2 and S_3
Listeria	Positive bacilli	S_1 and S_3
Corynebacterium	Positive bacilli	S_2 and S_4

DISCUSSION

Street food vendors are often the most affordable source of ready made meals for urban workers near places of work. Street foods are popular and are more convenient for workers, civil servants and students[13]. Such foods have a good chance to harbor a number of microorganisms and thus pose potential health risk. Beside local regulatory authority recently WHO and FDA recommended some important guideline to prevent food born illness. To produce quality food how far these measures are adopted in the developing country like Bangladesh does not go unquestioned. To address this issue a popular street snack, chanachur samples of one non-branded and three branded were collected and tested for determinations of microbial status. Among these samples, the most heavily contaminated one was street-vended, non-branded chanachur and contaminated with 11 different bacterial strains. The rest three branded chanachur were contaminated by 3 different bacterial strains/genus. Unhygienic condition of preparation areas and the vending areas as observed in case

of street-vended, non-branded chanachur may have contributed to the higher level of contamination. This may explain why the food samples collected from vending areas with poor hygienic conditions were more contaminated than those with good hygienic conditions. The use of clean water to wash utensil, which is replaced from time to time reduce bacterial contamination[14]. *Staphylococci* were the most prevalent organisms in this study before radiation constituting 5 (19%) of the isolates. This was followed by *Corynebacterium* 4 (15%), *Enterobacteria* 3 (11%), *Listeria* 3 (11%), *Bacillus* 2 (7%), *Clostridium* 2 (7%), *Streptococcus* 2 (7%), *Aerococcus* 2 (7%) and *Cardiobacterim* 1 (4%), *Erysipelothrix* 1 (4%), *Micrococcus*1 (4%), *Mycobacterium* 1 (4%). The highest frequency of occurrences of *Staphylococci* in this study might occur from food handlers through improperly washed hands and dirty fingernails by some of the vendors. *Staphylococcus* is salt tolerant and can grow in salty foods like ham and chanachur. As this bacterium multiplies in food and produces toxins in food it can cause serious health hazard.

The high microbial load in food may also connected with the poor hygienic practices observed in some of the food vending areas in this study such as improperly cleaned containers, mixing machines used for mixing chanachur with different ingredients, serving in dirty papers, vendors' dressing, unwashed hands etc. Coliform, a group of pathogen indicator bacteria are also present in high number in both non-branded and branded chanachur indicating that the hygienic quality of this ready-to-eat food was not up to the mark. Moreover, fungi can grow in such dry food and produce aflatoxin which is a deadly neurotoxin that can cause severe disorder of neurological system. Presence of high number of fungus is also another indication of unhygienic status of both non-branded and branded chanachur, though non-branded one scored the worst. As urban population increases and the consumption of street vended foods increases, there is the urgent need for an effective intervention to emphasis on the safety aspect of public health. Closed observations through inspection of food preparation, food packaging and serving, methods of food storage and other vending facilities confirmed that hygiene was compromised among the food vendors. WHO reported that, it is important to handle food in such a way that the microorganisms present do not have a chance to multiply and food can be prevented from becoming contaminated with other microorganisms by some practices e.g., wash and dry hands before preparing any food and after handling raw foods (meat, poultry, vegetables or fruits), ensure that food preparation areas and equipment are clean, protect kitchen areas or restaurants and food from insects, pests and other animals, people with gastrointestinal illness, such as vomiting or diarrhoea, should not handle food intended for consumption by others[15].

However, as the consumption of contaminated food can cause serious health problem, it is essential to eliminate or reduce the number of microorganisms present in food sample to a safe level. Besides taking some preventive measures in the production process, application of nuclear technique like irradiation has been proved to be very effective in the quality improvement of finished food product. Irradiation with gamma ray is a cold and effective process to eliminate the microbes from contaminated samples. In our study food sample chanachur of non-branded and different branded were treated with a series of gamma-radiation doses i.e. 2.0, 5.0, 10.0 and 15 kGy. After irradiation with the doses 5.0 and 10.0 kGy there was 1 to 3 log reduction of microbial count but not completely eliminated in different food samples. The radiation dose of 15 kGy was found sufficient to eliminate all kinds of viable microorganisms including pathogen and thus made the chanachur samples safe for consumption.

CONCLUSION

Street-vended non-branded open chanachur was contaminated with huge load of microorganisms including pathogens. Branded chanachur were also contaminated with the number of microorganisms that exceeded the satisfactory level. Contamination of chanachur samples with bacteria including pathogens indicate that GMP is not adopted properly in the production process. A number of control measures including irradiation can be taken to improve the quality of this food items. Moreover, the relevant agencies of the government must ensure and enforce strict compliance to GMP as well as Hazard Analysis Critical Control Points (HACCP) in all food production sectors considering the safety issue of public health.

Regular Paper

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