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Evaluation of Bacteriological Quality of Ready-to-eat Chicken Products by Total Viable Count Method

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Abstract

The present investigation describes the total viable count of ready-to-eat chicken products (chicken patties and chicken rolls) in Srinagar city during two seasons viz. autumn and winter. A total of 120 ready-to-eat chicken products comprising of 60 chicken patties and 60 chicken rolls were tested. The mean bacterial count of 60 chicken patties and 60 chicken rolls was 5.1281 and 4.9395 $\log_{10}$ cfu/g. *Bacillus cereus* strains were isolated from 25 of chicken patties and 22 of the chicken rolls resulting in prevalence of 41.66% and 36.67%, respectively.

Keywords: Bacillus cereus strains; total viable coun; chicken patties; chicken rolls

Introduction

Ready-to-eat (RTE) chicken products are those that are edible without any additional preparation to achieve food safety or may receive additional preparation for palatability, aesthetic, epicurean, gastronomic or culinary purposes. They also play an important role in the nutritional supply, providing an opportunity for consumers to meet their daily nutritional requirements (Hanashiro *et al*., 2004). However, questions have been raised about their safety and microbiological quality. The expanding population of highly susceptible people such as elderly and immunocompromised individuals and the high consumption of RTE foods due to changes in lifestyle and the global trade food distribution could be the reasons for observed increase of high risk on food poisoning (WHO, 2002). RTE foods may be contaminated with different pathogenic micro-organisms like *Bacillus cereus, Clostridium perfringens, Salmonella spp., Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus spp., Streptococcus spp.* etc. (Yeboah *et al*., 2010). Among various food-borne pathogens, *Bacillus cereus*, a gram positive, spore forming, aerobic, rod shaped bacterium has been regarded as the most prevalent pathogenic species present in the food. It grows over a temperature range of 4 to 55°C, pH of 2-11 and in high sodium chloride concentrations of approximately 7.5%. As a soil bacterium, *B. cereus* can spread easily to many foods such as vegetables, eggs, meat and dairy products and is known to cause 25% of food-borne intoxications in humans (FDA, 2007; Larsen and Jorgeusen, 1996).

Outbreaks of food poisoning due to Bacillus cereus have been described since the beginning of the last century with first confirmed report in Norway in 1948 (Hauge, 1955). Since then many food-borne outbreaks have been reported (Lund *et al*., 2000, Hussain *et al*., 2007). *Bacillus cereus* causes problems to the food industry both by deteriorating the products (Eneroth *et al*., 2001) and by endangering peoples' health upon consuming contaminated foods (Ghelardi *et al*., 2002).

Bacillus cereus causes two distinct types of gastrointestinal disorders in humans viz; an early "emetic syndrome" and a late "diarrheal syndrome" involving two different types of enterotoxins (Kramer and Gilbert, 1989). The emetic syndrome, a food borne intoxication is caused by preformed *B. cereus* emetic enterotoxin (BCEET)

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in foods. It has a rapid onset (1-5 hours) and is characterized predominantly by nausea and vomition, resembling closely to staphylococcal food poisoning (Adams and Moss, 2007). *Bacillus cereus* also produces a large array of other potentially toxic substances/metabolites including haemolysins, phospholipase-C, metalloproteases, collagenases and beta-lactamases (Turnbull *et al.*, 2004). Apart from gastroenteritis, it is also involved in a variety of non-gastrointestinal tract infections like meningitis, endopthalmitis, endocarditis, periodonitis, osteomyelitis, wound infection and septicemia in humans (Schoeni and Wong, 2005). It is also emerging as a potential pathogen of serious concern in animals owing to the increasing reports of its role in diseases like, osteomyelitis, middle ear infections, abortions and mastitis (Schiefer *et al*., 1976).

Although there is a growing demand for Ready-to-eat (RTE) chicken products, but no information is available regarding the bacteriological quality of these products in India. The present study was hence undertaken to Evaluate the Bacteriological Quality of Ready-to-eat Chicken Products by Total viable count Method in Srinagar City of Kashmir, India. Since this was the first investigation of Ready-to-eat (RTE) chicken products in this country, these results provide basic information about the microbiological quality. Keeping in view the importance of B. cereus in foodborne infections and intoxications and the increasing demand of RTE poultry products by general public, the present study was undertaken.

Materials and methods

Sampling

Bacteriological quality of 120 ready to eat (RTE) chicken products viz. chicken patties (60) and chicken rolls (60) collected from retail shops of five zones of Srinagar city was studied in two subsequent seasons viz. Autumn and Winter (Table 1) For this purpose, six samples of each product per zone per season were collected in sterile zip lock sachets and brought to Veterinary Public Health Laboratory in ice for processing within 2-3 hours. Estimation of total viable bacterial count of Bacillus cereus was done by using standard bacteriological techniques.

Table 1. Scheme of sample collection

Sample type	Season	East zone	West zone	North zone	South zone	Central zone	Total
		Nishat/ Shalimar	Qamarwari/Bemina	Hazratbal/ Zakura	Rambagh/ Jawahar nagar	Khayam/ Fateh Kadal	
Chicken Patties	Autumn	6	6	6	6	6	30
	Winter	6	6	6	6	6	30
Chicken Rolls	Autumn	6	6	6	6	6	30
	Winter	6	6	6	6	6	30

Evaluation of bacteriological quality

Total viable count

Determination of total viable bacterial count was done according to American Public Health Association (1992) using the pour plate method. Briefly 10 g of the samples were homogenized in 90 ml of buffered peptone water to make a 1:10 (10-1) dilution. Thereafter, tenfold serial dilution was made upto 6th dilution using peptone water as the diluents. From each of the selected dilutions,1 ml was inoculated in sterile petri dishes in triplicate. About 15 ml of sterilized nutrient agar media maintained at about 45±1°C was poured in the inoculated Petri plates. Sample dilution and media were mixed thoroughly and uniformly by alternate rotation and to and fro motion of plate on a leveled surface avoiding the formation of air bubbles. The plates were then kept in the incubator till solidification of the media and then inverted with their lids down, labelled and further incubated for 18-24 hours at 37°C. The total viable count/g of the sample was

calculated as:

TVC = average no. of colonies in the desired dilution x dilution factor

The TVC was expressed as cfu/g of the sample in $\log_{10}$ scale.

Results

Level of contamination

The mean viable bacterial count of chicken patties in autumn season was 5.7928±0.1673 cfu/g. The highest average counts were recorded in west zone (6.4268±0.3609cfu/g) and the lowest counts (5.2378±0.42cfu/g) were recorded in the Central zone. West and Central zones differed significantly ($p<0.05$) in bacterial counts of chicken patties. However, no significant difference was observed in counts of North, East and South zones (Table 2). While the highest average counts in winter were also recorded in the West zone (4.8285±0.2229 cfu/g), the samples from North zone revealed the lowest counts in the winter (4.1315± 2518 cfu/g). There was no significant difference in the viable counts of different zones in the winter season (Table 3). The mean viable count recorded in winter was 4.4635±0.1206 cfu/g . The counts were significantly lower than those recorded in the autumn season (5.7928±0.1673 cfu/g). The counts, however, varied significantly with respect to two seasons ($P< 0.05$).

Table 2. Total viable counts of chicken patties in autumn season

S. No.	Zone	Sample No.	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5	Sample-6	Mean±SE (log_{10} cfu/g)
1.	East	6	6.439	5.461	5.322	6.462	5.380	6.421	5.9142±0.2362AB
2.	West	6	6.462	5.419	7.438	6.415	5.447	7.380	6.4268±0.3609B
3.	North	6	5.398	6.462	5.228	4.272	6.301	5.362	5.5038±0.3255AB
4.	South	6	6.215	4.428	6.431	5.406	5.378	7.431	5.8815±0.4245AB
5.	Central	6	4.389	6.438	4.419	5.441	6.462	4.278	5.2378 ± 0.42A
	Total	30							5.7928± 0.1673a

Table 3. Total viable counts of chicken patties in winter season

S. No.	Zone	Sample No.	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5	Sample-6	Mean±SE (log10 cfu/g)
1.	East	6	5.176	4.362	4.279	5.240	4.212	5.301	4.7617±0.215A
2.	West	6	5.187	4.267	5.235	5.133	5.149	4.000	4.8285± 0.2229A
3.	North	6	4.143	5.146	3.204	4.041	4.079	4.176	4.1315± .2518A
4.	South	6	5.255	4.322	5.209	4.193	3.114	4.146	4.3732 ± 0.324A
5.	Central	6	4.225	5.292	4.250	3.139	4.322	4.107	4.2225± 0.2794A
	Total	30							4.4635±0.1206b

Mean viable counts of chicken rolls recorded in the autumn were 5.43±0.1733 cfu/g, whereas, the mean values of chicken rolls in the winter were 4.4489±0.1076 cfu/g. The counts varied significantly with respect to two seasons ($P<0.05$). The highest average counts in autumn were recorded in West zone (6.24±0.3076) and the lowest counts in the North zone (5.1498±0.3242). The results are presented in Table 4.

Chicken rolls from West zone in the winter season were highly contaminated with mean values as 4.68±0.2287cfu/g. The lowest viable counts were however were recorded in North zone (4.11±0.2514cfu/g) in winter. No significant difference was recorded in the viable counts of chicken rolls in the winter season with respect to different zones of Srinagar city (Table 5).

Table 4. Total viable counts of chicken rolls in autumn season

S. No.	Zone	Sample No.	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5	Sample-6	Mean±SE (log10 cfu/g)
1.	East	6	5.431	4.447	6.301	6.465	4.380	5.380	5.40 ±0.3604A
2.	West	6	5.431	6.423	5.396	7.419	6.415	6.371	6.24 ± 0.3076A
3.	North	6	6.380	5.431	5.235	4.230	5.301	4.322	5.1498±0.3242A
4.	South	6	7.204	4.392	5.398	4.387	5.363	4.398	5.19±0.4486A
5.	Central	6	4.367	6.403	4.380	6.403	5.431	4.285	5.211±0.4144A
	Total	30							5.43± 0.1733a

Table 5. Total viable counts of chicken rolls in winter season

S. No.	Zone	Sample No.	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5	Sample-6	Mean±SE (log10 cfu/g)
1.	East	6	4.190	4.322	4.283	5.204	4.185	5.322	4.584± 0.2162B
2.	West	6	4.176	4.225	5.209	5.146	4.124	5.230	4.68 ± 0.2287B
3.	North	6	3.117	4.114	4.152	5.061	4.064	4.152	4.11 ±0.2514B
4.	South	6	5.204	4.322	4.190	4.146	5.120	4.117	4.5165±0.2064B
5.	Central	6	4.167	5.230	5.176	4.120	3.301	4.097	4.348 ± 0.3004B
	Total	30							4.4489 ± 0.1076b

Discussion

Consumption of such ready-to-eat foods, the bacteriological quality of which is not monitored regularly has resulted into increased morbidity and mortality. Assessment of bacteriological quality of such foods is, therefore, of immense importance, as such foods are consumed without any further heat treatment. The bacteriological quality of ready-to-eat foods is expressed by estimation of its total viable bacterial and coliform counts giving a general idea about the hygienic measures taken during processing, handling and preparation of foods (Aberle *et al.*, 2001). The present work was undertaken to assess the level of microbial contamination in chicken patties and chicken rolls in two subsequent seasons' viz. autumn and winter in five different zones of Srinagar city.

The average bacterial load of chicken patties and chicken rolls irrespective of season and zones was 5.1281 and 4.9395 $\log_{10}$cfu/g, respectively. The level of bacterial load is directly associated with safety of food and various organizations have laid a permissible limit of bacterial load. Bureau of Indian Standards (BIS) has laid a standard permissible limit of bacterial load of less than 2 million per gram and Food and Agriculture Organization (FAO) of not more than 10^6/g in ready to eat food samples. Some of the samples, in the present study had a bacterial load of more than 10^6/g and in the few, the loads were approaching 107/g which is higher than the standard permissible limits laid by these organizations. Therefore, such ready-to-eat food products must be considered as high potential threat to public health as these food products are not subjected to further heat treatment unlike raw foods where the load is significantly reduced during heat treatment. Varying reports are available regarding the level of contamination of ready-to-eat products. Zaki *et al*. (1977) reported counts in excess of 105per gram in 46% of perishable foods in New York. Nichols *et al*. (1996) also reported counts in excess of 104 orgs/g in 12% samples of ready-to-eat doner kebab and counts in excess of 106 orgs/g in 1% of the samples. Kakar and Udipi (2002) found counts of 7.21±1.55 $\log_{10}$ cfu/g in chicken patties and chicken rolls in Mumbai. Similarly Counts of 2.06-2.80x10^6 cfu/g and 3.54x10^6 cfu/g in ready-to-eat chicken curry and tandoori chicken respectively were reported by Rindhe *et al*.

(2008), whileas, Yuksek *et al*. (2009) found aerobic counts of 5.7 x 10^4 cfu/g in ready-to-eat chicken donair in Turkey. The findings of the present work are in agreement with the findings of earlier workers. A significant difference was observed between west and central zones, with respect to bacterial load of chicken patties and chicken rolls which may be because of variability in the level of hygienic conditions in the two zones.

The bacterial counts in the different foods vary depending upon the ingredients used in the preparation and also the processing procedures followed during preparation. The average bacterial load in the chicken patties (5.1281 $\log_{10}$ cfu/g) was higher compared to the chicken rolls (4.9395 $\log_{10}$ cfu/g). This could be due to difference in the ingredients used in the preparation of these ready to eat foods. In chicken rolls, vinegar is used more often which has an inhibitory effect on growth of microorganisms (Ceylan and Fung, 2007).

The season also plays an important role in determining the bacterial load of foods, particularly those which kept in open as is generally experienced with ready-to-eat foods.The average bacterial load in chicken patties and chicken rolls were significantly lower in winter than in the autumn. The lower bacterial count in winter could be because of low ambient temperature compared to autumn. The higher temperature during the autumn allows the luxuriant growth of bacteria, increasing the number/g of the food particularly when the food is stored without refrigeration. However, the variability in the bacterial counts of the chicken patties and chicken rolls with respect to different zones was statistically insignificant.

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References

Aberle, E.D., Forrest, J., Gerrard, D.E., Mills, E.W., 2001. Principles of Meat Science (4th Ed). Hunt Publishing Co., Kendall, USA.

Adams, M.R., Moss, M.O., 2007. Royal Society of Chemistry Great Britain, England. Food Microbiology 3, 60-64.

American Public Health Association (APHA), 1992. Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association. Washington, D.C.

Ceylan, E., Fung, D.Y.C., 2007. Antimicrobial activity of spices. Journal of Rapid Methods and Automation in Microbiology 12, 1-55.

Eneroth A., Svensson, B., Molin, G., Christiansson, A., 2001. Contamination of pasteurized milk by *Bacillus cereus* in the filling machine. Journal of Dairy Research 68, 189-196

Food and Drug Administration (FDA), 2007. Bacillus cereus. In : Bacteriological Analytical Manual. 8th Edition, revision A, AOAC International, Gaithersburg, MD, USA, 1401-1408.

Ghelardi, E., Celandroni, F., Salvei, S., Barsoi, C., Baggiani, A., Senesi, S., 2002. Identification and characterization of toxigenic *Bacillus cereus* isolates responsible for two food-poisoning outbreaks. Fems Microbiology Le 208, 129-134.

Hanashiro, A., Morita, M., Matte, G.R., Matte, M.H., Torres, E.A.F.S., 2004. Microbiological quality of selected foods from a restricted area of Sao Paulo city, Brazil. Food Control 16, 439-444.

Hauge, S., 1955. Food poisoning caused by aerobic spore forming Bacilli. Journal of Applied Bacteriology 18, 591-95.

Hussain, S.A., Munshi, Z.H., Hussain, I., 2007. Food poisoning due to *Bacillus cereus*. A case report. Journal of Veterinary Public Health 5, 57-59.

Kakar, D. A., Udipi, S. A., 2002. Microbiological qualities of different varieties of chutneys sold in Mumbai city. Journal of Food Science and Technology 37(5), 509-511.

Kramer, J.M., Gilbert, R.J., 1989. *Bacillus cereus* and other Bacillus species. [Ed. M.P. Doyle] Food Borne Bacterial Pathogens, pp. 21-70.

Larsen, H.D., Jorgensen, K., 1996. The occurrence of Bacillus cereus in Danish pasteurized milk. International Journal of Food Microbiology 34, 179-186.

Lund, T., DeBuyser, M.L., Granum, P.E., 2000. A new cytotoxin from Bacillus cereus that may cause necrotic enteritis. Molecular Microbiology 38, 254-61.

Nichols, G., Monsey, H., Louvois, J., 1996. LACOTS/PHLS study of the microbiological quality of doner kebab meat. Kebab Report 3rd Final Report, pp. 1-15.

Rindhe, S.N., Zanjad, P.N., Doifode, V.K., Siddique, A., Mendhe, M.S., 2008. Assessment of microbial contamination of chicken products sold in Parbhani city. Veterinary World 1(7), 208-210.

Schiefer, B., Macdonald, K.R., Klavano, G. G., Van-Dreumel, A.A., 1976. Pathology of Bacillus cereus mastitis in dairy cows. Canadian Veterinary Journal 17, 239-43.

Schoeni, J.L., Wong, A.C.L., 2005. *Bacillus cereus* food poisoning and its toxins. Journal of Food Protection 68, 636-648.

Turnbull, P.C.B., Sirianni, N.M., LeBorn, C.I., Samaan, M.N., Sutton, F.N., Reyes, A.E., Peruski, L.F., 2004. MICs of selected antibiotics for *Bacillus anthracis, Bacillus cereus, Bacillus thuringiensis and Bacillus mycoides* from range of clinical and environmental

sources as determined by the Etest. Journal of Clinical Microbiology 42, 3626-3634.

World Health Organization (WHO), 2002. Global strategy for food safety : Safer food for better health. World Health Organization, Geneva Switzerland ISBN924154574.

Yeboah-Manu, D., Kpeli, G., Akyeh, M., Bimi, L., 2010. Bacteriological quality of ready-to-eat foods sold on and around University of Ghana Campus. Research Journal Microbiology 5, 130-136

Yuksek, N., Evrensel, S.S., Temelli, S., Anar, S., Sen, M.K., 2009. A microbiological evaluation on the ready-to-eat red meat and chicken donair kebabs from a local catering company in Bursa. Journal of Biological Environment Science 3(7), 7-10.

Zaki, M.H., Miller, G.S., Mclaughlin, M.C.,Weinberg, S. B., 1977. A progressive approach to the problem of foodborne infections. American Journal of Public Health 67, 162-163.