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# Microbiological quality evaluation of frozen food available in Peshawar city, Pakistan

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**Abstract:** This study was carried out to evaluate the microbiological condition of the frozen food samples found in the local market shops of Peshawar city. The studied microbiological parameters were Total Plate Count (TPC), Fecal Coliform, *E.coli, Staph. aureus* and Salmonella. TPC were found in the range of $3.0 \times 10^5$-$2.5 \times 10^2$, F. Coliform and E.coli were absent in all the analyzed sample, S. aureus were present in all the analyzed samples except one sample of Shrimp (S4) and Salmonell were present in 15% analyzed samples. It was observed from the study that some of the frozen food samples available in Peshawar City were heavily contaminated and are special concern for human consumption.

**Keywords:** Frozen food, fecal coliform, TPC, contamination.
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## INTRODUCTION

The microbiological quality of meat show that it is a good medium for the growth of microorganism, Biological contaminants such as bacteria, viruses, fungi, protozoa and helminthes constitute the major cause of food-borne diseases with varying degrees of severity, ranging from mild indisposition to chronic or life threatening illness, or both. In developing countries, such contaminants are responsible for food borne diseases such as cholera, campylobacteriosis, *E. coli* gastroenteritis, salmonellas, shigellosis, typhoid fever, brucellosis, amoebiasis and poliomyelitis The food and Agricultural Organization of the United Nations (FAO) and the World Health Organization (WHO) state that illness due to contaminated food is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity[1].

Fresh meat productions are marketed after having been cooled freshly or frozen in a particular way. In both situations the meats need to be marketed without getting sour and been corrupted and without any change in the quality and appropriate packaging. The microorganisms are very good indicators concerning whether the food has been processed in hygienic conditions. The number of aerobic /Coliforms bacteria is a good criterion in determining the hygienic quality of meat. Coagulase positive *Staph. aureus* is a bacteria that can be dangerous particularly in meat and meat products and that is important in terms of food poisoning. It has been emphasized in the standards and in the by law that *Salmonella* type bacteria is not to exist in food. When meat is frozen the number of the microorganisms decreases. However, yeast and mould can reproduce at -5$^o$C but the reproduction stops at -10$^o$C. Melted frozen meat creates suitable conditions for the reproduction of bacteria and increases the potential of food to get spoiled[2].

The microbiological safety of food is achieved by as far as possible ensuring the absence of pathogenic microorganisms and by all means preventing their multiplication[3]. The Hazard Analysis Critical Control Point (HACCP) concept is used to identify microbiological vulnerable points in the food production process and processing, to determine the most appropriate methods of control to be applied, usually such methods as improved handling techniques, monitoring of temperature and more intensive supervision[4].The objective of this study was to evaluate the microbiological status of frozen meat products available in departmental stores and local markets of Peshawar city, to determine its risk for public health.

## MATERIALS AND METHODS

### *Samples collection*

Twenty various frozen meat samples investigated in this study were collected from different markets in Peshawar City Pakistan and transferred in sterile food bags and analyzed in the same day and analyzed microbiologically (Total plate count, Fecal coliforms bacteria, *E. coli*, *Staph. aureus* and Salmonella.

### *Microbiological analysis*

Total 25 g frozen food sample had been weighed in sterile petri dishes for the counting of Aerobic plate count was determined by pour plate method[5]. Serial dilution ($10^{-1}$ to $10^{-8}$) of the frozen samples was made and aliquots of 1ml were added to each duplicate Petri dishes. Plate count agar was added to each Petri dish and incubated at 35$^o$C for 48 hours ±2, after incubation colony was count by colony counter and result was expressed as cfu/ml. Plate Count Agar (PCA) medium was used to determine the total bacteria number. Colony

counting was carried out after the 48-hour incubation at 37°C set, by pour plate method.

Total Coliforms bacteria and faecal coliforms bacteria count was determined by method[5]. Growth and appearance on Violet Red Bile Agar after an incubation period of 24h at 35°C were used for a presumptive count, and growth and gas production in 2% Brilliant Green Broth were used as the confirmatory test for coliforms. Simultaneously, the faecal coliforms bacteria was obtained on VRBA plates incubated for 24h at 44ºC, confirming typical colonies from these VRBA plates with growth and gas production in E.C. Broth over 24h at 44ºC.

*E. coli* was determined by the procedure[5], Positive tubes of EC medium were used for the determination of *E. coli.* Streak loopful from these tubes on L-EMB agar and incubated the plates for 18-24h at 35ºC. The plates were observed for *E. coli* colony (dark centered with or without metallic sheen). The typical colonies were confirmed by biochemical tests and also by kits (*E. coli O157:H7* latex test reagent kit Pro Lab. Canada).

To determine *Staph. aureus* dilution made for TPC were spread plated on manitol salt agar (MSA) (Oxoid Ltd. Hampshire, England). Typical yellow colonies were counted after 44hrs of incubation at 35ºC.

To isolate *Salmonella* spp., 25 g of samples were incubated in 225 ml buffered peptone water (Oxide) at 37ºC for 24 h. Subsequently 0.1 ml inoculated into Rappaport Vassiliadis Broth (Merck) and were incubated at 43ºC for 24-48 h. Streak plates were prepared on Salmonella Shigella Agar (Merck) at 24 and 48 h incubation times and incubated at 37ºC for 24-48 h. Pink- red colonies with black centers were inoculated onto Triple Sugar Iron Agar (Merck 1. 03915) and Lysine Iron Agar (Merck). Biochemical and serological (Oxide) tests were performed for the identification of Salmonella.

## RESULTS AND DISCUSSION

The microbiological analysis results of shrimp were shown in Table 1. The total plate count (TPC) of S1 sample was .8 x $10^3$ cfu/ml. The highest TPC were found in S3 i.e. 1.8 x $10^5$, followed S5 1.5 x $10^4$ cfu/g. The lowest 1.5 x $10^3$ cfu/g were found in S4. The S5 TPC was 1.5 x $10^4$cfu/g. Total plate count of frozen shrimp samples estimation of bacterial numbers in shrimp is frequently used to retrospectively assess microbiological quality or to assess the presumptive safety of the product. The high microbial load could deteriorate the product quality within short time. The Pinu[6] studied show that an average a high TPC 7.23 $\log_{10}$ cfu/g were found in shrimp samples of local market in Dhaka city.

No fecal Coliform and E.coli was found in any shrimp samples. In this study total Coliform were present in all the shrimp samples in high number. These results indicate that these samples have contained high standard in term of facal contamination. No S.aureus was found in S4, but S1, S2, S3 and S5 were found 75,65,43 and 58cfu/g. Salmonella were found in only two shrimp samples i.e. S1 and S2.

**Table 1:** Microbiological analysis of shrimp*.

| Lab. Code | TPC (CFU/g) | F.Coliform (MPN/g) | *E. coli* (MPN/g) | *Staph. aureus* (CFU/g) | Salmonella |
|---|---|---|---|---|---|
| | **Std.$10^5$-$10^6$ | <50 | Nil | <100 | Nil |
| S1 | 2.8 x $10^3$ | <0.3 | Nil | 75 | Present |
| S2 | 4.3 x $10^2$ | <0.3 | Nil | 65 | Present |
| S3 | 1.8 x $10^5$ | <0.3 | Nil | 43 | Nil |
| S4 | 1.5 x $10^3$ | <0.3 | Nil | Nil | Nil |
| S5 | 1.5 x $10^4$ | <0.3 | Nil | 58 | Nil |

**Mean of triplicate determinations

*Standard = Table C-3. FAO/ WHO Expert Consultation on Microbiological Specification for Foods: pre-cooked frozen shrimps.

**Table 2:** Microbiological analysis of chicken tenderlion*.

| Lab. Code | TPC (CFU/g) | F.Coliform (MPN/g) | *E.coli* (MPN/g) | *Staph. aureus* (CFU/g) | Salmonella (CFU/g) |
|---|---|---|---|---|---|
| | $10^5$-$10^6$ | <50 | Nil | 20<100 | Nil |
| CT6 | 2.5 x $10^2$ | <0.3 | Nil | 25 | Nil |
| CT7 | 5.6 x $10^2$ | <0.3 | Nil | 14 | Nil |
| CT8 | 5.2 x $10^2$ | <0.3 | Nil | 32 | Nil |
| CT9 | 6.4 x $10^2$ | <0.3 | Nil | 28 | Present |
| CT10 | 7 x $10^2$ | <0.3 | Nil | 36 | Nil |

*Mean of triplicate determinations

All the other samples were free from Salmonella contamination. Salmonella are usually transmitted to humans by eating foods contaminated with animal fees. Contaminated foods are often of animal origin, such as beef, poultry, milk or eggs, but all foods including vegetables may become contaminated. Many raw foods of animal origin are frequently contaminated, but fortunately, though cooking kill salmonella. The unwashed hands o an infected food handler, who forget to wash his or her hands with soap after using the bathroom, may also contaminated food.

The microbiological analysis results of chicken Tenderloin were shown in Table 2. The highest TPC was found in CT10 sample 690cfu/g and the lowest TPC was found in CT6 sample 250cfu/g. The other sample CT7, CT8 and CT9 found 470, 520 and 640 cfu/g TPC. Fecal Coliform and E.coli were absent in all the analyzed samples. Salmonella were also absent in the entire sample except present CT9. The study of Ismail and Belma[2] 2002 reported that TPC were found between 4.4 x $10^4$ – 3.6 x $10^6$ cfu/g, in frozen meat the Coliform was between 4.0 x $>10^2$-1.1 x $10^5$ cfu/g and 2g salmonella spp. Were isolated from the commercial frozen meat samples.

*Staph. aureus* were found in the entire examined sample. The results showed that high count were found in sample CT10 i.e.36cfu/g. The lowest were found in CT7. The other value 25, 32 and 28 were found in CT6, CT8 and CT9 respectively. The study[7] carried out on microbiological quality of ground beef samples showed that TPC, S.aureus, Coliform and E.coli were counted at the average of 3.4 x $10^4$, 1.6 x $10^1$, 2.0 x $10^1$ and 3.8 x $10^3$ cfu/g respectively. Salmonella spp. Were determined at the levels of 24.0%.

Microbiological analysis results of Beef Patties were found in Table 3. The results indicate that F. Coliform, E.coli and Salmonella were absent in all the examined samples. S.aureus results showed a variation. The highest number found which was 20cfu/g in BP15. The lowest S.aureus 04 cfu/g found in BP12. The other samples BP11, BP13 and BP14 have 06, 10 and 07 cfu/g. Vaderline[8] *et al* 1998 found Coliform number less than 2 each g in 523 samples (66.6%) and 2-10 each g 143 samples (18.1%) and 11-100 each 174 samples (9.4%) and more than 1000 each g in 15 samples (1.9%), Salmonella spp. were found in 3(0.38%) in 790 pieces and frozen as boneless.

The microbiological analysis results of Poultry Turkey were shown in Table 4. The results showed that F. Coliform, *E. coli* and Salmonella were absent in all the analyzed samples. The *S. aureus* results showed that highest count was found in sample PT19 and lowest count were found in PT16. The other samples PT17, PT18 and PT20 were 10, 22 and 06 cfu/g. Chang and Lan[9] (1984) found no Salmonella in 10 frozen beef sample in the microbiological analysis they did. In the study we carried out it was determined that some frozen food products investigated are of great risk in terms of Salmonella.

**Table 3:.** Microbiological analysis of beef patties*.

| Lab. Code | TPC (CFU/g) | F.Coliform (MPN/g) | *E.coli* (MPN/g) | *Staph. aureus* (CFU/g) | Salmonella (CFU/g) |
|---|---|---|---|---|---|
| | $10^5$-$10^6$ | <50 | Nil | 20-<100 | Nil |
| BP11 | 4 x $10^3$ | <0.3 | Nil | 06 | Nil |
| BP12 | 2.5 x $10^3$ | <0.3 | Nil | 04 | Nil |
| BP13 | 2.2 x $10^3$ | <0.3 | Nil | 10 | Nil |
| BP14 | 2.4 x $10^3$ | <0.3 | Nil | 07 | Nil |
| BP15 | 6.5 x $10^3$ | <0.3 | Nil | 20 | Nil |

*M of triplicate determinations

**Table 4:** Microbiological analysis of poultry turkey*.

| Lab. Code | TPC (CFU/g) | F.Coliform (MPN/g) | *E. coli* (MPN/g) | *Staph. aureus* (CFU/g) | Salmonella (CFU/g) |
|---|---|---|---|---|---|
| | $10^4$-$10^5$ | <50 | Nil | <100 | Nil |
| PT16 | 2.0x $10^2$ | <0.3 | Nil | 03 | Nil |
| PT17 | 3.0 x $10^5$ | <0.3 | Nil | 10 | Nil |
| PT18 | 5.0 x $10^3$ | <0.3 | Nil | 22 | Nil |
| PT19 | 4.5 x $10^2$ | <0.3 | Nil | 32 | Nil |
| PT20 | 6.0 x $10^4$ | <0.3 | Nil | 06 | Nil |

*Mean of triplicate determinations

Gomma[10] *et al* 2002 results showed that in case of meat product, the mean TPC was 2.3 x 105, Coliform was 3.2 x $10^2$, poultry products had a mean TPC of 6.8 x $10^5$, a mean Coliform count of 6.4 x $10^2$. Fecal coliforms were detected only in 60.0% of meat product samples and in 45.0% of poultry product samples. On the other hand Staph. Aureus was detected only in 10.0% of poultry product samples.

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