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Full Length Research Paper

Microbial flora of frozen chicken part varieties

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Forty five samples of four different parts of Chicken varieties were analyzed to determine their microbial flora; the samples were collected from freezer depots, open market and cold room depot. All the chicken parts examined (Gizzards, Wings, Laps and Legs) were contaminated with some bacterial species namely, *Bacillus, Escherichia, Sp. Staphylococcus, Corynebacterium, Micrococcus, flavobacterium* and *Alcaligene.* The yeast belong to the genera *Saccharomyces* and the *moulds Aspergillus sp.* The total aerobic bacteria counts for all the parts examined stored in the freezer was in the range of 1.4×10^2 cfu/g to 3.1×10^2 to 6.0×10^6 cfu/g and the ones stored in the cold room had a range of 1.5×10^1 cfu/g to 1.5×10^1 cfu/g. the coliform counts obtained for all the chicken parts ranged from 1.2×10^2 cfu/g to 3.2×10^2 cfu/g, 1.2×10^2 cfu/g to 7.2×10^3 cfu/g and 1.1×10^1 cfu/g to 1.4×10^2 cfu/g for chicken parts stored in the freezer, open market and cold room respectively, while the mould and yeasts count gave a range of 1.4×10^1 cfu/g to 1.5×10^2 cfu/g, 1.2×10^2 cfu/g to 7.2×10^3 cfu/g and 1.3×10^1 to $1.5x10^2$ cfu/g for freezer, open market and cold room respectively. These finding suggest that most of the frozen chicken parts stored in the open market may constitute sources of bacterial food poisoning consequently public health hazard.

Keywords: Chicken Parts, freezer depot, open market, cold room, microbial load.

INTRODUCTION

The skin of live birds may obtain a number of bacterial averaging 1.5×10^3 per square centimeter. This number probably reflect the natural flora of the skin plus other organisms that could be derived from feet, feathers and faeces. (Frazier and Weshoff, 1988). Contamination of the skin and lining of the body cavity occurs during washing, plucking and evisceration.

Bacterial number vary considerably on the surface of chickens. This variation however is greater between birds than is between different areas of the same birds.

The types of organisms isolated depends upon where the samples were taken and upon the stage of processing. (Frazier *et al., 1985*).

Fresh poultry products like meat are known to undergo deterioration due to microbial action, chemical and physical changes. In normal handling and storage of poultry meat, this deterioration changes are attributed to micro biological contamination and activity. Like all fresh (uncooked) foods, chicken carries natural microflora that may contain organisms potentially harmful to humans.

The microbial flora of table poultry is largely confined to the skin surface or visceral cavity isolates from poultry and poultry products could include members of the following general *Enterobacter, Alcaligenes, Escherichia, Bacillus, FLavobacterium, Micrococcus, Proteus, Pseudomonas, Staphylococcus, Corynebacterium* and *Salmonella.* (*Frazier et al., 1988*).

Poultry and poultry products are frequently contaminated with several types of microorganisms. This problem is even more severe under temperature-abused conditions as well as improper or inefficient refrigeration commonly observed in retail chicken sold in open markets.

Poultry can be kept in good condition for months if freezing is prompt and rapid and the storage temperature is low enough.

Poultry should freeze fast enough to retain most of the natural bloom or external appearance of a freshly dressed fowl. The storage temperature should be below

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17.8°C and the relative humidity above 95 percent to reduce surface drying. Most poultry is sharp-frozen at about 29°C or less in circulating air or on a moving belt in a freezing tunnel.

Other spoilage micro-organisms are introduced into the poultry products by the workmen during cutting and evisceration, through water, and air in the dressing, cooling and cutting room environment (Bhagirathi *et al*., 1982). However, various methods are used in the preservation of these poultry products in order to reduce the incidence of these organisms. These include ascepsis, use of heat, use of low temperature, chilling, freezing, preservatives such as aceptic, adipic, succinic etc. at pH 2.5 and use irradiation (Frazier and Westhoff, 1988). Despite these methods of preservation, contamination of poultry products remains the order of the day before it gets to the final consumer. It is therefore on the basis of the various bacteria contaminants associated with these products that the project was aimed at achieving the following objectives:

- To isolate the various microbial isolates associated with chicken parts stored at various conditions.
- To characterize and identify these micro-organisms.
- To speculate on the significance of these isolates on major contaminants of frozen chicken parts.

MATERIALS AND METHODS

Sample collection: A total of 45 samples of four chicken parts, which include gizzards, laps, legs and wings, were collected from the following locations:

Location 1: From chicken freezer depots Iddo market, Ojuwoye and Iyana Ipaja in Lagos metropolis, Nigeria.

Location 2: From open markets of Oshodi, Ojo Alaba market and Mile 12, Ketu markets in Lagos metropolis, Nigeria.

Location 3: Cold room of Egbeda stores, Bariga and Ketu markets in Lagos metropolis, Nigeria.

The samples collected were wrapped asceptically in sterile polythene bags. All the samples were transported immediately to the laboratory for analysis.

Preparation of homogenate of chicken samples: Using a pair of sterile plastic gloves, the various chicken parts was cut away from the bone with the aid of sterile knives. Twenty grams of each of the various chicken parts were separately grounded in a sterile mechanical blender. 90ml of sterile 0.1% peptone was added. The chicken meat was blended at medium speed and a slurry was obtained (Nwosu, 1986). Serial dilutions of each slurry samples was then subsequently made in each sterile test tube up to 10^{-10} dilution. From an appropriate dilution 10^{-2}, 1ml was then inoculated on the following media as follows:

1. Nutrient Agar: This was used for the enumeration of total bacteria isolates from the samples. The plates were incubated at 37°C for 24 – 48 hours.
2. MacConkey Agar: This was used for the enumeration of coliforms in the samples. The plates were incubated at 35°C for 24 – 48hours.
3. Potato – Dextrose Agar: This was used for the enumeration of mould and yeast isolates in the samples. The plates were incubated at 32°C for 24 hours for yeast isolates and 3 – 5 days for moulds.

Morphological and Biochemical Test

The various micro organisms were subjected to morphological and biochemical tests for their identification according to the combined specification of Cowan and Steel 1974, Beech *et al* 1968 and Westly *et al.,* 1982. The various biochemical characterization includes Gram staining, Catalase spore staining, starch hydrolysis, Nitrate reduction, Oxidase test, urease and sugar fermentation.

Identification of Fungi

Cultural Characteristics

Each mould isolates was cultured on potato dextrose agar and observed for the following pigmentation and character of the hyphae.

Microscopic Examination

Slide preparation of the mould were made, pieces of the young mycelium from the pheriphery of the culture was made with a sterile inoculating loop and put on a glass slide. The cut sections was flamed briefly to melt the agar and later stained with lactophenol cotton. Blue cover slips were placed over them and examined under the microscope.

The microbial isolates were counted and thereafter subcultured on fresh agar plates to ensure purity.

RESULTS

In the location 1 (chicken parts collected from freezer depots) total micro were as presented in Table 1. the result revealed that the total plate count for all the sample investigated, the gizzard sample has the highest level of contamination 2.4×10^2 cfu/g and the leg has the least 1.4×10^2 cfu/g. the coliform count was however very high in the lap (1.3×10^2 cfu/g) and least in the leg sample (1.2×10^1 cfu/g). (Table 1) The slight high

Table 1. Total Number of microflora isolate from chicken freezer depots for designated location 1

Type of Analysis	Sample Code / cfu/g			
	Gizzard	Wings	Laps	Legs
Total Plate count	$2.4x10^2$	$3.1x10^2$	$1.5x10^2$	$1.4x10^2$
Coliform count	$2.8x10^1$	$3.2x10^1$	$1.3x10^2$	$1.2x10^1$
Moulds and Yeast count	$1.5x10^1$	$1.5x10^2$	$1.3x10^2$	$1.4x10^1$

Table 2. Total number of microflora isolates from chicken in open market for designated location 2.

Type of Analysis	Sample Code / cfu/g			
	Gizzard	Wings	Laps	Legs
Total Plate count	$1.6x10^4$	$6.0x10^6$	$2.5x10^4$	$1.5x10^3$
Coliform count	$8.5x10^2$	$7.2x10^3$	$3.5x10^2$	$1.2x10^2$
Moulds and Yeast count	$2.4x10^2$	$3.5x10^2$	$4.2x10^2$	$1.5x10^3$

Table 3. Total number of microflora isolated from chicken in cold room for designated location

Type of Analysis	Sample Code / cfu/g			
	Gizzard	Wings	Laps	Legs
Total Plate count	$1.1x10^1$	$1.3x10^1$	$1.4x10^2$	$1.5x10^1$
Coliform count	$1.5x10^2$	$1.2x10^2$	$1.3x10^1$	$1.3x10^1$
Moulds and Yeast count	$2.4x10^1$	$2.5x10^1$	$1.5x10^1$	$1.5x10^1$

microbial count as observed might have been due to poor hygiene on the part of the workers.

For location 3, total plate counts was highest in the lap sample with 1.4×10^2 cfu/g and least in gizzard sample with 1.1×10^1 cfu/g. the coliform counts was highest in the gizzard sample with 1.5×10^2 cfu/g and least in laps and legs sample with 1.3×10^1 cfu/g each. The mould and yeast isolates was highest in the wing sample with 2.5×10^1 cfu/g and least in laps and leg samples with 1.5×10^1 cfu/g (Table 3).

At a glance, it was very obvious that in all the designated locations, the open market had the highest microbial load when compared to other locations. The high microbial load might have been as a result of the constant exposure of the chicken parts to the open environment.

From the studies, low microbial count was however experienced in locations designated 3 (cold room store). The low number experienced might have been due to the low temperature experienced from the cold room. International microbiological standards recommends limits of bacteria contaminants for foods (Amon, 1974, Refai, 1979), are in the range of 10^1 - 10^2 cfu/g of food for coliform organisms are less than 10^5 cfu/g of food for total bacteria plate counts and 10^1 cfu/g for mould and yeast count. The present study however revealed that all the frozen chicken under the different designated locations i.e. The freezer depots for all the chicken gave a range of $1.4 \times 10^2 - 3.1 \times 10^2$ cfu/g, the open market location designated as 2 gave a range of 1.5×10^3 cfu/g – 6.0×10^6 cfu/g and the designated location 3 i.e. cold room gave a range of 1.2×10^1 cfu/g – 2.5×10^1 cfu/g. The coliform count for location 1 gave a range of 1.2×10^1 - 3.2×10^1 cfu/g and location 2 gave a range of 1.2×10^2 – 7.2×10^3 cfu/g and for location 3 gave a range of $1.1 \times 10^1 - 1.5 \times 10^1$ cfu/g. The mould and yeast on the other hand for location 1 gave a range of 1.4×10^1 - 1.5×10^1 cfu/g and location 2 gave a range of $1.2 \times 10^2 - 7.2 \times 10^3$ cfu/g and for location 3 gave a range of $1.1 \times 10^1 - 1.5 \times 10^1$cfu/g. The mould and yeast on the other hand for location 1 gave a range of $1.4x10^1$ to $1.5x10^2$ to cfu.g and location II gave a range of $2.4x10^2$ to $1.5x10^3$ cfu/g and for location 3 $1.3x10^1$ to $1.5x10^2$ cfu/g. All the chicken parts stored under the different conditions designated as location, the open market was very high and therefore microbiologically unacceptable. The other locations of freezer and cold room gave results that were still within microbiologically acceptable standards. The high microbial flora experienced might have been as a result of poor hygiene, poor sanitary conditions, open tables, perching by flies and other organisms, spores of bacteria from the open environment, knives and tables on which these chicken parts have been placed.

A total of 14 bacteria species, 3 yeasts and 3 moulds species from the various frozen chicken parts examined were as shown in the table 4 and table 5. The bacteria

Table 4. Biochemical characteristic of the Micro flora from frozen chicken varieties

Labcode	Colony Morphology	Cell charact	End	Cat	Vo	Methl	Nit	Star	Oxi	Indo	Ure	AR	FR	GA	GL	LA	MA	MS	MT	SU	RA	*Probable Identity*
BCN1	Large White colonics Raised Mucoid and entire	Gram+ Rods	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	+G	+G	+	+	*Bacillus-subtilis*
BCN 2	Small Raised Puntform	Gram+ Rods	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	*Klebsiella-aerogenes*
BCN 3	Small white colonies	Gram- Rods	-	+	-	-	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+	*Pseudomas putida*
BCN 4	Yellow small flat and Irregular	Gram+ Rods	-	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	*Stephylocc-uis aereus*
BCN 5	Large white entire Mucoid	Gram- Rods	+	+	+	-	+	-	-	-	-	+	-	-	-	-	+	-	+	-	-	*Salmonella sp*
BCN 6	White colonies small flat	Gram+ Cocci	-	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+	*Staphylococcus epidermis*
BCN 7	Small white mucoid	Gram- Rods	-	+	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	+	-	*Proteus vulgaris*
BCN 8	Dirty white mucoid	Gram- Rods	-	+	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	*Escherichia coli*
BCN 9	Spreading white, large Raised mucoid irregular	Gram+ Rods	+	+	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	+	*Bccillus Cereus*
BCN 10	Small yellow Colonies	Gram+ Rods	+	+	-	-	-	-	-	-	-	+	-	-	+	-	+	-	-	+	-	*Corynebact-erium spp*
BCN 11	Large yellow Colonieentire	Gram- Rods	-	+					+	-	-	+	-	+	-	-	-	+	-	-	-	*Flavobacte-rium*
BCN 12	Small entire Mucoid irregular	Gram- Rods	-	+	-	+	-	-	+	-	-	+	-	+	+	-	-	+	-	-	-	*Alcaligene sp*
BCN 13	Light Red small colonies	Gram+ Cocci	-	+	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	*Micrococc-us sp*
BCN 14	Small white colories irregular	Gram- Rods	-	+	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	*Pseudomon-as capacia*
YN 1	Creamy colour	Ovoid shape										-	+G	+G	+G	+G	-	-	+G	+G	+G	*Saccharom-yces cerevisiae*
YN 2	Creamy colour											-	+G	+G	+G	+G	-	-	+G	+G	+G	*Saccharom-yces Cerevisiae sp*
YN 3	Round Mutilateral budding colour											+G	+G	+	-	+G	-	-	+G	+G	+G	*Rhodotorul*
YN 4	Milky colour	Ovoid shape											+G	+G	+G	+G	-	-	+G	+G	+G	*Saccharom-yces Cerevisiae*
YN 5	Round Mutilateral budding cells													+G	+G	-	+G	-	-	+G	-	*Candida spp*
YN 6	Cream Colour	Ovoid shape											+G	+G	+G	+G	-	-	+G	+G	+G	*Saccharomyces cerevisiae*

Table 5. Characterization of mould from frozen chicken

	Cultural Characteristics	Morphological	Probable Identity
MNl	Greenish, powdery Colonies with creamy Edges	Separate hyphae	Aspergillus sp
MN2	Greenish colour	finger-like sterigma	Penicillium sp
MN3	Dark green	short, non septate Conido phores	Aspergillus fumigatus

isolates were identified as the course of the study were basically the enteric organism and their presence in the frozen chicken is a source of faecal contamination. The presence of these organisms are also sources of diarrhea and or gastro intestinal disturbance to both adult and children when consumed and may lead to food intoxication. This is in agreement with the finding of other workers (Bryan *et al.,* 1981; Van steenberger *et al*., 1983; Bryan *et al.,* 1986) concerning frozen chicken stored under different conditions.

However, the result revealed a high microbial load and more species of pathogenic bacteria are obtained from samples kept in the open market. This might have been as a result of contaminations resulting from poor hygiene on the part of the sellers, from various contaminating insects and during evisceration of the chicken during processing.

The presence of yeast isolate of sacchromyces, cerevisiae, rhodotorula and candida sp (table 4) in all the sample stored under various condition might have been due to contaminations experienced during processing and storage.

Contaminated frozen chicken parts as a source of numerous infectious disease performed metabolites of these microbial isolate could be fatal. For instance, some Aspergillus spp isolate from the samples might have been introduced as spores from the atmosphere. The Aspergillus are known to produce Aflatoxin (jideani and Osuide, 2001).

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