

Original Paper

## Chemical and microbiological quality of commercial fresh and frozen chicken drumstick in Umuhia, Nigeria

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**Abstract**—Chicken meat, an excellent source of good quality proteins, is highly susceptible to microbial contaminations and heavy metals from feeds, processing, and retailing environments, thereby posing health risks to consumers. Therefore, the proximate composition, heavy metal concentrations, and microbial loads of commercial fresh and frozen chicken drumsticks, randomly sourced from poultry farms and groceries, respectively, within Umuahia metropolis, Abia State, Nigeria, were investigated to ascertain their qualities. A total of 40 (20 each of the fresh and frozen) chicken drumstick samples were processed and analyzed for proximate (moisture, fats, ash, proteins, fibers, and carbohydrates), heavy metal (cadmium, chromium, copper, manganese, nickel, zinc, and lead) contents, and microbial loads (total viable counts, *Escherichia coli* counts, *Salmonella* counts, *Staphylococcus* counts and fungal counts) and characterization using standard techniques. Results were analyzed statistically ( $p < 0.01, 0.05$ ). The fresh chicken drumstick samples had significantly higher ( $p < 0.01, 0.05$ ) levels of moisture ( $61.38 \pm 0.34\%$ ), proteins ( $22.30 \pm 0.02\%$ ), ash ( $5.27 \pm 0.17\%$ ) and microbial contents ( $0.24 \times 10^2$  CFU/g to  $2.42 \times 10^4$  CFU/g), when compared with the frozen drumstick samples with significantly higher ( $p < 0.01, 0.05$ ) contents of fats ( $3.70 \pm 0.13\%$ ), carbohydrates ( $12.55 \pm 0.27\%$ ) and heavy metals ( $0.047 \pm 0.017$  mg/kg to  $0.852 \pm 0.456$  mg/kg). This suggests that fresh chicken drumstick is more nutritionally beneficial, provided they are thoroughly treated with heat before consumption to prevent foodborne diseases from bacterial and fungal contaminations. Although the levels of heavy metals in both meat samples are below the toxic limits, consumers should be wary of bioaccumulation and biomagnification of heavy metals when constantly exposed to them. Hence, constant surveillance of chicken meat is

encouraged to monitor their exposure levels to these chemicals and biohazards.

**Keywords**—Bioaccumulation, Chicken drumsticks, Heavy metals, Microbial contaminations, Proximate.

### I. INTRODUCTION

Chicken is one of the most widely consumed meats all over the world [1], and serves as a main source of proteins for most people in Nigeria [2]. It is cherished and preferred by many because of its rich nutritional and delectable sensory qualities [1, 3], and has been named the most palatable animal protein [4]. Consumption of poultry meat and products has increased as a result of economic crises, driven by several factors, while consumers hold off from the more expensive beef, lamb meat, or meat products [5]. It is more economical due to its high meat yield, low shrinkage during cooking, and low cost [4]. According to Adu-Gyamfi *et al.* [1], chicken is not only an excellent source of good quality proteins; it is also highly susceptible to microbial contaminations and often implicated in foodborne diseases [6][63]. Chicken meats and chicken-based products are prone to microbial spoilages and could harbor pathogens, despite good adherence to best management conditions and practices [7, 8]. During the various stages of slaughtering and processing, all potential edible tissues are subjected to contaminations from a variety of sources within and outside the animal [9]. Quality of the food has roots in its raw or unprocessed state, the sanitary conditions under which the product is handled and processed, and the adequacy of subsequent packaging, handling and storage conditions of the products [10]. The microbiological quality of chicken as purchased by consumers, as with other meats generally, depends on factors such as the quality of the raw products, sanitation during processing and packaging, maintenance of

adequate cooling system from the processing to the retail level, and the consumer, and finally, sanitation during handling at the retail end [11]. Consumers are exposed to different deleterious effects through the consumption of meats with qualities marred by chemical or microbiological contaminants.

Over 30 genera of microorganisms have been reported known to contaminate poultry products [12, 13]. These may include several pathogens (*Campylobacter*, *Salmonella*, *Yersinia enterocolitica*, *Clostridium perfringens*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157:H7) which have together been known to be responsible for 3.9 million foodborne illnesses and 1600 deaths each year [10]. The chicken itself offers an excellent medium for the multiplication of most bacteria, including those that are not inhibited by low temperatures [1]. After the initial bacterial contamination of the meat and the constitution of the initial microbiota, the storage conditions and the various treatments applied form the outcome of this microbiota in terms of population and diversity [6, 14, 15]. Storage at low temperature is selective for some microbial populations but mostly favor the growth of psychrotrophic and psychrophilic bacteria. A study by Abdu and Abubakar [6] in Kano showed that the frozen chicken was more highly contaminated with aerobic mesophilic and coliform counts than the freshly slaughtered ones. The difference in the population of the microorganisms from different parts of frozen and freshly slaughtered chicken, therefore, raises some scientific curiosities on the variables around it, including natural micro-flora, the different environments and personnel, materials used including water during processing, transportation, and storage and general handling.

Despite its nutritional benefits and good advantages in comparison with beef meat, the quality of poultry meat may be affected by the contaminations from toxic metals through various anthropogenic activities [16]. Chicken may be contaminated with toxic elements such as arsenic, cadmium, or lead as a result of coming into contact with the materials on the farm, factory, or while moving through the marketing channels. Chicken meat may sometimes still carry metals and other elements either naturally found in air, water, soil, and poultry feed or as a result of human activities such as industrial and agricultural processes [4]. Entry of undesirable substances into the food chain is mainly due to environmental pollution [5]. Food chain contamination has on the other hand been a common route of exposure to heavy metals for humans [17]. Chickens, either free-range or under intensive rearing systems, are exposed to a vast array of heavy metals and trace elements through polluted air inhalation or contaminated feed ingestion. Poultry feed contains high concentrations of minerals which are directly added to the ration to satisfy the body requirements of the bird, but in many cases, they are more than the needs of the bird [4, 18]. The metals of particular concern with harmful effects on health are often referred to as “heavy metals”. Some of them include mercury, lead, cadmium, tin, copper, cobalt, and arsenic. These toxic elements are known to induce widespread adverse health effects [5, 19]. Contaminations by such elements pose risks to human health, because, as metals

bioaccumulate up the food chain, they can biomagnify and can cause various adverse health hazards when consumed constantly [20]. Certain chicken tissues are analyzed for residues of contaminants such as pesticides, toxic elements, or persistent organic pollutants. Although residues of pesticides such as organophosphorus, fungicides, herbicides, and carbamate compounds in poultry meats and products are always low and most times negligible due to the elimination of these compounds to a certain extent [21, 22], other residues still accumulate to levels where they pose toxicity concerns. Organic arsenic compounds (roarsone, arsanilic acid, nitarsone, and carbarsone) have been widely used in the poultry sector for long years to prevent some diseases, accelerate growth, increase feed efficiency, and increase pigmentation of the meat [5]. Some of these compounds in residual amounts in poultry meat and eggs have important deleterious effects such as genotoxic, immunotoxic, carcinogenic, teratogenic, or endocrine-disrupting effects [23]. In recent times, many researchers have delved into the concentrations of heavy metals in meats (including chicken) and other foods to check for their health risks [4, 18, 24]. This study, therefore, evaluated the macronutrients and the chemicals and the microbiological loads of commercial chicken drumsticks, the commonest raw forms of this popular meat, within Umuahia, South East Nigeria.

## II. MATERIALS AND METHODS

### A. Study area

The study area was Umuahia, the capital city of Abia State, Southern Nigeria. The geographical coordinates are within longitudes 7°22' and 7°33' E and latitudes 5°26' and 5°34' N (Fig. 1). The climate conditions are 70 % and 29 – 31°C relative humidity and temperature respectively [25].

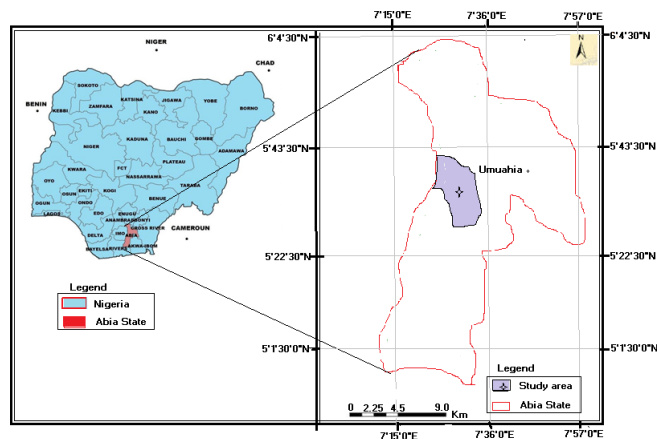


Fig. 1 Location map of Abia State showing Umuahia, the study area.

### B. Sample collection

Selected live chickens were randomly sourced from poultry farms located within Umuahia; processed manually to obtain the thighs (fresh parts used) (20 samples) needed. The frozen samples (20 thigh parts) were purchased from major Grocery shops within the Umuahia metropolis. Both sampling sites were commonly patronized by customers/consumers of chicken. All

the samples were transported in ice-packed well-sealed stomacher bags to the Laboratory for processing and analyses.  
*C. Proximate analysis of the chicken samples*

#### Moisture content determination

Five grams (5 g) of each of the chicken meat samples was weighed out using analytical balance into a dried, cooled, and pre-weighed dish. The samples in the dishes were then put into a hot air oven (Gallenkamp, size 2, SG97/04/070, England) set at 105° C and allowed to dry for 6 h. When this time elapsed, the samples were then transferred into a desiccator with the aid of a laboratory tong and then allowed to cool for 30 min. After the cooling, they were weighed again and their respective weights were recorded accordingly. The moisture content was calculated from the difference in weight [26]. The difference in weight was calculated as a percentage of the original sample as follows:

$$\% \text{ Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad (1)$$

Where:

$W_1$  = Initial weight of the empty dish

$W_2$  = Weight of the dish + wet sample, and

$W_3$  = Weight of the dish + dried sample.

#### Fat content determination

The fat contents of the meat samples were determined according to the method described by Onwuka [26]. Two hundred and fifty milliliters (250 mL) boiling flasks were washed with water, dried in an oven set at 105° C for 30 min, cooled in a desiccator, and then used for each sample. The flasks were each firstly labeled, weighed with an analytical balance, and then filled with 250 mL of petroleum ether. Then, a five grams (5 g) weight of each of the samples was weighed out with an analytical balance into a correspondingly labeled thimble. The extraction thimbles were in each case tightly plugged with cotton wool. The Soxhlet apparatus was then assembled and allowed to reflux for 6 h. When this time elapsed, the thimble was removed and the petroleum ether was recovered in the setup and drained into another container for re-use. The flasks were removed in each case and then dried in an oven at 105° C for 1 h. After drying, they were transferred into a desiccator and allowed to cool and be weighed. The percentage of fat was calculated for each sample as:

$$\text{Fat (\%)} = \frac{W_2 - W_1}{W_s} \times 100 \quad (2)$$

Where:

$W_1$  = weight of the empty flask

$W_2$  = weight of flask + oil after drying and

$W_s$  = weight of the sample extracted

#### Protein content determination

The protein contents of the chicken meat samples were determined according to the method described by Onwuka [26], with slight modifications. Half a gram (0.5 g) of each sample

was mixed with 10 mL of concentrated sulphuric acid ( $H_2SO_4$ ) in a Kjeldahl digestion flask. A 0.8 g portion of Kjeldahl catalyst was added to each of the samples; a small quantity of anti-bumping granules was added to prevent frothing and then digested (heated) inside a fume cupboard until a clear solution was obtained for each sample. Also, a blank was made by digesting the above reagents without any sample in it. Then, for each sample, the digest was carefully transferred with several steps of washings into a 100 mL volumetric flask and made up to the 100 mL mark with distilled water. A 10 mL portion of the digest was mixed with an equal volume of 40 % sodium hydroxide (NaOH) solution in a Markham distilling unit. The resultant mixture was distilled and the distillate was collected into a 100 mL conical flask containing 10 mL of 4 % boric acid solution and three drops of mixed indicators (bromocresol green and methyl red). A total of 50 – 75 mL of the distillate was obtained and titrated with 0.01 N hydrochloric acids (HCl) solution. Titration was done from the initial green color to a deep red end-point. The nitrogen content of each sample was then calculated as:

$$\% \text{ Nitrogen} = \frac{N \times 0.01401 \times T}{W} \times 100 \quad (3)$$

Where:

$W$  = weight of sample analyzed,

$N$  = Normality of HCl titrant,

$T$  = Titre value of the sample minus titre value of the blank (given as  $T = V_s - V_b$ ).

Where;

$V_s$  = total volume of acid required to titrate sample and  $V_b$  = volume of acid required for blank. The crude protein content of each sample was then obtained as:

$$\text{Crude protein (\%)} = N \times 6.25 \text{ (i.e. conversion factor)}$$

#### Determination of ash content

This was determined according to the method described by Onwuka [26]. Two grams (2 g) of each of the samples were weighed out with the aid of an analytical balance into a dried cooled and weighed crucible in each case. The samples were then charred by placing them on a Bunsen flame inside a fume cupboard to drive off most of the smoke for 30 min. The samples were thereafter transferred into a pre-heated muffle furnace already at 550° C with the aid of a long laboratory tong. The samples were allowed to incinerate in the furnace for 3 h until a light grey to white ash resulted. Samples that remained black or dark in color after this time had elapsed were moistened with a small amount of water to dissolve salts, dried in an oven, and then incinerated again. After incineration, the crucibles were then transferred into a desiccator with a laboratory tong for cooling. Once they were cooled, they were each weighed again and recorded accordingly. The ash content was then calculated as:

$$\text{Percentage of ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \quad (4)$$

Where:

$W_1$  = weight of empty crucibles

$W_2$  = weight of crucible + food sample before incineration and

$W_3$  = weight of crucible + ash

#### The determination of crude fibre content

This was determined according to the method described by Onwuka [26]. Five grams (5 g) of each of the defatted samples (during fat analysis) were used in this determination. For each of the soups, the defatted sample was boiled in a 500 mL flask containing 200 mL of 1.25 %  $H_2SO_4$  solution under reflux for 30 min. When this time elapsed, the sample was washed with several portions of hot boiling water using a multifold muslin cloth to trap the residual particles. The residual particles were carefully transferred qualitatively back to the flasks and 200 mL of 1.25 % NaOH solution was then added into the flask. Again, the sample was boiled for 30 min and washed as before with hot water. Then, residual particles were carefully transferred into a weighed crucible and then dried for 3 h in an oven set at 105°C. The dried sample was then transferred into a desiccator for cooling for about 20 min before being weighed again. After weighing, the sample was transferred into a muffle furnace set at 550°C for 2 h (until they were incinerated). Finally, when the incineration had been completed, they were cooled in a desiccator and weighed again. The crude fiber content for each sample was then calculated as follows:

$$\text{Crude fibre (\%)} = \frac{W_2 - W_3}{W_1} \times 100 \quad (5)$$

Where:

$W_1$  = weight of the original sample

$W_2$  = weight of crucible + sample after washing and drying in the oven, and

$W_3$  = weight of crucible + sample as ash

#### Total carbohydrate content

The carbohydrate content of each of the samples analyzed was determined by difference [26]:

$$\% \text{ Total carbohydrate} = 100 - \% (\text{moisture} + \text{ash} + \text{crude fibre} + \text{crude protein} + \text{fat}) \quad (6)$$

#### D. Heavy metal analysis of the chicken samples

Two grams (2 g) of the chicken meat samples were weighed out using analytical balance into a pre-digestion tube and homogenized in 10 mL of concentrated  $HNO_3$  at 135° C until the contents became clear. The clear content was further mixed with Nitric acid ( $HNO_3$ ), Hydrochloric acid ( $HClO_4$ ), and hydrogen peroxide ( $H_2O_2$ ) in a ratio of 10:1:2. Thereafter, the content was allowed for digestion at 135° C for 1 h, gradually evaporated, and cooled at room temperature. The cooled digest was homogenized in 1 M  $HNO_3$  and filtered using Whatman filter paper (No. 1). Furthermore, 1 M  $HNO_3$  was added to the digested filtrate and diluted to 25 mL before analysis of the selected heavy metals Cadmium (Cd), Chromium (Cr), Copper (Cu), Manganese (Mn), Nickel (Ni), Zinc (Zn), and Lead (Pb) using graphite furnace atomic absorption spectrophotometer (GBS Scientific Equipment Sens-AAS 1175, Australia [27].

#### E. Microbiological (Bacterial and Fungal) analysis of chicken samples

Each of the chicken drumstick samples was weighed; 25 g was homogenized in 225 mL sterile peptone water (0.1 %) and serially diluted to  $10^6$  [28]. An aliquot part of a well-mixed portion of the selected dilutions ( $10^2$ ,  $10^4$ , and  $10^6$ ) of the samples was aseptically cultured (spread plate method) on sterile petri dishes of Nutrient Agar (Oxoid, UK), MacConkey Agar (Oxoid, UK), Eosin Methylene Blue Agar (Oxoid, UK), *Salmonella-Shigella* Agar (Oxoid, UK), Mannitoal Salt Agar (Oxoid, UK) and Sabouraud Dextrose Agar (Oxoid, UK) for enumeration and isolation of total heterotrophic bacteria, coliform, *Escherichia coli*, *Salmonella* and *Staphylococcus aureus*. All the cultures were incubated at 36 – 44° C, 24 - 48 h (bacteria) and 28 – 30° C, 48 – 72 h (fungi) as the case may be for good growth and colony formation. The specific bacterial and fungal colonies were observed and enumerated using a colony counting chamber and expressed as colony-forming units per gram of chicken sample (CFU/g). Representative colonies of each bacterium and fungus were subcultured onto fresh media to obtain pure cultures, which were subjected to a battery of phenotypic characterizations as described for bacterial and fungal species [29-31].

#### F. Data analysis

The data generated from this study were analyzed using Microsoft Excel version 2007 and ANOVA for differences in means with  $p < 0.01$  and  $p < 0.05$  considered statistically significant for all the comparisons. Mean values that were significantly different were separated using Duncan Multiple Range Test.

### III. RESULTS

#### A. Proximate analyses of chicken samples

The data obtained from proximate analyses shown in Table I revealed significantly higher ( $p < 0.05$ ) contents of moisture ( $61.38 \pm 0.34$  %), protein ( $22.30 \pm 0.02$  %), and ash ( $5.27 \pm 0.17$  %) in fresh drumstick samples compared to the frozen samples, which had significantly greater ( $p < 0.01$ ) amount of fat ( $3.70 \pm 0.13$  %) and carbohydrate ( $12.55 \pm 0.27$  %) contents. The crude fiber was not detected in both samples.

TABLE I. PROXIMATE ANALYSIS OF CHICKEN DRUMSTICK

Proximate content (%)	Fresh Chicken	Frozen Chicken
Moisture	$61.38^* \pm 0.34$	$60.02^* \pm 0.30$
Fat	$2.45^{**} \pm 0.01$	$3.70^{**} \pm 0.13$
Protein	$22.30^{**} \pm 0.02$	$21.30^{**} \pm 0.06$
Ash	$5.27^{**} \pm 0.17$	$2.54^{**} \pm 0.09$
Fiber	$0.00 \pm 0.00$	$0.00 \pm 0.00$
Carbohydrate	$8.61^{**} \pm 0.18$	$12.55^{**} \pm 0.27$

Values are mean  $\pm$  standard deviation of duplicate determinations.

\*Means are significantly different at  $p < 0.05$ . \*\*Means are significantly different at  $p < 0.01$ .

### B. Heavy metal analysis of chicken samples

The results of the heavy metal analysis (Table II) showed significantly higher ( $p < 0.05$ ) contents of Cd ( $0.094 \pm 0.077$  mg/kg), Cu ( $0.117 \pm 0.027$  mg/kg), Mn ( $0.390 \pm 0.272$  mg/kg), Ni ( $0.852 \pm 0.456$  mg/kg) and Pb ( $0.074 \pm 0.034$  mg/kg) in the frozen chicken samples compared with the fresh samples. The metal Cr (0.047) was higher ( $p < 0.01$ ) in the frozen samples, while the level of Zn was not significantly different ( $p > 0.05$ ) in both samples.

TABLE II. ANALYSIS OF HEAVY METALS IN CHICKEN DRUMSTICK SAMPLES

Heavy metal (mg/kg)	Fresh chicken	Frozen chicken	Toxic level [32]
Cd	$0.019^{**} \pm 0.007$	$0.094^{**} \pm 0.077$	0.012 – 0.04
Cr	$0.029^* \pm 0.015$	$0.047^* \pm 0.017$	0.01 – 0.3
Cu	$0.067^{**} \pm 0.022$	$0.117^{**} \pm 0.027$	0.05 – 0.8
Mn	$0.390^{**} \pm 0.272$	$0.117^{**} \pm 0.040$	4.0 – 4.8
Ni	$0.577^{**} \pm 0.300$	$0.852^{**} \pm 0.456$	0.3 – 0.5
Pb	$0.024^{**} \pm 0.006$	$0.074^{**} \pm 0.034$	0.01 – 0.1
Zn	$0.825 \pm 0.269$	$0.828 \pm 0.457$	5 – 40

\*Means are significantly different at  $p < 0.05$ . \*\*Means are significantly different at  $p < 0.01$ .

### C. Microbiological analysis of chicken samples

The microbial quality of the chicken drumstick samples analyzed from Umuahia is represented in Table III. The fresh

drumstick samples had significantly higher ( $p < 0.05$ ) loads of total viable bacterial counts (TVBC), *Escherichia coli* counts (EC), *Salmonella* counts (SC), *Staphylococci* counts (StC), and fungal counts (TFC).

TABLE III. MEAN COUNTS OF BACTERIA AND FUNGI OF CHICKEN DRUMSTICK SAMPLES

Microbial load	Fresh drumstick sample	Frozen drumstick sample
TVBC	$2.42 \times 10^{4a}$	$0.61 \times 10^{2b}$
EC	$2.50 \times 10^{2a}$	$0.01 \times 10^{2b}$
SC	$0.24 \times 10^{2a}$	$0.05 \times 10^{2b}$
StC	$1.02 \times 10^{3a}$	$0.25 \times 10^{2b}$
TFC	$1.45 \times 10^{2a}$	$0.15 \times 10^{2b}$

\*Values are mean  $\pm$  standard deviation of triplicate determinations; values with similar alphabet across each row are not significantly different ( $p < 0.05$ )

Phenotypic characterization and identification of the representation isolate revealed the presence of seven (7) bacterial and four (4) fungal genera (Tables IV and V). The predominant bacterial isolates were *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Klebsiella* spp., *Proteus* spp., *Bacillus* spp. and *Pseudomonas* spp. (Table IV).

TABLE IV. PHENOTYPIC CHARACTERIZATION AND IDENTIFICATION OF BACTERIAL ISOLATES

Parameters	A	B	C	D	E	F	G
Colonial morphology	Colorless, small, round, Smooth, colonies	Golden-yellow, round, pinpointed smooth, convex colonies	White, Transparent shiny, circular, colonies.	White, opaque, Round, smooth, convex, shiny, colonies	Cream, round, shiny, swarming colonies	White, opaque, Large, rough colonies	blue/green colored opaque smooth colonies
Grams stain	(-) rods	(+) cocci	(-) rods	(-) rods	(-) rods	(+) rods	(-) rods
Shape/ arrangement	Short rods, singly, paired	Clusters, paired chains	Single, short-paired chains	Singly, pairs short chains	Singly, pairs short chains	Singly paired chains	Singly, paired chains
Spore test	(-)	(-)	(-)	(-)	(-)	(+)	(-)
Catalase test	(+)	(+)	(-)	(+)	(+)	(+)	(+)
Oxidase test	(-)	(-)	(-)	(-)	(-)	(+)	(+)
Coagulase test	(-)	(+)	(-)	(-)	(-)	(-)	(-)
Motility test	(+)	(-)	(+)	(-)	(+)	(+)	(+)
Indole test	(+)	(-)	(-)	(-)	(+)	(+)	(-)
Methyl red	(+)	(+)	(+)	(-)	(+)	(-)	(-)
VP test	(+)	(+)	(+)	(-)	(+)	(+)	(+)
Citrate test	(-)	(+)	(+)	(+)	(-)	(+)	(+)
Urease test	(-)	(-)	(-)	(-)	(+)	(-)	(+)
Lactose test	(+)	(+)	(-)	(+)	(-)	(+)	(+)
Sucrose test	(+)	(+)	(-)	(+)	(-)	(+)	(+)
Glucose test	(+)	(+)	(-)	(+)	(-)	(+)	(+)
Mannitol test	(+)	(+)	(-)	(-)	(+)	(+)	(+)
Organism	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella</i> spp.	<i>Klebsiella</i> spp.	<i>Proteus</i> spp.	<i>Bacillus</i> spp.	<i>Pseudomonas</i> spp.

TABLE V. PHENOTYPIC CHARACTERIZATION AND IDENTIFICATION OF FUNGAL ISOLATES

Characteristics	H	I	J	K
Cultural/ macroscopic	Grey black (top view) Dark (brown reverse) mycelia	Dark blue/green, wide- spreading fluffy colonies	White fluffy, cottony fast spreading colonies with black spots	White/cream fast- growing loose, fluffy cottony mycelia
Morphological/ Microscopic	Septate-hyphae, transparent with columnar head	Septate-branched hyphae with enlarged conidiophores	Non-septate hyphae	Non-septate hyphae
Organisms	<i>Aspergillus</i> spp.	<i>Penicillium</i> spp.	<i>Rhizopus</i> spp.	<i>Mucor</i> spp.

The most frequent bacterial and fungal isolates were recorded from the fresh drumstick samples (Fig. 2 and 3). Among the bacterial isolates, *S. aureus* occurred most in both samples (Fig. 2) with percentages of 25.7 % (fresh samples) and 10.8 % (frozen samples). *Proteus* spp. (2.7 %) and *E. coli* (4.1 %) occurred the least, respectively. While *E. coli*, *Salmonella* sp., and *S. aureus* were present in both samples, *Bacillus* spp. was

not detected in the fresh samples as against *Klebsiella* spp., *Proteus* spp., and *Pseudomonas* spp., which were absent in the frozen counterpart. For the occurrence of fungi, the four (4) isolates were recorded in both fresh and frozen drumstick samples except for *Rhizopus* spp. which was not detected in the frozen samples (Fig. 3).

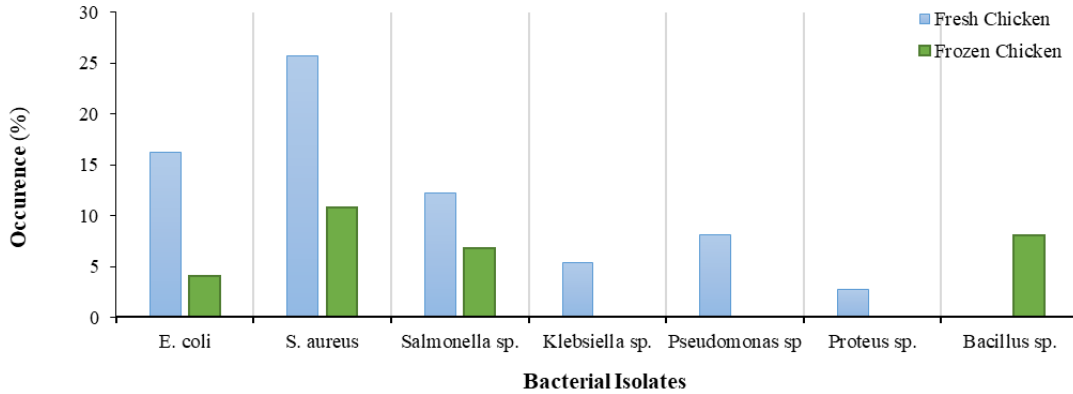


Fig 2. Occurrence of bacterial isolates in chicken samples from Umuahia metropolis

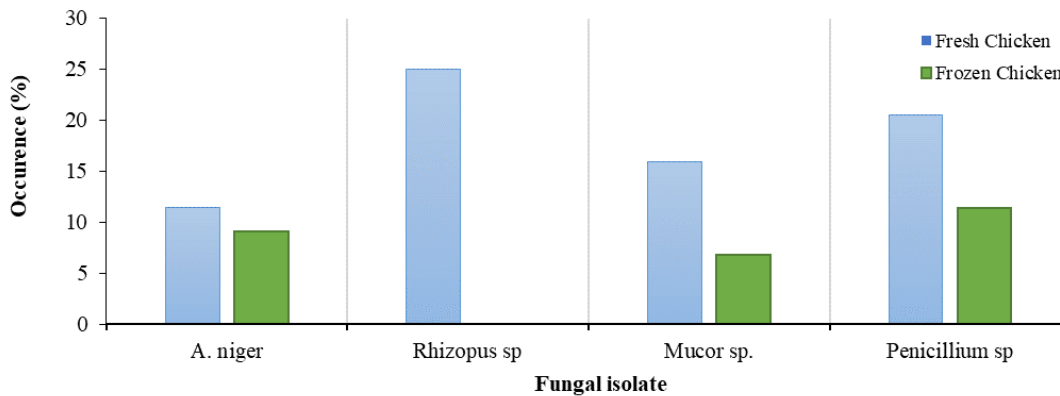


Fig. 3. Occurrence of fungal isolates in chicken samples from Umuahia metropolis

#### IV. DISCUSSION

The fresh drumstick samples had higher contents of moisture, proteins, and ash than the frozen samples. The temperature and duration of freezing must have impacted the proximate contents of the meat as reported previously [33].

Higher moisture content in food encourages microbial growth and spoilage and thus fresh chickens are prone to a higher rate of spoilage. Proteins supply amino acids and other essentials for good growth, tissue repairs, and proper development of the cells. Ash contents imply the levels of mineral nutrients, which

are also essential for the proper functioning of the body. The frozen chicken drumsticks on the other hand had higher levels of crude fats/lipids and carbohydrates. Unfortunately, higher consumption of animal fats may predispose some individuals to higher risks of hyperlipidemia and its attendant effects. Similarly, overconsumption of carbohydrates leads to insulin-related ailments. Hence, findings from proximate analyses of the fresh and frozen drumstick samples suggest that the consumption of fresh drumsticks is nutritionally better than the frozen counterpart. Microbiologically, high carbohydrate content in fresh meat samples encourages the activities of glycolytic spoilage microbial species on those samples and may prompt the risk of spoilage of those samples by glycolytic microbial agents on eventual contamination. The proximate composition obtained in this study was comparable to the works of Hammuel *et al.* [34], whose fresh chicken proximate contents were moisture ( $71.58 \pm 0.31$  % to  $71.93 \pm 0.23$  %); proteins ( $21.46 \pm 0.78$  % to  $23.96 \pm 0.21$  %), ash ( $0.81 \pm 0.03$  % to  $1.12 \pm 0.02$  %), fat ( $4.2 \pm 0.13$  % to  $4.49 \pm 0.10$  %), and carbohydrate ( $0.31 \pm 0.02$  % to  $0.66 \pm 0.05$  %). The moisture and ash contents of the fresh and frozen chicken drumsticks were higher than those reported for the liver, kidney, and intestine of a cow, sheep, and goat, which ranged from 15.96 to 38.95% (moisture), 0.81 to 1.76 %, (ash) [35]. However, beef had higher moisture ( $69.45 \pm 0.19$  to  $71.22 \pm 0.19$  %) and crude fat ( $6.36 \pm 0.12$  % to  $7.21 \pm 0.11$  %) contents [34]. This finding further lays credence to the global preference for chicken meat over other sources of animal products [1].

Findings from the heavy metal analysis suggest that the frozen drumsticks generally had higher levels of all the heavy metals tested in this study. When compared with the recommended toxic levels for heavy metals in meat, their concentrations were all within the permissible limits, suggesting little or no risk of predisposition to heavy metal toxicity. Nutritionally different classes of food harbor diverse biologically useful elements, known as micro-nutrients. They are needed by the cells in a small amount for active cellular activities and among these are manganese, selenium, cobalt, chromium, copper, zinc, and iron. Lack of these nutrients affects the optimal activities of the cells, tissues, and organs and thus are needed optimally by animals and humans in their diets. Moreover, some of these micronutrients and other non-essential elements, for example, cadmium, nickel, lead, and mercury among others, pose serious toxicity to exposed cells when consumed above the recommended limits. The toxicity associated with heavy metals includes metabolic, renal, kidney, nervous, gastrointestinal, liver, and ocular dysfunctions and is linked especially to Pb, Ni, Cd, and Cu [36]. Studies have shown that some of the metals (Zn, Cu, and, Mn) are essentials for good growth and development of the live chicken and thus are added as feed supplements for this purpose [37]. This must have added to the observed relatively high levels of these metals over others in this study. These heavy metals find their way into the food chain via feeds, water, industrial effluents, agrochemicals, fossil fuel combustions, and other anthropogenic activities [36]. Through these sources, humans get exposed to these heavy metals. Most of the frozen chicken

retailed in the country are imported from industrially reared products and might be exposed to heavy metal pollution via the feeds, or during processing, packaging, and transportation. This might have accounted for the higher concentrations of heavy metals recorded from frozen chicken drumstick samples.

The viable counts were relatively similar to a range of  $1.44 \times 10^4$  CFU/g to  $4.38 \times 10^4$  CFU/g in raw chicken meat from Lagos, Nigeria [38], but lower than  $1.66 \times 10^7$  CFU/g to  $1.34 \times 10^7$  CFU/g from different locations in Kaduna, Nigeria [34],  $5 \times 10^6$  CFU/g from Hisar, India [39] and  $4.3 \times 10^6$  CFU/g to  $3.6 \times 10^7$  CFU/g from different cities in Bangladesh [40-42]. On the other hand, loads of fungi in fresh ( $1.45 \times 10^2$  CFU/g) and frozen ( $<15$  CFU/g) chicken drumsticks were relatively lower than those from Warri [28], Lagos [38], Egypt [43], and Ghana [44]. Some possible reasons for the observed variations are factors such as the year of study, designs, and sampling techniques employed. Generally, raw meats are nutrient-rich products that are constantly exposed to microbial contaminations when poorly processed or grossly handled or stored in unclean containers and environments [45]. The general standard acceptable limit for microbial loads in fresh meat, according to the International Commission on Microbiological Specifications for Foods (ICMSF), is TVC of below  $10^6$  CFU/g at  $35^\circ$  C [46], because further thermal processing along with the immune system will reduce, eliminate and mitigate their effects when consumed. Therefore, the microbial loads obtained for fresh and frozen meat in our study could be considered as not too risky, provided that the raw meats are well cooked before consumption. The bacterial and fungal counts of the frozen samples were generally lower, reemphasizing the need for storage of fresh meat at relatively lower temperature, which obviously reduces the effects and activities of food-borne organisms. Our finding is however in disagreement with the findings of Abdu and Abubakar [6], who reported that frozen chicken meats were more contaminated than freshly slaughtered chicken meat. The differences in the results could be attributed to the variations in the processing and handling of the raw products in the different production/retailing locations.

Considering the levels of specific pathogens in the samples, results from this study indicated a mean *Staphylococcus* count (StC) yielded the highest, followed by total fungal counts (TFC), *Salmonella* counts (SC) and total *Escherichia coli* counts (EC) being the least in both samples. The differences obtained from the counts were statistically significant ( $p < 0.05$ ). Microbiologically, a high load of *Staphylococcus* indicates gross handling, while high *Escherichia coli* counts (*E. coli*) indicate recent fecal contamination of processing water. Similarly, the presence of *Salmonella* spp. indicates poor sanitary, feeding, and storage containers. In recent different strains of *Salmonella* from raw chicken meat have been found to demonstrate widespread antimicrobial resistance due to their abilities to produce extended beta-lactamase and biofilms [47-49]. Hence, the presence of *Salmonella* spp. is of public health importance and needed greater attention. Molds are major environmental contaminants. It, therefore, suggests that the contaminations of meat samples observed in this study were



mostly due to poor handling of the products, during processing and retailing, and the storage environment. Similar findings had been reported [38, 43, 44, 50-53].

The fungal isolates were mostly the species of *Aspergillus*, *Penicillium*, *Rhizopus*, and *Mucor*. The presence of *E. coli*, *S. aureus*, *Salmonella* spp., *Klebsiella* spp., (bacteria) and *Aspergillus*, *Penicillium*, *Rhizopus*, and *Mucor* (fungi) had been commonly isolated as reported previously [28, 38, 43-45, 50, 54, 55]. This further confirms their predominance in chicken meat and related products globally. Among the bacterial isolates in this study, *E. coli*, *Salmonella* spp., and *S. aureus* were present in both samples. These isolates are of public health importance because they are commonly implicated in food-borne diseases, especially among the young, elderly, and immune-compromised individuals. In recent times, the prevalence of these pathogens in chicken meat has risen beyond measure. For example, studies have shown that chicken meats from Cambodia were heavily contaminated by *S. aureus* (46.2 %) and *Salmonella* (40.4 %) [50]. Also, reports from local markets in Indonesia recorded huge microbial contamination of several chicken meats by *Salmonella* spp. (85 %) and *E. coli* (90.03 %) [57, 58]. Findings from this study further corroborated our study on the prevalence of *S. aureus*, *Salmonella* spp., and *E. coli* in chicken meat. In this study, *Bacillus* spp. was not detected in the fresh samples, as against *Klebsiella* spp., *Proteus* spp., and *Pseudomonas* spp. which were absent in the frozen counterpart. This presence of *Bacillus* sp. in the frozen sample could be due to the presence of endospores which enhances their survival rates in extreme conditions, such as the low temperature of refrigerators and freezers [59]. The presence of *Klebsiella* spp., *Proteus* spp., and *Pseudomonas* spp. in fresh chicken samples could be traced to contaminations via the gut contents of chicken, feathers, and rinsing water [45]. Fresh chicken meats, apart from serving as the vehicle for transmission of *S. aureus*, *Salmonella* spp., and *E. coli* could also harbor and transmit *Klebsiella* spp., *Proteus* spp., *Bacillus* spp. and *Pseudomonas* spp. to exposed humans [60-62]. The fungal isolates in this work are consistent with the workers [44, 58], who identified the same organisms as the major contaminants of poultry products. Unlike the bacteria isolates, some fungi, under appropriate conditions, synthesise mycotoxins, which are heat-stable and deleterious to humans and animals [45]. Hence, the presence of mycotoxin-producing fungi such as *Aspergillus* and *Penicillium* calls for stricter hygienic practices during meat processing and retailing as well as heating of the meats before consumption to prevent or reduce their effects.

## V. CONCLUSION

This study has shown that fresh and frozen chicken meats have varying levels of proximate, trace, and heavy metals, bacterial, and fungal loads. The proximate contents of the fresh chicken drumstick samples had significantly higher concentrations of moisture, protein, ash, and microbial loads when compared with the frozen drumstick samples with significantly higher contents of fats, carbohydrates, and heavy metals. From the results, therefore, the fresh chicken

drumsticks are more nutritionally beneficial, provided they are thoroughly treated with heat before consumption to prevent foodborne diseases from bacterial and fungal contaminations. Although the levels of heavy metals in both drumstick samples were below the toxic levels, consumers should be wary of bioaccumulation and biomagnification of heavy metals when constantly exposed to them. Hence, constant surveillance of chicken meat is encouraged by health workers to monitor the exposure levels to these heavy metals.

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