
Storage and Cooling Effects on Levels of Protein Carbohydrates, Fats, Minerals and Microbes in Guinea Fowl (*Numida meleagris*) Meat Sold in Kaduna Metropolis, Nigeria

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Abstract: The effect of storage and cooling time (0, 1, 3, 7 and 28 days) on the nutritional quality of guinea fowl (*Numida meleagris*) was assessed in terms of crude protein, moisture, ash, fat, carbohydrate, minerals (calcium, zinc, iron and potassium) and microbial content. Crude protein was determined using Kjeldahl method, moisture, ash and fat content were determined using AOAC methods, carbohydrate was estimated by difference and the minerals using AAS methods (for Calcium, Zinc and Iron) and AES (for Potassium). The result of crude protein, moisture and fat content were observed to decrease as storage time increases. Moisture content ranged from 67.70 to 66.50%, ash content from 0.98 to 1.01%, fat content from 2.31 to 2.19%, crude protein content from 20.20 to 19.13% and carbohydrate content from 8.79 to 11.17%. The ash content fluctuates between the same ranges while the carbohydrate content increases with increased in storage time. The minerals content decreases with increase in storage time and the microbial analysis shows freezing inhibited the growth of bacteria which is the main cause of meat spoilage. The study showed that the meat samples could be kept in a frozen state without spoiling but the nutritional content where altered as storage time increased.

Keywords: *Numida meleagris*, Meat, Poultry, Storage and Cooling Effects

1. Introduction

In most developing countries, including Nigeria, fresh meat forms a significant proportion of meat intake [1]. It is either eaten cooked or processed into other forms to avoid associated spoilage. The main causative factor of such spoilage has been linked to unavailability of necessary storage facilities and favourable ambient temperature that usually prevail in developing countries that are in tropical regions [2]. Meat has long been considered a highly desirable and nutritious food, but unfortunately it is highly perishable because it contains the nutrients needed to support the growth of many types of microorganisms [3]. Due to its unique biological and chemical nature, meat undergoes progressive deterioration from the time of slaughter until consumption. In

general, the metabolic activity of the ephemeral microbial association which prevails in a meat ecosystem under certain aerobic conditions, or generally introduced during processing, leads to the manifestation of changes or spoilage of meat [4]. These changes or spoilage are related to the type, composition and population of the microbial association and, the type and the availability of energy substrates in meat. Indeed the type and the extent of spoilage is governed by the availability of low-molecular weight compounds such as glucose, lactate existing in meat [5]. A consortium of bacteria, commonly dominated by *Pseudomonas spp.*, is in most cases responsible for spoilage of meat stored aerobically at different temperatures (-1 to 25°C); the *Pseudomonas spp.* can grow under refrigeration temperatures [6, 7]. It is established that under aerobic storage, three

species of *Pseudomonas*, *P. fragi*, *P. fluorescens* and *P. lundensis*, are the most important spoilage organisms. The population of *Pseudomonas* to the level of 107-8 CFU/g, has been attributed to slime and off-odours formation. However, in practice both these characteristics become evident when the *Pseudomonas* have exhausted the glucose and lactate present in meat and begin to metabolise nitrogenous compounds such as amino acids.

A common method of preserving meat in Nigeria is by refrigerating. Frozen storage causes several reactions between different meat components such as oxidative reactions that mainly occur due to the presence of oxidative catalyst in high concentration. Free radicals formed react with side chains of proteins that produce protein free radicals which on reaction with oxygen gives peroxy radicals. This has been suggested by several authors that hydroperoxides decompose to form carbonyl derivatives while sulphhydryl

group oxidation leads to formation of disulphide cross linkages or to the formation of mixed disulphide conjugates with glutathione, cysteine or other low molecular weight mercaptans [8, 9]. Hence, this study was carried out to determine the storage and cooling time effect on the levels of protein, carbohydrate, fat, ash, moisture and minerals in guinea fowl, a meat commonly consumed in Kaduna, Nigeria.

2. Materials and Methods

2.1. Study Area

Figure 1 shows the map of study area, Kaduna North in Kaduna metropolis, which is located between latitude 10°34' N to 10°35'N and longitude 7°25'E and 7°36'E.

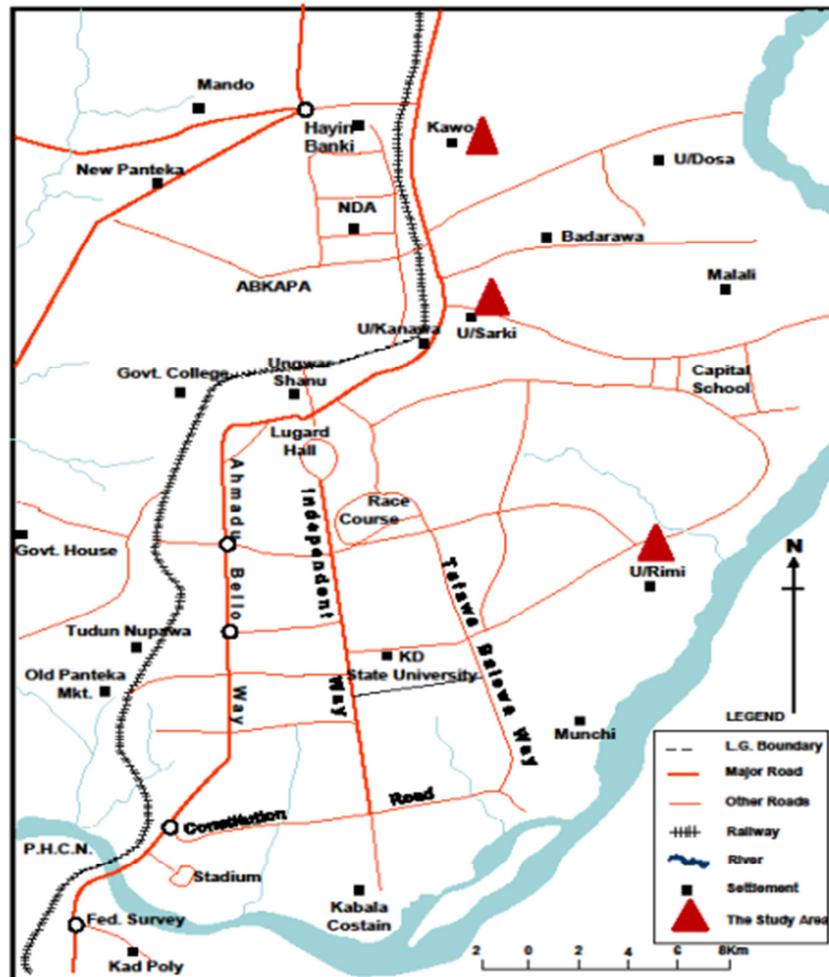


Figure 1. Map of Study Area.

2.2. Sample Collection and Preparation

Guinea fowls (*Numida meleagris*) were obtained from Kawo, Unguwan Sarki and Unguwan Rimi markets in Kaduna metropolis. Each sample was slaughtered, de-feathered, washed and de-boned, and then the lean part was then finely minced. The samples were stored in a clean

plastic polyethylene bag and were kept in a freezer at 17.7°C. Each sample was analysed for protein, carbohydrate, ash, moisture content, minerals (calcium, iron, zinc and potassium) contents and microbial determination when fresh (i.e. before refrigerating) and after 1, 3, 7 and 28 days of freezing.

2.3. Methods of Analysis

2.3.1. Determination of Nitrogen and Crude Protein

The meat sample (2.0g) was weighed and placed in a Kjeldahl flask, 1.14g of a mixed catalyst consisting of copper sulphate, sodium sulphate and selenium dioxide and 20cm³ concentrated sulphuric acid were added to the sample in the Kjeldahl flask. The flask was heated in the digester and boiled until the solution became clear. Heating continued for about 30 minutes more, after heating, the flask was allowed to cool by gradually adding approximately 100cm³ distilled, de-ionized water in a volumetric flask. To an aliquot (10cm³) of the digest, 10cm³ of 40% NaOH solution was added and then connected to Kjeldahl distillation apparatus and heated to boiling. The distillate was condensed into a conical flask containing 10cm³ of 2% boric acid and 2 drops of methyl red indicator was added and the alkaline distillate was titrated against 0.1mol/dm³ hydrochloric acid [10].

$$\%Nitrogen = \frac{100(A \times B \times 0.014)}{C} \quad (1)$$

Where A = Hydrochloric acid used in titration (cm³), B = Molarity of HCl

C = Weight of sample (g)

$$\text{Crude protein (\%)} = \%Nitrogen \times 6.25$$

2.3.2. Fat Determination

The fat content was carried out using the procedure described by the Association of Official Analytical Chemists using soxhlet extractor apparatus [11]. 2.0g of the meat sample was weighed into porous extraction thimble and weighed. The thimble was placed in an extractor and was connected to flask containing 350cm³ of petroleum ether (with boiling point 40°C to 60°C) for 5 hours at condensation rate of 5-6 drops per second under reduced temperature and pressure and refluxing at 80°C. The top was closed with cotton to minimize the escape of the extraction solvent. The fat content in the samples were exhaustively extracted in the solvent. The content of the flask was weighed and later the solvent was distilled off and the flask was reweighed. The percentage fat was then calculated using the following formula:

$$\%Fat = \frac{Wt\ of\ the\ Ether\ Extract}{Wt\ of\ the\ Sample} \times 100 \quad (2)$$

2.3.3. Determination of Moisture content

Meat sample (2.0g) was weighed into petri dish and placed on steam bath and heated uncovered at 102°C for 2 hours. The sample was reweighed and the whole process was then repeated until constant weight was obtained [11]. The percentage moisture content was calculated using the formula:

$$\%M.C = \frac{Loss\ in\ Weight}{Weight\ of\ Sample} \times 100 \quad (3)$$

2.3.4. Determination of Ash Content

Meat sample (2.0g) was weighed into crucible and placed

in muffle furnace, the temperature was set at 550°C for 8 hours. The content was re-weighed again, when cooled the whole process was repeated until constant weight was obtained [11]. The percentage ash content was calculated using the formula:

$$\%A.C = \frac{Wt\ of\ Ash}{Wt\ of\ the\ Sample} \times 100 \quad (4)$$

2.3.5. Determination of Carbohydrate

The carbohydrate was determined by difference. The protein, fat, ash and moisture content of food determined were added and the sum was then subtracted from the total 100%. [12].

2.3.6. Mineral Analysis

The minerals of interest: potassium, calcium, zinc and iron were determined using the method described below:

The meat sample (2.0g) was weighed into a 50cm³ digestion tube, 20cm³ of acid mixture H₂SO₄, HNO₃, HClO₄ (2:1:1) were added to the sample and put in fume cupboard and digested with a hot plate until a clear colourless solution was observed. The digested sample was allowed to cool and carefully filtered into a 100cm³ volumetric flask using Whatman No. 40 filter paper and made up to the mark. Then it was transferred to a labelled plastic sample bottles and carried to the Atomic Absorption Spectrophotometer (Thermo Scientific Model: iCE vl.30) for calcium, zinc and iron determination while Flame Emission Spectrophotometer was used for potassium determination [13].

2.3.7. Microbial Determination

After slaughtering and pre-treatment, the meat sample was washed with sterile distilled H₂O. Then Nutrient agar was prepared (38g in 1000cm³ of distilled water) and sterilized in an autoclave for 15 mins. The sterilized agar was placed in petri dishes to solidify. The sterile water was used to wash the samples. Sterile swap stick was then used to collect the water from the meat and smear it on the solidified agar and the control which contains only the solidified agar where incubated for 24 hours at 37°C for bacterial growth and the procedure was repeated for the frozen samples [11].

2.4. Statistical Analysis

Data obtained from various analysis were analyzed using Microsoft Office Excel 2013. The data were expressed in terms of descriptive statistics while the figures were presented with Mean values as (Mean ± SD). Analysis of Variance (ANOVA) was used to test for significance, p-value less than 0.05 were considered as Significant, while p-values greater than 0.05 were not significant.

3. Results and Discussions

The results as presented in Table 1 showed that the moisture content is slightly affected by duration of cooling

time. The results on moisture content before refrigerating and the 28th day after refrigerating are 67.70 and 66.50% respectively. It showed slight decrease with cooling time, meaning as the number of cooling days increases the moisture content decreases. Freezing alters both the content and the supply of moisture in meat tissue. The decrease in moisture content during cold storage may be attributed to the decreases in water holding capacity and loss of small drip [14, 15]. Water content may be affected by the absorption of moisture during the chilling process and by any type of phosphate treatment, which favours moisture retention [16]. The result of protein level showed slight decrease during cold storage. As the number of days increases the protein content decreases. From Table 1, before refrigerating has the highest protein content with 20.20% compared to the remaining days. These effects might be due to bacterial decomposition and escaping of some soluble nitrogen compounds [14, 15]. Ash in food determines largely the extent to which the dietary minerals would be available in a food and the rate at which food substances would make available amount of energy locked in it [17]. The feed type determines the ash content of the meat. Regarding cold storage, the ash content of the samples show no significant changes during storage. The carbohydrate content of the meat samples shows day 28 has the highest while before refrigerating has the lowest. The carbohydrate content of the samples increased during refrigerating, which increased from 8.77% before refrigerating to 11.20 after 28th day and these effects might be due to reduction of moisture level from slaughter plus the freezing period of the meat (as seen from the moisture results of this work). However George and Aguiar reported that reindeer meat can be frozen for up to a year without compromising quality in their work to determine the effect of reindeer meat quality of frozen storage for 12 months [19]. The fact that the moisture, ash, fat, protein and carbohydrate content for all the cooling days are within the USDA and ADHS recommended food storage times cold and dry refrigerated and frozen foods which is one year shows that the meat is still safe for consumption [20].

Table 1. Proximate analysis.

Days	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)
0	67.70 ± 0.13	1.02 ± 0.54	2.31 ± 0.50	20.20 ± 0.02	8.76 ± 0.56
1	67.69 ± 1.47	1.01 ± 0.55	2.30 ± 0.23	20.10 ± 0.15	8.90 ± 2.06
3	67.56 ± 0.24	1.00 ± 0.74	2.28 ± 0.19	19.86 ± 0.02	9.30 ± 0.69
7	66.89 ± 1.72	0.99 ± 0.75	2.23 ± 0.31	19.51 ± 0.37	10.38 ± 2.12
28	66.50 ± 1.20	0.98 ± 0.50	2.19 ± 0.28	19.13 ± 0.55	11.19 ± 2.11

Values are (mean ± SD)

Table 2. Mineral Analysis.

Days	Calcium (mg/g)	Zinc (mg/g)	Iron (mg/g)	Potassium (mg/g)
0	2.26 ± 0.25	0.04 ± 0.01	1.55 ± 0.06	3.60 ± 0.23
1	2.24 ± 0.26	0.04 ± 0.00	1.53 ± 0.03	3.30 ± 0.16
3	1.49 ± 0.26	0.03 ± 0.00	1.51 ± 0.04	2.70 ± 0.16
7	1.33 ± 0.29	0.02 ± 0.00	1.49 ± 0.03	2.60 ± 0.12
28	0.42 ± 0.16	0.01 ± 0.00	1.45 ± 0.07	1.80 ± 0.84

The result as presented in Table 2 shows that the calcium content is slightly affected by duration of cooling time. Before freezing has the highest calcium content (2.26mg/g) which decreases to (0.42mg/g) after 28 days of freezing. Zinc content is slightly affected by duration of cooling time. Day zero has the highest calcium content 0.04mg/g which decreases to 0.01mg/g at day 28 after freezing. The reduction could be attributed to drip loss and dehydration that is associated with frozen storage. Zinc is a trace element involved in many body reactions which help in construction and maintenance of DNA, formation, growth and repair of body tissues, hair, skin, bones nails and teeth. It is also important in the reduction of pneumonia and diarrhoea deaths among children under five years of age in developing countries. Zinc also play a role in immunity, chronic diseases and apoptosis [21]. Iron content from the analysis decreases as storage time increases from day zero to day 28th. Iron which is a trace metal has many functions in the body hence very important in maintaining healthy immune system for blood to work efficiently and transports oxygen as oxyhaemoglobin. Deficiency of iron is manifested as fatigue especially among long distance athletes. Severe deficiency of iron causes deficiency anaemia which affects many people globally especially pregnant women in developing countries [22]. Potassium from the study shows decrease with increase storage time. At day zero 3.60mg/g and at day 28 is 1.80mg/g. Also, this reduction could be attributed to drip loss and dehydration that is associated with frozen storage.

Microbial analyses were conducted on the fresh and frozen meat samples for the days and a control. The control which contains only the nutrient agar were negative while the fresh and the frozen samples were positive with microbial growth. Bacterial growth is the main cause of meat spoilage [23]. Freezing appears to decrease the number of viable microbes present in meat. During freezing however, microbial spoilage is effectively terminated as the microbes become dormant. Unfortunately, they regain their activity during thawing.

4. Conclusion

From the results, it can be concluded that the levels of protein, fats, moisture, ash and minerals in guinea fowl decreases as the period for freezing preservation increases while that of carbohydrate increases. Guinea fowl meat can be kept in frozen state without spoiling but the nutritional content is altered as storage time increases. Freezing appears to decrease the number of viable microbes present. However, the microbes become dormant during freezing.

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