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Physicochemical properties and microorganisms isolated from dried meat obtained in Oja- Oba market in Ilorin, Nigeria

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ABSTRACT

The physicochemical properties and microorganisms isolated from dried meat samples obtained from the Oja-Oba market in Ilorin metropolis during the rainy season were investigated. Meat samples were stored under two storage conditions (cupboard and refrigerator) before they were analysed using potato dextrose agar and nutrient agar for fungi and bacteria load of the dried meat samples. Analytical studies on the nutritional contents (moisture content, total amino acid content, titratable acidity and protein content) of the dried meat samples stored under a period of five weeks revealed that the moisture content of dried meat samples stored in the cupboard increased from 35% to 65%, while those in the refrigerator had an initial increase from 50% to 55% for the first two weeks of storage and became constant through the period of storage. The total amino acid content ranged from 0.2128-0.5040mg/g and 0.2464-0.5376mg/g for both samples respectively. Titratable acidity ranged from 0.04mg/g-0.10mg/g and 0.07mg/g-0.14mg/g for both samples respectively. The increase in protein content recorded for both meat samples ranged from 5.79-14.02mg/g and 5.11-13.52mg/g respectively. Bacteria and fungi isolated include Staphylococcus aureus, Micrococcus luteus, Neisseria sp., Acinetobacter sp., Aspergillus niger, Aspergillus flavus, Penicillium sp. and Rhizopus sp. The total bacterial count increased in the dried meat stored in the cupboard (2.5×10^{-7} to 3.3×10^{-7}cfu/ml) while it decreased in the meat samples stored in the refrigerator (2.0×10^{-7} to 1.4×10^{-7}cfu/ml).

Keywords: Dried meat, Bacteria, Fungi, Storage, Oja-Oba market.

INTRODUCTION

Meat is one of the best source of proteins, vitamins and minerals which are essential nutrients required for proper growth and maintenance. Meat has been defined as the flesh of animals which are suitable as food [1]. This includes all processed or manufactured products which might be prepared from these tissues i.e. meat may be fresh, cured, dried or otherwise processed.

In Nigeria there is the preferential consumption of different types of meat by communities and this may be due to a combination of a number of factors bordering on religious belief, culture, adaptability, food habits, age, sex, socio-economic facts and individual variation. [2] reported that goat meat is most popular among the Igbos of East central states of Nigeria. Chicken is also a major source of protein for the South East population where there are varieties of poultry farms and abundant market [3]. Despite the differences in acceptability, cow meat appears to predominate all over Nigeria though sheep, goat and poultry meat are widely accepted [2].

Meat is one of the highly perishable foods because of its high nutritional contents, enzymatic action and the presence of microorganisms (bacteria, yeasts moulds) which may result in oxidative rancidity, discolouration, mouldiness, off flavour, sliminess etc. The major source of these deteriorative changes being microorganisms, this renders the meat unacceptable and unfit for human consumption [1].

Methods of preserving meat include the use of high temperature e.g. canning, low temperature e.g. chilling, freezing and pasteurization, drying e.g. hot air drying, wind and sun drying, smoking, use of radiation and the use of chemical preservatives. It has been reported that drying reduces the moisture content to a level that prevents the growth of microorganisms especially fungi and bacteria [4]. Meat smoked and dehydrated has a good shelf life and are not open to spoilage unless rehydrated. All handling and storage methods are therefore primarily concerned with minimising microbial contamination and retarding microbial growth and activity.

The amino acid content of meat includes Isoleucine, Leucine, Lysine, Methionine, Cystine, Phenylalanine, Valine, Threonine, Tryptophan, Arginine, Histidine, Alanine among others which makes it better than other foods as source of protein [5]. Meat has also been discovered to be rich in essential Vitamins A, $B_{1,}$ $B_{2,}$ $B_{6,}$ Nicotinic acid, Folic acid, Ascorbic acid and vitamin D. Some of these vitamins are water soluble and therefore get lost during cooking. Minerals like sodium, potassium, calcium, magnesium, copper, iron, phosphorus and zinc are also found in meat [5].

Microorganisms commonly associated with meat include majorly the psychrophiles of the genera *Pseudomonas, Lactobacillus, Moraxella, Acinetobacter*, *Microbacteria, Brochotrix, Klebsiella* and *Vibrio* [6]. The Mesophiles include *Salmonella* spp, *Escherichia coli*, *Clostridium perfringens,* and the Thermophiles include *Streptococcus faecalis;* others include members of the genera *Flavobacterium*, *Bacillus, Leuconostoc*, *Proteus, Micrococcus* and *Achromobacter*.

The common moulds on meat are the genera *Cladosporium*, *Sporotrichum*, *Oospora*, (*Geotrichum*) *Thamidium*, *Mucor*, *Penicillium*, *Alternaria* and *Monilia*. The yeasts found on meat are majorly of the asporogenous genera and include *Torulopsis*, *Rhodotorula* and *Candida.* Meat being highly nutritious and having high moisture content with nearly neutral pH serves as a good culture medium for most microorganisms and as such, it is classified among perishable foods whose contamination with spoilage organisms is almost unavoidable [7]. This makes meat preservation more difficult than other kinds of food.

Methods of preserving meat include the use of high temperature e.g. canning, low temperature e.g. chilling, freezing and pasteurization, drying e.g. hot air drying, wind and sundrying, smoking, use of radiation and the use of chemical preservatives. Meat smoked and dehydrated has a good shelf life and are not open to spoilage unless rehydrated. All handling and storage methods are therefore primarily concerned with minimising microbial contamination and retarding microbial growth and activity.

Dried meat is commonly prepared in the North from where it is transported to other parts of the country for consumption. These meat being transported from the northern parts of Nigeria where there is a relatively lower relative humidity in jute bags located in lorries to the south might contain cells and spores of microorganisms which would grow again when the environmental conditions favour their growth and hence cause meat spoilage. According to [8] there was an increase in microbial load of organisms on dried meat samples after six months of storage due to moisture absorption from the environment.

In most cases, dried meats which are on display in the market are found to be visibly mouldy (high level of discolouration). Previously, researchers [9, 10, 11], have reported the presence of the insect fauna of dried fish in Nigeria. [12] isolated a number of mould species from the dried fish in Nigeria. [13] investigated the mycoflora of dried salted tropical fish. However, little or no information is available on the mycoflora of dried meat, hides and skin in Nigeria [14]. This project is therefore based on the isolation of the microorgsnisms involved in the spoilage of locally dried and stored meat. Studies on their physicochemical properties under different storage conditions were also carried out.

MATERIALS AND METHODS

Collection of Samples

The dried meat samples were purchased at the Oja-Oba market in Ilorin town (Nigeria). They were put into sterile cellophane bags and taken to the laboratory for study.

Storage of Samples

The dried meat samples were stored in (a) The refrigerator (4^0C) and (b) Cupboard (room temperature 25^0C)

Preparation of Media

The culture media used such as Nutrient agar, Potato Dextrose Agar and Nutrient Broth were prepared according to the manufacturers' instruction.

Isolation of Microorganisms

A seven fold serial dilution of the meat was carried out. One gram of the meat sample was aseptically weighed and macerated by blending. This was dispersed in 9 ml of sterile distilled water in a sterile test tube to give a 10^{-1} dilution. Further decimal dilutions were made until 10^{-7} dilution was obtained. One millilitre from 10^{-7} dilution was taken to seed by pour plate method. Nutrient agar was used for the isolation of bacteria while Potato dextrose agar containing drops of 0.1% streptomycin was used for fungal isolation. The plates were incubated at 37^0C for 24-48 hours for bacterial isolation and 25^0C for 48-72 hours for fungal isolation respectively. The colonies subcultured were purified by repeated subculturing until a pure culture was obtained. Pure cultures were inoculated on agar slants as stock cultures. Characterization of Isolates

After obtaining pure isolates, The 1987 Bergey's manual of Determinative Bacteriology was used for identification of the isolates.

Bacteriological Analysis

Methods of microbiological investigation of bacterial isolates adopted include: Gram Staining, Spore staining, Capsule staining, Motility test**,** Catalase test, Voges Proskauer test, Citrate utilization, Indole production, Methyl red test, Carbohydrate fermentation test, Starch hydrolysis, Gelatin liquefaction, Urease test, Oxidase test, Oxygen relationship as described by [15].

Characterization of Fungal Isolates

The fungal isolates were identified based on their colonial morphology, microscopic examination of each fungal culture was made in the vegetative and sporulation stages. Characterization such as size, shape and septation of hyphae were also made. A clean slide with a drop of lactophenol blue in its centre was used. A portion of the mycelium at the edge of the colony at growing edge/sporulating head was taken. This was put in the drop of lactophenol blue using a flamed inoculating wire. It was then leased and covered with cover slip. This slide was then mounted in the microscope, focused and observed at X40 objective lens. Appropriate drawings were made upon observation.

Physico-Chemical Analysis of the Dried Meat Samples

The physicochemical analysis run on the dried meat samples include the following: Determination of moisture contents, Determination of pH, Determination of total titratable acidity, Determination of protein content, Estimation of total amino acid [16]. These results were adequately represented in various schematic illustrations.

RESULTS

A total of eight organisms were isolated from the dried meat in this study. Four of the organisms were bacteria and four were fungi. The bacterial isolates which were identified on the basis of their colonial, cellular morphology and also biochemical characteristics include: *Staphylococcus auereus, Micrococcus luteus, Neisseria* sp, and *Acinetobacter* sp which were denoted as Isolates B_1 to B_4 respectively. Colonial morphology and microscopic examination were used for the fungal isolates and they include: *Aspergillus niger*, *Penicillium* sp *Rhizopus* sp. and *Aspergillus flavus,* which were tagged Isolates F_1 to F_4 respectively.

Table 1: The frequency of bacterial isolates on the dried meat (Tinko) stored under different storage conditions for a period of 5 weeks

Storage period (wks)	Microorganisms	Isolate Code	Refrigerator	Cupboard
1	*Staphylococcus aureus*	B_1	+	+
2	*Micrococcus luteus*	B_2	+	+
3	*Neisseria* sp	B_3	_	+
4	_	_	_	_
5	*Acinetobacter* sp	B_4	+	_

(R) Refrigerator (C): Cupboard (+): Isolated (-): Not Isolated

Table 2: The frequency of fungal isolates on the dried meat (Tinko) stored under different storage conditions for a period of 5 weeks

Storage period (wks)	Microorganisms	Isolate Code	Refrigerator	Cupboard
1	*Aspergillus niger*	F_1	+	+
	Aspergillus flavus	F_2	+	+
2	*Penicillium* sp	F_3	+	+
3	*Rhizopus* sp	F_4	-	+
4	_	_	_	_
5	*Rhizopus* sp	F_4	-	+

(R) Refrigerator (C): Cupboard (+): Isolated (-): Not Isolated

The frequency of occurrence of microbial isolates from the dried meat samples stored in the refrigerator and cupboard are shown in tables 1 and 2. *Aspergillus flavus* and *Aspergillus niger* were isolated from the dried meat samples from the onset of the experiment (day zero) before

storage. *Penicillium* sp was isolated after one week while *Rhizopus* sp was isolated from the 4th week of storage in the cupboard. The bacterial species that occurred at the day zero was *Staphylococcus aureus* and maintained a consistency in its occurrence while *Micrococcus luteus* was isolated on the 2nd week of storage from the refrigerator and cupboard. *Neisseria* sp. was isolated on the 3rd week of storage in the cupboard while *Acinetobacter* sp was isolated on the 5th week of storage from the refrigerator (Table 1).

Table 3: Total Bacterial counts obtained from dried meat samples (Tinko) stored under different storage conditions

Period of Storage (weeks)	Cupboard cfu/ml (10^{-7})	Refrigerator 4^0C cfu/ml (10^{-7})
1	2.5	2.0
2	2.8	1.9
3	3.0	1.8
4	3.2	1.5
5	3.3	1.4

The total bacterial counts of the meat samples stored under different storage conditions (cupboard & refrigerator) are shown in Table 3. Meat samples stored in the cupboard yielded the highest bacterial count (3.3 x 10^{-7}) cfu/ml for the period of storage compared to the meat stored in the refrigerator. However, there was a decrease from (2.0 to 1.4) x 10^{-7} cfu/ml in the bacterial counts stored in the refrigerator as the storage period increased while an increase was recorded for the dried meat stored in the cupboard (2.5 to 3.3) x 10^{-7} cfu/ml (Table 3).

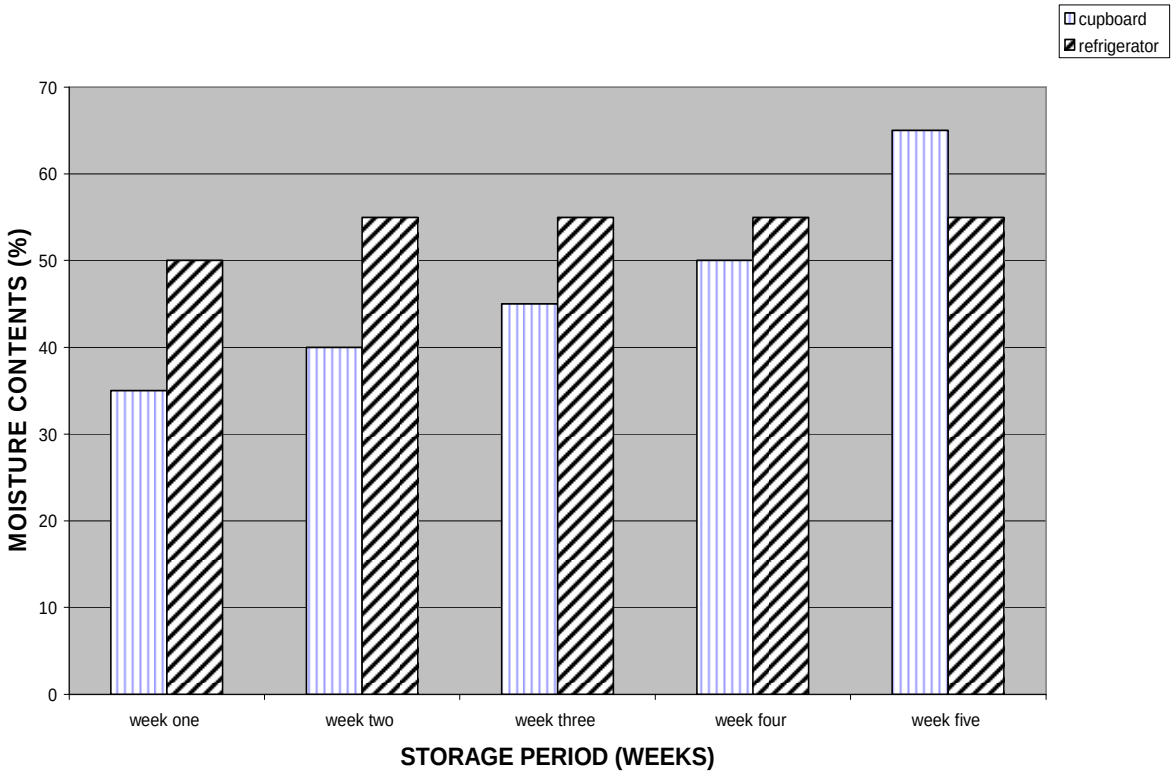

Fig. 1 Moisture content of dried meat in Refrigerator and Cupboard during the period of Storage

Changes in the moisture content of dried meat samples under different storage conditions for a period of 5 weeks.

The dried meat stored in the cupboard had increasing moisture content as the storage period increased (35 – 65%) while the meat stored in the refrigerator during the second week of storage

has an increase in moisture content (50 – 55%) and became quite constant in the storage period increased (Fig. 1).

Changes in the pH of dried meat samples under different storage conditions for a period of 5 weeks.

It was observed from the readings that there was an increase in pH of dried meat stored in the refrigerator and cupboard for the first two weeks of storage but the pH started decreasing as from the third week of storage. Generally, the pH dropped with increasing time of storage (Fig. 2).

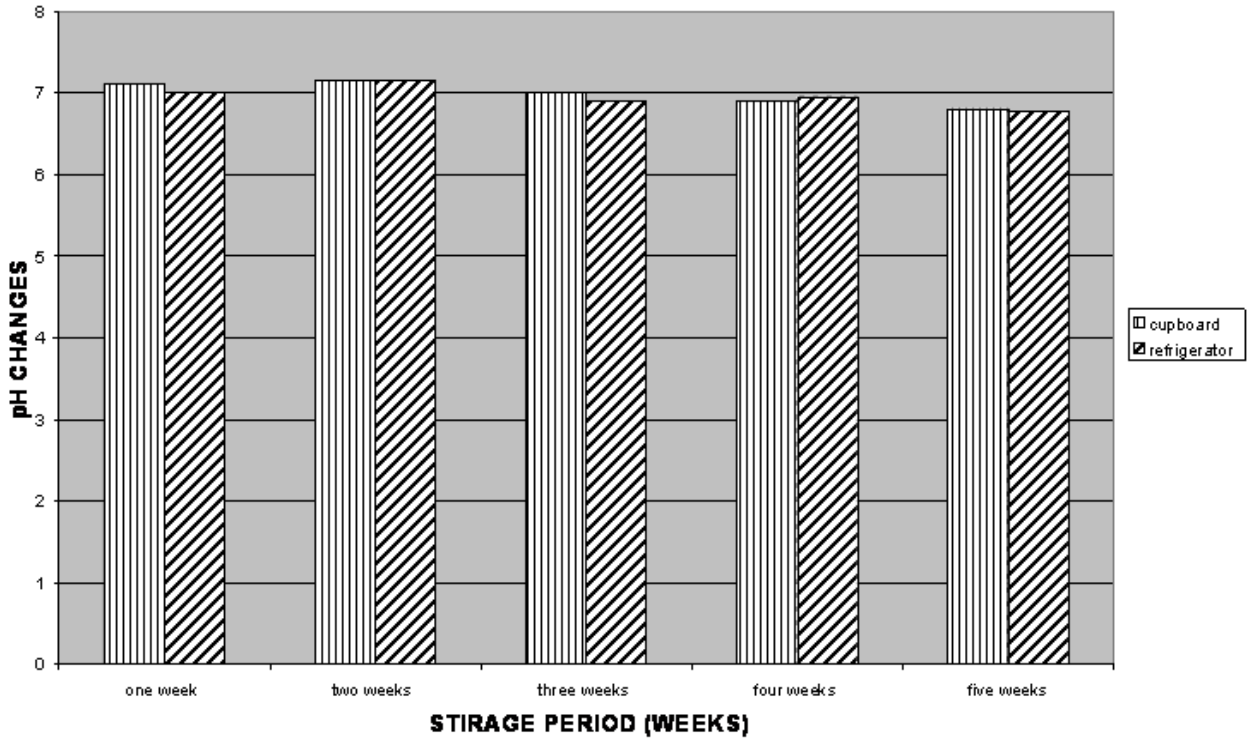

Fig. 2: pH changes of dried meat in Refrigerator and Cupboard during the period of Storage

Table 4. Estimation of total amino acid of dried meat samples under different storage conditions for a period of 5 weeks

Period of Storage	Cupboard	Refrigerator (4^0C)
1	0.2128	0.2464
2	0.2912	0.3472
3	0.3360	0.4144
4	0.3920	0.4480
5	0.5040	0.5376

The amino acid content of the meat samples stored in the cupboard increased from (0.2128 – 0.5040)mg/g sample within 5 weeks of storage while the dried meat stored in the refrigerator had a higher increase in amino acid content from (0.2464 – 0.5376) mg/g.

Estimation of total titratable acidity of dried meat samples under different storage conditions for a period of 5 weeks.

As the period of storage increased, the total titratable acidity increased in the meat samples stored in the cupboard (From 0.04 to 0.10mg/g) and also from (0.007 to 0.14mg/g) in the meat samples stored in the refrigerator (Fig. 3).

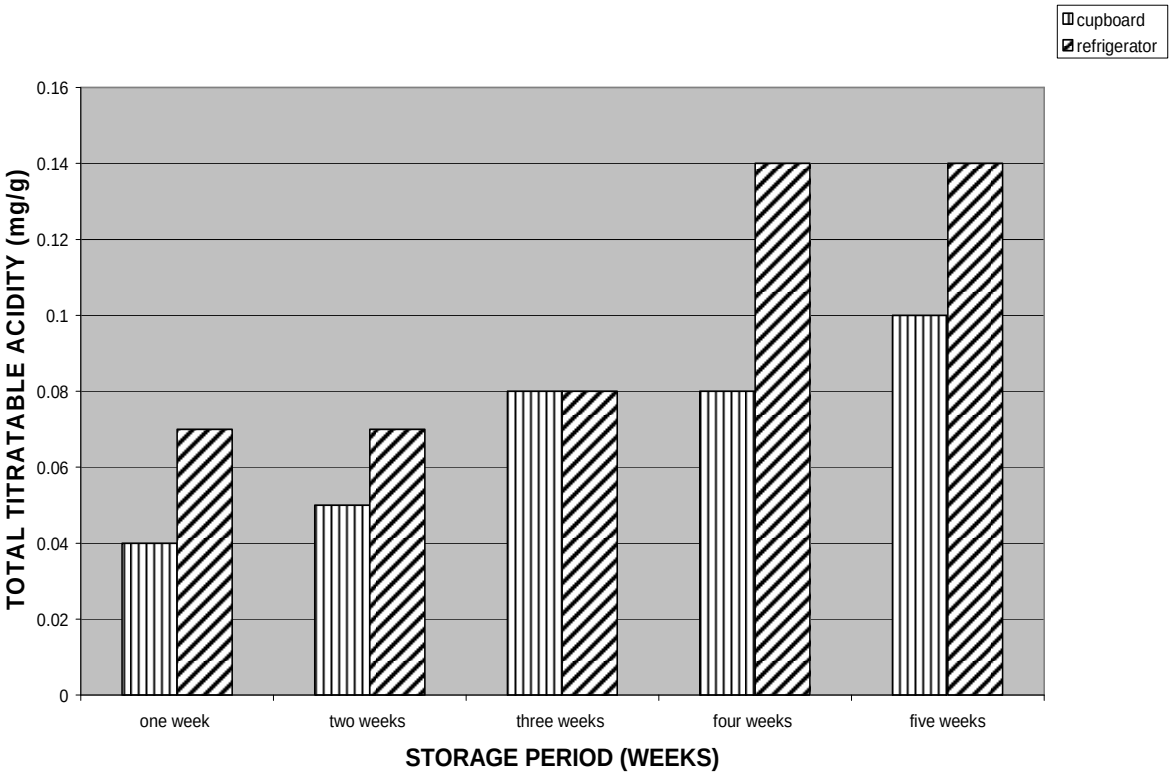

Fig. 3: Total Titratable acidity of dried meat in Refrigerator and Cupboard during the period of Storage

Average Changes in protein content of dried meat sample under different storage conditions.

There was generally an increase in protein content of the dried meat samples during the period of storage in the cupboard and refrigerator (Fig. 4).

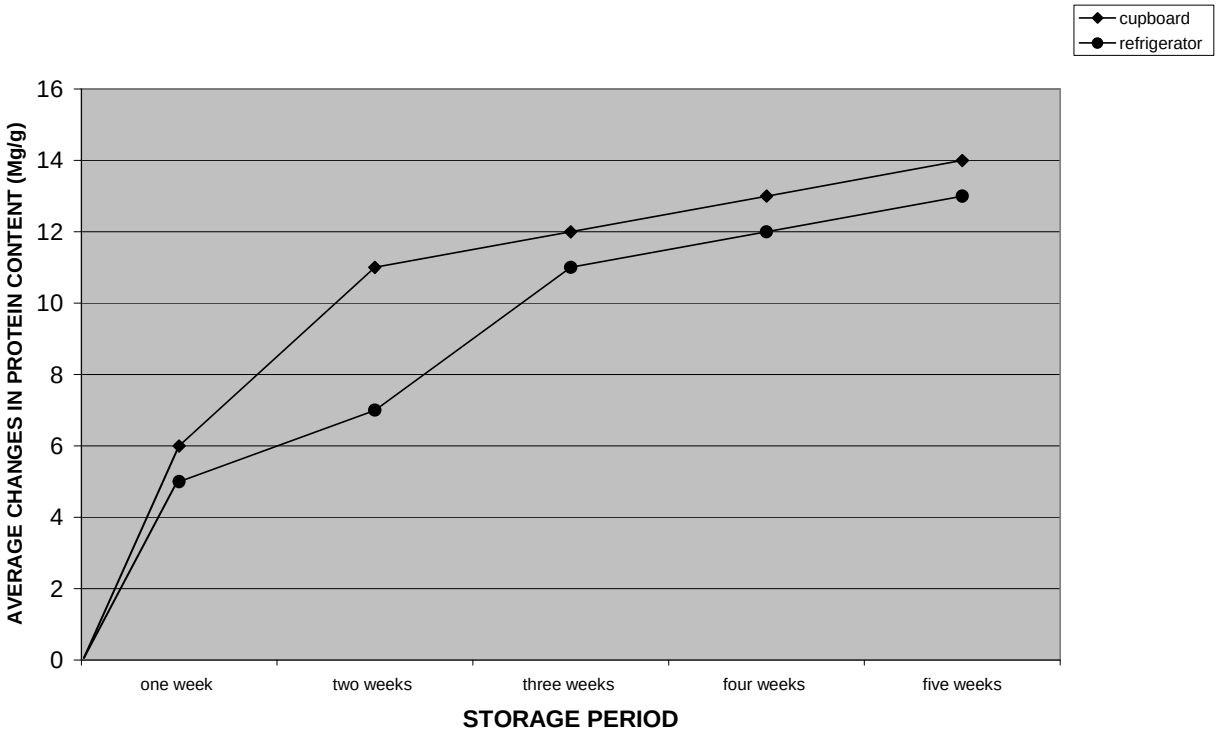

Fig. 4: Changes in protein content of dried meat samples stored under different Storage condition

DISCUSSION

The results obtained showed that the dried meat samples were contaminated with microorganisms despite the dried and hard texture of the meat. Bacteria isolated from the dried meat were *Staphylococcus aureus, Micrococcus luteus, Neisseria* sp *and Acinetobacter* sp. Fungi isolated include *Aspergillus niger, Aspergillus flavus, Rhizopus sp and Penicillium* sp. Some of the isolated microbes are directly or indirectly involved in spoilage although some may be present as opportunistic contaminants which may not play a role in the spoilage of the dried meat samples. According to [17], microbial growth on meat is higher prior to processing but is reduced by drying processes. This may explain the reduction in the types of microorganisms that were isolated from the samples. This is also in accordance with a research work conducted by [18] on Portuguese fermented sausage.

Staphylococcus aureus have been found to be relatively resistant to drying which is a property that favours their transmission from one host to another [19, 20], they are however able to grow in concentrations of sodium chloride up to 15%. Likewise, *Acinetobacter* sp can also tolerate drying and high salt concentration fairly well [21].

Micrococcus has been known to produce discolouration and putrefaction in meat [22]. Its occurrence in meat is as a result of forceful release as in coughing, sneezing or talking by meat handlers since it is a normal resident in the respiratory tract and also an aerobic organism [22]. [5] attributed the presence of *Neisseria* sp in meat as one of the bacterium frequently found on processed and fresh meat.

Aspergillus among the fungal isolates has been associated with disease conditions [23]. Most of the dried meats produced from the North are transported to the South in small packages such as woven sisal bag, jute sacks and baskets. Most of these become mouldy before reaching the market; some are displayed for sale in small containers, while some others are heaped carelessly on the floor in unventilated stores. The mouldy ones were either coated with oil or washed and re-dried in the sun [14]. Their findings also revealed that *A. flavus, A. niger* and *R. arrhizurs* are some of the fungal isolates of dried meat. Meat contamination by mold could also be as a result of contact of meat with soil [24].

The results of the analysis of changes in the pH of the dried meat samples showed that they are quite neutral the first two weeks and dropped as the storage period increased. [25] suggests that a high pH favours microbial growth and that most bacteria will grow best at neutral pH 7 although they can still tolerate ranges from pH 5 (acidic) to pH 8 (basic).

The moisture content of the dried meat sample stored in the cupboard and refrigerator were generally high during the five weeks of storage. An increase in moisture content was recorded for the meat samples in the cupboard which suggests that the meat absorbed more moisture from the atmosphere during storage which might be as a result of the high humidity in the environment and hence there is increase in the water activity of the meat sample, according to the United States Department of Agriculture–Food Safety and Inspection Service (USDA-FSIS) a water activity (a_w) of 0.70 is recommended to prevent mold growth [26]. There was a general increase of amino acids and protein content in the meat sample stored in the refrigerator and the cupboard. Previous reports has shown that meat loses its moisture content on drying resulting in an increase in the concentration of protein and other nutrients per unit weight than in their fresh counterparts [7,27] .

CONCLUSION

This study revealed that dried meat sold in Oja-Oba market are contaminated with microorganisms but they are not in any way of lesser nutritional quality and should not be written off as meat of the lesser quality than fresh meats. Proper handling during processing and at sales point is the best approach for reducing microbial contamination [1]. Caution should be exercised in consuming dried meat raw until it is reheated to inactivate or destroy microorganisms on it. The habit of traders coating mouldy dried meat with oil should be discouraged through proper enlightenment on the disadvantages of this as a possible source of microbial contamination since the oil itself could be found to have its own mycoflora. During transportation, clean jute bags should be used and the bags well protected from rain to prevent rehydration. In addition, the habit of displaying the dried meat for sale in dirty containers or heaping carelessly on the floor in unventilated stores should also be discouraged. There's however, the need for further investigation on the processing, handling, storage and nutritional qualities of dried meat with a view to finding actual sources of infection and possible means of preventing it with more work carried out on the microorganisms associated with dried meat.

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