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Full Length Research Paper

Studies on the microorganisms associated with dried meat (Tinko) sold in Ilorin, Nigeria

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Dried meat (Tinko) samples obtained from the Oja-Oba market in Ilorin metropolis during the rainy season were investigated for their microbial flora and the sensitivity of the bacterial isolates to different chemotherapeutic antibiotics under two different storage conditions (Cupboard and Refrigerator) for 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. Bacteria and Fungi isolated include *Staphylococcus aureus, Micrococcus luteus, Neisseria* sp., *Acinetobacter* sp., *Aspergillus niger, Aspergillus flavus, Penicillium* sp. and *Rhizopus* sp. Bacterial isolates showed resistance to selected antibiotics (erythromycin, tetracycline, chloramphenicol, gentamycin, streptomycin, ofloxacin, nitrofurantoin, ciprofloxacin, azithromycin and clindamycin) except for *Neisseria* sp. which was sensitive to the antibiotics. 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).

Key words: Antibiotics, dried meat, bacteria, fungi, storage.

INTRODUCTION

Meat is defined as the flesh of animals which are suitable as food (Forrest et al., 2001). 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.

Drying meat and fruit is one of the oldest methods of preservation known to humankind. What was probably an accidental discovery allowed humans to both store food for long periods of time, as well as having an easily carried nutritionally dense source of nutrition to take with them on journeys. There are several kinds of fried meat with almost the same method of preparation and preservation. 'Jerky' is a type of fried meat which derived its name from the Native (South) American Quechua term 'ch'arki' (which means 'dried meat'), and was well received in Europe by the Spanish in the 1500's when it was introduced during the early conquest of the Americas.

Biltong is another kind of cured meat that originated in South Africa. Many different types of meat are used to produce it, ranging from beef through game meats to fillets of ostrich from commercial farms. It is typically made from raw fillets of meat cut into strips following the grain of the muscle, or flat pieces sliced across the grain. It is similar to beef jerky in that they are both spiced, dried meats, but differ in their typical ingredients, taste and production process. The word biltong is from the Dutch bil ('rump') and tong ('strip' or 'tongue') (Eric, 2006). The most common names for dried meat in Nigeria are Tinko, Kilishi and Kundi, majorly prepared by the Northerners in Nigeria. Others include 'Ndariko', 'Jiorge' and 'Banda' which is prepared from meats of donkeys, asses, horses, camel, buffalo and wild-life.

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,

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sex, socio-economic facts and individual variation. Goat meat is most popular among the Igbos of East central states of Nigeria. Despite the differences in acceptability, cow meat appears to predominate all over Nigeria though sheep, goat and poultry meat are widely accepted (Obanu, 1986).

Meat deterioration begins soon after it has been slaughtered due to chemical changes, enzymatic action and the action or presence of microorganisms (bacteria, yeasts and moulds) which may result in oxidative rancidity, discolouration, mouldiness, off flavour, sliminess etc., the major source of these deteriorative changes are the microorganisms and these render the meat unacceptable and unfit for human consumption (Forrest et al., 2001). All handling and storage methods are therefore primarily concerned with minimising microbial contamination and retarding microbial growth and activity.

Meat preservation through smoking is achieved through the combination of drying and deposition of naturally produced chemicals resulting from the thermal breakdown of wood. Smoking may be done at a temperature of 29°C (85°F) or at a higher temperature of about 80°C which is referred to as cold which are required to as cold smoking and hot smoking, respectively.

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 (Fakolade and Omojola, 2008) 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 meat which is on display in the market is found to be visibly mouldy (high level of discolouration). Previously, researchers (Rollings and Hayward, 1962; Rollings et al., 1963; Osuji, 1975.) have reported the presence of the insect fauna of dried fish in Nigeria. Earlier work revealed that a number of mould species has been isolated from dried fish in Nigeria (Okafor, 1968). Also (Phillips and Wallbridge, 1976) 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 (Akano, et al., 1983). This research is based on the isolation of the microorganisms' involved in the spoilage of locally dried and stored meat and the sensitivity of the bacterial isolates to different chemotherapeutic antibiotics.

MATERIALS AND METHODS

Samples

The dried meat (Tinko) 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°C) and (b) Cupboard (room temperature 25°C)

Preparation of media, isolation techniques and characterization of isolates

The materials used such as glass wares were properly sterilized in the oven (Gallenkamp) at 160°C for 1h. All the media used were prepared according to the manufacturer's instructions and then autoclaved at 121°C for 15 min. The isolation and characterization of bacterial and fungal isolates were carried out according to the methods described by (Fawole and Oso, 2004). The Bergey's Manual of Determinative Bacteriology (Bergey and Holt, 1994) was used for identification of bacterial isolates.

Disk diffusion tests

Sensitivity of the isolated organisms (Bacteria) to ten different antibiotics was carried out using the Kirby-Bauer method (Bauer et al., 1966). The plates were duplicated and then incubated for about 16-18 hours. Mean values of two readings of diameters of zones of inhibition were obtained to the nearest millimetre. The antibiotics disks were obtained from the Microbiology Department of University of Ilorin Teaching Hospital.

Determination of moisture content

The moisture content of the dried meat (Tinko) samples were determined weekly for a period of five weeks using the method described by (A.O.A.C. 1990).

RESULTS

A total of eight organisms were isolated from the dried meat (Tinko) 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 aureus, 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. *Neisseria* sp was more susceptible to the antibiotics compared to the other isolates. Ciprofloxacin with a disc content of 1 µg had the widest zone of inhibition in all the four bacterial isolates Table 4.

DISCUSSION

Massive growth of microorganisms on the dried meat

Table 1. Frequency of bacterial isolates on 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	+	_

Key: (R) Refrigerator, (C): Cupboard, (+): Isolated , (-): Not Isolated.

Table 2. Frequency of fungal isolates on 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	-	+

Key (R): Refrigerator, (C): Cupboard, (+): Isolated, (-): Not Isolated.

Table 3. Total Bacterial counts of dried meat samples (Tinko) stored under different storage conditions for a period of 5 weeks.

Period of Storage (weeks)	Cupboard Cfu/ml (10^7)	Refrigerator 4°C Cfu/ml (10^7)
1	2.5	2.0
2	2.8	1.9
3	3.0	1.8
4	_	_
5	3.3	1.4

Samples is prior to processing but the microbial population is reduced by the drying process. This is in accordance with a research work conducted by (Venia et al., 2006) on Portuguese fermented sausage. The total bacterial count obtained from the dried meat samples stored in the cupboard and refrigerator is shown in Table 3. There was an increase in bacterial counts in the dried meat stored in the cupboard as the storage period increased while there was a slight decline in the bacterial counts of dried meat stored in the refrigerator, this correlates with the findings of (Patterson and Gibbs, 1978; Ikeme, 1990; Ayres, 2007) who found that spoilage of meat can be fairly rapid in the absence of refrigeration and that refrigeration slows or stops microbial growth Table 2.

S. aureus have been found to be relatively resistant to drying which is a property that favours their transmission from one host to another (Nester et al., 2007; Prescott et al., 2008), they are however able to grow in concentrations of sodium chloride up to 15%.

According to Table 1, *S. aureus* and *M. luteus* were isolated from the week 1 and 2 of storage in the refrigerator and cupboard. The presence of these bacterial agrees with the work of (Jawetz and Melnik, 1993; Levy et al., 1993) which reported that some common source of food contamination includes *Staphylococcus* sp and *Micrococcus* sp.

Aspergillus among the fungal isolates has been associated with disease conditions (Talaro, 2009). 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 antibiotic sensitivity of bacterial isolates in this study is largely linked to structural difference in the composition of gram negative and gram positive bacterial cell walls. It is possible that the antimicrobial properties of the antibiotics in which the microorganisms are

Table 4. Sensitivity of bacterial isolates to some selected antibiotics.

Bacterial isolates	Antibiotics and zone diameter (mm)									
	E	**T**	**C**	**G**	**S**	**F**	**CP**	**OF**	**Z**	**D**
Staphylococcus aureus	7.5	9.5	10.5	9.0	11.0	16.0	36.0	23.5	12.0	31.0
Micrococcus luteus	6.5	9.0	11.0	18.5	8.5	-	14.0	11.5	5.0	9.0
Neisseria sp	24.0	21.0	20.0	21.5	5.0	7.0	25.0	23.0	25.0	16.5
Acinetobacter sp	3.5	13.0	9.0	18.0	8.0	6.0	24.5	23.0	-	18.0

Mean Values of the two readings.

Key and concentration of antibiotics; -: No growth' E: Erythromycin 5 µg, OF: Ofloxacin 10 µg, T: Tetracycline 50 µg, F: Nitrofurantoin 200 µg, C: Chloramphenicol 50 µg, CP: Ciprofloxacin 1 µg, G: Gentamycin 10 µg, Z: Azithromycin 15 µg, S: Streptomycin 25 µg, D: Clindamycin 10 µg.

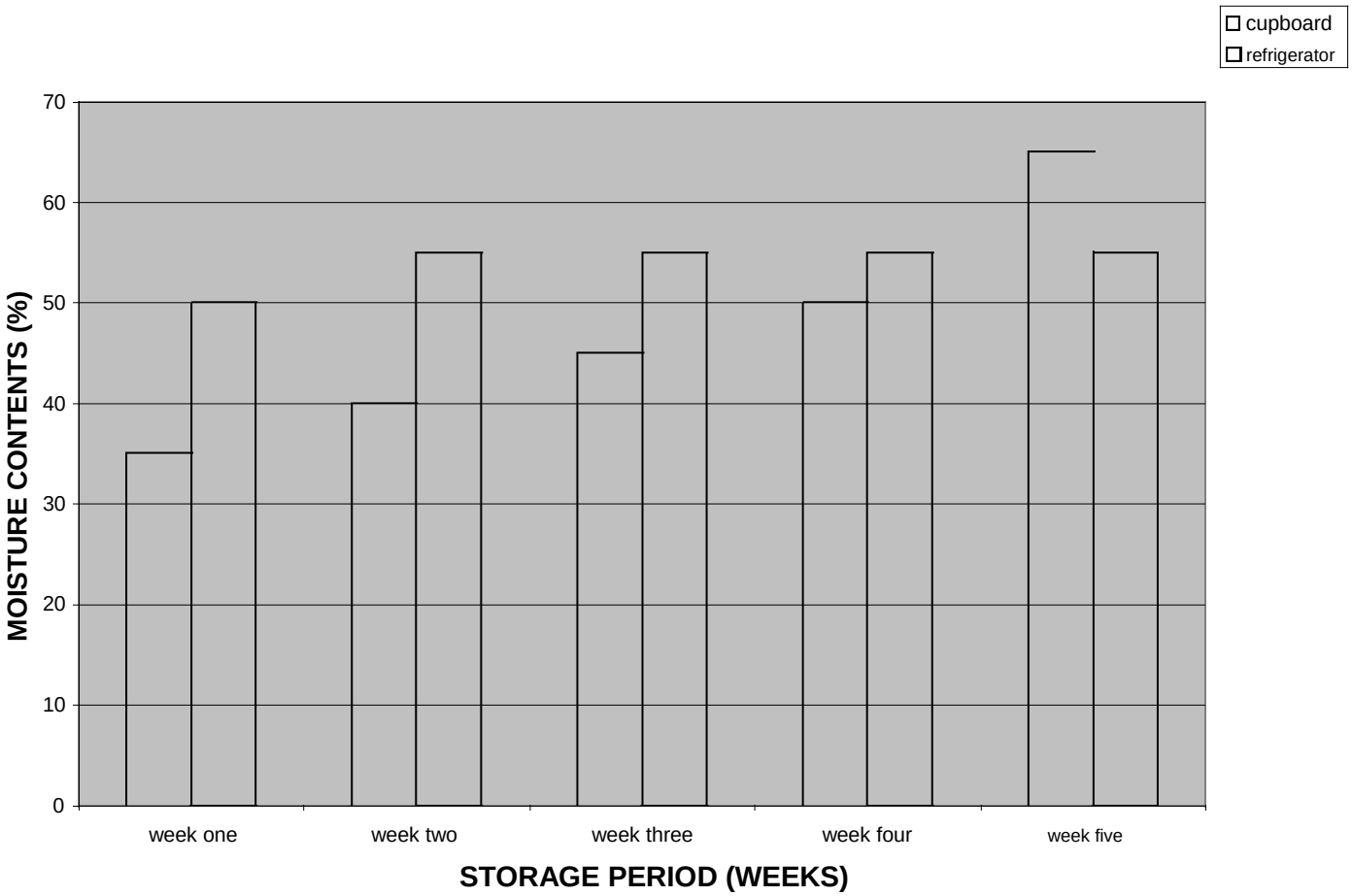

Figure 1. Moisture content of dried meat in refrigerator and cupboard during storage period.

susceptible are active on the peptidoglycan component of the cell wall (Jawetz, 1989). The low susceptibility or high resistance to the majority of antibiotics by the microbial isolates from the dried meat (exception of *Neisseria* sp) due to the barrier effect in their outer membrane may also act for the apparent resistance of the microorganisms. The varying zones of inhibition obtained may be due to their different diffusion rates (Irobi et al., 1996).

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 (Figure.1) 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 (USDA,

2004).

According to (Kolawole et al., 2010) any food substance with high moisture will favour the growth of microorganisms at a high growth rate.

Conclusion

In conclusion, dried meat under storage are not of lesser nutritional quality and should not be written off as meat of the lesser quality than fresh meats, therefore , meat for dying should be of good bacteriological quality without previous development of appreciable numbers of microorganisms or of undesirable flavour. It should be realized that the use of proper sanitation is the best approach for reducing microbial contamination, there's no substitute for good sanitation in the meat industry as rightly pointed out by (Forrest et al., 2001). This could be done by providing portable clean water as slaughter houses and retail markets in order to improve the sanitary conditions of the meat and subsequent method of drying and or smoking which should be done in a neat environment with proper and very clean handling as this permits easier preservation by keeping microbes away as much as possible.

From the results obtained, dried meat is very economical and easy way of preserving meat with no appreciable loss of its nutritional qualities, and because of its hard and dry texture, there's reduced number and types of microorganisms that can thrive on them, when compared to the microflora of fresh meat and fish therefore, the outcome of this study points to the imperativeness of good sanitary practices during the period of dried meat storage before sale to consumers. Also, 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 microflora.

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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