



EFFECTS OF STORAGE ON THE PROXIMATE, MINERAL COMPOSITION AND MYCOFLORA OF “TINCO” DRIED MEAT SOLD IN OSHODI MARKET, LAGOS STATE, NIGERIA

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ABSTRACT

The effect of storage on proximate, mineral composition and mycoflora of “Tinco” dried meat were investigated during 18 weeks of storage. Eight fungi namely *Rhizopus nigricans*, *Mucor* sp., *Aspergillus flavus*, *Aspergillus niger*, *Fusarium moniliforme*, *Absidia candidus*, *Aspergillus glaucus* and *Penicillium* sp. were isolated using washing, serial dilution and direct plating methods. The result of the proximate (g/100g) showed that ash increased from 2.05±0.01 to 3.07±0.40, crude protein 69.98±0.15 - 74.56±0.80 and carbohydrate 4.08±0.05-4.48±0.15 while moisture content decreased from 17.50±0.10 to 11.88±0.05, fat 7.15±0.10-4.93±0.25 and fibre 1.28±0.01 - 1.09±0.02. The result of the mineral analysis (mg/100g) also revealed that the following parameters increased during storage Na 29.00±0.05 - 33.5±0.40, K 33.57±0.02 - 47.39±0.10 and Mg 28.00±0.05 - 30.42±0.04 while calcium decreased from 24.10±0.01 to 20.66±0.01, Zinc 3.01±0.01 - 0.71±0.02 and Iron 5.88±0.02 - 2.50±0.02. However, copper, lead, manganese and mercury were not detected in the sample. In this study, *Penicillium* sp. was the most predominant contaminant in this area. Therefore, proper hygiene practices should be observed during handling and marketing.

KEYWORDS: Storage, Proximate, Mineral, Mycoflora, “Tinco” (dried meat)

INTRODUCTION

Meat is one of the most popular and nutritious food items which come from flesh of animals that are suitable as food (Forest *et al.*, 2001). Meat and other animal products make valuable contributions to diets of developing countries due to its high nutritional qualities (Olusola *et al.*, 2010). Different kinds of meat exist as a result of their methods of preparation and preservation. Biltong is a type of cured meat which originated from South Africa and produced from different types of meat ranging from beef through game meats to fillets of meat cut into strings. Jerky is another kind of dried meat which derived its name from native (South) American Quechua.

In Nigeria, the most common name for dried meats are Tinko, Kilishi and Kundi majorly prepared by Northerners. Others include Ndariko, Jiorge and Banda prepared from meats of donkey, asses, horses, camel, and buffalo (Okaka *et al.*, 2006). There is a preferential consumption of different types of meat by communities which may be due to a combination of factors bordering on religious belief, culture, adaptability, food habits, sex, socio-economic factors and individual variations (Obanu, 1986). Meat surface is usually heavily contaminated with a wide range of microorganisms due to its chemical composition which includes; water content, peptides, sugars, amino acids, nucleosides, mineral and vitamins. This composition makes the meat a suitable medium for the growth of microorganisms (Ostry and Reprich, 2001). In this part of the world, meat is preserved by sun-drying after cooking or smoking. Dried meat “Tinco” can be preserved by regular sun-drying, salting and prevention of moisture being absorbed into the meat product. Traditionally, dried meat microbiology involves a natural

development of wild fermentation in which microbial successions occur, uniform salting over the entire surface is most critical to inhibit pathogens and spoilage organisms (Okaka *et al.*, 2006).

Moreover, dried meats displayed in the market are found to be visibly mouldy (high level of discolouration). Most of these meats are produced from the Northern part of Nigeria and are transported to the South in small packages such as woven sisal bags, jute sacks and baskets. They invariably become mouldy before reaching the market. The presence of moulds in the meat products usually causes a decrease in their biological and nutritional values due to the enzymatic degradation of meat components (Amusa *et al.*, 2002).

The fungi that invade stored products are grouped into field and storage fungi. These moulds or yeasts could be spotted in different forms and colours on dried meats (Lowry and Gill, 2008). Earlier work by Okafor (1968) revealed that a number of mould species has been isolated from dried meat and fish in Nigeria. Also, Berwal (1991) and Oladejo and Adebayo-Tayo (2011) reported that numerous strains of microscopic filamentous fungi isolated from the surface of various meat products showed in-vitro ability to produce toxic substances called mycotoxins and which may remain in the product for years, long after the mould has died.

However, this study was carried out to investigate the effect of storage on mycoflora, proximate and mineral composition of “Tinco” dried meat from Oshodi Market, Lagos State, Nigeria.

MATERIALS AND METHODS

Collection of samples

Seventy six pieces of dried meat samples were bought from Oshodi market. The dried meat samples were chosen randomly from different sacks. The samples were tied up tightly in polythene bag each to prevent cross contamination and stored.

Isolation of fungi from the stored mouldy “Tinco” dried meat

Direct plating

Eight pieces of the dried meat were randomly picked and examined for external mouldiness. The mouldy parts of meat were aseptically picked using a sterile dissecting forceps and placed on potato dextrose agar (PDA) plate and incubated at 28°C for 5 to 7 days as described by Arotupin and Akinyosoye (2001). The fungi cultures were subcultured until pure colonies were obtained by successive hypha tip transfer (Fagbohun *et al.*, 2011). The cultures were examined under the microscope for fruiting bodies, hyphae to determine the common fungi present. The procedure above was carried out in duplicates.

Dilution plate method

This method was used to determine the type of fungi present in the stored dried meat. Meat samples were randomly picked and one gram of the sample was sterilized with ethanol. This was grinded with 10ml of distilled water using sterile ceramic mortar and pestle. This was shaken thoroughly and 1 ml of suspension was pipetted into a sterile test tube containing 9 ml of distilled water. This was thoroughly mixed together. The sample was serially diluted and 1 ml each of aliquots of 10^{-5} and 10^{-6} were added to molten PDA plates. The plates were swirled gently to obtain thorough mixing and were allowed to solidify and incubated at room temperature for 5 to 7 days. The fungal colonies were counted every 24 h. Successive hyphae tip were transferred until pure cultures of each of fungus was obtained.

Washing method

This was carried out by weighing one gram each of another sets of mouldy meat samples randomly picked into 10ml of sterile distilled water in a beaker. This was shaken thoroughly and drops of suspension of contaminated water were introduced into petri dishes containing potato dextrose agar. This was evenly spread on the agar plate with aid of a sterile glass spreader. The plates were incubated at 28°C for 5 to 7 days and were observe for visible fungal growth (Ahmad *et al.*, 2006).

Identification of mycoflora

The fungi were identified by their cultural and morphological features (Alexopoulous *et al.*, 1996). The isolates were examined under bright daylight for the colour of the culture and further examinations were carried out.

Needle mount preparation method

The method of Fagbohun *et al.* (2011) was used whereby fragments of the sporing surface of the initial culture was taken midway or between the centre and the edge of the colony. This was teased out in drop of alcohol on a sterilized glass slide using a botany needle. The fragments

were stained by adding a drop of lactophenol blue. A cover slip was applied and the preparation was examined under X10 and X40 objective lens of the microscope.

Slide culture technique

From a plate approximately 2 mm deep, 1 cm² PDA was cut and placed on a sterile glass slide. Fungus was inoculated into the four vertical sides using a sterile needle. A sterile cover slip was placed on it so that it overlapped the medium on all sides. The preparation was placed on a suitable support in a Petri dish containing blotting paper soaked in 20% glycerol in water. The preparation was kept moist at 28°C until adequate growth was observed. After removing the medium with scalpel, the fungus adhering to both cover slip and slide was examined (Crowley *et al.*, 1969). A drop of alcohol was added followed by a drop of lactophenol blue and the preparation was covered and examined under the low power objective of microscope.

Proximate analysis

The proximate analysis of the samples for moisture, ash, fibre and fat were done by the method of AOAC (2005). The nitrogen was determined by micro-Kjeldahl method as described by Pearson (1976) and the percentage nitrogen was converted to crude protein by multiplying with 6.25. All determinations were performed in triplicates.

Mineral analysis

The mineral was analyzed by dry ashing the samples at 550°C to constant weight and dissolving the ash in volumetric flask using distilled water, deionized water with a few drop of concentrated HCl. Sodium and potassium were determined by using a flame photometer (Model 405 Corning, UK) with NaCl and KCl standards. Phosphorus was determined colorimetrically using Spectronic 20 (Gallenkap, UK) as described by Pearson (1976) with KH_2PO_4 as standard. All other metals were determined by atomic absorption spectrophotometer (Pekin-Elmar Model 403, Norwalk CT, USA). The detection limits had previously been determined using the methods of Varian Techtron (1975) as Mn 0.01, Cu 0.005, Co 0.05, Zn 0.005, Fe 0.02, Mg 0.002, Ca 0.004, Na 0.001ppm (all for aqueous solution). The optimum analytical range was 0.5 to 10 absorbance units with coefficient of variation of 0.05-0.04% phosphovanadomolybdate method using a Spectronic 20 colorimeter (Galenkamp, London,UK) (AOAC, 2005). All the proximate values were reported in g/100g while the minerals were reported as mg/100 g. All determinations were done in triplicates. All chemicals used were analytical grade (BDH, London).

Statistical Analysis

Statistical analysis (Oloyo, 2001) was carried out to determine the overall mean, overall standard deviation and standard error of mean of each sample.

RESULTS AND DISCUSSION

The summary of the isolation of mycoflora from “Tinco” dried meat during 18 weeks storage period using various methods are shown on Table I.

TABLE I: Summary of microorganisms isolated from “Tinco” dried meat using various methods.

Fungi Isolated	Washing method (% Occurrence)	Direct Plating (% Occurrence)	Serial Dilution (% Occurrence)
<i>A. niger</i>	25.00	50.00	25.00
<i>A. flavus</i>	75.00	63.50	25.00
<i>F. moniliforme</i>	25.00	62.50	25.00
<i>Mucor</i> sp.	0.00	0.00	25.00
<i>R. nigricans</i>	0.00	0.00	25.00
<i>Penicillium</i> sp.	87.50	100.0	81.50
<i>Absidia candidus</i>	50.00	63.50	50.00
<i>A. glaucus</i>	37.50	0.00	25.00

TABLE II: The summary of the results of proximate analysis of “Tinco” dried meat during storage (g/100g).

Weeks of Storage	Ash	MC	CP	Fat	Fibre	CHO
Before storage	2.05±0.01	17.50±0.10	69.98±0.15	7.15±0.10	1.28±0.01	4.08±0.05
3 weeks	2.19±0.02	16.58±0.03	69.59±0.20	6.53±0.23	1.26±0.02	3.86±0.20
6 weeks	2.32±0.10	16.53±0.02	70.24±0.24	6.34±0.33	1.23±0.00	3.35±0.10
9 weeks	2.45±0.03	16.37±0.10	72.53±0.10	5.39±0.05	1.21±0.01	2.04±0.02
12 weeks	2.05±0.01	15.04±0.16	72.63±0.30	5.27±0.01	1.18±0.02	3.40±0.01
15 weeks	2.88±0.20	12.35±0.26	73.65±0.09	5.21±0.06	1.15±0.00	4.81±0.30
18 weeks	3.07±0.40	11.88±0.05	74.56±0.80	4.93±0.25	1.09±0.02	4.48±0.15
Overall mean	2.43	15.18	71.88	5.83	1.20	3.90
Overall S.D	0.40	2.21	1.95	0.84	0.07	0.91

Determinations were carried out in triplicates and the mean for each sample are presented with their standard error.

Legend

S.D – Standard Deviation

The result showed that a total number of eight fungi namely *R. nigricans*, *Mucor* sp., *A. niger*, *A. glaucus*, *Penicillium* spp., *F. moniliforme*, *Absidia candidus* and *A. flavus* were isolated in their respective frequencies. This result is in agreement with the findings of Anderson (1995) who reported a 90% and 4% occurrence of *Penicillium* sp and *Mucor* sp respectively in dried meat and other meat products. Similarly, Cvetnic and Pepeijnjak (2006) reported 20% incidence of *A. flavus* and *A. parasiticus* in smoked meat products, pork, lacon and ham. Also, Oladejo and Adebayo-Tayo (2011) reported the isolation of *A. niger* (36.23%), *A. flavus* (30.43%), *A. fumigatus* (13.04%), *A. candidus* (10.14%) and *A. piperis* (10.14%) from “Banda” dried meat. Moreover, Laciakova *et al.* (2004) reported the isolation of toxigenic genera of *Aspergillus* spp., *Pencillium* spp and *Fusarium* spp in dried meats. Microbial contamination of meat and meat products must not exceed level which could adversely affect the shelf life of the product. If it does, it renders the meat useless, unwholesome and hence not fit for human consumption. Mycotoxins which are usually produced in-vitro by these moulds, when ingested can produce both acute short term and long term toxicities ranging from death to chronic interferences with pulmonary systems and the alimentary tract in man and laboratory animals (Amusa *et al.*, 2002).

From this study, it can be deduced that *Penicillium* sp. had the highest occurrence (100%) in “Tinco” dried meat stored for eighteen weeks. This is in agreement with the findings of Oladejo and Adebayo-Tayo (2011) who reported *A. niger* to be predominantly present in “Banda” meat samples under study with the frequency of occurrence of 36.23%. Also, it established that types and occurrence of fungi species isolated from stored products

are dependent on growth conditions and methods of isolation (Fakolade and Omajola, 2008).

The summary of the proximate analysis (g/100g) of “Tinco” dried meat during eighteen weeks of storage is shown on table II. The result revealed that ash increased from 2.05±0.01 to 3.07±0.40, crude protein 69.98±0.15 - 74.56±0.80 and carbohydrate 4.08±0.05 - 4.48±0.15. This result is in agreement with the findings of Oladejo and Adebayo-Tayo (2011) who reported an increase in ash content (8.29-23.86), crude protein (21.68-54.16) and crude fat (1.86-6.21%) of “Banda” dried meat collected in July. Also, Torres *et al.*, (1994) reported that ash content at the end of storage of dried meats differs significantly to that at the onset. However, Fagbohun (2012) reported that ash content of non-infected pods (10.7) and beans (8.0) were depleted when infected with *Phytophthora palmivora* to 9.3 and 7.8 in cocoa pods and beans respectively. Moreover, increase in ash obtained could be due to the condiments used during the boiling of the meat for the kundi production. It may also be due to the resultant dirt on the meat pieces during sun-drying on the ground in the open market. Rodolfo *et al.* (2000) and Bilgrami and Dube (2001) found out that fungi increase the protein content of the samples on which they grow. Protein increase could as well be from slight protein synthesis by proliferation of microorganisms and synthesize enzyme protein. More so, in this study, moisture content decreased from 17.50±0.10 to 11.88±0.05, fat 7.15±0.10-4.93±0.25 and fibre 1.28±0.01 - 1.09±0.02. This result agreed with the work of Onifade and Jeff-Agboola (2003) who found the moisture content of healthy *Cocos nucifera* to decrease from 36.4g/100g to 10.4g/100g in infected samples. Similarly, Omokolo *et al.* (1996) found carbohydrate content of healthy pods (91.0) to decrease to 13.2 in infected cocoa pods. Decrease in water content in this

study could be attributed to the fact that infecting fungus utilizes the moisture content for its survival and growth. The shelf life of any product is influenced by the amount of water present in it (Pearson, 1976).

The summary of the mineral constituents (mg/100g) of "Tinco" dried meat during storage is shown on Table III. The result showed that the following parameters increased during storage; Na 29.00±0.05 - 33.5±0.40, K 33.57±0.02 - 47.39±0.10 and Mg 28.00±0.05 - 30.42±0.04. The result is similar to the findings of Oladejo and Adebayo-Tayo (2011) who reported an increase in Na (0.35-1.55), K (0.09-0.35) and Magnesium (0.12-0.21) in "Banda" dried meat during storage. However, Ca decreased from 24.10±0.01 to 20.66±0.01, Zn 3.01±0.01 - 0.71±0.02 and Fe 5.88±0.02 - 2.50±0.02. This is in agreement with the findings of Aziz *et al.* (2000) who reported that *A. flavus* depleted Zn and Fe from an infected crushed corn. Meat is a major source of iron. Iron in meat has a high bioavailability, the main reservoir being as a component of the haem protein Myoglobin. Iron deficiency is the most common nutritional deficiency in the world (Warriss,

2003). More than 90% of the iron in the body is combined with proteins, e.g haemoglobin, which contain about 3.49/kg of the element. It has a major role in host biochemical reactions, particularly in connection with enzymes of the electron transport Chain (Cytochromes). Electrons are transported by the oxidation and reduction activity of bound iron (McDonald *et al.*, 1995). High zinc concentration has been found in the skin, hair and wool of animals. It is an activator of several enzyme systems and involves in cell replication and differentiation, particularly in nucleic acid metabolism. It is also involved in production, storage and secretion of hormones, in immune system and electrolyte balance (Mgbabu, 2011). This study revealed that mould infections on "Tinco" dried meat has highly devastating effects since Zn and Fe are important minerals in meat products. However, copper, lead, manganese and mercury were not detected in the sample. This is in contrast to the findings of Oladejo and Adebayo-Tayo (2011) who reported the detection of copper in the stored samples.

TABLE III: The summary of the results of mineral analysis of "Tinco" dried meat during storage (mg/100g).

Weeks of Storage	Na	K	Ca	Mg	Mn	Zn	Fe	Cu	Pb	H
Before storage	29.00±0.05	33.50±0.02	24.10±0.01	28.00±0.05	ND	3.01±0.01	5.88±0.02	ND	ND	ND
3 weeks	29.50±0.10	34.41±1.00	24.36±0.02	28.05±0.03	ND	2.70±0.02	5.88±0.10	ND	ND	ND
6 weeks	29.65±0.04	38.54±1.00	25.69±0.06	28.75±0.01	ND	1.84±0.01	3.85±0.02	ND	ND	ND
9 weeks	30.10±0.20	39.87±0.05	26.07±0.03	28.80±0.02	ND	1.81±0.03	3.82±0.01	ND	ND	ND
12 weeks	30.35±0.05	41.26±0.00	27.10±0.01	30.54±0.03	ND	0.95±0.01	2.75±0.02	ND	ND	ND
15 weeks	33.50±1.00	46.33±0.42	26.59±0.03	31.48±0.06	ND	0.73±0.02	2.63±0.04	ND	ND	ND
18 weeks	33.55±0.40	47.39±0.10	20.66±0.01	30.42±0.04	ND	0.71±0.02	2.50±0.02	ND	ND	ND
Overall mean	30.81	40.19	24.94	29.43	-	1.68	3.90	-	-	-
Overall mean	1.91	5.35	2.18	1.37	-	0.93	1.46	-	-	-

Determinations were carried out in triplicates and the mean for each sample are presented with their standard error.

ND – Not Detected

S.D – Standard Deviation

CONCLUSION

From this study, it can be concluded that the highest contaminant of "Tinco" dried meat in this region is *Penicillium* sp. Therefore, special attention should be paid to the microbial investigation to minimize the threats posed to public health. Also, good sanitary practices must be followed and microbiological standards must be adhered to by checking production procedures and handling until the dried meat reaches the consumer's table. Dried meat sellers, especially the illiterates should be enlightened about good hygiene practices.

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