Proximate and Microbiological evaluation of the West African dried Meat product, *Kilishi* sold in three major cities of Nigeria

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Abstract: The proximate and microbial evaluation of *Kilishi*, a West African dried meat product was carried out in this study, samples collected from three major cities in Nigeria were evaluated from May- November 2013. The data were subjected to analysis of variance using SPSS version 16, while means were separated using Duncan Multiple Range Test at P<0.05. The result on the microbial analysis shows that the growth response of fungi isolated in *Kilishi* obtained from Ibadan and Minna cities were non- significantly different from each other but significantly different from of fungi isolated in *Kilishi* obtained from Lagos city. The location effect is highly significant (p< 0.01) for crude protein and ash content but non significantly different for moisture content while the location effect were significant for the ether extract. The ANOVA showed that *Kilishi* obtained from Lagos and Ibadan cities was similar in crude protein with a value ranging from 19.44 – 23.55. This study shows that ash content was highest in *Kilishi* obtained from Ibadan city and significantly different from *Kilishi* obtained from Minna and Lagos cities. There are non significant differences in the ether extract of *Kilishi* obtained from Minna and Ibadan cities. The result of correlation coefficient of location, day after inoculation and replicate shows that the growth area was positive and highly correlated with day after inoculation (p<0.01; r=0.43) but non associated positively with replicates. Fungi species isolated from the samples were *A. niger, A. flavus, A. fumigatus, Fusarium oxysporum, Neurospora crassa, Penicillum notatum and P. chrysogenum*. The highest occurring fungus in all the locations were *Aspergillus* sp. and significant amount of Aflatoxin B1 from mouldy *Kilishi* was also detected. The result of this study shows the potential of *Kilishi* as a high protein product. However, the increase in crude protein content found in *Kilishi* obtained from Lagos and Ibadan cities are related to the growth of fungi isolated from the samples. The presence of these fungi is unusual and it compromises the safety of the *Kilishi* for human consumption Therefore, proper hygiene practices should be observed during handling and marketing process for food safety.
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Introduction

Meat is one of the most highly perishable items of all important foods, because of its abundance of nutrients and moisture content that favor the colonization and multiplication of microorganisms. Its consumption is gradually on high demand due to the ever-increasing population of developing countries. Meat products enhance diets due to its high nutritional qualities (Olusola *et al*., 2010). In Nigeria the most common dried varieties meat known are *Kilishi*, *Tinco* and *Kundi* mainly prepared by the Northerners. Others include, *Ndariko*, *Jiorge* and *Banda* prepared from meats of donkey, asses, horses, camel, and buffalo after removing the bones (Okaka *et al*., 2006).

Kilishi is a rich nourishing snack, and source of supplementary animal protein formulated using hurdle technology, a concept described by Leistner, (1974). The availability and affordability of meat to a wide range of the Nigerian population is still far from expected (Abdullahi *et al.,* 2004). The desirable qualities of meat *Kilishi* include; ease for bulk transportation, fortified nutrient levels and its long shelf life while the main method of meat preservation transferred by the medieval Arabic sources to West Africa was that of sun drying (Alonge and Hiko, 1981). The preparation of the this meat delicacies derived from animal is by partially drying thin sheets of quality lean beef in the sun followed by soaking in a slurry of plant ingredients before a second period of sun drying and briefly roasting (Igene *et al.,* 1990; Musonge and Njolai, 1994). The crude manner of processing of *Kilishi* makes it highly susceptible to serious microbial contamination which jeopardizes its safety for consumption. It contains about 46% meat and 54% non-meat ingredients. A finished product contains about 50% protein, 7.5% moisture, 18% lipid

and 9.8% fibre /ash respectively (Igene, 1988; Igene *et al*, 1993). The common gift from the northerners to visitors especially the youth corps members from other parts of Nigeria is *Kilishi*. The addition of spices to *Kilishi* ingredients is also of health importance as this-could be a check to stomach disorders, rheumatics and act as relaxers of the alimentary system (Ketiku, 1975).

However, the formulations of the ingredients, infusion time and duration of the solar drying stages depending on the environmental conditions are the major sources of microbial contamination during the production process. Contamination can occur through attack of the meat and tissue enzymes by microorganisms. This causes the development of free acidity and oxidation of unsaturated bonds that changes the organoleptic properties of the meat. The presence of fungi in meat products as a result of little or no packaging for buyers may render it useless and unhygienic for human consumption. This study was therefore carried out to assess the variations in fungi associated with spoilage of Kilishi and its nutrient compositions.

2. Materials And Method

Study area and sample collection

This study was conducted at the Mycology/pathology unit of the Department of Botany, University of Ibadan, Ibadan, Nigeria. Ibadan is located in the Southwestern part of Nigeria approximately between Latitude N 7^o 261 Longitude E 3^o 531 and an Altitude of 190m. The city ranges in elevation from 150m in the valley area to 275m above sea level. Ibadan has a tropical wet and dry climate with mean monthly temperatures fluctuating between 23^o C to 30^o C and humidity is usually from 55% to 75%. Meat used for this study was purchased from an open markets in Ibadan, Lagos and Minna cities of Nigeria. These samples were collected in sterile nylon and transported to the laboratory immediately.

Fungi Evaluation Methods

Dilution plate method

Two grams of each of the meat products samples was picked and swabbed with ethanol to remove contaminant. This was blended with 10ml of distilled water using sterile blending machine. This was vigorously shaken and 1 ml of sample was pipetted into a sterile McCartney bottles containing 9 ml of distilled water. The sample was serially diluted and 1 ml each of aliquots of 10^{-6} and 10^{-7} were added to molten PDA plates. The plates were allowed to solidify and incubated at 30 °C for 3-5 days. The fungal colonies were counted every 24hours. Successive hyphae tip were transferred until pure cultures of each of fungus was obtained.

Direct plating

The sliced parts of meat were aseptically picked using a sterile dissecting forceps and placed on potato dextrose agar (PDA) plate supplemented with streptomycin sulphate (0.05g/1000cm-3) to prevent bacterial contamination (Jonathan and Fasidi,2001). Incubation was done at 30 °C for 3-5 days. The fungi cultures were sub cultured until pure cultures were obtained by repeated hypha tip transfer. Microscopic examination of pure culture was carried out under the light microscope for hyphae slides which were prepared in triplicates.

Analysis of Nutrient Composition of raw meat and *Kilishi*

The crude protein, ether extract, ash content, crude fibre and moisture content of the *Kilishi* products were determined according to AOAC (2005). The experimental plates were arranged in triplicates. Screening for aflatoxin B1 was also carried out using the proceedure of AOAC Offical methods of analysis (2005).

Data analyses

The data obtained were subjected to Analysis of Variance (ANOVA) using SPSS version 16.0. Duncan Multiple Range Test (DMRT) was further used to separate treatment means where there was significant difference. Tables, plates and graphs were also used to illustrate results as appropriate.

3. Results And Discussion

Proximate composition

Table 1 shows that the mean effect of location is highly significant ($p<0.01$) for crude protein and ash content but non significant for the moisture content, the location effect is significant for Ether extract, all the parameters analysed were highly significant at replicate level (s). A value of 50.02% was analysed for crude protein found in traditional *Kilishi* after roasting as reported by Igene *et al.* (1990). However, Badau *et al.,* 1997 reported that the major part of the protein comes from the groundnut cake which has 55.85%. The less crude protein recorded for *Kilishi* obtained from Minna might also be due to the loss of some soluble protein as a result of heating intensity during the production process. This is also similar to the work of Mbofung, 1993 who reported loss of some soluble protein due to the production process of *Kilishi.*

The highest ether extract observed in *Kilishi* obtained from Lagos might be as a result of addition of peanut as one of the infusion ingredients. The ash content observed in the *Kilishi* obtained from Minna and Ibadan might also be due to the contribution of the ash content of the curing ingredient especially the spices. Also, No significant differences were observed in the moisture content of the various *Kilishi* obtained

from all the locations. However, lower moisture contents of 7.5% and 4.2% were recorded by Igene *et al.,* (1990) and Abubakar *et al.,* (2011), respectively in Northern Nigeria with a drier climate.

There were no significant differences between the crude protein of *Kilishi* obtained from Ibadan and Lagos cities but significantly different from *Kilishi* obtained from Minna city. For the Ash content of the *Kilishi*, all the locations were significantly different from each other and for the ether extract, *Kilishi* obtained from Ibadan and Minna cities were not significantly different from each other but significantly different from *Kilishi* obtained from Lagos city while the moisture content of the *Kilishi* obtained from the three locations were non-significantly different from each other (Table 2). *Kilishi* obtained from Lagos and Ibadan were similar in crude protein with a value ranging from 19.44 – 23.55. The similarity in crude protein content observed in *Kilishi* obtained from Lagos and Ibadan might be as a result of various ingredients that were used during the production process. Rodolfo *et al*. (2000) and Bilgrami and Dube (2001) found out that fungi increase the protein content of the samples on which they grow. The potential of *Kilishi* as a high protein food product has been demonstrated in this study. No significance differences were observed in the ether extract of *Kilishi* obtained from Minna and Ibadan with a mean value of 1.97- 2.04. Igene *et al.,* (1990) recorded ether extract values of 17.8% while Abubakar (2011) reported 26.1 % ether extract but the type of muscle used for their *Kilishi* was not specified. The average moisture content of the *Kilishi* obtained from Lagos, Minna and Ibadan cities indicates that the products were well dried.

The effect of replicates of the proximate analysis were shown in table 3, Replicate 1 is significantly different from replicate 2 and 3 for the crude protein while the ash content for replicate 1 is significantly different from rep 3 and 3 which are significantly different from each other. Also ether extract for replicate 1 was significantly different from replicate 2 and replicate 3 which are also significantly different from each other and the moisture content of replicate 1 is significantly different from replicate 2 and 3 which are also significantly different from each other. The analysis of variance shows that ash content was highest in *Kilishi* obtained from Ibadan and significantly different from *Kilishi* obtained from Minna and Lagos. The higher ash content observed in the *Kilishi* obtained from Ibadan *might* be due to the addition of the ash content of the curing ingredient especially the spices, differences in ash content might also be as a result of smoking process during production. Pruthi, 1980 gave the ash content of clove powder to be 5.26% and red pepper to be 6.17%. Torres *et al.*, (1994) reported that ash content at the end of storage of dried meats differs significantly to that at the onset.

Identification of fungal isolates showing their appearance.

Table 4 of this study showed that six different fungi species were isolated from *Kilishi*. Fungi isolated from *Kilishi* obtained from Minna includes *Aspergillus flavus, A. niger*, *A. fumigatus and Fusarium oxysporum*; Fungi isolated from *Kilishi* obtained from Lagos are *A. niger, Neurospora crassa, A. flavus and Penicillum notatum*, while fungi isolated from the *Kilishi* obtained from Ibadan were *Fussarium oxysporum, A. flavus and P. chrysogenum.* The frequency of occurrence shows that *Aspergillus* spp. had the highest frequency of occurrence among all the locations where the *Kilishi* were collected. The microorganisms isolated from the *Kilishi* were fungi capable of growth at a very low moisture content namely *Aspergillus flavus, A. niger, A. fumigatus, Fusarium oxysporium, Penicillum chrysogenum, Penicillum notatum and Neurospora crassa.* This work agrees with the findings of Fonkem *et al.,* 2010 who reported the presence of xerophilic moulds in *Kilishi*, Anyawu *et al* 2010 also isolated *A. niger* from *Kilishi* sold in Abuja. This work was also in accordance with Faleye *et al.,* 2012 who reported *Aspergillus flavus, Aspergillus niger, Fusarium* sp, *Aspergillus* sp and *Penicillium* sp from Tinco dried meat. Fakolade *et al.,* 2012 also reported the presence of *Aspergillus flavus, Aspergillus Niger. Penicillium* sp, *Fusarium* sp in dried meat. The result of correlation coefficient of location, day after inoculation and replicate shows that the growth area was positive and highly correlated with day after inoculation ($p<0.01$;$r=0.43$) but non associated positively with replicates, location was also non correlated with day after inoculation and replicate (Table 5). The mean effect of the location and day after inoculation is highly ($p<0.01$) significant for growth area, but the effect of replicate and interaction of location X replicate, location X day after inoculation, day after inoculation X replicate and location X day after inoculation X replicate are ($p<0.05$) non significant for the growth area (Table 6). A significant amount Aflatoxin B1 from mouldy *Kilishi* was also detected. Figure 1 show that *Kilishi* obtained from Lagos shows the highest Aflatoxin B1 content of 86.81% followed by Ibadan which has the total of 79.67 % while Minna shows the lowest Aflatoxin B1 content of 73.24%. The percentage crude protein of *Kilishi* obtained from Lagos shows 34.29 while *Kilishi* obtained from Ibadan shows 29.70. The *Kilishi* obtained from Minna shows the least percentage value of 13.14. Plates a-d shows the growth of the fungi isolated in PDA plates.

Table 1: Mean Square of % Crude protein, %ash, % ether extract and % moisture content in *Kilishi* collected from three different states.

Source of variation	df	% CP	%AS	%EE	%MC
Location	2	173.16**	6.06**	0.38*	0.00^{ns}
Replicate	2	220.42**	6.02**	3.75**	850.76**
Error	4	11.21	0.02	0.06	13.27
Total	9				
Corrected Total	8				

% CP =%Crude protein, %AS = %Ash, % EE =% ether extract, % MC =% Moisture content *.** P< 0.01 highly significant, P< 0.05 significant, ns= Non significamt

Table 2: Comparism of the proximate contents of *kilishi* from the different locations.

Location	%CP	%AS	%EE	%MC
Ibadan	23.55^{a}	75.00^{b}	2.04^{b}	50.00^{a}
Minna	8.83^{b}	6.75^{c}	1.97^{b}	50.00^{a}
Lagos	19.44^{a}	9.50^{a}	2.62^{a}	50.00^{a}

% CP =%Crude protein, %AS = %Ash, % EE =% ether extract, % MC =% Moisture content. Means with the same letter in the same column are not significantly different at P< 0.05 using Duncan's Multiple Range Test (DMRT

Table 3: Effect of replicates on the proximate analysis of *Kilishi*

Replicate	%CP	%AS	%EE	%MC
1	25.84^{a}	9.33^{a}	3.33^{a}	66.84^{a}
2	8.70^{c}	6.50^{c}	1.10^{c}	33.16^{c}
3	17.27^{b}	7.91^{b}	2.20^{b}	50.00^{b}

% CP =%Crude protein, %AS = %Ash, % EE =% ether extract, % MC =% Moisture content.Means with the same letter in the same column are not significantly different at P< 0.05 using Duncan's Multiple Range Test (DMRT)

Table 4: Identification of fungal isolates showing their appearance.

Fungi isolated	*Occurrence*	*Appearance*
Aspergillus flavus	*Minna*	Its yellowish-green, consisting of a dense felt of conidiophores
Aspergillus niger	*Minna*	It is darkly pigmented with flask-shaped
Aspergillus fumigates	*Minna*	It appears in chains with white mycelium and blue green spores.
Fusarium oxysporum	*Minna*	It forms a pigment on the colonies in agar, from none to purple to violet mycelium white to purple.
Neurospora crassa	*Lagos*	It grows slowly with a marked difference in morphology
Aspergillus flavus	*Lagos*	It appears in yellowish-green colour with varying shapes
Penicillum notatum	*Lagos*	They are dark green in colour with powdery appearance.
Aspergillus niger	*Lagos*	It appears in black colony, It appears cottony, white to yellow and then it turns black
Fusarium oxysporum	*Ibadan*	They grow fast, and are brightly colored. they grow from whitish to yellow, brownish, pink, reddish or lilac shades.
Penicillum chrysogenum	*Ibadan*	It has pellet appearance with blue green in colour with a yellow pigmentation at two weeks after subculturing.
Aspergillus flavus	*Ibadan*	It forms yellow green colour and brownish with age

Table 5: Correlation matrix of fungi growth in *Kilishi*

AREA	LOCATION	DAI	REP
AREA	0.03^{ns}	0.43^{**}	0.32^{ns}
LOCATION		0.00^{ns}	0.33^{ns}
DAI			0.00^{ns}

*, ** significant at P < 0.05 and P < 0.01respectively; Non-significant at P < 0.05 and P < 0.01 respectively

TABLE 6: Growth stages of fungi in *Kilishi* to interactive effect of location, days after inoculation and replicate

Source of variation	df	AREA
Location (LOC)	2	72.14**
Days after Inoculation (DAI)	4	41.21**
Replicate (REP)	2	0.02ns
LOC x REP	4	0.20ns
LOC x DAI	8	7.82ns
DAI x REP	8	6.93ns
LOC x DAI x REP	16	7.22ns
Error	60	4.52
Total	105	
Corrected total	104	
CV%		

*P<0.05= significant; ** P<0.01= highly significant, ns= non- significant. DAI= Days after inoculation REP+ Replicate

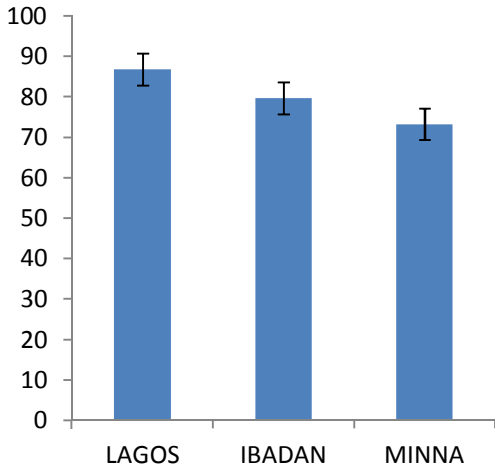


Figure 1: Aflatoxin B1 content in three weeks old *Kilishi* from three different locations

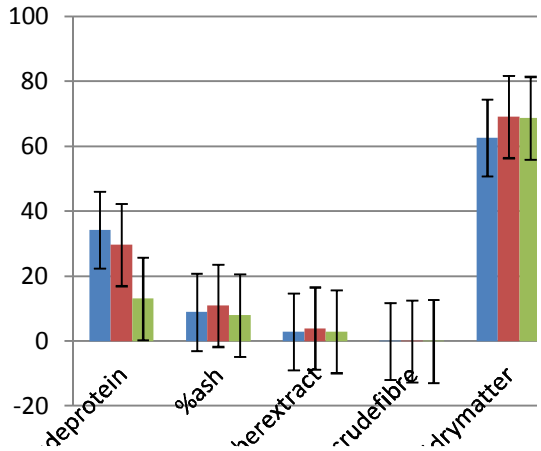


Figure 2: The percentage of the proximate analysis of *Kilishi* from three different location on dry basis

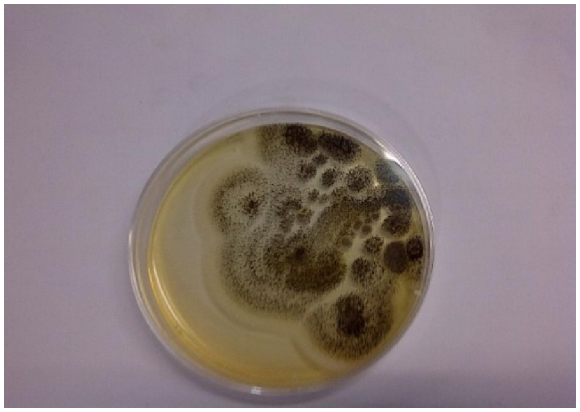


Plate. a: *Aspergillus niger*.,

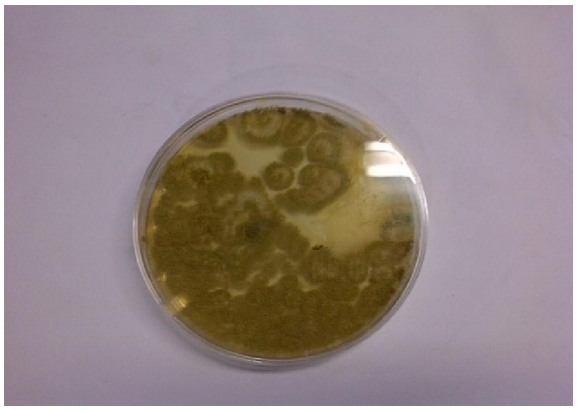


Plate b: *Aspergillus flavus*

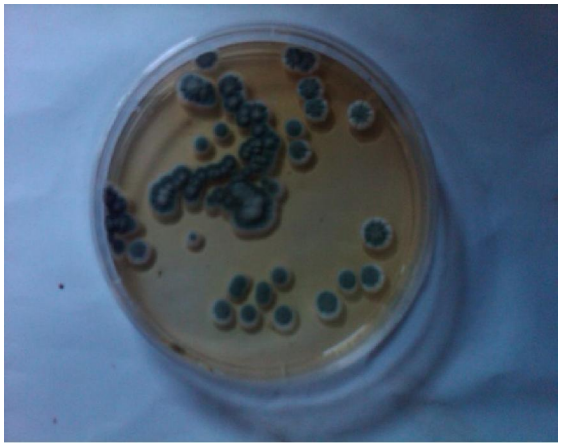
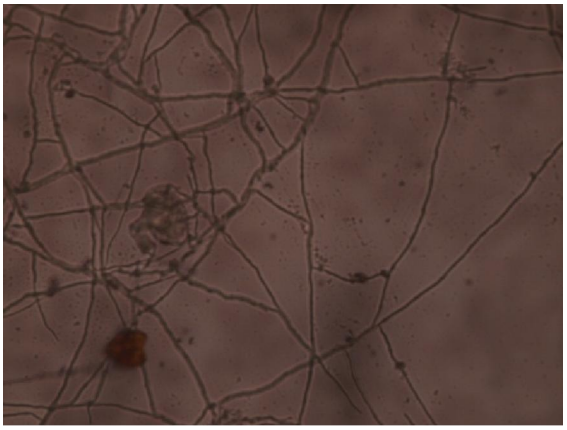
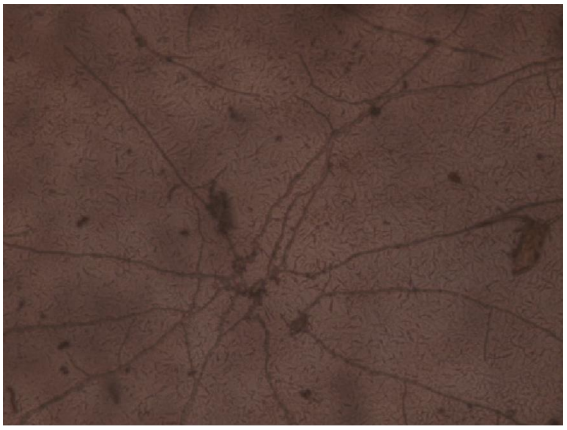


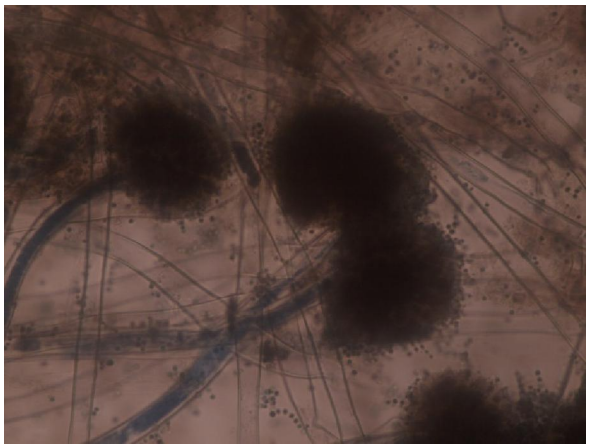
Plate c*: Penicillum* sp

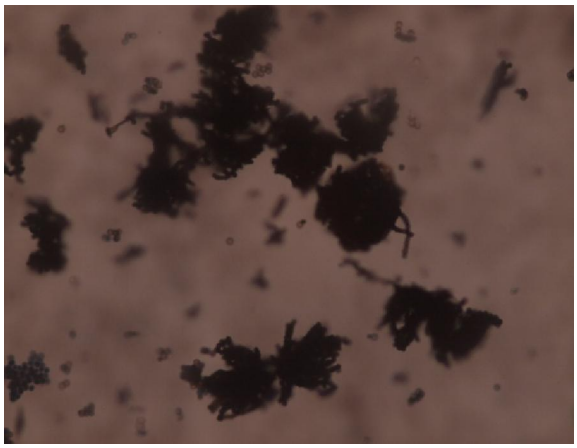
Plate d: *Neurospora crassa*

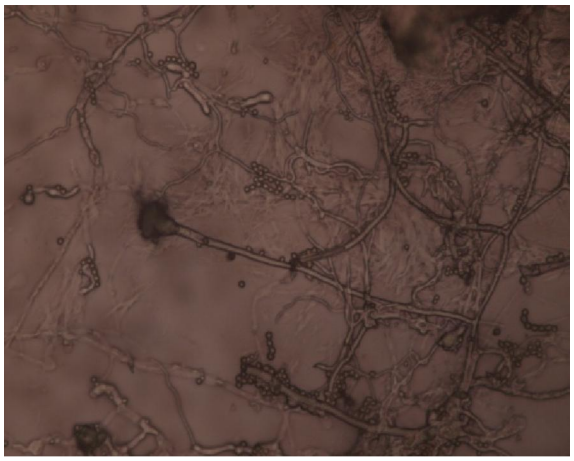
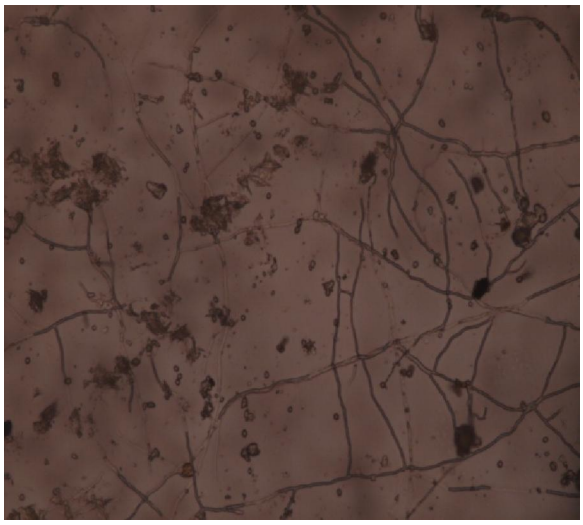
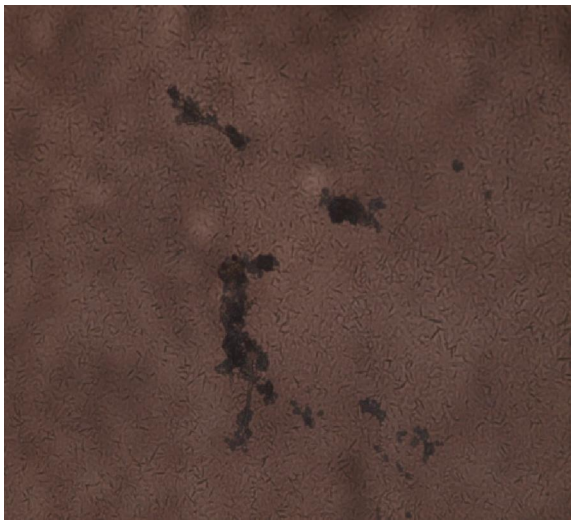
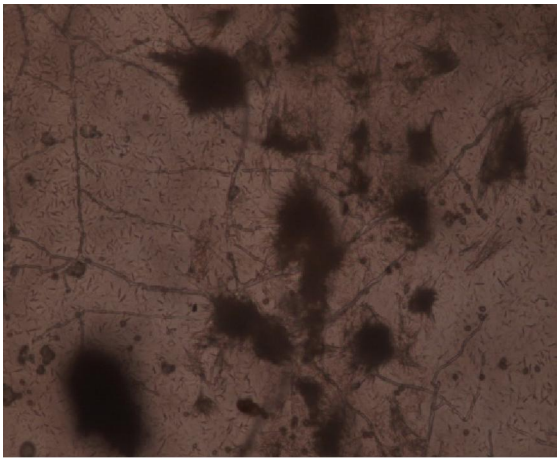
Conclusion









The proximate compositions, crude protein, ash content, ether extract, moisture content of *Kilishi* were influenced positively by the locations. It is therefore concluded that the processing of *Kilishi* differs from one location to another, an acceptable processing method should be encouraged for improving the nutritional qualities of *the* meat product, also only meat of good microbiological quality should be used for processing, and measures should be properly taken during transportation to avoid contamination as this could be done by using new package techniques such as aluminum foil paper, sterile polythene bags and use of brown envelope should be totally discouraged to avoid re-absorption of moisture. From the findings, fungi isolated from the *Kilishi* might be due to condiments used, drying stages, infusion time and roasting period. Good precautionary measures must be taken to prevent or greatly reduce its contamination by micro-organisms. The issue of meat products safety from a number of potential harmful fungi is a serious concern, since mycotoxins produced by toxigenic fungi have the ability for life threatening diseases. There is should be an urgent need to address the food safety, it is suggested that preparation of *kilishi* should be further explored as a means of avoiding meat spoilage most especially during glut in supply meat products in the market.

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