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MYCOLOGICAL ASSESSMENT OF BEEF SOLD AT SELECTED RETAIL POINTS IN OWO, ONDO STATE, NIGERIA

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Abstract
The mycological quality of six samples of fresh beef sold at selected retail points in Owo, Ondo State was assessed using conventional methods. The fungal counts for the samples ranged from 8.0 ×105 to 57 x 105 sfu/g with Poly gate sample on the morning of day 10 scoring the lowest count of 8.0 x 105 sfu/g while Ikare junction sample on day 1 scored the highest count of 57.0 x 105 sfu/g. The Ikare-junction sample recorded the second highest count with 52.0 x 105 sfu/g on day 6 (evening) followed by Oke Ogun (50.0 x 105 sfu/g) on day 6 (morning). The fungal isolates were identified as: Aspergillus niger, A. fumigatus, Fusarium oxysporum, Rhizopus stolonifer, Penicillium oxalicum, Mucor plumbeus, Botrytis cinerea, Cladosporium herbarum, and Aureobasidium pullulans. The samples were grossly contaminated with some pathogenic moulds considered as major spoilage of meat, and may constitute public health hazards by production of wide variety of mycotoxins which are carcinogenic when consumed by humans. Beef handlers and consumers should be educated on the adverse effects of lack of proper personal and environmental hygiene and the need for efficient heat treatment of meat before cosumption.
Keywords: Beef, Fungi, Owo, Pathogenic, Retail

Introduction

Beef is one of the most popular and known meat that comes from the flesh of cattle which is suitable as food for humans (Olusola and Omojola, 2010). Meat is one of the most perishable of all important foods as it contains sufficient nutrients needed to support the growth and development of microbes (Magnus, 2001). The chief constituents of meat are water, protein, fat, phosphorus, and iron. Vitamins are also present in meat, hence are considered as one of the most nutritive sources of protein available to man. Most meat has a high water content corresponding to water activity of approximately 0.99 which accommodates microbial growth (Rao et al., 2009), and it is considered to be spoiled when it is unfit for human consumption. Meat is subject to changes by its enzyme, microbial activities, and oxidation of its fat content. Microorganisms grow on meat causing visual, textural, and organoleptic changes when they release metabolites (Jackson et al., 2001). Flesh from healthy animals is usually sterile however, it has been pointed out that during slaughter, evisceration, and cutting, microbial contamination occurs chiefly from the exterior of the animal and its intestinal contents.

External contamination of meat is a constant possibility from bleeding to consumption (Lawrie, 2004). Among the factors that affect microbial growth in meat are intrinsic properties (physical and chemical properties of meat) and extrinsic (environmental factors), however the factors having the greatest influence on the growth of microorganisms in meat and meat products are the storage temperature, moisture content and oxygen availability. Foodborne microbiological hazards may be responsible for many cases of illness each year and are thus an important food safety challenge. To lower the incidence of foodborne disease caused by microbial proliferation, efforts must be taken by appropriate authorities to maintain standard hygienic procedures from farm to fork during the processing of food animals.

The preservation of meat as a perishable food usually is accomplished by a combination of preservation methods which greatly lengthen the quality of meat. The presence of molds in meat products usually cause a decrease in their biological and nutritional values. These molds or yeasts could be spotted in different forms and colors on beefs (Lowry and Gill, 2008). Earlier work by Okafor (2008) revealed that many mold species have been isolated from beef and fish in Nigeria. Also, Berwal (2001); 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. The mycological quality of meat products plays an important role in an increasing public health issue all over the world. Microbial analysis should be carried out on meat aimed for human consumption to protect public health because a healthy citizenry makes a healthy nation. It is imperative to note that the tremendous growth in the consumption of beef in Owo, Ondo State necessitated the current study. This research therefore examines the mycological assessment of beef sold at different retail points in Owo, Ondo State Nigeria.

Materials and Method

Sample Collection

Samples of beef were obtained from meat vendors at six popular retail points (morning and evening) in Owo, Ondo State. The samples were immediately

wrapped in sterile aluminum foil to prevent contamination and then taken to the Microbiology Laboratory of Science Laboratory Technology Department, Rufus Giwa Polytechnic, Owo for assessment.

Sample Preparation
The beef samples were thoroughly pulverized using a previously sterilized pestle and mortar; 10g were transferred into 90 ml of sterile peptone water, and then shaken thoroughly to make a homogenous mixture (these served as stock solutions for each sample). Serial dilutions were made using 1ml from the stock homogenate and 9ml of the sterile distilled water. Several dilutions were made, up to 5 folds (i.e. 105) for each sample, to obtain discrete colonies as described by Banwart (2012).

Fungal Isolation
All glassware used in the analysis was sterilized in a hot oven at 170 ± 5 °C for at least two hours, while the media and distilled water were sterilized by autoclaving at 121 °C for 15 min at 15 psi (Abdullahi et al., 2019; Igwegbe et al., 2019). The isolation of fungi was carried out according to the agar dilution method as described by Pittet (2008) using Potato Dextrose Agar (PDA). and the pour plate method was used to make the viable counts (Jay et al., 2005; Igwegbe et al., 2019). In this method, one 1ml of the inoculums was mixed thoroughly in molten plate count agar held in a hot water bath at 47 ± 2 °C. The agar was allowed to set; the plates were inverted and then incubated at 28°C for 72 – 120 hours for fungal counts (including mesophilic and thermophilic spore formers).

Fungal isolates were sub-cultured on Sabouraud Dextrose agar (Oxoid, UK) and incubated for 5 to 7 days at 25°C for purification. Fungi were identified by using taxonomic schemes based on microscopic observation and cultural appearance. The total fungal count for each plate was expressed as spore-forming units per gram of sample (sfu/g). {Each genus or species identified was then expressed as a percentage (%) of the total isolated fungi.} The total colonies of fungi were enumerated and results were reported in mean and average fungal counts as described (Pitt and Hocking, 2007; Dachoupakan et al., 2009).

Identification of Mold
Identification of fungal genera and the determination of each species of fungi were done using the keys of Klich (2002) for Aspergillus spp. and Pitt and Hocking (2007) for Penicillium sp. This was done by observing both the microscopic characteristics and morphology of the colonies on the SDA medium.

Analysis of data
The analysis of data was carried out on percentage (%) basis

Results and Discussion

Results
Table 1 presents the fungal counts on the beef samples analyzed for ten (10) consecutive days (morning and evening). The fungal counts for all samples ranged from 8.0 to 57 x 105 sfu/g with the Poly gate sample on the morning of day 10 scoring the lowest count of 8.0 x 105 sfu/g on day 1 while the Ikare junction sample on day 1 scored the highest count of 57. 0 x 105sfu/g. It was also noted that Ikare-junction recorded the second highest count with 52.0 x 105 sfu/g at on day 6 (evening) followed by Oke-ogun (50.0 x 105 sfu/g) on day 6 (morning). On days 3 and 8, Ijebu scored the second lowest count (9.0 x 105sfu/g) in the morning. All the samples from the locations recorded very high counts which show they are heavily contaminated with varying degrees of pathogenic fungi.

Table 2 indicates the percentage (%) occurrence of fungal count. Samples from Ikare Junction recorded the highest percentage of 18.90% while those from Poly gate recorded the lowest (14.85%).

Table 3 revealed the probable fungal isolates according to their morphological characterization. The isolates were Aspergillus niger, Aspergillus fumigatus, Fusarium oxysporum, Rhizopus stolonifer, Penicillium oxalicum, Mucor plumbeus, Botrytis cinerea, Cladosporium herbarum, and Aureobasidium pullulans making a total of nine (9) fungi species.

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Table 1: Fungal count on the fresh beef samples from selected retail outlets in Owo (× 10 ^{5} *sfu/g*)

Location	Day 1 M	Day 1 E	Day 2 M	Day 2 E	Day 3 M	Day 3 E	Day 4 M	Day 4 E	Day 5 M	Day 5 E	Day 6 M	Day 6 E	Day 7 M	Day 7 E	Day 8 M	Day 8 E	Day 9 M	Day 9 E	Day 10 M	Day 10 E
Emure	22	38	15	38	18	31	13	31	16	23	40	47	13	28	21	26	16	38	21	32
OkeOgun	19	27	11	27	15	35	15	19	21	34	50	33	16	36	18	28	11	32	17	40
EhinOgbe	13	21	12	28	22	31	12	28	15	27	38	35	12	35	13	46	14	32	21	36
Ikare Junction	32	57	13	37	14	36	21	28	17	32	41	52	18	31	11	37	17	39	17	29
Ijebu	19	41	21	33	9	22	15	34	19	28	34	47	12	37	9	26	20	25	16	39
Poly Gate	33	46	23	30	12	26	21	17	22	35	43	41	12	28	11	26	13	27	8	21

Key: M = Morning; E = Evening.

Table 2: Summary and Percentage composition of fungal count on the fresh beef samples from selected retail outlets in Owo (× 10 ^{5} *sfu/g*)

Location	Morning	Evening	Total	Percent (%)
Emure	195	322	517	16.87
Oke-Ogun	214	311	525	17.13
Ehin-ogbe	172	319	491	16.02
Ikare junction owo	201	378	579	18.90
Ijebu	175	322	497	16.22
Poly- gate	158	297	455	14.85
Total	**1,115**	**1,949**	**3,064**	**100**

Table 3: Morphological Characterization of the Fungal Isolates

Isolate	Description	Organism
1	Velutinous colonies that covers the plate surface with mycelia bearing closely packed, dark brown to black conidia with a yellow reverse	*Aspergillus niger*
2	The colonies are wrinkled, velutinous, and dense with white mycelia bearing dark bluish-green conidia. The reverse is greyish.	*Aspergillus fumigatus*
3	The colonies are floccose, dense with mauve mycelia bearing yellowish pink spores arranged at the center of ring layers. The reverse is pale pink.	*Fusarium oxysporum*
4	Fluffy colonies filled the Petri dish with floccose white mycelium-bearing white prominent sporangia that turned black after 5 days. The black spores were concentrated at the sides of the Petri dish. The plate reverse is plain.	*Rhizopus stolonifer*
5	The colonies are centrally velutinous with pale yellow mycelia bearing abundant greyish green. The reverse is brown.	*Penicillium oxalicum*
6	The colonies are spread across the Petri dish possessing deep grey coloured sporangia with colourless reverse.	*Mucor plumbeus*

7	The colonies cover the whole plate showing irregular white floccose mycelia which changed to grey colour after 5 days. The plate reverse is pale.	*Botrytis cinerea*
8	Colonies are velutinous to floccose and slightly wrinkled with a distinctive olive color. The reverse is greenish-grey	*Cladosporium herbarum*
9	Colonies appeared low and mucoid, faintly pink, becoming grey to black in areas at 7 days; reverse in similar colours. Conidia are borne on denticles directly from hyphae and yeast-like conidia	*Aureobasidium pullulans*

Discussion

This study assessed the pathogenic fungi present in beef samples from selected retail outlets in Owo. It was observed that the counts in evening samples were higher than that of morning in most of the samples. The highest fungal count was found at Ikare junction followed by Oke-ogun and Emure in that order while the lowest count was recorded at Poly-gate (Table 2). This signifies the high level of contamination of the samples from these locations. The high level of fungal loads found on the samples may be due to the prevailing contaminated air around the retail point. Fungal spores are known to be dispersed through air. The air around the retail point may have gathered spores of these fungi from the environment (Zukal and Incze, 2010). The organisms present in the beef samples depicts a deplorable state of poor hygienic and sanitary practices employed in the slaughtering and processing of fresh beef.

In a similar research, Ismail et al. (2005) examined fungal contamination of beef carcasses and could isolate 34 fungal genera, represented by 62 species and one variety of which Aspergillus, Cladosporium, and Penicillium were recorded in high incidence.

Also, Farghaly et al. (2004) studied the contamination of meat stored in home refrigerators and eleven mold genera could be identified and the most common genera were Aspergillus, Penicillium, and Cladosporium. Omorodion and Odu (2014) analyzed three different meat samples namely; beef, chicken, and pork obtained from Creek Road Market, Mile 3 Market, and Rumokoro Market in Benin City for their microbiological quality using differential, selective, and routine media. Thirteen fungal isolates covering three genera were isolated and characterized as Aspergillus spp., Mucor spp., and Penicillium spp.

This present study implies that beef produced from the sampled locations is not safe enough for consumption because several species of fungi were isolated from the meat samples. These fungi isolates are capable of producing mycotoxins which when ingested by consumers may cause serious health defects. It is generally recognized that illnesses due to the consumption of contaminated foods are perhaps the most widespread health problems in the contemporary world and an important cause of reduced economic productivity. In developed countries, it is estimated that one-quarter to one-third of the population is made ill each year because of foodborne diseases. In developing countries, including Nigeria, the burden is much more severe; therefore there is a need to regularly survey the food preparation, handling, and storage techniques to ensure the safety of the food on one hand, and the health of the consumers on the other hand.

Conclusion and Recommendations

In conclusion, this work showed that the examined beef samples were contaminated with several moulds, especially of the genus Aspergillus. Many of these molds are capable of producing mycotoxins such as aflatoxins, ochratoxins, and fumonisins that are hazardous to humans when consumed.

The following recommendations are hereby made from the findings of this study:

1.) The presence of isolated pathogenic fungi in the beef samples should receive particular attention;

2.) Beef handlers and sellers should be educated on the adverse effects of lack of proper personal hygiene and environmental sanitation;

3.) Veterinary personnel should not only carry out ante-mortem and post-mortem examinations on meat animals but should also do so on carcasses at sales points to safeguard public health;

4.) Further regulatory and educational efforts are needed to improve the safety of beef in the sampled metropolis; and

5.) Buyers should not be allowed to touch beef yet to be bought by them with bare hands.

6.) Adequate heat treatment of meat should be encouraged before consumption.

References

Abdullahi, F.S., Igwegbe, A.O., Bello, A.B., Abashe, S., Adam, I.K. & Badau, M.H. (2019). Assessment of Microbial Quality of Balangu-Dipping Water from Six Outlets in Wudil Town in Kano State, Nigeria. *Int. J. Food Sci. Technol*. 9(2): 1 – 12.

Banwart, G. (2012). Basic food microbiology. Springer Science & Business Media, USA.

Berwal, D. (2001). Basic Food Microbiology. West Pat ConnecticuitAvi Publishing Company Inc.

Dachoupakan, C., Ratomahenina, R., Guiraud, J.P., Baccou, J.C. & Schorr, S. (2009). Study of the phenotypic and genotypic biodiversity of potentially ochratoxigenic black aspergilli isolated from grapes. *International Journal of Food Microbiology*. 132:14-23.

Farghaly, R.M., Gherbawy, Y.A.M.H. & Yosef, M.S. (2004). Contamination of meat stored in home refrigerators in Qena (Egypt). *Czech Mycol*. 56: 53-62.

Igwegbe, A.O., Idakwo, P.Y., Yusuf, H.L., Agbara, G.I., Maijalo, A.I. & Abubakar, F. (2019). Effects of sodium citrate and garlic on organoleptic properties, proximate composition, free fatty acid, and thiobarbituric acid levels of treated smoke-died meat stored at ambient temperature. *CPQ Med*. 5(5): 2 – 14.

Ismail, M.A., Abou-Elala, A.H., Nassar, A. & Michail, D.G. (2005). Fungal contamination of beef carcasses and the environment in a slaughterhouse. *Food Microbiol*. 12: 441-445.

Jackson, D. & Mcgowan, C.H. (2001). Diet management effects on carcass attributes and meat quality of young goats. *Small Ruminant Research*. 28(1):93-98.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005). Modern Food Microbiology, 7th Edition, Springer Science + Business Media Inc., New York, USA.

Klich, M.A. (2002). Introduction; Economic and medical importance of Aspergillus. *Identification of common Aspergillus species*. 2: 1-16.

Lawrie, R.A. (2004). The preservation effect of smoke on meat. Meat Science, New York: Pergaman Press Inc. Maxwell House Fair View Park –Elmford.

Lowry, P.O. & Gill, C.O. (2008). Temperature and water activity minimal for growth of spoilage moulds from meat. *Journal of Applied Microbiology*. 1(56): 193-198.

Magnus, P. (2001). *Meat Composition. Food Science and Technology* (4th ed.). London: Gohumunary Pub.

Okafor, N. (2008). Fungi associated with mouldy dried fish. *Nig. J. Sci.* 6(2): 500-549.

Oladejo, D.A. & Adebayo-Tayo, B.C. (2011). Moulds, Proximate, mineral composition, and mycotoxin contamination of Banda ("Kundi/Tinco") sold in Ibadan, Oyo State, Nigeria. *AUJ.T*. 15(1): 32-40.

Olusola, F.P. & Omojola, A.B. (2010). Relevance of dried meat product (Kundi), an Intermediate Moisture Meat (IMM), for food security. Pp 23-24.

Omorodion, N.J.P.N. & Odu, N.N. (2014). Microbiological quality of meats sold in Port Harcourt Metropolis, Nigeria. *Nat Sci*. 12(2): 58-62.

Pitt, J. & Hocking, A. (2007). Fungi and Spoilage (2nd Ed.). London: Blackie Academy and Professional.

Pittet, A. (2008). Natural occurrence of mycotoxins in foods and feeds: an updated review. *Revue Medicine Veterinary*. 149: 479 – 492.

Rao, V.A., Thulasi, G. & Ruban, S.W. (2009). Meat quality characteristics of non-descript buffalos as affected by age and sex. *World Applied Science Journal*. 1058-1065.

Zukal, E. & Incze, K. (2010). Drying. In: Handbook of Food Processing/ Chapter 11. Fidel T. (Ed.). Wiley-Blackwell: A John Wiley and Sons, Inc. Publication, Iowa, USA.