APR -20

Meat Speciation Assessment of '*Tinko'* Meat Sold in Lagos Market

O.B. Omodunbi, O.O. Olusola and A.T. Adeshola
Department of Animal Science, University of Ibadan, Ibadan, Nigeria
Corresponding author: O.B. Omodunbi; E-mail: omodunbioluwatoyin@yahoo.com; Tel: +2348023442118

Abstract
The fraudulent substitution of high quality meat with cheaper counterpart is a common practice prevalent in the meat industry. *Tinko* is one of the most popular traditional hard-smoked and sun-dried meat products, mostly from cattle and transport beasts widely consumed in Nigeria and neighboring countries. This product can however be adulterated by the first stage processors. Meat speciation can be used to validate the animal species from which *Tinko* is produced. This study was therefore carried out to assess the state of *Tinko* sold in Lagos markets. Four batches each of *Tinko* were purchased from Oyingbo, Mile 12 and Agege markets in Lagos state and subjected to DNA extraction using Dellaporta DNA extraction protocol to determine the meat species. Amplified fragment and purification of gene was also carried out and were sequenced using standard procedure. The result of gene analysis showed that the *Tinko* sampled from the three open markets in Lagos were made from donkey (a work animal) as against the processors' claim that Tinko sold was made from beef carcass. In conclusion, speciation revealed that most *Tinko* meat sold in the sampled open markets were not beef as believed by consumers. Therefore, monitoring program should be carried out to control meat adulteration and ensure declaration of meat species by processors.

Keyword: *Tinko*, beef carcass, meat speciation, Lagos state

Introduction

Meat is defined as the flesh of animals used as food. The diverse nutrient composition of meat makes it an ideal environment for the growth and propagation of meat spoilage micro-organisms and common food - borne pathogens. It is therefore essential that adequate preservation technologies are applied to maintain its safety and quality (Aymerich *et al*., 2008). The processes used in meat preservation are principally concerned with inhibiting microbial spoilage although other methods of preservation are sought to minimize other deteriorative changes such as colour and oxidative changes (Faustmann and Cassens, 2001). Meat are processed into intermediate moisture meat (IMM) which are heterogeneous group of foods, resemble dry foods due to its resistant to bacterial spoilage, but is known to contain too much moisture to be considered as dried food. It has 30 - 40% moisture content, stable shelf life, reduced water activity and it is good enough for eating without further hydration. *Tinko* is an IMM that is highly palatable and could be eaten with or without hydration (Fakolade and Omojola, 2004). It is one of the most popular traditional hard-smoked or sun-dried meat products, mostly from cattle and transport beasts (donkeys, horses, camels, buffaloes and elephants), widely consumed in Africa.

The fraudulent substitution or adulteration of high quality meat with their inferior/cheaper counterpart is a common practice prevalent in the meat industry all over the world (Sharma, 2015). Adulteration of processed meat products with inexpensive meat are usually done for higher economic gain by processors, as it is more difficult to detect adulterant in cooked or ground meat than in fresh or intact meat (Ayaz *et al.,* 2006). Declaration of meat species used for processed meat products is very relevant to consumers and it is highly encouraged due to economic, health and religious implications (Miguel, 2004).

This study was therefore carried out to determine the occurrence of meat specie adulteration and substitution in a locally processed meat product sold in Lagos markets.

Materials and Methods

Sample preparation: Four batches each of *Tinko*were purchased from Oyingbo, Mile 12 and Agege markets in Lagos state. The meat samples were labeled for identification, oven dried at 60^{o}C and washed with ethanol to reduce the microbial organisms and mold from the samples. The samples were again oven dried, ground into powder and stored for further analysis.

DNA extraction: DNA was extracted from individual samples using a modified Dellaporta DNA extraction protocol (Dellaporta *et al.,* 1983). Briefly, each sample was homogenised in 1000 µl of extraction buffer.

Macerated samples were poured into a sterile microtube, 33 µl of 20% Sodium Dodecyl Sulphate (SDS) was added, vortexed and incubated in water bath at 65^{0}C for 10mins. At room temperature, 160µl of 5M potassium acetate was then added, vortexed and centrifuged at 12000rpm for 10 minutes. Supernatant were collected in another microtube and equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added, vortexed and centrifuged at 12000rpm for 10 minutes. Supernatant where collected in another microtube tube and 450µl of cold iso-propanol was added, mixed gently and kept at -20^{o}C for 60 minutes. Samples were then centrifuged at 13000rpm for 10 minutes to sediment the DNA after which supernatant was gently decanted. DNA pellet was then washed with 500µl of 70% ethanol by centrifuging at 10000rpm for 10 minutes. Ethanol was decanted and DNA air-dried at room temperature. Pellet was then re-suspended in 50µl of Tris EDTA buffer to preserve and suspend the DNA. The re-suspended DNA were then kept at -20^{o}C till further analysis.

Polymerase Chain Reaction: PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaqcolourless reaction, 3 µl of 25mM $MgCl_2$, 1µl of 10mM of dNTPs mix, 1µl of 10 pmol each COI-AF GCCTCCAACTCAAAATACGC and COI AR GTCAAATGGGGCTCGGT primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water. PCR was carried out in a GeneAmp 9700 PCR System Thermal cycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30s, 55°C for 30s and 72°C for 30s; and a final termination at 72°C for 10mins. It was there after chilled at 4^{o}C.

The integrity of the amplified sample which is about 240kb gene fragment was checked on a 1.5% Agarose gel ran to confirm amplification. The prepared buffer was used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. 2µl of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed to confirm spreading at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel.

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Bio systems using manufacturers' manual while the sequencing kit used was that of Big Dye terminator v3.1 cycle sequencing kit. Bio-Edit software and MEGA 6 were used for all genetic analysis.

Results and Discussion

Meat speciation: The result from the meat speciation analysis revealed that 98 to 99% of the gene sequence of supposed beef *Tinko* marched that of the *Equusasinus africanus* (donkey). This means that the sampled *Tinko* from the three open markets were not produced from beef as claimed by the producers. This result is in line with a survey conducted by Hsieh *et al*. (1995) on species substitution in raw and cooked meat samples from over 500 Florida retail markets. The fraudulent adulteration of expensive meat type with cheap meat is a practice that has been observed all over the world. The Nigerian meat processing industry is largely unorganized; hence adulteration or substitution of meat in meat products is likely to be practiced. It was estimated that about 25-30% of meat products sold in India is adulterated (Sharma, 2015) and that of Lagos might not be an exception as beef is one of the costliest meat available in the country and may be adulterated with donkey or camel meat, which are close substitutes.

Gene Sequencing

Sample A: 99% identical to *Equusasinusafricanus* (donkey)

GCCTACCGGGGCGGGTACCCACGTCCCGTTTCATATGAAGTAACTCTAGCAATTATTCTACTCTCAGTCCTC
CTAATGAGCGGATCATTCACACTATCAACACTCATAATTACCCAAGAATACTTATGATTAATCTTCCCATCATG
ACCCTTAGCCATAATATGATTCATCTCAACATTGGCCGAAACCAACCGAGCCCCATTTGACC

Sample B: 99% identical to *Equusasinusafricanus* (donkey)

CCTTGGGAAGGCGGGTAGACAAACCATTTCGTATGAAGTAACTCTAGCAATTATTCTACTCTCAGTCCTCCT
AATGAGCGGATCATTCACACTATCAACACTCATCATTACCCAAGAATACTTATGATTAATCTTCCCATCATGA
CCCTTAGCCATAATATGATTCATCTCAACATTGGCCGAAACCAACCGAGCCCCATTTGACCA

Sample C: 98% identical to *Equusasinus africanus* (donkey)
GTTGCCTACCGGGGCGGGTACCCACGTCCCGTTTCATATGAAGTAACTCTAGCAATTATTCTACTCTCAGTC
CTCCTAATGAGCGGATCATTCACACTATCAACACTCATCATTACCCAAGAATACTTATGATTAATCTTCCCATC
ATGACCCTTAGCCATAATATGATTCATCTCAACATTGGCCGAAACCAACCGAGCCCCATTTGACCGTTGGGA
CCCCCG

Conclusion and Recommendation

It can be concluded that first stage processors of *Tinko* used donkey meat to make *Tinko* obtained from Oyingbo, Mile 12 and Agege markets in Lagos state. This is against the stated claims by these processors that beef was used. Relevant authorities are therefore called on to assess the extent of meat adulteration in the country, through wider market surveys and screening of processed meat products and declaration of meat species by processors to consumers should be ensured.

References

Ayaz, Y., Ayaz, N.D. and Erol, I. (2006). Detection of species in meat and meat products using enzyme-linked immunosorbent assay. *Journal of Muscle Foods,* 17: 214-220

Aymerich, T., Picouset, P.A. and Monfort, J.M. (2008). Decontamination technologies for meat products. *Meat Science,* 78: 114-129.

Dellaporta, S.L., Wood, J. and Hicks, J. B. (1983). A plant DNA mini preparation: version II. *Plant Molecular Biology Reporter,*1(4): 19-21.

Fakolade, P.O. and Omojola, A.B. (2008). Proximate composition, pH and microbiological evaluation of *Kundi* (dried meat) products from beef and camel meat. *Proceedings of Conference on International Research on Food Security, Natural Resource Management and Rural Development,* Tropeng University of Hohenheim, October 6-9.

Faustmann, C. and Cassens, R.G. (2001). The biochemical basis for discolouration in fresh meat: a review, In *Journal of Muscle Food,*1(3):217-243.

Hsieh, Y.H.P., Woodward, B.B. and Ho, S.H. (1995). Detection of species substitution in raw and cooked meat using immunoassays. *Journal of Food Protection,* 58: 555-559.

Miguel A., Teresa, G., Isabel, G., Lius, A., Pab, E.H. and Rosario, M. (2004). PCR identification of beef, sheep, goat, and pork in raw and heat treated meat mixtures. *Journal of Food Protection,* 67:172-77.

Sharma, B.D. (2015). *Fraudulent substitution of meat and its recognition: meat and meat product technology (including poultry products technology),* 1st Ed. Japee Brothers Medical Publishers Limited, New Delhi.