FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 8 No. 3, June, 2024, pp 302 - 310
DOI: https://doi.org/10.33003/fjs-2024-0803-2514

DNA-BASED IDENTIFICATION OF PROCESSED MEAT ADULTERATION WITHIN KADUNA METROPOLIS

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ABSTRACT

Processed meat adulteration poses significant risks to consumer's health and trust, with fraudulent practices like meat substitution, filler addition, and false labeling compromising product quality and authenticity. Insufficient regulatory oversight exacerbates these issues, necessitating stricter regulations, improved inspection procedures, and increased consumer awareness to ensure the safety and transparency of processed meat products. This study employed a DNA-based approach to detect adulteration in processed meats using primers specific to cattle cytochrome C oxidase, pig cytochrome C oxidase, and chicken 12S rRNA. Forty-four processed meat samples, including beef meat pie (8), chicken meat pie (8), beef minced meat (9), chicken minced meat (9), pork steak (5), and pork balangu (5), were randomly collected from stores in four Kaduna Metropolis markets: Central Market, Sabon Tasha, Barnawa, and Kawo. Results showed that 25.0% of beef meat pies and 77.8% of beef minced meat were adulterated, with an overall adulteration level of 52.9% for processed beef. For processed chicken, 37.5% of chicken meat pies and 44.4% of chicken minced meat were adulterated, yielding an overall 41.2% adulteration rate. All ten processed pork samples were authentic. Central Market samples had a 40% adulteration rate, with 50% involving beef and 50% chicken. Sabon Tasha showed 9.1% beef adulteration. Barnawa had 30.8% adulteration, with 25.0% beef and 75.0% chicken. Kawo recorded a 70.0% adulteration rate, with 42.9% beef and 57.1% chicken. These findings demonstrate the effectiveness of DNA-based methods in detecting processed meat adulteration.

Keywords: Multiplex PCR, DNA, Adulteration, Primer, Processed meat

INTRODUCTION

Meat is the consumable portion of an animal that primarily consists of fat, muscle, and connected tissues, and is utilized as food (Ndife et al., 2022). It is a prominent protein source in various food products, serving as a protein-rich dietary ingredient with a protein content ranging from 15% to 22%. Additionally, meat possesses a comprehensive composition of essential amino acids (Orkusz, 2021). Processed meat products are popular as most of them are acceptable in taste and contain the nutrients needed for fulfilling daily nutrient intake, particularly essential amino acids and iron (Gómez et al., 2020). Processed meat products have gained popularity due to their generally agreeable taste and provision of vital nutrients, such as essential amino acids and iron, which are crucial for meeting daily dietary requirements (Ballin, 2010). The consumption of meat and meat products is steadily increasing in the majority of global regions, particularly in developing nations. In the context of Turkey, it has been calculated that the annual per capita meat intake amounts to 12 kg. In a similar vein, there has been a rise in the pricing of beef and other meat products. The meat product in Turkey is still regarded as a luxury item, with a price that is twice as high as that of chicken. Meat adulteration and fraud have become prevalent as a result of rising pricing, the globalization of the food sector, and increasing processing (Keyvan et al., 2017). Meat, fish, and animal products are the fourth most prevalent food group consumed by households in Nigeria, accounting for 88.9% of the total consumption. With a consumption rate of 97.2%, it falls behind cereals and flours, oils and fats, and vegetables. In comparison to other food categories, meat, fish, and animal goods exhibited the highest average weekly household expenditure, amounting to N1,359 per week (Keyvan et al., 2017).

Meat is extensively consumed in Nigeria as a protein source to offset the predominantly starch-based diet. The majority of consumers buy meat on a regular basis in tiny portions of 2kg or less, which is an adequate amount for preparing a single-family supper. A diverse range of meat products is procured and consumed throughout the nation (Olaoye et al., 2010). The intake of meat in Nigeria is influenced by several demographic factors, including economic, health, social, and religious considerations. Religious, age, sex, socio-economic characteristics, individual variance, and income have been identified by a research team as significant determinants influencing meat consumption trends in Nigeria (Ojewola & Onwuka, 2001). In the Muslim northern region of the country, pork is not well-liked. However, chicken and turkey are the preferred meats for Christian celebrations. For Muslim celebrations throughout the country, chicken, beef, mutton, and turkey are commonly used. It is worth noting that beef seems to be the most commonly consumed meat in Nigeria (Aborisade & Carpio, 2017).

The heightened insecurity and insurgency in the northern region of Nigeria, where most red meat is produced have significantly disrupted the production, processing, and sales of this product in the country. In addition, the escalating cost of gasoline and the ongoing conflicts between ranchers and farmers are factors that contribute to the shortage of meat. These problems have resulted in extensive fraudulent activities within the manufacturing sector (Adenuga & Montowska, 2023). The fraudulent activity involves the replacement of costly raw materials in processed meat products with more affordable alternatives sourced from other origins. For instance, this can be observed in the substitution of beef with chicken or pork in processed beef products (Unajak et al., 2011).

Meat adulteration has the potential to alter the halal status of meat and give rise to health risks as a result of the presence of allergic chemicals (Cahyadi et al., 2020). The authenticity of food plays a crucial role in enhancing customers' confidence in food goods. Currently, the utilization of DNA as a molecular approach is extensively employed for the purpose of food verification. DNA possesses greater informational value compared to proteins and may be readily extracted from

even minute quantities of organic matter (Hellberg & Morrissey, 2011). The efficacy, specificity, and sensitivity of the DNA-based approach in species identification within processed food products have been well-established (Cai et al., 2021).

In the conventional approach, the detection of adulteration in meat products is accomplished by the utilization of HPLC, electrophoretic techniques, and ELISA-based approaches. Numerous limitations are involved with these approaches; nonetheless, polymerase chain reaction (PCR) methods were created with the aim of enhancing specificity and sensitivity. Multiplex PCR is one of several PCR-based detection technologies that have been developed to enhance the efficiency of PCR techniques for the identification of animal species in processed meat products. This very sensitive technique employs several primers within a single PCR tube, hence minimizing the expenses and duration associated with examining a substantial quantity of samples (Cai et al., 2021). The verification of food authenticity using this methodology relies on the utilization of nuclear or mitochondrial markers. The utilization of mitochondrial markers, such as Cyt b, CO III, and ATPase subunit 8/6, has been extensively investigated and employed due to their substantial abundance inside a single cell and their higher likelihood of stability in processed products compared to nuclear markers (Kumar et al., 2015; Qin et al., 2019). The mitochondrial markers known as cytochrome oxidase sub Unit I (COI) and 12S rRNA are widely recognized as efficient tools in DNA barcoding for the identification of animal species (Wang et al., 2019). The advantages of multiplex PCR are its simplicity, sensitivity, cost-effectiveness, and reliability (Kumar et al., 2015).

The prevalence of processed meat adulteration in Kaduna Metropolis remains undocumented and unquantified, posing a potential risk to public health and consumer rights. The current regulatory frameworks and enforcement procedures may be insufficient in addressing this issue due to a lack of reliable detection methods. Consequently, this study evaluated the efficiency of a DNA-based method in assessing the extent of adulteration in processed meat products sold within this area.

MATERIALS AND METHODS

Study Area

Geographically, Kaduna lies somewhere between 7°22′ and 7°31′E, on the Greenwich meridian, and 10°20′ and 10°37′N, on the equator. The metropolitan area includes parts of several local governments, including Igabi, Chikun, Kaduna North, and Kaduna South. With a population of around 764,084, it experiences two distinct seasons: the rainy season (March–October) and the dry season (approximately four months) (Akpu et al., 2017).

Sampling sites

The four (4) main markets in Kaduna metropolis namely, the central market, Kawo, Barnawa, and Sabon Tasha were used as sampling areas, as shown in Figure 1.

Figure 1: Map of Kaduna metropolis showing the sampling area.

Sample collection

The study involved the random collection of forty-four (44) samples of processed meat, specifically meat pie, minced meat, and pork steak, from stores located in four local governments within the Kaduna Metropolis. These markets were central market, Sabon Tasha, Barnawa and Kawo, as indicated in Table 1. This sample functioned as the experimental treatment group. A control group consisting of raw meat samples from cattle, poultry, and pig was employed to assess the specificity of primers.

Table 1: List of samples used in this study

Serial number on gel image	Sample Type	Settlement Area
1	Beef Minced Meat	Central market
2	Beef Minced Meat	Central market
3	Beef Minced Meat	Central market
4	Beef meat pie	Central market
5	Beef meat pie	Central market
6	Beef meat pie	Sabon tasha
7	Beef Minced meat	Sabon tasha
8	Beef meat pie	Sabon tasha
9	Beef meat pie	Barnawa
10	Beef meat pie	Barnawa
11	Beef Minced meat	Barnawa
12	Beef Minced meat	Barnawa
13	Beef Minced meat	Kawo
14	Beef Minced meat	Kawo
15	Beef minced meat	Kawo
16	Beef meat pie	Kawo
17	Beef meat pie	Kawo
18	Chicken minced meat	Central market
19	Chicken Minced meat	Central market
20	Chicken Meat pie	Central market
21	Chicken Meat pie	Central market
22	Chicken Meat pie	Central market
23	Chicken meat pie	Sabon tasha
24	Chicken Minced Meat	Sabon tasha
25	Chicken Minced meat	Sabon tasha
26	Chicken meat pie	Kawo
27	Chicken meat pie	Kawo
28	Chicken Minced meat	Kawo
29	Chicken Meat pie	Kawo
30	Chicken Minced meat	Kawo
31	Chicken Minced meat	Barnawa
32	Chicken Meat pie	Barnawa
33	Chicken Minced meat	Barnawa
34	Chicken Minced meat	Barnawa
35	Pork balangu	Sabon tasha
36	Pork balangu	Sabon tasha
37	Pork balangu	Sabon tasha
38	Pork steak	Sabon tasha
39	Pork steak	Sabon tasha
40	Pork balangu	Barnawa
41	Pork steak	Barnawa
42	Pork balangu	Barnawa
43	Pork steak	Barnawa
44	Pork steak	Barnawa

Primers

Primers used in this study were obtained from relevant published literatures (Table 2). The primers were specific to the following genes, cattle cytochrome C oxidase, pig cytochrome C oxidase and chicken 12S rRNA.

Table 2: Primers used in this study

Species	Primer Sequence	PCR product size (bp)	Gene	Reference
Cattle (*Bos taurus)*	Forward 5' GAACTCTGCTCGGAGACGAC 3' Reverse 5' GGTACACGGTTCAGCCTGT 3'	255	Cytochrome C oxidase	Spychaj *et al.* (2016)
Pig (*Sus scrofa*)	Forward 5' GGAGCAGTGTTCGC 3' Reverse 5' TTCTCGTTTTGATGCGAATGCATTAT 3'	294	Cytochrome C oxidase	Spychaj *et al*. (2016)
Chicken (*Gallus gallus*)	Forward 5' CCTAGCCCTAAATCTAGATACC 3' Reverse 5' TTTTGAGGGTGACGGGCGGTG 3'	420	12S rRNA	Spychaj *et al*. (2016)

DNA Extraction

DNA was extracted from both raw and processed meat samples using the BIONEER AccuPrep Genomic DNA Extraction Kit, following the manufacturer's instructions. Initially, 50 mg of each sample was homogenized using a mortar and pestle and placed into a 1.5 ml tube. Subsequently, 200 µl of Tissue Lyse Buffer (TL) was added. The mixture was then supplemented with 20 µl of Proteinase K and 10 µl of RNase, followed by vortexing to mix thoroughly. The sample was incubated at 60°C for 1 hour, or until complete tissue lysis was achieved. After incubation, 200 µl of GB Buffer was added and vortexed briefly to mix.

Next, 400 µl of absolute ethanol was added to the mixture and mixed well by pipetting. The resulting lysate was carefully transferred into the upper reservoir of the binding column tube fitted in a collection tube, ensuring the rim remained dry. The tube was closed and centrifuged at 800 rpm for 2 minutes. The flow-through was discarded, and the collection tube was reused.

To wash the column, 500 µl of WA1 Buffer was added, avoiding the rims, and centrifuged at 800 rpm for 2 minutes. The flow-through was discarded, and the collection tube was reused. A second wash was performed with 500 µl of WA2 Buffer, which was then discarded. The column was centrifuged at 13,000 rpm for 1 minute to completely remove any remaining ethanol, ensuring no droplets clung to the bottom of the binding tube.

Finally, the binding column was transferred to a new 1.5 ml tube for elution. Between 50-200 µl of Elution Buffer was added to the binding column, incubated at room temperature (15-25°C) for at least 1 minute, and centrifuged at 800 rpm for 3 minutes to elute the DNA.

Multiplex PCR and Gel Electrophoresis

To the PCR tube, 0.5µl each of the forward and reverse primer, 1µl of multiplex master mix, and 2µl of template DNA, and 15µl of distilled were added. PCR tube for negative control consisted of 0.5µl of each of the forward and reverse primer and 17µl of deionized water. PCR tube for positive control comprised of 0.5µl of each of the forward and reverse primer, 2µl of template DNA, and 15µl of distilled water. The PCR cycle conditions were as follows, pre-denaturation at 94^0C for 5 minutes, denaturation at 94^0C for 30 seconds, annealing at 55^0C for 30secs and extension for 5 minutes at 72^0C. A total of 35 PCR cycles were performed. After which, gel electrophoresis was performed images were captured.

Sequencing and Basic Local Alignment Search

PCR products were sequenced using Sanger sequencing. The National Center for Biotechnology Information (NCBI) database was searched using the Basic Local Alignment Search Tool (BLAST) to determine sequence similarity.

Data Analysis

Descriptive statistics were used to summarize the data. The percentages of adulterated samples were calculated, and comparisons were made between different meat types and markets. The data were presented in tabular and picture formats to illustrate the extent of adulteration and facilitate easy comparison. Prevalence was calculated with the formula below:

$$Prevalence = \frac{Number\ of\ positive\ samples}{Total\ Number\ examined} \times 100$$

RESULTS AND DISCUSSION

Assessment of primer specificity

Results of primer specificity check revealed that all three (3) primers used in this study (cattle cytochrome c oxidase, chicken 12S rRNA, pork cytochrome c oxidase) were specific to the target sequences (Figure 2).

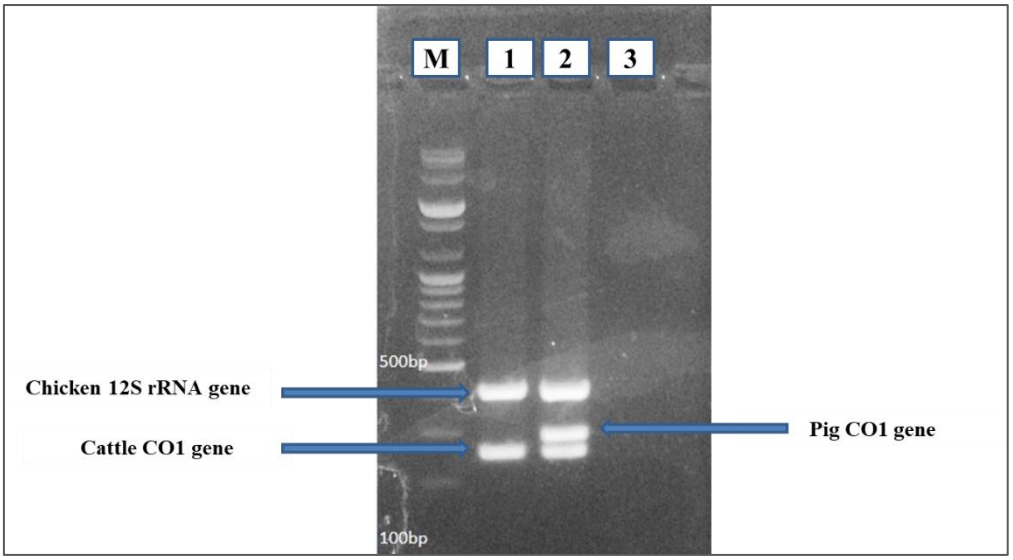

Figure 2: Gel electrophoresis image for primer specificity check. M: Molecular ladder, 1: DNA from cattle and chicken mixed with PCR primer for cytochrome C oxidase 1 gene from cattle and pork and 12S rRNA gene from chicken

Detection of Different types of Meat Sources in the Processed Meat Products

Gel electrophoresis revealed that all 17 samples of processed beef products contained beef. Also, all 17 samples of chicken products contained chicken while the 10 samples of processed pork were observed to contain pork (Figure 3, 4, 5).

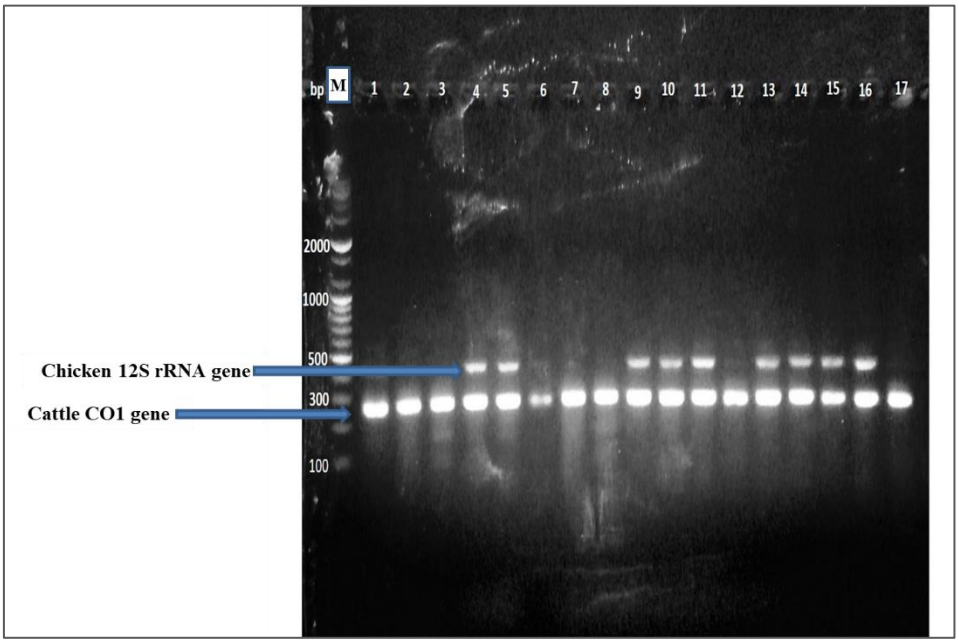

Figure 3: Gel Electrophoresis image of PCR products obtained from multiplex PCR of 17 processed cattle meat samples. M: Molecular ladder, 1-17: DNA from processed cattle meat sample. (1) beef minced meat, (2) beef minced meat, (3) beef minced meat, (4) beef meat pie, (5) beef meat pie, (6) beef meat pie, (7) beef minced meat, (8) beef meat pie (9) beef meat pie, (10) beef meat pie, (11) beef meat pie, (12) beef minced meat, (13) beef minced meat, (14) beef minced meat, (15) beef minced meat, (16) beef meat pie, (17) beef meat pie

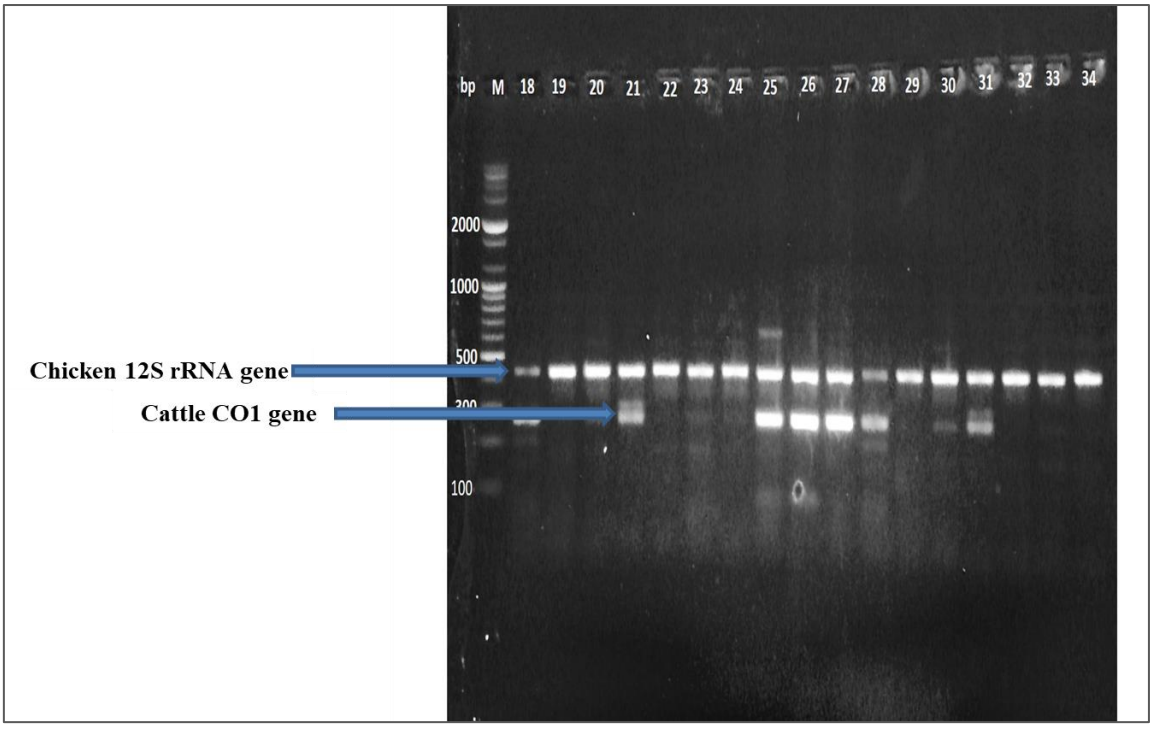

Figure 4: Gel Electrophoresis image of PCR products obtained from multiplex PCR of 17 processed chicken meat samples. M: Molecular ladder, 18-34: DNA from chicken meat samples; (18) chicken minced meat, (19) chicken minced meat, (20) chicken meat pie, (21) chicken meat pie, (22) chicken meat pie, (23) chicken meat pie, (24) chicken minced meat, (25) chicken minced meat, (26) chicken meat pie, (27) chicken meat pie, (28) chicken minced meat, (29) chicken meat pie, (30) chicken minced meat, (31) chicken minced meat, (32) chicken meat pie (33) chicken minced meat, (34) chicken minced meat

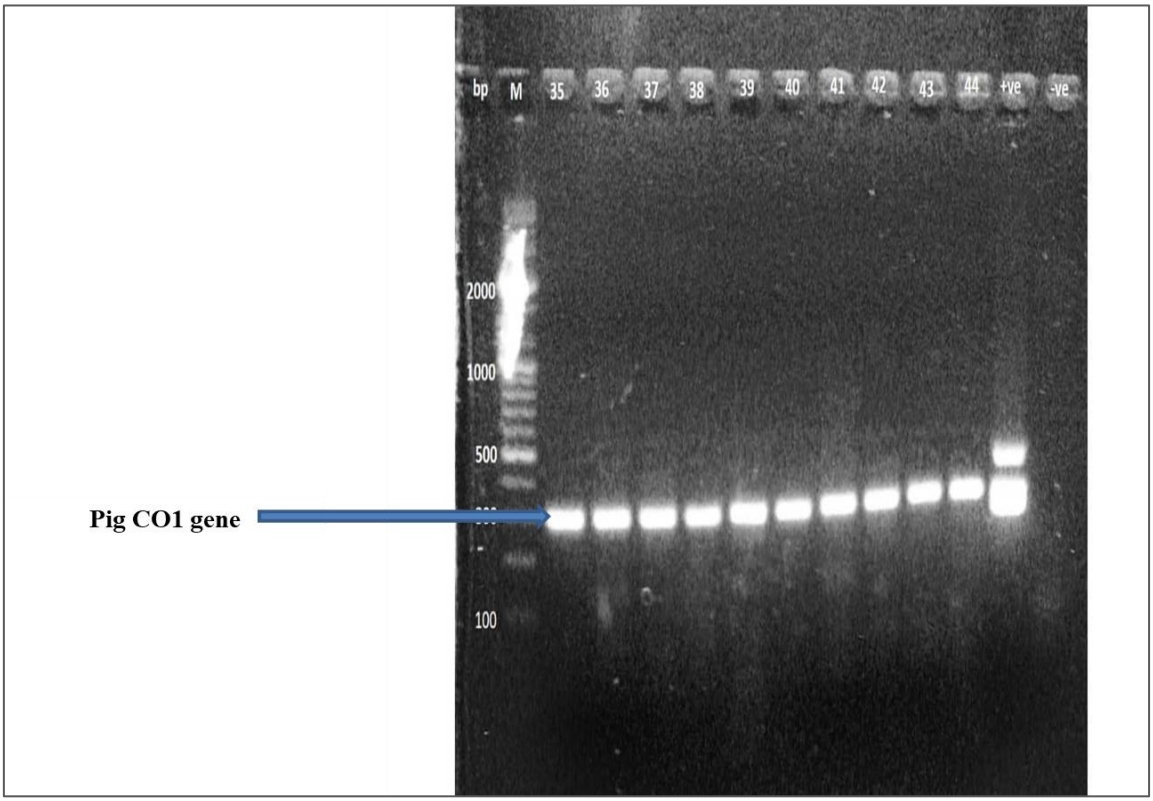

Figure 5: Gel Electrophoresis image of PCR products obtained from multiplex PCR of 10 processed pig meat samples. M: Molecular ladder, 35- 44: (35) pork steak, (36) pork steak, (37) pork steak, (38) pork steak, (39) pork steak, (40) pork balangu, (41) pork balangu, (42) pork balangu, (43) pork balangu, (44) pork balangu, +ve: positive control

Adulteration of processed meat products

Based on results of this study 2 samples of beef meat pie was adulterated with chicken while 7 samples of beef minced meat were adulterated with chicken (Table 3, Figure 3). Of the 8 samples of Chicken meat pie, 3 were adulterated with beef while 4 samples of chicken minced meat were adulterated with beef (Table 3, Figure 4). However, there was no adulteration in all 10 samples of processed pork examined (Table 3 and Figure 5).

Table 3: Processed meat adulteration

Meat product	Number examined	Beef detected	Chicken detected	Pork detected
Beef meat pie	8	8	2	0
Beef minced meat	9	9	7	0
Chicken meat pie	8	3	8	0
Chicken minced meat	9	4	9	0
Pork steak	5	0	0	5
Pork balangu	5	0	0	5
Total	44	17	17	10

Prevalence of processed meat adulteration

It was observed that 25.0% of beef meat pie was adulterated while 77.8% of beef minced was adulterated yielding an overall adulteration level of 52.9% (Table 4). On the other hand, chicken meat pie had 37.5% adulteration while 44.4% adulteration was observed in chicken minced meat culminating in overall adulteration level of 41.2% (Table 5). Processed pork was observed to be free of adulteration.

Table 4: Adulteration of processed beef

Beef product	Number of samples examined	Adulteration (Number/%)
Meat pie	8	2 (25.0)
Minced meat	9	7 (77.8)
Total	17	9 (52.9)

Table 5: Adulteration of processed chicken

Chicken product	Number of samples examined	Adulteration (Number/%)
Meat pie	8	3 (37.5)
Minced meat	9	4 (44.4)
Total	17	7 (41.2)

Prevalence of processed meat adulteration in sampling locations

Of the 10 samples of processed meat products obtained from central market, an overall adulteration level of 40.0% was ascertained. This comprised of 50% adulteration with beef and 50% adulteration with chicken (Table 6). However, there was no adulteration with pork. Samples obtained from Sabon Tasha recorded 9.1% adulteration consisting entirely of beef. With respect to Barnawa, 30.8% of the samples examined were adulterated comprising of 25.0% beef adulteration and 75.0% chicken adulteration (Table 6). Adulteration of processed meat products with pork was not observed in this market. Kawo recorded 70.0% adulteration which was made up of 42.9% adulteration with beef and 57.1% adulteration with chicken. Also, there was no adulteration of processed pork samples (Table 6).

Table 6: Prevalence of processed meat adulteration in sampling locations

Sampling location	Number of samples examined	Over all adulteration (Number/%)	Adulteration with beef (Number/%)	Adulteration with chicken (Number/%)	Adulteration with pork
Central market	10	4 (40.0)	2 (50.0)	2 (50.0)	0 (0.0)
Sabon Tasha	11	1 (9.1)	1 (100.0)	0 (0.0)	0 (0.0)
Barnawa	13	4 (30.8)	1 (25.0)	3 (75)	0 (0.0)
Kawo	10	7 (70.0)	3 (42.9)	4 (57.1)	0 (0.0)

Discussion

Food fraud encompasses various deceptive practices, including ingredient substitution, undisclosed chemical addition, dilution, adulteration, and mislabeling. Such fraudulent activities are widespread in global markets, and Nigeria is no exception (Otu et al., 2019). In Nigeria, food fraud is a common and well-recognized issue, especially among those who frequent local markets. This problem is particularly pressing in Nigeria, where food fraud is a persistent reality. Annually, around 200,000 Nigerians die from food poisoning, and over 90,000 require hospitalization due to foodborne infections (Adenuga & Montowska, 2023). Locally produced and unbranded products are especially susceptible to fraudulent manipulation. Meat is notably vulnerable, particularly during processing, where determining the origin, cut, or quality can be challenging. While meat is readily available in Nigeria, it remains a costly commodity, particularly in a volatile economy where minor changes in production costs quickly escalate food prices. Recently, the northern region of Nigeria, the primary hub for red meat production, has experienced significant disruptions due to escalating instability and conflict. The availability of meat in Nigerian markets is also affected by rising fuel prices and ethnoreligious conflicts among ranchers, farmers, animal traders, and butchers (Adenuga & Montowska, 2023). The

Nigerian meat industry has seen a rise in fraudulent activities, including mislabeling, selling expired meat, marketing meat of dubious origin, and smuggling meat products.

Globally, meat fraud incidents have been documented, such as the European horsemeat scandal (Smith & McElwee, 2020), the Chinese rat meat scandal (Dai & Jiang, 2013), and the Brazilian rotten meat controversy (Adenuga & Montowska, 2023). These incidents have significantly impacted media coverage and heightened consumer concerns about meat authenticity. Consumers now actively seek to verify the authenticity of the meat products they purchase (Rhymer et al., 2019). In Nigeria, incidents of meat fraud are underreported, and media coverage does not sufficiently raise consumer awareness about the associated risks. The reasons for this lack of attention are speculative. Some suggest it is due to poverty and a lack of consumer knowledge, while others point out that despite the growing food safety issues resulting in numerous deaths, a significant portion of the population remains unaware of these hazards within the food sector (Onyeaka et al., 2021).

In 2018, allegations emerged in Bayelsa State, located in the South-South region of Nigeria, where chicken meat was allegedly substituted with vulture meat and donkey carcasses. The perpetrators reportedly supplied this meat to restaurants, bars, and canteens at significantly reduced prices, making it difficult for end consumers to identify the species they were consuming (Adenuga & Montowska, 2023). In Edo State, four individuals were arrested for illegally selling donkey meat. The Nigerian government has recently banned the slaughter of donkeys, the consumption of donkey meat, and the illegal export of donkey hides to protect the species from extinction. Consequently, donkey meat is now illegal for human consumption in Nigeria. Additionally, suya, a popular Nigerian beef kebab, has been implicated in fraudulent practices, with butchers supplying meat from deceased animals to suya makers. In one instance, seven individuals from the same household in Abia State died after consuming tainted meat (Adenuga & Montowska, 2023). These cases highlight the lack of awareness among meat processors regarding the harmful effects of their cost-cutting practices on consumer health. The perceived disregard for public health and consumer autonomy is exacerbated by the indifference of government officials responsible for enforcing food safety standards (Adenuga & Montowska, 2023).

CONCLUSION

This research successfully identified instances of processed meat adulteration using a DNA-based method. Utilizing cytochrome C oxidase (COX) primers unique to cattle, pig, and chicken, alongside 12S rRNA primers specific to chicken, we examined the potential for adulteration in processed meat products. Forty-four (44) processed meat samples were randomly collected from stores in four locations within the Kaduna metropolis: Central Market, Sabon Tasha, Barnawa, and Kawo. The samples included beef and chicken meat pies, minced meat from both animals, pork steak, and pork balangu. The results revealed significant adulteration, with 52.9% of the 17 processed beef samples containing chicken and 41.2% of the processed chicken meat samples containing beef. The processed pork samples were found to be authentic. These findings underscore the effectiveness of DNA-based methods in detecting adulteration in processed beef products.

Future research should broaden testing to include a wider range of meat products and geographic locations for a comprehensive understanding of meat adulteration practices. Enhancing DNA-based detection methods, incorporating next-generation sequencing, will improve sensitivity and accuracy. Longitudinal studies are needed to monitor trends in meat adulteration and evaluate regulatory interventions. Investigating the public health implications and developing risk mitigation strategies is crucial. Additionally, assessing the impact of consumer education campaigns on awareness and meat adulteration detection will help reduce its prevalence. Analyzing and improving current policy and regulatory frameworks will ensure robust food safety and quality standards.

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