Investigating the Presence of Contamination in Beef and Chicken from Fast-Food Restaurants, Grocery Stores, and Fine-Dining Restaurants

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Abstract

The growing and major issue associated with mislabelling and contamination in the meat industry has resulted in a significant threat to food safety. This lack of transparency in the production of meat and packaging of meat administered to consumers has resulted in negligence and poor food safety practices which has contributed to the sale of contaminated meat to consumers due to sellers having to follow less protocols and thus are able to increase their profits more by deceiving others. Our research centralized on examining beef, and chicken meat samples from a grocery store, a fine-dining restaurant, and a fast-food restaurant. We isolated DNA of nine animal species and then added a multiplex PCR and finally analyzed our PCR results using gel electrophoresis. By comparing fragment sizes, we determined the authenticity of the samples. Our findings showed that meat from both the fast-food restaurant and grocery store sources showed signs of contamination with goat meat, more specifically for grocery store beef sample #1, beef sample #2, and fast-food beef sample #3, while no contamination was detected from meat obtained from the fine-dining restaurant source. It is also worth mentioning that grocery store beef sample #1 and grocery store beef sample #2 had some chicken contamination. Therefore, our study suggests that fine-dining restaurants have a lower incidence of meat contamination than fast-food restaurants and grocery stores, possibly due to better hygienic practices by employees. It is also worth mentioning that this was a single study with a small sample size.

Introduction

The food service industry faces a significant risk of cross-contamination, where raw food can easily contaminate other surfaces, hands, and foods, leading to foodborne illnesses (Kirchner et al., 2021). This risk is further compounded by the increasing number of people eating out, making food safety a serious concern for food service establishments (Kirchner et al., 2021).

Identifying the species of meat in processed products can also be challenging, increasing the risk of species substitution (Hellberg et al., 2017). The processing of meat, such as grinding or smoking, can alter their appearance, making it difficult to identify the species. This is especially concerning given the varying prices of different meat and poultry species (Hellberg et al., 2017).

Furthermore, the rapid growth of the fast-food industry has raised concerns about the hygiene and safety practices of food service establishments (Amoah et al., 2018). As the number of fast-food consumers increases, food control officers are increasingly concerned about the hygiene requirements outlined in the code of hygienic practices for food service establishments (Amoah et al., 2018). Unfortunately, many fast-food vendors lack knowledge of food safety and hygiene practices, which can lead to foodborne illnesses (Amoah et al., 2018). A study found that a significant percentage of fast-food operators have no background in catering education, highlighting the need for proper training and education in basic hygiene practices to ensure the safety and health of consumers (Amoah et al., 2018).

We conducted a small-scale DNA analysis of beef and chicken samples from fast-food restaurants, fine-dining restaurants, and grocery stores to verify the authenticity of these meat products. We compared the results with Matsunaga et al. (1999) to ensure accuracy. We anticipate that meat contamination levels in grocery stores will be lower due to better hygiene practices and less meat handling compared to fast-food restaurants (Amoah et al., 2018). Fast-food restaurants are more likely to have issues with hygiene and food safety standards, making their meat more susceptible to cross-contamination compared to grocery store meat. While contamination can occur during food preparation in fine-dining restaurants, we predict that they will have less meat contamination compared to fast-food restaurants due to better handling and hygiene practices but more contamination compared to grocery stores due to more meat handling (Kirchner et al., 2021).

Methods

The methods used in this study were based on the protocol described by Bhagat et al. (2022) for meat species identification using DNA analysis. We collected and analyzed beef and

chicken samples from three different venues: one fine-dining restaurant, a large grocery chain, and a fast-food restaurant. The overall procedure for determining the meat composition of the samples involved isolating the DNA, performing the polymerase chain reaction (PCR), and then carrying out gel electrophoresis.

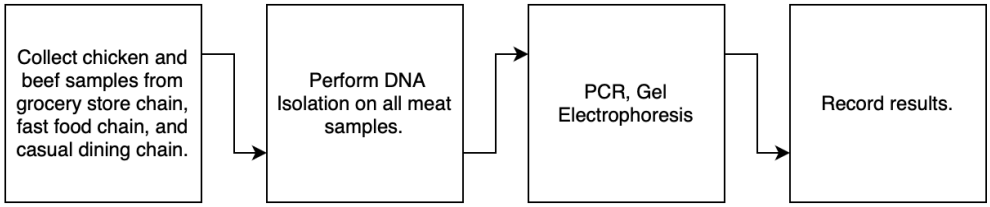

Figure 1. Outline of experimental method procedure, in line with protocol described in Bhagat et al. (2022)

After sample collection, the DNA was isolated before performing the PCR and visualizing the results via gel electrophoresis. A total of 18 meat samples were collected, six from each venue. Samples were labeled as follows: 1) beef samples from fast food restaurant: MB1, MB2, MB3; 2) beef samples from the grocery store: SB1, SB2, SB3; 3) beef samples from fine dining restaurant: EB1, EB2, EB3; 4) chicken samples from fast food restaurant: MC1, MC2, MC3; 5) chicken samples from the grocery store: SC1, SC2, SC3; and 6) chicken samples from fine dining restaurant: EC1, EC2, EC3.

To isolate the DNA, we used the "Total Nucleic Acids Purification Protocol" for tissue samples that come with the MasterPure Complete DNA and RNA Purification Kit (Epicenter, 2012). The resulting DNA was stored at 4°C for a day. The PCR protocol was derived from Matsunaga et al. (1999). For this multiplex PCR approach, the forward primers (goat, chicken, beef, sheep, pig, and horse) were added. To minimize error, 720 µL of PCR master mix was

prepared to distribute among all samples. The master mix was comprised of deionized water (108 µL), 50% glycerol (150 µL), 10X PCR buffer (75 µL), 10 mM dNTP (15 µL), 25 mM $MgCl_2$(45 µL), and 1000U/200 µL Taq polymerase (15 µL). The forward, goat, chicken, cattle, sheep, pig, and horse primers were added in the amounts of 30 µL, 6 µL, 90 µL, 18 µL, 90 µL, 18 µL, and 60 µL, respectively. To each PCR tube, 24 µL of the master mix was transferred, and 1 µL of the DNA samples were then added, with 1 µL of deionized water used as the negative control. The DNA pellets in each sample were resuspended into the TE buffer before being added to the master mix. This was more difficult for certain samples (namely the beef samples), requiring more prolonged mixing to resuspend the pellets. The PCR was then run using the following cycle: 95°C for two minutes, 35 repeats of 95°C for 30 seconds, then 60°C for 30 seconds, then 72°C for 30 seconds, and finally 72°C for five minutes. Samples were then stored at 4°C for a week.

To visualize the results, we utilized gel electrophoresis. To each of the PCR samples, 5 µL of 6X loading buffer was added. Then, 15 µL of each sample was added into the wells, and the samples were run on a 2% gel (2 g of agarose per 100 mL TAE buffer) at 120V for two hours. An ultra-low range order ladder solution consisting of a 10 µL ladder, 10 µL 6X loading buffer, and 40 µL of deionized water was used to interpret the results.

Results

The fragment sizes corresponding to the DNA of goat, chicken, cattle, sheep, pig and horse are 157, 227, 274, 331, 398, and 439 base pairs (bp), respectively (Matsunaga et al., 1999).

Our results, shown in Figure 2 below, showed bands for fast food restaurant beef sample 3 (MB3) at around 150 bp, which most closely corresponds to goat DNA which has a band of 157 bp. Both grocery store beef sample 1 and sample 2 (SB1 and SB3, respectively) had bands both

around 150 bp (goat DNA) and around 240 bp, which most closely corresponds to chicken DNA. All chicken samples had bands at around 240 bp (chicken). The chicken sample bands were the thickest of all samples. In addition to the band at 240 bp, fast food restaurant chicken sample 2 also produced a band at 150 bp. Apart from MB3, SB1 and SB2 samples, the beef samples did not show any bands identifying the type of meat DNA they contained. Our control, which did not contain any DNA, did not produce any bands either.

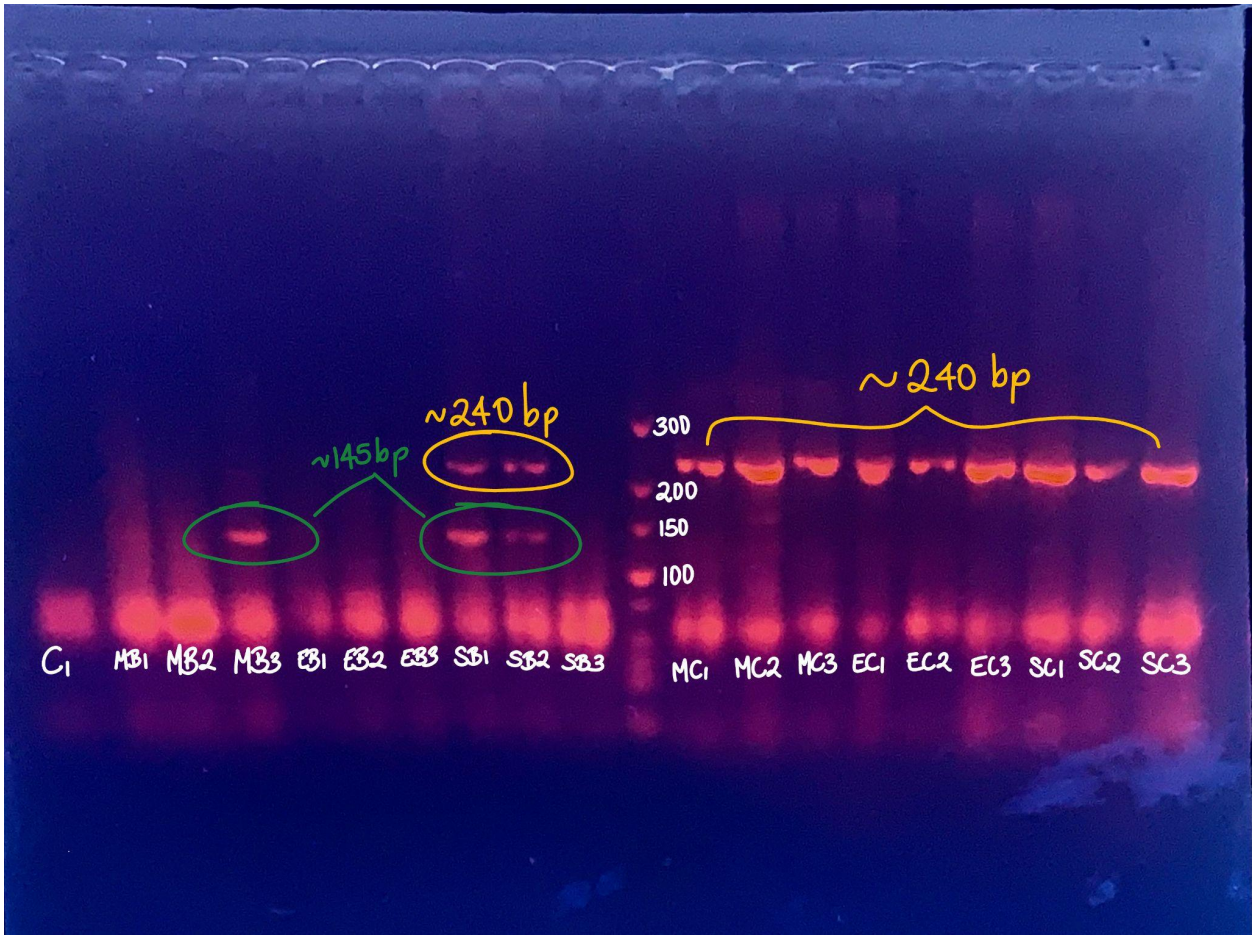

Figure 2. The results of gel electrophoresis using 2% agarose gel and an ultra-low-range DNA ladder solution. The letters on the bottom correspond to the sample type (see Methods).

Discussion

In this study, we analyzed the meat composition of beef and chicken at a fast-food restaurant, fine-dining restaurant, and grocery store in order to determine the presence of meat contamination in each of these meats. To view the composition of these samples, the assay developed by Matsunaga et al. (1999) was utilized, and gel electrophoresis allowed for the visualization of the DNA of the beef and chicken samples. We predicted that meat contamination would be greatest in fast-food restaurants, followed by fine-dining restaurants, and grocery stores. However, our results provided evidence that meat contamination was greatest in grocery stores, followed by fast-food restaurants, and fine-dining restaurants.

In the fast-food restaurant samples, it was observed that none of the three beef samples displayed any bands that indicated that those species were in fact beef samples. On the other hand, sample 3 (MB3) contained some traces of goat meat. The fast-food restaurant chicken samples all displayed bands consistent with the presence of chicken, with sample 2 (MC2) bearing a single faint band indicating the presence of goat meat. This was an observation of interest, as the fast-food restaurant where the meat was obtained does not offer goat meat on their menu. This decreases the likelihood of cross-contamination occurring at the restaurant due to immediate contact with goat meat despite previous studies noting the lack of consistent basic hygiene, such as hand washing and cleaning of utensils within fast-food restaurants (Amoah et al., 2018). However, their company's website mentions the sourcing of their meat, including beef, from over 100 different Canadian suppliers. According to Innovation, Science and Economic Development Canada (2022), four of the five top goat importers to Canada also distribute beef and chicken, one of them being among the largest beef suppliers in Canada, leading to a plausible explanation of contamination occurring at facilities prior to exportation.

However, this does not eliminate the possibility of contamination by unhygienic practices by staff.

In the grocery store samples, no bands were visible in grocery store sample 3. However, two bands were visible in grocery store beef sample 1 and grocery store beef sample 2. The higher band appears to correspond more closely to chicken DNA rather than beef DNA, thus indicating chicken contamination. The second, lower band suggested the possibility of goat contamination. The grocery store where the beef and chicken samples were obtained do not sell goat, however this does not eliminate the possibility of cross-contamination at the factory where this meat was obtained.

Chicken samples from all three sources did not display signs indicating meat contamination, whereas meat contamination was seen in three of the nine beef samples. A study conducted by Chung, S. M. & Hellberg, R. S. (2020) on the presence of cross-contamination in ground meat products found that partial cleaning, more commonly utilized by processing staff, of the grinding equipment still yielded <1% contamination of the previous species processed to the following species ground. As all of our beef samples were processed, ground beef, and the majority of our chicken samples were whole chicken breasts, this may provide an explanation as to why meat contamination was seen in the beef samples and not in the chicken samples.

It is important to note that none of the beef samples displayed bands that would allow us to correctly identify them as beef samples. As the beef samples were run in the same gel as the chicken samples, and all of the chicken samples from the various sources displayed bands indicating the presence of chicken DNA, we can eliminate the possibility of there being an error with the gel used. The exact cause behind the lack of bands indicating beef DNA could not be determined, but possible explanations include too high of a concentration of DNA, as the

samples of beef used were quite large, and problems with the primers, specifically the beef primer, as bands appeared indicating chicken DNA for all chicken samples, but all beef samples lacked bands indicating beef DNA, which is unlikely.

Additionally, the usage of pseudo-replicates for each of the samples limits our ability to extrapolate our findings to other fast-food restaurants, fine-dining restaurants, and grocery stores, as contamination may have been isolated on the single meat samples obtained from each source, rather than replicates obtained from different locations or even different menu items. Other reasons behind this meat contamination we found may be due to problems associated with the meat processing facilities where cross-contamination could have occurred when packaging and sorting out the meat. Thus, future studies may look into a wider variety of sources to obtain their samples and compare meats that go through similar processing.

Conclusion

Meat from both fast-food restaurants and grocery store sources showed signs of contamination with goat meat, while no contamination was detected from meat obtained from fine dining restaurant sources. While this could be a sign of intentional mishandling, it is also possible that the contamination was accidental, considering that goat meat is more expensive than beef or chicken. Regardless of whether the contamination was a result of intentional mislabelling or accidental mishandling, our results showed the differences in food products given to customers in our chosen eateries, and that food fraud continues to be a major issue.

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