Comparison of Meat Contamination in Chicken, Beef, and Pork Samples From Grocery Store and Fast-Food Restaurant Sources

Kartik Bhagat, Mouaz Imdakem, Lyam Lee, Navkiran Takhar

Abstract

The mislabelling, adulteration and contamination of meat products are part of a growing issue in the world of food safety. Due to the decreasing transparency behind the source, production and packaging of meat, the industry is seeing an increase in negligence and poor food safety practices that result in the sale of adulterated and contaminated meat to consumers. Our research examined meat samples obtained from pig, beef and chicken products that were purchased from fast-food restaurants and grocery stores. We isolated DNA and used a multiplex PCR approach with primers from horse, chicken, goat, sheep, beef and pork. The PCR results were then analyzed with gel electrophoresis where a comparison of fragment sizes was used to determine the authenticity of the samples. We observed two cases of chicken contamination in the beef and pig meat samples purchased from fast-food restaurants, whereas the grocery store-purchased meat showed no signs of contamination. Therefore, our study found that grocery store-sourced meat was more accurately labelled, likely due to better hygiene and less handling by employees.

Introduction

The importance of both accurately labelling meat products to maintain a high food safety standard and preserving the trust of consumers cannot be understated. Negligence behind the purchasing, handling and labelling of meat in fast-food restaurants and grocery stores has become a breeding ground for meat contamination and adulteration (Phillips et al., 2006). These malpractices are a product of cutting costs, improper training, and potentially attempting to purposefully mislabel. Verifying the cleanliness and handling of food that is being distributed to customers is enforced by government bodies through food safety inspectors; however, meat mislabeling can often be overlooked. In this study, we conduct a small-scale DNA analysis of chicken, beef and pork samples taken from fast-food restaurants and grocery stores. The results were compared with data from Matsunaga et al. (1999) to affirm the authenticity of these meat products. Our investigation is a small, but rather important aspect of food inspection that allows us to better understand the magnitude of an issue like food mislabeling and adulteration.

Research conducted by the Canadian Food Inspection Agency in 2019 found a 20% mislabelling rate of sausages purchased in Canada (Shehata et al., 2019). The inclusion of unlabelled meat in all-beef sausages is an indication that mislabeling due to negligence and fraud was still a prominent issue in Canadian markets. The lack of proper training and enforcement of food safety regulations by fast-food workers is also a large issue that continues to be propagated by negligence and oversight by management in restaurants (Dundes & Swann, 2008).

We expect to find less meat contamination in grocery store meats as a result of better hygiene practices and less meat handling compared to fast food restaurants. Since fast-food restaurants are more likely to handle meat and have issues with hygiene and food safety standards (Phillips et al., 2006), we predict that their meat will be more prone to cross-contamination compared to grocery store meat which is generally packaged by individual manufacturers and undergoes less handling.

Methods

The overall procedure for determining the meat composition of the samples was collecting samples of varying meat products from different sources, isolating the DNA, performing the polymerase chain reaction (PCR), and then carrying out gel electrophoresis (Figure 1).

A total of 24 meat samples were collected where half were sourced from a fast-food restaurant and the other half from a grocery store. Three types of meat were collected from each source (chicken, beef, and pork) and there were four replicates of each sample. The chicken samples were obtained from breaded chicken cutlets from both sources. The beef samples were roast beef and beef flank steak from the fast-food and grocery store sources, respectively. The

pork samples were pepperoni and pork tenderloin, from the fast-food and grocery store sources, respectively.

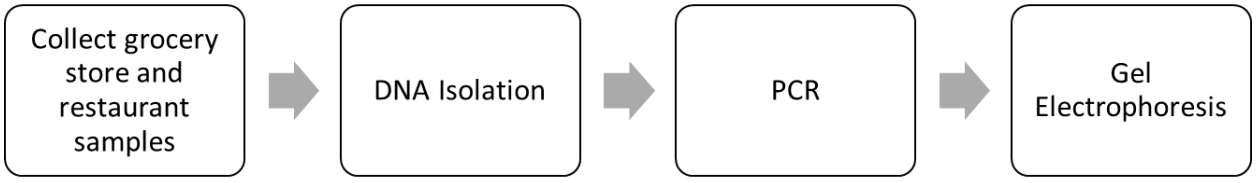

Figure 1. Experimental design. After sample collection, the DNA was isolated before performing the polymerase chain reaction and visualizing the results via gel electrophoresis.

To isolate the DNA, we used the "Total Nucleic Acids Purification Protocol" for tissue samples that comes with the MasterPure Complete DNA and RNA Purification Kit (Epicentre, 2012). The resulting DNA was stored at 4°C until further use.

The PCR protocol was derived from Matsunaga et al. (1999). For this multiplex PCR approach, the primers (forward meat, goat, chicken, beef, sheep, pig, and horse) were added in a ratio of 1:0.2:3:0.6:3:0.6:2, where the values in the ratio are based on 20 pmol of primer per 50 µL of PCR solution. 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 grocery store beef, grocery store

chicken, and fast-food restaurant chicken 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 (Figure 2). Samples were then stored at 4°C until further handling.

95°C: 2 minutes
95°C: 30 seconds
60°C: 30 seconds } ×35
72°C: 30 seconds
72°C: 5 minutes
4°C: overnight

Figure 2. PCR cycle indicating the temperatures of the denaturation, annealing and extension steps.

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 were 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, comprised of 10 µL ladder, 10 µL 6X loading buffer and 40 µL of deionized water was utilized to interpret the results. Fragment sizes were determined and compared to sizes expected for each meat type, as given in Matsunaga et al. (1999).

Results

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

The agarose gel results in Figure 3 showcase our amplified PCR products through bands at different base pair sizes. All four replicates of the fast-food restaurant purchased pork samples displayed two bands at 398 and 274 bp. However, one of the replicates also had an additional band at 227 bp. The band at 398 bp was the darkest. The fast-food restaurant-purchased breaded chicken sample showed single bands at 227 bp for all four replicates. Three of the four replicates of the fast-food restaurant-purchased roast beef sample showed a band at 274 bp. Of those three replicates, one also had an additional, fainter band at 227 bp. The furthest right of the four replicates did not show any bands.

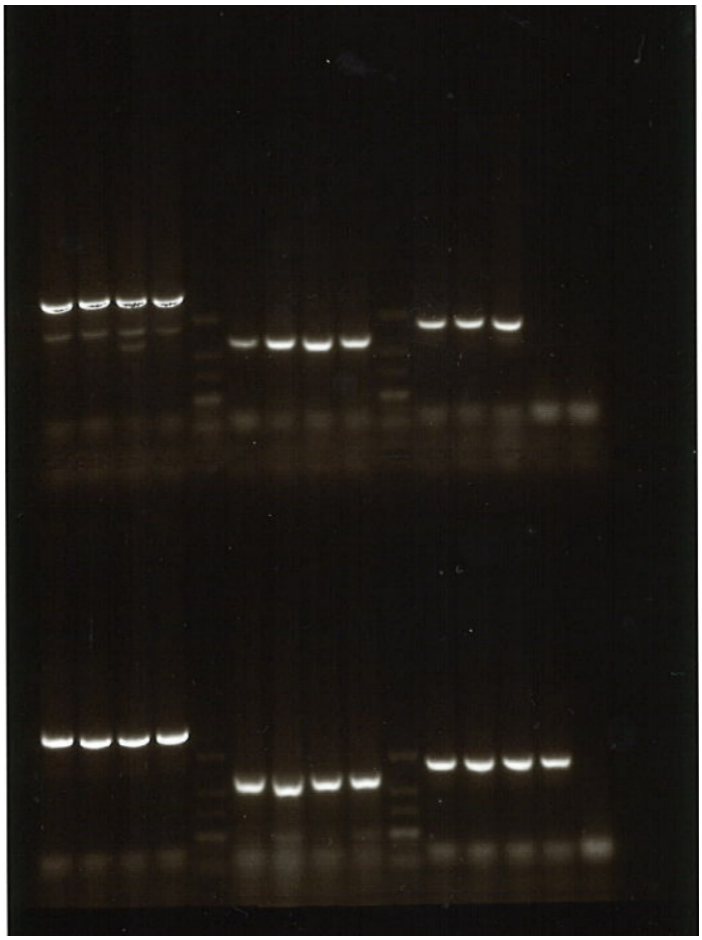

Figure 3. The results of gel electrophoresis after running at 120 V for two hours. The top lanes represent the three fast-food meat types, grouped into four samples each, with the left, middle

and right groups representing pork, chicken and beef respectively. Ladders are placed between these groupings. The bottom lanes are similarly categorized, except with grocery store meat variants.

The agarose gel results for the grocery store-purchased pork tenderloin sample displayed a single band at 398 bp for all four replicates. The grocery store purchased breaded chicken samples displayed a single band at 227 bp for all four replicates. The grocery store purchased flank steak samples displayed a single band at 274 bp for all four replicates.

Discussion

In this study, we analyzed the meat composition of pig, beef, and chicken products from restaurant and grocery store sources to investigate the differences in contamination levels. The gel electrophoresis results indicate the presence of different meat types through bands at varying fragment sizes. The addition of six different types of meat primers (goat, chicken, cattle, sheep, pig and horse) allowed us to amplify one or more of those types of meats if present in our samples (Matsunaga et al., 1999).

In the fast-food restaurant samples, the breaded chicken sample only showed a single band which indicated that the sample was correctly labelled and had no contamination or adulteration. The pepperoni samples showed up to three bands at different fragment sizes which indicated the presence of three types of meats, namely pig, beef, and chicken. It is important to note that during sample collection, when asked for a pork meat sample, the fast-food restaurant employee provided pepperoni, stating that it was 100% pork. However, upon consultation with the restaurant's official website, we found that their pepperoni product contains both pork and beef. This explains the bands seen at 274 bp. Although the presence of cattle meat in the pepperoni can be attributed to the false claim made by the fast-food restaurant staff, the gel

results also indicated the presence of chicken within one of the replicates of the pepperoni samples. Since this was only present in one of the replicates, it is likely a result of cross-contamination by the employee who dispensed all of the meat samples using the same gloves or contamination during the procedure. Two of the fast-food restaurant roast beef sample replicates indicated the presence of only beef and one replicate indicated the presence of both beef and chicken. Since the samples were taken from a similar area on the same piece of meat, this indicates a high likelihood of contamination occurring while trimming the meat for DNA isolation in the lab. However, it is also possible that it occurred as a result of improper handling by the employee as they observed them using the same pair of gloves. One of the replicates also showed no bands despite having a visible DNA pellet which suggests that this could be due to incomplete resuspension of the DNA pellet in the buffer or due to an error in loading the DNA into the agarose gel wells. This is likely a procedural error and unlikely to be due to the product as the other beef samples displayed single bands.

In the grocery store meat samples, we observed no contamination, unlike the fast-food restaurant meat samples. For all four replicates of the pork tenderloin, breaded chicken and flank steak samples, we observed single bands at the expected base pair sizes of the meats that they were being labelled and sold as.

These results are in line with our expectation that grocery store meats have less contamination compared to fast-food restaurant meat. Our prediction also stated that this discrepancy in contamination of meat would be due to a difference in hygiene practices and handling of meat. During sample collection, we observed a noticeable difference in the hygiene standards between the restaurant and grocery store sources. While the grocery store meat was pre-packaged, the fast-food restaurant meat was placed in open containers and separated from

other ingredients and meats using metal storage containers. The fast-food restaurant meat was also handled extensively by the workers and, notably, the same gloves were used to handle all three meat samples. Fast-food restaurant staff are shown to be informed on hygiene practices and food safety regulations; however, these practices are generally ignored, leading to an increased risk of cross-contamination (Amoah et al., 2018). This discrepancy in handling practices means that fast-food restaurants have a significantly higher likelihood of meat contamination through the negligence of hygiene practices (Phillips et al., 2006). Grocery stores have a lower likelihood of contamination due to the preparation and packaging of meats in factories with stricter hygiene regulations.

One of the flaws of this experimental design was that the four replicates of each sample were not collected from different parts of the meat sample. This allowed us to account for potential errors during the DNA isolation, PCR, and electrophoresis steps. However, it did not allow us to account for the fact that contamination could vary across different areas of the meat.

Another limitation is that the meat sources were derived from only one location of a single fast-food restaurant. Therefore, this does not reflect meat contamination across all locations of this fast-food restaurant, or across fast-food restaurants in general. A more robust comparison would either focus on multiple locations of a single fast-food restaurant (to account for variation between branches of the same chain) or multiple different fast-food restaurants (to make a general comparison of fast-food compared to grocery stores). Similarly, the grocery store samples were derived from two types of stores. Further experimentation could take a better approach by, again either focusing on different locations of one grocery store or multiple kinds of grocery stores.

Lastly, the experimental design could have been improved by obtaining meat samples that better were aligned between the two sources. For instance, pork tenderloin is very different from pepperoni and undergoes different levels of processing. Therefore, the comparison of our sampled meat products is not as robust because it is possible that grocery store pepperoni could have given a contaminated result.

Conclusion

The purpose of our investigation was to examine the level of meat contamination in different types of meat from grocery stores and fast-food restaurants. As expected, the results show higher levels of meat contamination in the fast-food samples when compared with similar types of meats from grocery stores. This may be attributed to differences in meat handling and hygiene practices. However, the intentional adulteration and mislabelling of meat are also increasing problems. We hope that our research sheds light on the rising issues of adulteration and contamination of meat and encourages more investigation into the negligence and malpractices that plague this industry.

Acknowledgements

We would like to thank the University of British Columbia (UBC) for the opportunity to conduct our research. We would like to acknowledge that UBC is situated on the traditional, ancestral, and unceded territory of the xʷməθkʷəy̓əm (Musqueam) people – we thank them for allowing us to learn on their land. We would also like to thank Dr. Celeste Leander and Miriam Fenniri for their support and guidance throughout this process and for assisting in the refinement of our experimental design. Additionally, we would like to acknowledge the lab technicians, Mindy Chow and Jarnail Chandi for their assistance in preparing our reagents and agarose gel matrix, as well as for providing their expertise on the overall procedure.

References

Amoah, M., Adonu, R. E., & Paintsil, E. (2018). The level of awareness of fast food operators on food safety and hygiene practices. *OALib*, *05*(04), 1-7. https://doi.org/10.4236/oalib.1104392

Dundes, L., & Swann, T. (2008). Food safety in fast food restaurants. *Journal of Human Resources in Hospitality & Tourism*, *7*(2), 153-161. https://doi.org/10.1080/15332840802156881

Epicentre. (2012, June). *MasterPure™ Complete DNA and RNA Purification Kit*. https://biosearchtech.a.bigcontent.io/v1/static/manual_NAEXPU-001_masterpure-complete-dna-and-rna-purification-kit

Matsunaga, T., Chikuni, K., Tanabe, R., Muroya, S., Shibata, K., Yamada, J., & Shinmura, Y. (1999). A quick and simple method for the identification of meat species and meat products by PCR assay. *Meat Science*, 51(2), 143-148. https://doi.org/10.1016/s0309-1740(98)00112-0

Phillips, M. L., Elledge, B. L., Basara, H. G., Lynch, R. A., & Boatright, D. T. (2006). Recurrent critical violations of the food code in retail food service establishments. *Journal of Environmental Health*, 68(10).

Shehata, H. R., Naaum, A. M., Chen, S., Murphy, T., Li, J., Shannon, K., Awmack, D., Locas, A., & Hanner, R. H. (2019). Re-visiting the occurrence of undeclared species in sausage products sold in Canada. *Food Research International*, *122*, 593-598. https://doi.org/10.1016/j.foodres.2019.01.030

Siswara, H. N., Erwanto, Y., & Suryanto, E. (2022). Study of meat species adulteration in Indonesian commercial beef meatballs related to halal law implementation. *Frontiers in Sustainable Food Systems*, *6*. https://doi.org/10.3389/fsufs.2022.882031