Under The Bun: An Analysis of Meat and Meat Alternative Integrity in Vancouver Restaurants

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Abstract

Meat product mislabeling and meat contamination of plant-based meats can significantly impact consumer trust, public health, and market integrity, underscoring the necessity for accurate meat labeling. This study aims to assess the authenticity of beef and plant-based burger patties from various fast-food chains through the analysis of DNA. Following DNA isolation, Polymerase Chain Reaction (PCR) was performed to identify six different types of meat: cow, pig, chicken, sheep, goat and horse. Subsequent gel electrophoresis was performed to identify specific targeted DNA segments. However, gel electrophoresis yielded only a single band among all 17 samples, indicating possible chicken cross-contamination. PCR failure may be attributed to altered DNA base pairs from the heat of cooking, which suggests analyzing raw meat in future studies may yield more accurate assessments.

Introduction

Correct labeling of meat products and plant-based meat (PBM) is an important step in ensuring a level of trust between consumers, producers, and retailers. The authenticity of such products is something that consumers rely on when making health-related, religious, and lifestyle choices. However, the occurrence of fraudulent labeling has been a cause for concern. For instance, a scandal took place in the EU in 2013 when an investigation into the authenticity of meat products revealed that many of said products contained horse meat without disclosing this information on the label (O’Mahony, 2013). The lack of transparency that led to such scandals in the past warrants an analysis of meat and PBM products currently available on the market.

Mislabelling of meat species mostly occurs in ground or processed meat products in order to decrease the cost of production, a possible incentive for mislabelling. Ayaz et al. conducted a study in Türkiye established that 22% of tested meat products were mislabelled (2006). Among the mislabelled products, various types of sausage and salami showed particularly high levels of fraudulent labeling (Ayaz et al., 2006). A study from Bangladesh found that up to a third of the beef products in the Bangladeshi market may be mislabelled (Afifa et al., 2021). It further concluded that chicken and buffalo were the most common substitutes, noting that both of these species are considered cheaper alternatives when compared with authentic beef (Afifa et al., 2021). In conjunction such findings suggest that consumers are not only misinformed but may also be overpaying for mislabelled meat products.

Another area of concern is the potential for meat contamination of PBMs. In recent years many restaurants started offering PBMs as an alternative to meat products as the demand for such substitutes is slowly but steadily on the rise (Zhao et al., 2022). However, it remains to be seen if these products are fully plant-based, considering that meat contamination could occur due to the proximity of meat and PBM during the cooking process at restaurants. Previous studies have shown that, especially in fast-food restaurant settings, meat products were cross-contaminated with each other (Bhagat et al., 2022), which means that contamination between meats and PBMs is also possible. Some examples of potential contamination risks are the fact that meat and PBMs may be kept on the same countertop, refrigerated in the same fridge, and cooked on the same grill.

The Canadian government has strict regulations for meat product labeling. These regulations include the requirement to disclose every animal species contained in a meat product on the label (Government of Canada, 2022). Such regulations are imperative in order to provide transparency for consumers. However, testing is needed in order to ensure that restaurants follow these regulations. This need is the primary motivation behind our study, which aims to explore meat species authenticity. The evaluation was conducted by identifying the species of animal found in beef hamburger patties from 3 restaurants, and PBM hamburger patties from 2 restaurants. The results were then compared to the corresponding labels in order to evaluate their accuracy.

Methods

Sampling

Burger patties were obtained from three different fast-food restaurants. A total of five burger samples were obtained; three patties advertised as beef-based and two patties advertised as plant-based. Each burger patty sample was mechanically degraded using toothpicks and then placed into individual Eppendorf tubes. Two replicate samples were isolated from different sites on each patty and placed into their own tubes. Since ground meat consists of a homogenized arrangement of tissues, we determined that sampling from multiple sites on the same patty would be a suitable replicate to increase confidence in any findings being significant. Samples were labeled according to the following convention: S(Restaurant #) - (Replicate #); the human sample was labeled “S6” and the negative control “H2O.” All samples were stored in the refrigerator to maintain DNA integrity until DNA isolation procedures. Lastly, cells containing human DNA were collected from a member of the team via a buccal swab.

DNA Isolation

300 µL of Cell Lysis Solution with Proteinase K was added to each labeled tube and tubes were incubated at 65℃ for 15 minutes, with intermittent vortexing every 5 minutes. After a 5-minute ice rest, 150 µL of “Protein Precipitate Reagent” was added to each tube, vortexed for 10 seconds and then centrifuged at a maximum speed of 13,000 -14,000 RPM for 10 minutes to precipitate proteins. The DNA-containing supernatant was carefully transferred to new Eppendorf tubes, and 500 µL of cold isopropanol was added to each tube. Following centrifugation at a maximum speed of 13,000-14,000 rpm for 10 more minutes, the isopropanol was carefully removed. Subsequent rinses with 500 µL of ethanol were performed twice to eliminate residual salts. The DNA pellet was obtained after leaving the pellets to evaporate residual ethanol overnight.

Master Mix Preparation

First, 30 µL of Tris-EDTA (TE) Buffer was added to each dry pellet. Then, a total of 17 PCR tubes were labeled: 15 for the burger patty samples, one for the human DNA sample, and one for negative control (containing distilled water). Master Mix was prepared in an Eppendorf tube according to the BIOL 342 Teaching Team’s Master Mix recipe for meat PCR analysis (see Table 1). Finally, 24 µL of the prepared Master Mix was distributed into each PCR tube, and subsequently, 1 µL of the respective DNA samples was added to the designated tubes for the PCR process. The Master Mix was prepared for 24 PCR tubes to ensure there was sufficient volume in case of experimental errors.

Table 1. Meat PCR Master Mix recipe

Item	Individual Tube Volume (µL)	Master Mix Volume (µL) X20
dH2O	3.6	72
10X PCR Buffer	2.5	50
10 mM dNTPs	0.5	10
25 mM MgCl2	1.5	30
50% Glycerol	5.0	100
5’ Primer 10 uM (Meat forward (SIM))	1.0	20
3’ Primer 10 uM (Goat ‘G’)	0.2	4
3’ Primer 10 uM (Chicken ‘C’)	3.0	60
3’ Primer 10 uM (Cattle ‘B’)	0.6	12
3’ Primer 10 uM (Sheep ‘S’)	3.0	60
3’ Primer 10 uM (Pig ‘P’)	0.6	12
3’ Primer 10 uM (Horse ‘H’)	2.0	40
Taq Polymerase (1000U/200uL)	0.5	10
Total	**24**	**480**
DNA (at ~700 ug/mL)	**1.0**	N/A

PCR

The T100™ Thermo Cycler was set to 95°C for 2 min, then cycled 35 times as follows: denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, then extension at 72°C for 30 seconds. The machine remained at 72°C for 5 minutes following the 35th cycle and was left overnight at 4°C. Samples were then frozen to preserve integrity until gel electrophoresis.

Gel Electrophoresis

Following amplification, 5 uL 6X Loading buffer was added directly to each PCR sample and then loaded onto a 3% Agarose gel. When loading S3-1 onto the well, the pipette tip was inserted into the gel causing a majority of the sample to disperse across the gel. It was swiped away promptly, and then the remaining S3-1 sample was reloaded successfully into its respective well. When sampling S6, we were not able to draw a full 20 uL sample, we loaded approximately 10 uL instead. All other samples were loaded equally at 20 uL.

Samples ran on the gel in a Biorad PowerPac 300 at 50 V for 10 minutes allowing them to leave the wells. Then, 150 V for 70 minutes. At this point, the gel was visualized briefly and then ran under 200 V for another 20 minutes.

Results

DNA Isolation

DNA isolation techniques produced DNA pellets from all meat-containing samples. A DNA pellet was also obtained from the human sample. No pellets were obtained from the H2O negative control.

Gel Electrophoresis

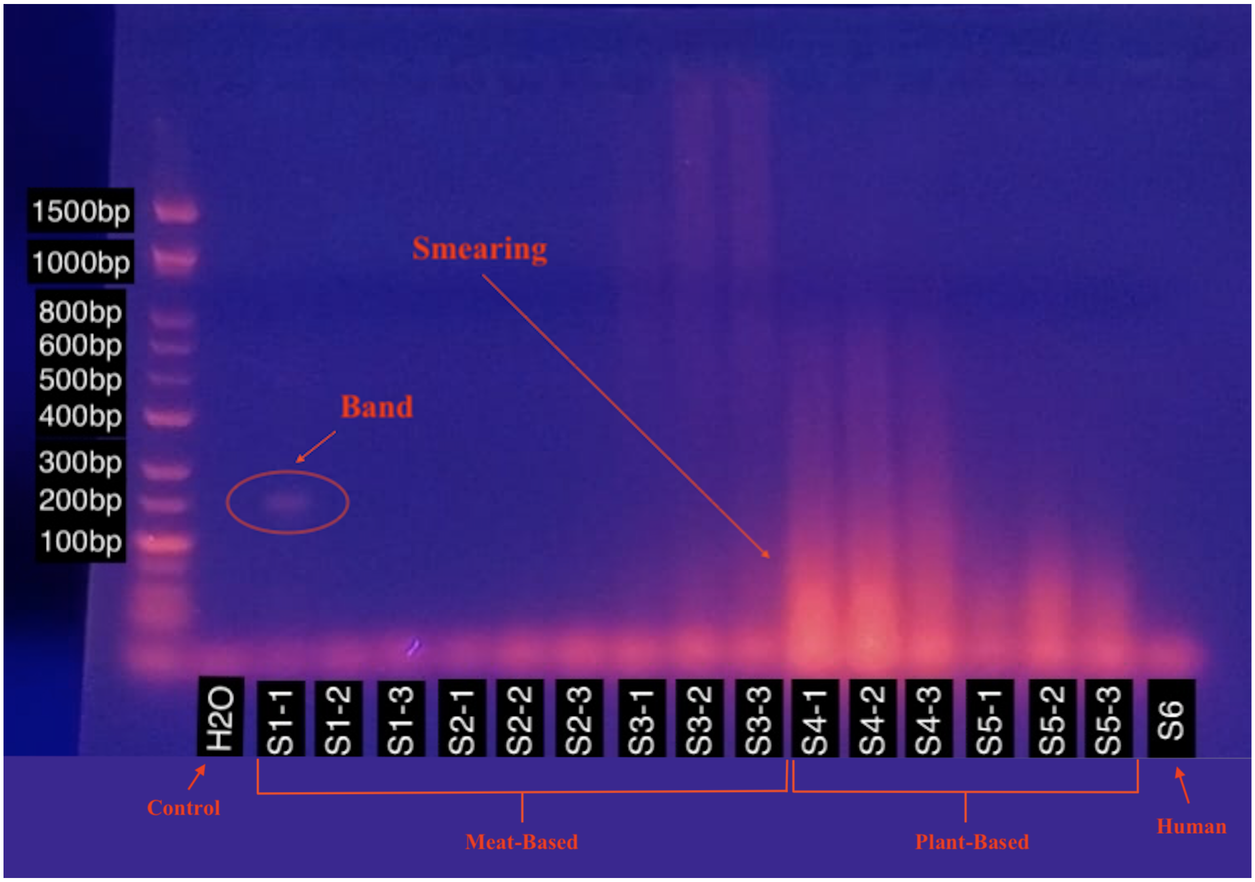

Figure 1. Gel electrophoresis final results. Gel electrophoresis results yielded one band at ~215 base pairs on S1-1. No other bands were observed for any other samples. Samples S1, S2, and S3 were meat-based samples, while S4 and S5 were plant-based samples. There is significant smearing of plant-based samples observed.

Discussion

To frame our discussion below is a brief summary of our results: Smearing was observed in all plant-based samples and sample 3 (beef), no bands in sample 6 (human), negative control (water), or sample 2, and finally one band produced in sample 1 (S1-1). Plant-based sample smearing may have been caused by the extensive chemical processes pea-based meat alternatives undergo in order to achieve a similar texture to meat. The combination of physical and chemical alteration of the plant proteins may have precipitated such patterns (Webb et al., 2023). It could also be a result of inadequate protein digestion or leftover proteases in the plant-based samples that caused smearing (Kurien & Scofield, 2019). Due to the presence of smearing, we consider the results of the plant-based patty analysis inconclusive. Meanwhile, the smearing in sample three was unexpected since it was derived from a beef patty. We considered the leaking of S3-1 during well loading may have caused it, especially if the proteins from sample three were not completely digested (Kurien & Scofield, 2019), but it seems unlikely since S2-3 is directly adjacent to it and has no smearing. As expected, the human sample (S6) and negative control (H2O) produced no bands; S6 was sampled from a vegetarian labmate and successfully returned no indication of meat.

The band of S1-1 at approximately 215 bp offered an opportunity for meat identification. A band of this size might be either chicken (~227 bp) or goat (~157 bp) based on the work of Matsunaga and colleagues analyzing the effects of cooking to PCR-amplified meat products (Matsunaga et al., 1999). Upon reviewing S1's restaurant's menu, we found that they offer an assortment of chicken burgers which could make cross-contamination a plausible explanation; this same restaurant does not offer goat products. It is also unlikely to be goat meat since chicken usually costs less than goat, therefore meat fraud would presumably be economically unreasonable. With this taken into account, it is suspected that the cooking surface used to prepare Sample 1 was cross-contaminated with chicken.

Lastly, the absence of banding across the remaining beef samples may be attributed to DNA integrity degradation which resulted from high-temperature cooking. A recent publication found that cooking alters the DNA of plants and meat resulting in cytosine nucleotides transforming into uracil along with the oxygenation of guanine nucleotides (Jun et al., 2023). The team also determined that for beef, extended cooking time and higher temperatures are more likely to cause more degradation to the DNA (Jun et al., 2023). Since the burgers from restaurant number one utilize flame grills to cook their patties, it is very likely that heat-induced DNA degradation caused the dearth of beef-sized banding. This could have caused the PCR primer pairing to fail and result in little to no amplified DNA from the beef samples.

Our failed attempt at producing bands for beef samples is consistent with the results of a number of colleagues; one from a team investigated meat authenticity in dog food and another working with university dining hall foods. Our lab technician found the presence of genomic DNA in our beef samples prior to PCR amplification (Figure A.1), suggesting that DNA degradation may have limited the ability of the RNA primers to attach and amplify sample DNA. Further analysis regarding the occurrence of cross-contamination during the cooking process is encouraged in order to verify meat authenticity.

To enhance the accuracy and reliability of meat DNA analysis, several factors should be considered in future studies. Firstly, standardizing DNA sample sizes would ensure consistent protein and DNA quantities. Inconsistent sample sizes may result in varied protein amounts, hindering effective protein digestion by proteinase and potentially causing smearing during gel electrophoresis. Secondly, considering the use of raw meat samples or investigating cooking methods that minimize DNA degradation during sample preparation is essential. By understanding the impact of cooking temperatures and durations on the damage done to DNA, as outlined in the recent paper by Jun et al. (2023), future studies can improve the reliability and accuracy of meat integrity assessments.

Conclusion

Our study, aimed at assessing the authenticity of burger patties, revealed significant challenges regarding DNA integrity following the cooking process and subsequent PCR analysis. Despite successful DNA isolation, the cooking process likely induced DNA base pair alterations, hindering the primers from binding targeted sequences, leading to failure of PCR and no detectable amplified DNA segments on the gel. This obstruction resulted in yielding only a single band among 17 samples during the gel electrophoresis, indicating possible chicken cross-contamination on a beef patty. These limitations highlight the necessity for alternative methods in meat analysis, especially emphasis on examining raw meat analysis to prevent DNA base pair alterations. Addressing these limitations is crucial in refining methodologies to enable more precise assessments of meat integrity, ultimately contributing to accurate labeling and reinforcing consumer confidence in meat authenticity.

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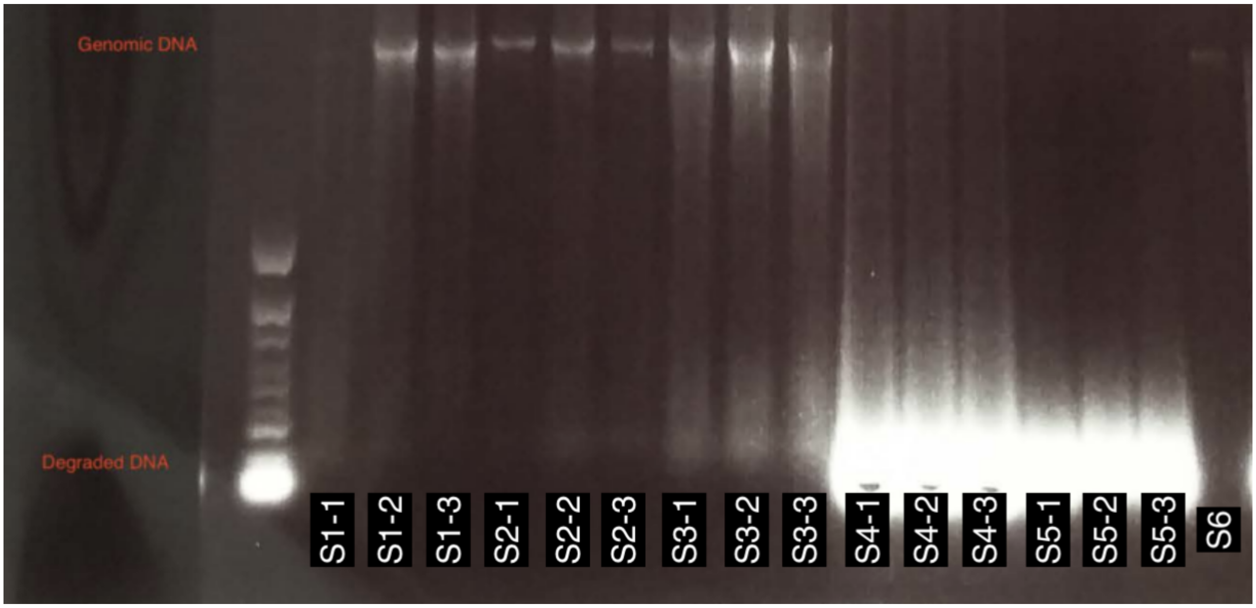
Appendix A

Figure A.1



Note. Produced after running 5 uL of extracted DNA on a 1% gel at 120 V for 1.5 hrs. Chow, M. (2023). *Genomic DNA* [Photograph].

Appendix B

Table B.1

RNA Primers	Nucleotide Sequence	Number of Base Pairs
Meat forward (SIM)	GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA	N/A
Goat (reverse)	CTCGACAAATGTGAGTTACAGAGGGA	157 bp
Chicken (reverse)	CTAGAAAAGTGTAAGACCCGTAATATAAG	227 bp
Cattle (reverse)	CTATGAATGCTGTGGCTATTGTCGCA	274 bp
Sheep (reverse)	CTATGAATGCTGTGGCTATTGTCGCA	331 bp
Pig (reverse)	GCTGATAGTAGATTTGTGATGACCGTA	398 bp
Horse (reverse)	CTCAGATTCACTCGACGAGGGTAGTA	439 bp