Meat Contamination in Dumplings

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Abstract

Accurate labelling of meat products is essential for public health. Mislabeled or cross contaminated meat products endanger the health of people with allergies. This study aims to evaluate the accuracy of meatballs in dumplings by using polymerase chain reaction (PCR), DNA separation, and gel electrophoresis. 30 samples were tested with 5 replicates for each of the 6 dumpling types (vegetarian, chicken, lamb, pork, beef + pork, and lamb + pork samples). In addition, a negative control containing distilled water was included to ensure reliability. The result of our study shows the pork samples did not contain pork DNA and goat DNA was detected in five of the six lamb meatball samples but lamb specifically refers to sheep. These findings suggest that contamination or mislabeling occurred in processing dumplings. The vegan samples confirmed the authenticity of its label because no meat DNA was detected in all vegan meatballs. Our results emphasize the importance of strict quality regulations in the food industry to ensure accurate product labelling and prevent cross-contamination during the process.

Introduction

Accurate labelling of meat products is important for consumer trust and public health. Meat products that do not meet the ingredient list were not only cheating consumers, but also endangering the health of people with allergies or dietary restrictions. Nowadays, the food industry is very global, which means it is crucial to ensure that companies comply with labelling regulations. However, previous research has demonstrated that the food industry frequently experiences intentional mislabeling and incidental contamination, especially when processed and blended meat products such as meatballs and dumplings (Adenuga & Montowska, 2023).

Polymerase Chain Reaction (PCR) and gel electrophoresis (Gel) are reliable tools for food authentication, which have been used to identify the species-specific DNA in complex mixtures (Ballin et al., 2009). For example, Demirhan et al.,detected pork DNA in vegetarian label products (2011). These techniques provided an effective approach to ensuring the authenticity in food labelling. Therefore, this study focused on identifying contamination in meatball samples by DNA isolation and amplification of species-specific

markers.

The research aimed to determine whether the meatballs complied with its declared ingredient label and to identify any unlabeled meat varieties. The expectation was that all samples should strictly conform to their ingredient lists(no contamination). The findings would have important implications for food safety that highlighted the need for stricter quality control measures and regulations.

Methods

There would be one negative control which used distilled water only. And a total of 30 examples, 5 replicates of each 6 types of dumplings (vegetables, chicken, lamb, pork, beef + pork, lamb + pork). The information for the samples was collected in the table.

Table 1: Sample information included the assigned sample ID, the brand name of the dumping, the product name on the cover, the sample collected date and the main ingredient in the dumpling.

Sample ID	Brand Name	Product Name	Sample Collected Date	Main Ingredient

DNA Isolation

1. Labelled each tube with a distinct symbol. Cut each meat sample into small pieces, and used a toothpick to place the sample in a sterile 1.5 ml Eppendorf tube. Mashed the meat. Sterilzed the scissors and tweezers between each use.
2. Added 300 uL of "Cell Lysis Solution with Proteinase K" to each tube. Then incubated the tubes at 65°C for 15 minutes, vortex each tube every 5 minutes until the solution looks cloudy.
3. Placed samples on ice for 5 minutes then added 150 uL of "Protein Precipitate Reagent" to each tube. Vortexed for 10 seconds.
4. Centrifuged the tubes at maximum speed for 10 minutes. After, transferred the

supernatant to a new tube. Discarded the old tubes.

5. Added 500 uL ice-cold isopropanol to the supernatant in each new tube. Carefully inverted the tube 30 - 40 times. Then centrifuged the tubes at maximum speed for 10 minutes.
6. Used a micropipette to remove the isopropanol, leaving the pellet at the bottom. Added 500 uL of ethanol to the pellet, then pour off the ethanol. Repeated this step once more.
7. Left the tube cap open overnight to allow any remaining ethanol to evaporate.

PCR

1. Added 30 µL of TE buffer to each tube containing the DNA pellet. Mixed gently by pipetting up and down until the pellet dissolves completely.
2. Prepared Master Mix (MM). Prepared extra to account for spills. Kept all reagents on ice throughout the procedure while preparing the MM. Added the components in order from the largest amount to the smallest, with the Taq polymerase being added at the end by the lab supervisor. Mix the MM using a micropipette.

Table 2: Master Mix components and their corresponding amount. Each component was timed by 36 for 30 test samples, 1 negative control and an extra account for spills.

COMPONENT	**AMOUNT (µL)**	**MM (x 36) (µL) (samples + controls)**
10X PCR buffer	2.5	90
10 mM dNTPs	0.5	18
25 mM MgCl2	1.5	54
5’ Primer 10µM (Meat forward (SIM))	1.0	36

3’ Primer 10µM (Goat “G”)	0.2	7.2
3’ Primer 10µM (Chicken “C”)	3.0	108
3’ Primer 10µM (Cattle “B”)	0.6	21.6
3’ Primer 10µM (Sheep “S”)	3.0	108
3’ Primer 10µM (Pig “P”)	0.6	21.6
3’ Primer 10µM (Horse “H”)	2.0	72
Taq polymerase (1000U/200ul)	0.5	18
dH_2O	8.6	309.6
Total:	24	864
DNA (If ~700ug/mL)	1.0	36

3. Labelled each PCR tube with a unique symbol. Pipetted 24.0 uL of the MM into each tube then added 1.0 uL of the DNA sample to the corresponding tube. For the negative control added 1.0 uL of distilled water.
4. Loaded the PCR tubes into the PCR machine and recorded the layout of the tubes on paper.
5. Programed the PCR Machine:
    - 95°C for 2 minutes.
    - 95°C for 30 seconds.
    - 60°C for 30 seconds.
    - 72°C for 30 seconds.
    - 72°C 5 minutes.

- Repeated Cycles: 35 cycles.
- 4°C overnight.
- Stored in the freezer.

Gel Preparation (3% agarose gel)

1. Added 3 g agarose and 100 ml SB buffer into an Erlenmeyer flask. Microwaved the mixed compounds until the agarose completely dissolved and the solution became clear.
2. Stopped heating if any bubbles appeared. Added 10 uL GelRed into the solution and shake to mix. Then used the microwave to reheat the solution and poured the solution into the bottom of the gel mold.
3. Placed the combs (1.0 mm) into the gel mold to create the wells. Then covered the gel tray with aluminium foil and set for 1 hour.

Gel Electrophoresis

1. 1 uL of 6X loading dye was added to each sample tube and pipetted to mix. Changed the pipette tip after each use.

2. Pipetted 5 uL of each sample onto the wells of the gel. And loaded a DNA ladder in the well for size reference.

3. After samples have been loaded, run the gel at 120V for an hour. Once the electrophoresis was complete, examined the gel under UV light. Recorded the results in the table.

Table 3: Gel electrophoresis results included the sample ID and corresponding presence of the DNA that appeared in the gel marking it as yes or no. If nothing was present in the gel marked as N/A.

Sample ID	Pig DNA Present (Y/N)	Chicken DNA Present (Y/N)	Cattle DNA Present (Y/N)	Sheep DNA Present (Y/N)	Horse DNA Present (Y/N)	Goat DNA Present (Y/N)

The observations were recorded in the lab notebook while doing the experiment. The data were analyzed by using the chi-squared test to test the significance of the result. Whether the observed distribution of meat types in the dumplings aligned with the expected distribution outlined in the ingredient table.

Results

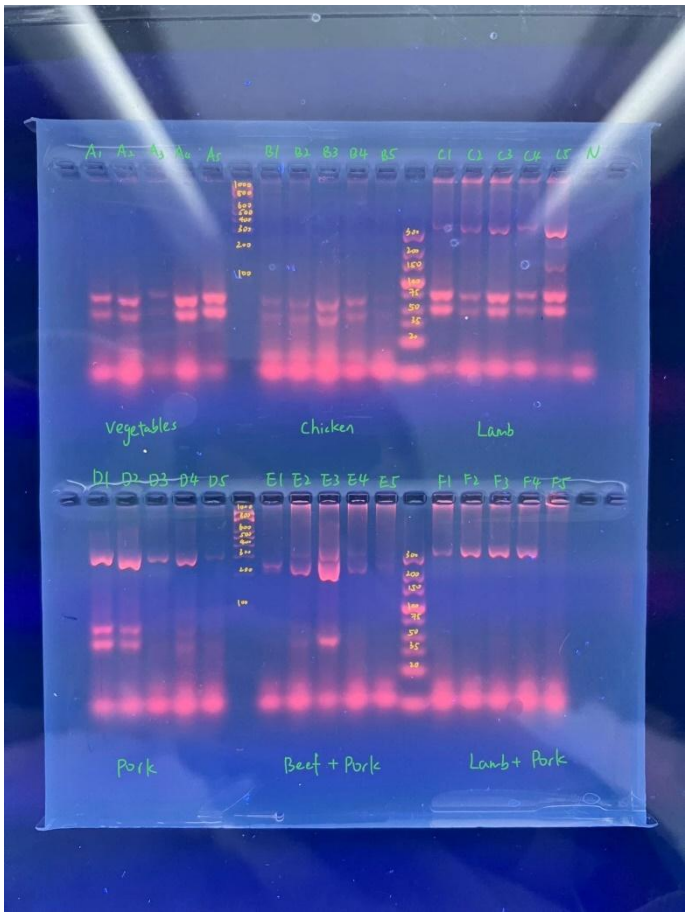

Figure 1: The gel electrophoresis results for dumpling meat ingredient analysis. The bands with the orange indications are the gel ladder for size reference. 1000dp to 100dp is the big ladder, and 300dp to 20dp is the small ladder. Group A1-A5 are vegetables, B1-B5 are chicken, C1-C5 are lamb, D1-D5 are pork, E1-E5 are beef + pork, and F1-F5 are lamb + pork. "N" represents the negative control group with distilled water only.

The results from the gel electrophoresis showed the polymerase chain reaction (PCR) in most of the groups amplifies the targeting sequences successfully. The targeting meat sequences included Goat (157 bp), Chicken (227 bp), Cattle (274 bp), Sheep (331

bp), Pig (398 bp), and Horse (439 bp).

The PCR product is analyzed on a 3% agarose gel, stained with GelRed, and visualized under UV light. One big DNA ladder and 1 small ladder have been added into lane 6 and lane 11 in each row.

Figure 1 shows that group A had no bands falling into the targeted region (157-439 bp). For group B, a faint band around 227 bp were shown in B1, B2, and B3. For group C, all samples showed a distinct band at approximately 331 bp, consistent with the expected size. In C1, C2, and C5, a faint band at approximately 157 bp was observed. Additionally, in C5, a faint band around 274 bp was shown. For group D, a band around 227bp was observed in D2, and a band around 274bp was observed in D1, D3, D4 and D5. For group E, all the samples observed a band around 227bp. For E3, E4, and E5, a band was observed around 274bp. For group F, all the samples except F5 were observed in a band around 331 bp. Bands around 75 bp and 50bp were observed in all groups A, B, C and D and in E2, E3, and E4.

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        Pearson's Chi-squared test

data:  observed
X-squared = 26.19, df = 5, p-value = 8.196e-05
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Figure 2: Results for the Chi-squared test. A high X-squared value (26.19) indicates a larger difference between the observed and expected frequencies. The degree of freedom (df) is the total number of sample groups minus one (sample group = 6). The p-value (8.196e-05) to compare the significance of the hypothesis.

The test statistic is 26.19 and p-value was 8.196e-05, which means the result was statistically significant.

Discussion

The results reveal critical insights into the accuracy of meat labelling in dumpling products. Based on our findings, we observed heavy contamination or mislabeling in lamb, pork, beef + pork and lamb + pork dumpling samples. Goat DNA was

detected in three out of five lamb dumpling replicas. Lamb only refers only to sheep meat, which is completely different in texture and nutrition from goats (Gruevska, 2023). In addition, chicken DNA is detected in one-fifth of the pork dumpling replicas, and cattle DNA is detected in four-fifths of the pork dumpling replicas. Moreover, chicken DNA is detected in all beef + pork dumpling replicas. Additionally, no pork DNA is detected in any of the samples labeled pork. These findings are particularly concerning because contamination or mislabeling occurred in most of our experimental groups, raising questions about the authenticity of these products. In contrast, the vegetarian samples do not show any contamination, matched its label and confirmed the integrity of its ingredient claims.

The data is analyzed by Chi-squared test to compare the observed contamination rates to expected ingredient compositions. Based on the high test statistics (26.19) and the p-value (8.196e-05), which is lower than the significant level of 0.05 (Figure. 2). The result shows that the differences between observed and expected frequencies of meat DNA are statistically significant, which means the differences are unlikely to occur by random chance. It means that cross-contamination or mislabeling issues are consistent in the samples.

The detection of different DNA in the dumplings with labels may be due to cross contamination during production. Shared equipment and inadequate sanitation practices may contaminate DNA with unintentional sources. Cross-contamination is a common problem in industrial food production, especially when equipment that processes different types of meat is inadequately sterilized (Ballin et al., 2009). This view is consistent with our findings because different types of meat are always processed in the same facility, which can lead to meat DNA contamination. A study from Chapman University (2015) highlighted that poor hygiene in meat processing plants can lead to cross-contamination, where DNA from unexpected sources can be detected in the final product.

The absence of pork DNA in pork dumplings was unexpected, which could be attributed to the following several reasons. First, the products may be mislabeled and contain no pork at all. In some cases, products are deliberately mislabeled to reduce costs (Demirhan et al., 2011). Second, some experimental manipulation contamination may have affected the accuracy of our results, such as DNA isolation and processing. Some experimental tools may also introduce foreign DNA or degrade pork DNA in the sample. This includes inadequate disinfection of scissors, tweezers or knives.

Another possibility is that the pork primers used in our PCR protocol are not effective.Too short annealing time may be a cause of primer failure.If the annealing time is too short, the primer may not have enough time to bond to the template of the pork DNA, resulting in the failure to display strips of pork in the video game (Lorenz, 2012).In addition, the pork gene sequences in the dumpling are different from those used in the design primer, that is, from genetically different pig breeds, in which case the primers may fail to amplify the target DNA, resulting in PCR failure (Kim et al., 2002).Gel run time may also be a consideration for false readings in pork dumplings.Due to the large DNA segments of pork, the banding of pork is not clear due to the short electrophoresis time or the use of high voltage (Voytas, 2001).

Changes in DNA concentration and purity may also have an impact on the results. Some samples do not have bands on the gel (e.g. B4, B5, F5), which may be due to insufficient DNA concentration (< 0.25 ng) or impurities inhibited PCR amplification (Matsunaga et al., 1999). Low DNA concentrations, residual ethanol and protein contamination can significantly reduce PCR efficiency (Matsunaga et al., 1999), which emphasizes the need for strict quality control during DNA extraction and purification. Furthermore, research has shown that the detection and quantification of DNA in meat products using PCR methods depends heavily on proper primer concentration and sample preparation to avoid false negatives (Cai et al., 2017).

The presence of primer dimers (75 bp and 50 bp) observed in many groups' gels also indicates suboptimal reaction conditions. High primer concentrations or inadequate annealing temperatures could have led to non-specific binding and amplification. These issues may affect the accurate interpretation of the results and should be addressed in future experiments by optimizing PCR parameters (Garafutdinov et al., 2020).

In addition, the presence of banding may also affect gel readings, leading to inaccurate interpretation of banding. The presence of banding smears may be caused by overload of DNA samples or by running the gel at high voltage (Voytas, 2001).

Our findings are consistent with earlier research. The study conducted by Adenuga and Montowska revealed extensive mislabeling and contamination in meat products (2023). However, there are some disparities between the findings and earlier research. For example, our findings reveal no pork DNA in pork-labeled goods. It contrasts the findings of Demirhan et al. (2011), who discovered that pork DNA was commonly found in mislabeled items. These disparities could be related to differences in sample sources or manufacturing methods.

Conclusion

Our research concludes that there are discrepancies between the observed meatball DNA results and their ingredient lists. Specifically, no pork DNA was found in pork-labeled samples, goat DNA was detected in lamb samples, and chicken DNA was present in all beef labeled meatballs. These findings do not support our expectation that all samples meet their ingredient lists, which may be because of cross-contamination or mislabeling. It highlights the need for the food industry to implement stricter quality control measures, such as DNA testing and improved hygiene. Future studies need to focus on improving PCR protocols in order to improve the specificity and dependability of DNA detection techniques.

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