Meat species analysis of food served at UBC owned food vendors using PCR and Gel analysis.

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Abstract

Species identification of meat is achieved through PCR and Gel Electrophoresis, where a certain fragment of the DNA is amplified, and the DNA sample is loaded into a gel to be pulled into bands by an electric current. Mislabeling of meat has been found in a variety of meat products sold in commercial markets in Italy, Turkey, and the US, while a few studies have investigated the proper labeling of meats in Canadian commercial markets. This study aims to identify meat species in UBC dining hall foods listed on the phone application NutriSlice, and to investigate cases of food mislabeling through DNA isolation, PCR, and Gel Electrophoresis. UBC dining halls claim to have halal certified beef and fresh chicken, which sets the expectation for minimal mislabeling, especially that no pork DNA be found in chicken and beef samples. Out of the 15 samples (5 chicken, 5 beef, 5 pork) collected, 2 pork samples are found to show bands of ~400bp, matching the size of their respective meat species; the other 13 samples have shown no bands and thus lead to inclusive results. Through DNA isolation, PCR, and gel experiments, this study has found no mislabeling in 2 samples and inconclusive results in 13 samples.

Introduction

Proper labeling of food is a concern worldwide, including Asia, Europe and North America (Murugaiah et al. 2009), for it ensures food safety and encourages individuals to adhere to their lifestyles and religious beliefs. While searching with the keywords “meat mislabeling” and “PCR,” the chances of mislabeling are found to vary across different sources of meat retailers (Kane and Hellberg 2016). A 2016 study in the United States concludes that meats from online specialty meat distributors have higher rates of mislabeling than the combined chances of meat mislabeling coming from local butchers and local supermarkets (Kane and Hellberg 2016). In addition, a 2015 study from Italy on 72 processed meat products reveals a 57% chance of mislabeling, where meats are either partially or entirely substituted for less expensive meat species (Di Pinto et al. 2015). The cases of meat mislabeling found across various countries suggest insufficient support for ensuring food safety and discourages certain lifestyles, core values, and religious practices, thus underlining the necessity for greater attention regarding the authentication of meat species on markets.

Meat species identification is achieved through DNA isolation and PCR, where cooked or raw meat samples are mixed with Taq polymerase to amplify certain segments of the DNA (Ulca et al. 2013). The results from PCR experiments are vulnerable to factors including heat and contamination (Lorenz 2012), which can result in a lack of bands from gel electrophoresis, rendering the results inconclusive.

Meat species authentication studies have been published by researchers from countries across the globe, including Japan (Matsunaga et al. 1999), Switzerland (Wolf et al. 1999), Turkey (Ulca et al. 2013), and the US (Kane and Hellberg 2016), but few studies are found to examine meat mislabeling in Canada, which rationalizes the motivation to investigate meat species served in Canadian dining halls. This study collects meat samples from dining halls of University of British Columbia, and attempts to identify meat species through DNA isolation, PCR, and gel electrophoresis.

Due to the difference in heating, processing, and preparation of meats in each sample, the extent of DNA degradation (Lorenz 2012) may vary, thus introducing risks for lack of bands and inconclusive results from PCR and gel experiments. Therefore, meat species identification in this study is based on samples that yield results from PCR and gel experiments.

This study expects no pork DNA, or no bands at 395 bp (Afifa Khatun et al. 2021) be found in chicken (227bp) and beef (472bp) samples, because of the claim in halal certification of beef burgers and freshness of chicken served in UBC residence dining halls (Residence dining – food at ubc vancouver, 2023). Additionally, the correct labeling requires no bands of any other meat species to be found other than the band corresponding to meat species specified in the labels. The 2015 Italian study on the meat species identification of processed meats (Di Pinto et al. 2015) provides inspiration into the analysis of processed meat samples in this study. In case that there are meat mislabeling found in our samples, we expect the lower rates of mislabeling in the least processed meats (e.g. whole cuts and slices of meat), and higher rates of mislabeling in the highly processed meats (e.g. sausages, salami).

Methods

Sample Collection:

Meat samples were collected from UBC owned food vendors whose nutritional information was available on Nutrislice UBC. A total of 15 samples were collected over a week. Samples were collected from four places; Harvest, Open Kitchen (Orchard Commons residence), Feast (Totem Hall residence) and Gather (Place Vanier

residence). Specific dishes were selected based on whether you could immediately tell what the meat was in the dish and all samples were collected from a unique menu item.. Samples collected were at least 0.5cm in length. And kept in 1.5mL Eppendorf tubes. They were labeled with unique ID numbers and kept in a freezer until DNA extraction.

DNA extraction:

Samples were cut/shredded into smaller pieces using sterile toothpicks or scissors. Samples were transferred to a new sterile Eppendorf tube (1.5mL) , the volume of minced samples in the new tube was below the 0.5mL mark on the tubes. These new tubes were labeled with new ID numbers.

300 µl of cell lysis solution with proteinase K was added to all samples. They were then incubated in a water bath at temperature 65°C. Samples were vortexed every five minutes. This was repeated until the solutions turned cloudy. It is recommended to wash the samples with deionized water before adding the cell lysis solution, dyes/spices from the dishes may make it hard to interpret when the solution turns cloudy. The samples were then removed from the water bath and placed in ice for five minutes. We were supposed to vortex the solution here for 15 seconds, but accidentally missed this step, which could generate errors.

The samples were then centrifuged for 9.5 minutes at maximum speed to separate all of its components into layers. Here we were supposed to let the machine run for 10 full minutes, so there could be errors introduced.. It is recommended to gently remove the upper layer of fat (using sterile toothpick) before extracting the supernatant from the Eppendorf tubes; it is likely to break into small pieces and contaminate the DNA and produce errors. Sample #B2.2 was dropped and may show unexpected results during electrophoresis.

500 µl of ice cold isopropanol was added to the tubes and gently shaken 30 - 40 times. Do not vortex here, it could break the DNA extracted. Then the samples were centrifuged for 10 full minutes. After which the isopropanol was poured out of the tubes. In case the DNA (solid at the bottom of the tube) is moving, it is recommended to use a pipette to remove the isopropanol. The DNA extracted then was washed twice with 500 µl of ethanol. The ethanol was poured off and the tubes were left open to dry overnight. They were then resuspended using a 30 µl TE buffer and left in -20°C.

Gel Preparation:

3 g of agarose powder was added to 100 ml of TAE buffer. The mixture was microwaved at 1 min intervals, swirling between each minute until the solution turned clear. A drop of GelRed was added before the solution cooled down. This was just enough for a proper gel but wells could hold only 10 µl samples.

PCR and Electrophoresis:

Master mix:

The recipe for MM is below shown in Table 3.1. A big batch for 20 samples was made, first adding the deionized water (dH_2O) then the rest of its components. All components were kept in ice before preparation. Add 24 µl of MM solution to PCR tubes. Tubes are labeled sample ID.

Component	Volume per tube/ µl	Total volume x20/ µl
10X PCR buffer	2.5 µl	50 µl
10mM dNTPs	0.5 µl	10 µl
25 mM $MgCl_2$	1.5 µl	30 µl
5' Primer 10µM (Meat forward (SIM)	1.0 µl	20 µl
3' primer 10µM (Goat "G")	0.2 µl	4 µl
3' Primer 10µM (Chicken "C")	3.0 µl	60 µl
3' Primer 10µM (Cattle "C")	0.6 µl	12 µl
3' Primer 10µM (Sheep "S")	3.0 µl	60 µl
3' Primer 10µM (Pig "P")	0.6 µl	12 µl
3' Primer 10µM (Horse "H")	2.0 µl	40 µl
Taq polymerase 1000U(/200ul)	0.5 µl	10 µl
50% Glycerol	5.0 µl	100 µl
Sterile dH_2O	3.6 µl	72 µl
Total	24 µl	480 µl

Table 1.0: Master Mix recipe used for our experiment. Volume per tube is for 1.0 µl of DNA at 10ng/ul concentration

PCR:

1.0 µl of resuspended sample DNA was added to the PCR tubes with MM. These tubes were kept on ice before transferring to the PCR machine. Once in the PCR machine, the samples were first kept at 95°C for 2 minutes then cycled through 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, 35 times. Then kept at 72°C for 5 minutes then finally at 4°C overnight.

Electrophoresis:

5 µl 6X loading dye was added to all the samples, without touching the sample or the PCR tubes. Using a new tip every time, the sample was mixed and 10 µl of sample loaded onto the wells of the gel. The gel used for this experiment was a 3% (3g agarose/100 ml TAE buffer). The samples were first run at 60V for 10 mins, then the voltage was increased to 187 V for 22.5 mins (this was to check if the samples were moving at all). The voltage was then decreased to 120V for 30 mins and finally at 122V for approximately 2 hours.

Results

Out of the 15 samples, only 2 produced bands. These were sample #B2.2 and #B2.14 which were chorizo and BBQ pork tenderloin from ramen. Bands, shown in figure 1.0 below, are both 400 bp~ (which signifies pork[1]).

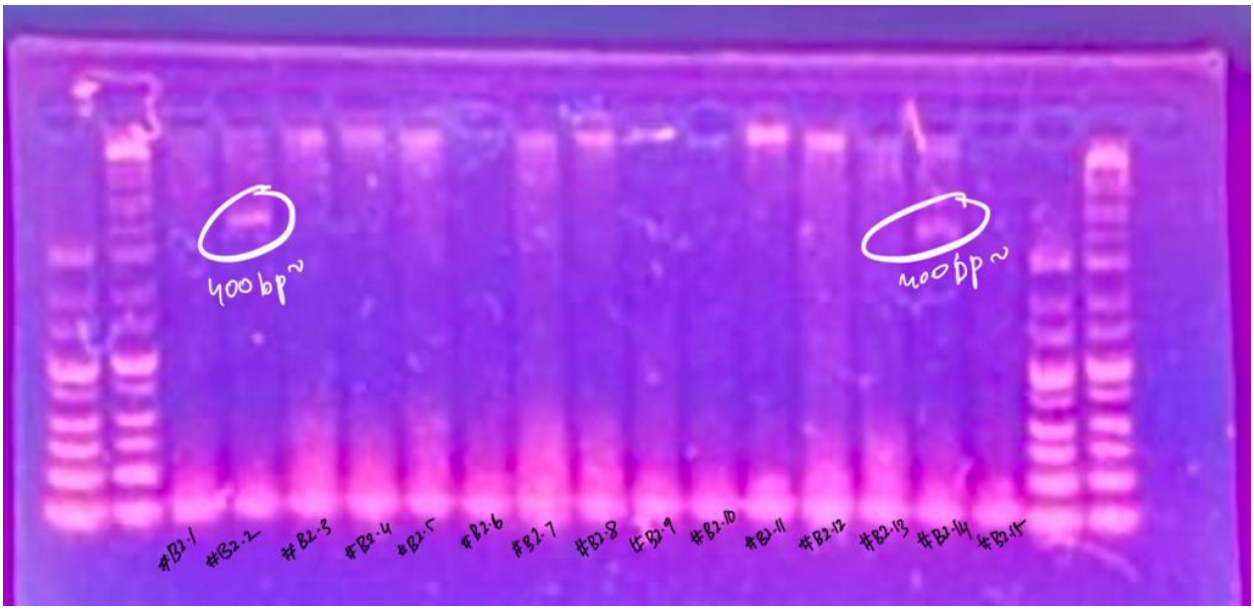

Figure 1.0: Gel electrophoresis results of 15 meat samples. Lanes 1-2 and 18-19 are "ultralow" ladders and the mixes ladders; lanes 3-17 are gel bands of meat samples.

Discussion

In formulating the hypothesis for our experiment, we considered several factors that led us to anticipate a lack of variation in the meat primers compared to the meats obtained from the dining halls. A factor that led us to developing this hypothesis was the stringent regulations surrounding meat labeling in Canada. Canada has established robust laws to prevent mislabeling practices in the food industry. For instance, if a food product, such as a hotdog, contains 99% beef and 1% pork, it is mandated by Canadian law to be accurately labeled as a beef and

pork hotdog[3]. This legal framework ensures transparency in food labeling and provides consumers with accurate information about the composition of the products they purchase. Additionally, we took into account the specific policies of the University of British Columbia (UBC), where our experiment was conducted. UBC maintains a strict stance against mislabeling, further supporting our prediction of minimal variation in the meat primers compared to the meats served in the dining halls[13]. The university's commitment to upholding high standards in food quality and accurate representation aligns with the broader regulatory environment in Canada. By considering both the national regulations and the internal policies of UBC, we aimed to create a hypothesis that reflects the commitment to transparency and accuracy in food labeling. This not only informed the design of our experiment but also gave us confidence in our prediction that the meats would exhibit consistency with their respective primers. We analyzed the results, and found valuable insights into the effectiveness of these regulatory measures in ensuring the accuracy of meat labeling within the Canadian and university contexts.

The PCR result of our experiment only had two successful bands, the Chorizo collected from Open Kitchen (Sample #B2.2), and the BBQ pork from Vanier (Sample #B2.12). Both of these bands appear around the 400 bp region, which is consistent with pork meat. This confirmed our hypothesis that there was no mislabelling of the meat found at UBC catering services.

An article from 2009, published in the National Library of Medicine, found that when meats are cooked, significantly less DNA can be extracted from it compared to raw meats[11]. The paper found that cooking the meats to high temperatures gives bands smaller than 200 bp. This would not show up on our Gel Electrophoresis. We expect we were able to extract DNA from the chorizo, as chorizo is cured and not cooked. This would mean the DNA of the pork would not be affected. However, we have not been able to find any research regarding DNA extraction of cured meats. The other sample we were able to get DNA from was the BBQ pork. We suspect we were able to get DNA from pork because pork is cooked an internal temperature of 62ºC, while the beef we collected samples from, ground beef and stewing beef, is cooked for a longer time to an internal temperature of 71ºC, and chicken is cooked to a minimum internal temperature of 74ºC[14].

We suspect we were not able to get DNA from the other three pork samples, B2.6 and B2.9 which were both pulled pork, and B2.12 the corndog, due to their method of cooking and the type of meat cut used. Pulled pork is a fatty cut of meat usually cooked on low for 8 hours, or on high for 4-5 hours. The long cooking time will affect the ability for DNA to be extracted, and research has found that it is harder to extract DNA from fatty

meats/tissues[12]. We suspect the corndog meat is ultra processed, making it hard to extract any DNA from this sample as well.

After seeing these research articles, looking at the samples we have taken of beef and chicken, it is not surprising we were unable to get DNA from these samples. The beef cuts used were Beef Chuck and ground beef, both have very long cook times, and they are fatty meats. The chicken is lean; however, cooked to the highest internal temperature, also unsurprising we were unable to extract DNA.

Discussion Table 1: Analysis of Sample Meat Type and Cook Time

Reassigned Sample ID	**Name of sample**	**Ingredient we test for**	**Fat content of Meat**	**Cook time/ Temp**	**Dna Extraction Expected?**
#B2.1	Chicken Pesto Sandwich	Chicken	Lean	Short / 74ºC	No
#B2.2	Chorizo	Pork	Fatty	Cured	Yes
#B2.3	Meatballs	Beef	Fatty (Ground Beef)	Long / 71ºC	No
#B2.4	Beef Burger (Halal)	Beef	Fatty (Ground Beef)	Long / 71ºC	No
#B2.5	Chicken Skewer	Chicken	Lean	Short / 74ºC	No
#B2.6	Pulled Pork	Pork	Fatty (Pork Butt)	Long / 62ºC	No
#B2.15	Massaman Beef Curry	Beef	Fatty (Chuck Beef)	Long / 71ºC	No
#B2.8	MeatBalls (Halal)	Chicken	Lean	Short / 74ºC	No
#B2.9	Pull Pork Burrito Bowl	Pork	Fatty (Pork Butt)	Long / 71ºC	No
#B2.10	Beef Stroganoff Pasta	Beef	Fatty (Beef Chuck)	Long / 71ºC	No
#B2.11	Butter Chicken	Chicken	Lean	Short / 74ºC	No
#B2.12	CornDog	Pork	Fatty	Short / 62ºC	No
#B2.13	Beef Sausage Roll	Beef	Fatty (Ground Beef)	Long / 71ºC	No
#B2.14	BBQ Pork Ramen	Pork	Lean (Tenderloin)	Short / 62ºC	Yes
#B2.7	Chicken Burger	Chicken	Lean	Short / 74ºC	No

** *Short = Less than an hour*

We suspect any inconsistencies in our results would be due to human error, not mislabelling of meats. During the experimental process we have noted a few sources of error. The first being contamination during collection of the samples. The samples were collected from the dinning hall and placed in the sample vials using a new set of utensils for each sample. However, when gathering the samples many different meats were at the table at the same time, for example, when gathering the pork from the pulled pork (Sample #B2.9), the beef from the beef stroganoff (Sample #B2.10), the chicken from the butter chicken (Sample #B2.11), and the beef from the beef sausage roll (Sample #2.13) all samples were present and being collected at the same time. This could have led to cross contamination with utensils, this was kept to a minimum; however, any variation could be a result of this. The second contamination could have come from the sauces the various meats came in. For example, the pulled pork had a BBQ sauce that was washed off before DNA isolation; however, the sauces were not labeled with what meats may have been contained, this could lead to variation in the primer if the BBQ sauce contained beef DNA for example. We suspect the DNA concentration of any meats in the sauces would not be enough to affect the PCR; however, if there are variations this could be a reason.

Conclusion

Thirteen out of fifteen experimental results were inconclusive, as no bands appeared on the Gel Electrophoresis. Two out of thirteen samples did show bands; both of these samples were identified as pork and exhibited a consistent pattern with the ladder. This confirmation supports our hypothesis that there was no mislabeling of UBC Food Catering Services' meats.

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Appendix

UBC nutrislice website: https://ubc.nutrislice.com/

Sample Data Table 3:

Reassigned Sample ID	Collection Sample ID	Collection date	Name of sample	Ingredient we test for	Location
#B2.1	N1	25/10/2023	Chicken Pesto Sandwich	Chicken	Harvest
#B2.2	N2	5/11/2023	Chorizo	Pork	Open Kitchen (Orchard)
#B2.3	N3	8/11/2023	Meatballs	Beef	Open Kitchen (Orchard)
#B2.4	N4	8/11/2023	Beef Burger (Halal)	Beef	Open Kitchen (Orchard)
#B2.5	N5	8/11/2023	Chicken Skewer	Chicken	Open Kitchen (Orchard)
#B2.6	N6	9/11/2023	Pulled Pork	Pork	Feast (Totem)
#B2.15	N7	9/11/2023	Massaman Beef Curry	Beef	Feast (Totem)
#B2.8	N8	9/11/2023	MeatBalls (Halal)	Chicken	Feast (Totem)
#B2.9	O1	3/11/23	Pull Pork Burrito Bowl	Pork	Feast (Totem)
#B2.10	O2	3/11/23	Beef Stroganoff Pasta	Beef	Feast (Totem)
#B2.11	O3	3/11/23	Butter Chicken	Chicken	Feast (Totem)
#B2.12	O4	3/11/23	CornDog	Pork	Feast (Totem)
#B2.13	O5	3/11/23	Beef Sausage Roll	Beef	Feast (Totem)
#B2.14	O6	03/09/23	BBQ Pork Ramen	Pork	Gather (Vanier)
#B2.7	O7	03/09/23	Chicken Burger	Chicken	Gather (Vanier)

Annotated Bibliography

BIOL 342 annotated bibliography

https://docs.google.com/document/d/1fCiZL5nYtDB_H7WDcLqzdQiZxgHSreskThAKlX6atVU/edit

OBSERVATIONS

#B2.9 → Yellow cloudy color in bottom layer, clear cloudy layer on top