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Research article

Method development for identification of the chicken, beef and pork processed food

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Abstract

Food adulteration is an addition of impure ingredients to food that cannot be seen by the naked eye unless it is tested and investigated thoroughly. In the market, people are buying canned and processed meat for consumption but little did they know the content. In this study, we examined the protein profile of canned meat by using SDS-PAGE method that can show the differences of protein profile of three types of canned meat and processed meat that is chicken (Gallus gallus), pork (Sus scrofa) and beef (Bos taurus). A simple approach by extracting protein from meat product and followed by protein purification and SDS-PAGE protein profiling was established. We find the purification process is critical in order to obtain a good profiling of the food's protein as the meat product was seasoned and therefore salt composition in the food interfered the SDS-PAGE separation. Using this newly developed method, we were able to identify the origin of meats in food.

Introduction

Canning is a method of preserving food in which the food contents are processed and sealed in an airtight container. Canning provides a shelf life typically ranging from one to five years, although under specific circumstances it can be much longer. Other than that, consumers do not know the halal content in any of the canned food whether it is adulterated. There are several kinds of canned meat in the market which are halal. The species of the meat could not have been identified. The manufacturing techniques remain basically the same, no matter from which animal species the raw materials derived [1]. However, some meats which are marketed as halal, such as beef or chicken meat might be intentionally or unintentionally adulterated. There are several cases of adulteration of meat in Malaysia, one of these examples are, 112 over 143 of meat products were mislabeled. 49 samples over 58 sample beef products were adulterated. As for chicken: 62 samples over 84 samples were adulterated. This was the first record of investigation in mislabeling of meat products in Malaysia [2]. Another case of food adulteration in Malaysia is about Cadbury chocolate controversy occurred in between end of May 2014 to early June 2014 when a government officer leaked the information concerning DNA porcine contamination in Cadbury products [3]. Therefore, it is useful to determine the source of the meat, in which we accomplished it by the protein profiling analysis in view that the main component of meat is protein. Sodium chloride, dissolved in water, is used in the canning process has turned out to be one of interferences that lead to the failure of successful protein profiling of canned meat, therefore we have improvised a protein purification step in order to achieve the desired result[4].

Experimental

Materials and method

Canned meat preparation

Canned meat was removed from the can and prewashed with distilled water followed by soaking the meat until there are no obvious oil remaining. A ratio of 1:2 of meat to extraction buffer was used to obtain the whole crude protein.

TRIS extraction

In TRIS (trisaminomethane) extraction method, 500 µL of 40 mM TRIS extraction buffer was added to 250 mg of meat sample followed by homogenizing the sample by using a small hand homogenizer. The supernatant is then collected for further precipitation method [5].

Trichloroacetic acid Acetone precipitation (TCA)

The extracted protein sample was concentrated and desalted. First, 200 µL of acetone with 20 mM of DTT is added to a 100 µL sample extract and vortexed. Then, 100 µL of 10% TCA is added to the sample and vortexed.

The sample is placed at -20°C for 2 hours. The sample was then spun at 16,000 g for 15 minutes at -4°C. The supernatant is discarded. The pellet is washed twice with acetone with 20 mM DTT and was briefly centrifuged at each washing. Finally, the pellet was resolubilized in TSE buffer that contains 40 mM Tris, 10% SDS, 0.1% of ampholyte and 0.1 mM EDTA[6].

Chloroform methanol precipitation

In chloroform methanol precipitation[7], 400 µL of methanol is added to 100 µL of protein extract and vortex thoroughly. Next, 100 µL of chloroform is added and vortexed. A volume of 300 µL of H_2O is added to the mixture and vortexed thoroughly. Then the mixture is centrifuged for 5 minutes at 14,000 g resulting in three layers: a large aqueous layer on top, a circular flake of protein in the interphase, and a smaller chloroform layer at the bottom. The top aqueous layer is removed carefully, not to disturb the protein flake. Next, 400 µL methanol is added and vortexed and followed by centrifugation for 5 minutes at 20,000 g, which will bring down the pellet protein precipitate against the tube wall. The methanol solution is removed as much as possible. Care must be taken where not to touch the pellet. The sample is dried under vacuum and the protein was reconstituted with TSE buffer (40 mM Tris, 10% SDS, 0.1% of bioampholyte and 0.1 mM EDTA).

Result and Discussion

Results

The purity of the samples will affect the resolution and migration of proteins in SDS-PAGE, in this study, we have tested two protein precipitation methods to produce the extract with good purity, this is because the canned meats are prepared differently by different manufacturers and therefore, a comprehensive cleaning on the non-meat ingredients is highly desired in order to produce a high reproducible result.

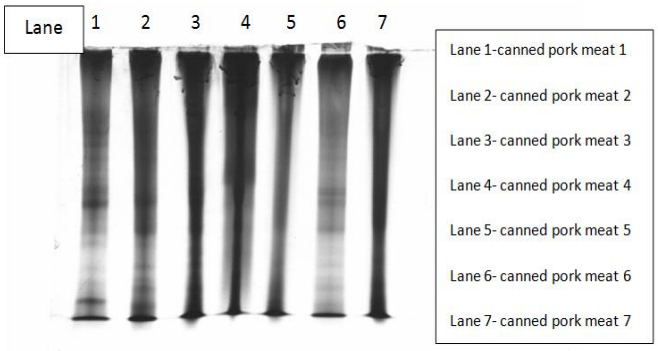

Figure 1. Tris Buffer-Pork meat extract followed by TCA Acetone precipitation

Figure 1 shows the different canned pork meats which were extracted by Tris buffer followed by TCA Acetone precipitation. Protein bands can be visualized in the gel however the clarity of the bands was interfered by the impurities which obstructed the visibility of the bands in some of the pork samples, the inconsistency in the band pattern of each pork samples is caused by the amount of the impurities in each canned pork meat as these canned food contained different ingredients but were subjected to identical protein extraction and purification procedure in our laboratory, the result indicates that we cannot have a standardized purification method for all the samples as one sample may need a more thorough cleaning than the other depending on their respective ingredients.

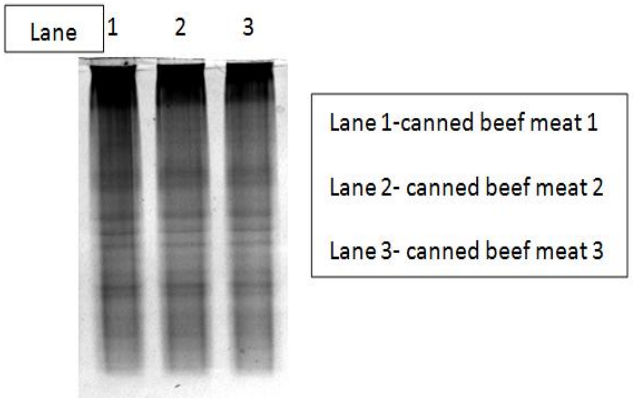

Figure 2. SDS-PAGE of canned beef meat extract followed by chloroform/methanol precipitation

Figure 2 shows four canned beef meat sample subjected to TRIS buffer extraction followed by chloroform /methanol precipitation. Smearing was observed throughout the gel and the bands at the top of the gel cannot be detected while the bands with lower molecular weights can be identified.

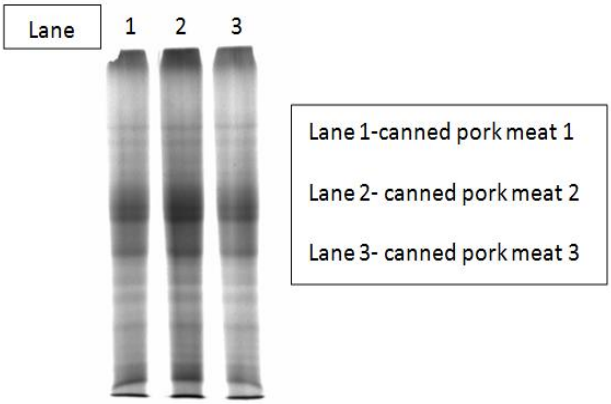

Figure 3. SDS-PAGE on canned pork meat extract followed by chloroform/methanol precipitation

Figure 3 shows the protein band pattern of canned pork meat extract followed by chloroform/ methanol precipitation. The protein bands can be visualized clearly although smearing appears in each lane. The smear has affected the sharpness of the protein bands.

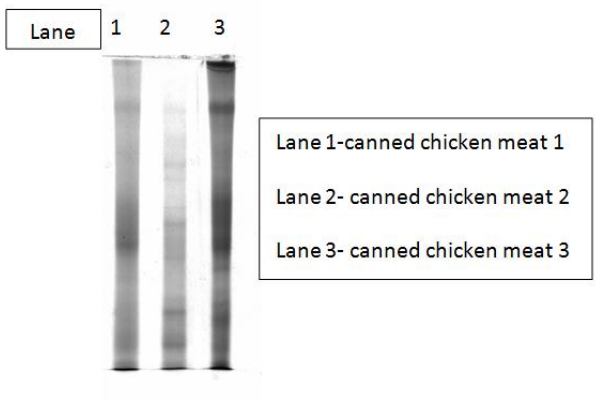

Figure 4. SDS-PAGE on canned pork meat extract followed by chloroform/methanol precipitation

Figure 4 show the SDS-PAGE protein profile of three canned chicken extract followed by chloroform/ methanol precipitation purification. In general, the protein content for these three chicken meats is much lower than those of pork or beef. Therefore, by using the similar meat's weight resulted in less protein recovered from the chicken meat. Although the lane smearing is observed, the protein bands can be identified.

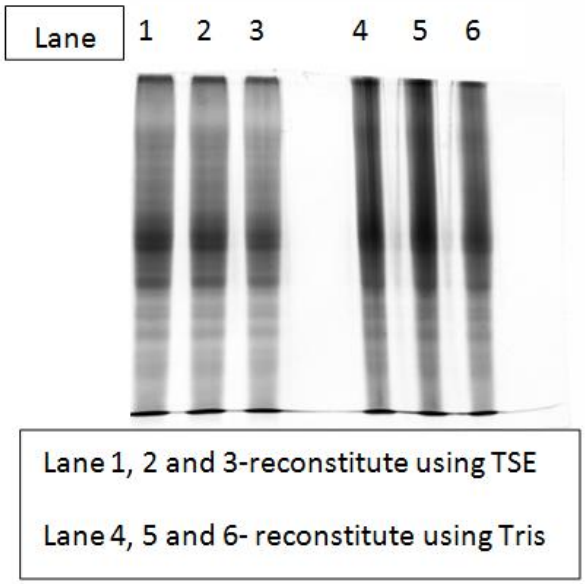

Figure 5. Comparison of the reconstitution buffers used after chloroform/methanol precipitation

Figure 5 shows the two different types of buffers TLB and TRIS buffer that were used to reconstitute the precipitate formed after chloroform/methanol purification on canned pork meat extract. More bands were detected in protein reconstituted in TSE buffer as shown in lane 1, 2, and 3 than the TRIS buffer shown in lane 5, 6, and 7. Thus TSE buffer that contains Tris, SDS and EDTA, which increases the solubility of precipitated proteins have been chosen for the subsequent study.

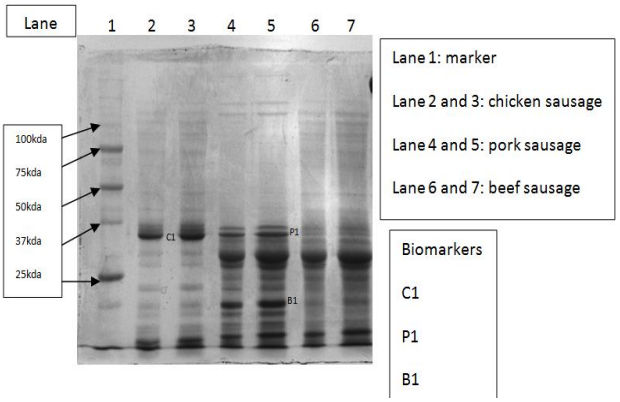

Figure 6. SDS-PAGE of processed chicken, pork and beef meat by using chloroform/ methanol precipitation

Figure 6 shows the processed meat in the form of sausages. The protein profile of the chicken sausage is uniquely different from those of pork and beef especially at the MW range of 25 KDa to 37 KDa. Although the profiles for pork and beef are relatively similar, they are not identical. This is especially true at lower MW protein bands (>20 KDa). In addition, the authors also identified the unique bands for each of the meat origins (as labelled in Figure 6), where C1 is the protein band that is unique to chicken. Whilst two bands at P1 and B1 were presented with different degree of bands intensity between pork and beef. The band's intensity of pork is consistently darker than those of beef.

Discussion

Consumption of adulterated food in Malaysia will be a big issue in term of religion. The usage of pork and lard is a serious matter in Islam because food containing ingredients from pig sources are haram (unlawful or prohibited) for Muslims to consume. Thus, methods were developed by using polymerase chain reaction (PCR) to examine the source of animal. There are limitations on using PCR itself where in spite of its ability to fulfil the task [8]. PCR often encounters problems like jumping PCR that may lead to questioning of the authenticity of the amplified sequences. Jumping PCR is the integration of broken fragments into a longer strand to permit the "conventional PCR. Moreover, the performance of the PCR technique is also affected by any or unknown contamination [9, 10].

In the analysis of protein involved highly processed meat products, we are facing challenges of the highly denatured proteins and also the presence of high level of foreign ingredients and salts used in preparing the food. Although thorough washing of the meat before extraction may help, it can only use to remove the ingredients that trapped on the meat surfaces while those invaded into the meat was not able to fully remove. The degree of protein denaturation of meat sample is very much depended on how the meat was processed, this has added into the difficulty in getting a conserved protein profile between

different meat products. In addition, the impurities or ingredients in the canned meat products and the denatured proteins will reduce the visualization of the protein bands where smearing effect is anticipated. This has led to the need in developing not just a protein extraction method but a protein purification method became a necessity.

The methodology to obtain high resolution protein separation of complex mixtures by SDS-PAGE has remained essentially unchanged since its introduction four decades ago[11]. It is routinely used to probe the presence and purity of proteins, their approximate molecular mass. In this study, we aimed to develop a simple yet accurate method for identification of meat sources from processed meat products. The method involved a simple step of protein extraction and purification followed by SDS-PAGE protein separation method. A protein extraction step by TRIS buffer was applied in this study, the meat sample was mixed with TRIS buffer and homogenized, the mixture was centrifuged and the supernatant was recovered, the use of TRIS buffer will enable us to extract most of the aqueous soluble proteins. However, a further purification of supernatant is required as the meat are cooked in gravy which contained high level of salts and ingredients that are not suitable for SDS-PAGE, two purification methods, namely TCA/acetone precipitation and chloroform/methanol precipitation. In the TCA/acetone precipitation (Figure 1), the sample loaded lanes appeared to be smeared, which indicate interference of impurities unto the protein bands, therefore TCA/acetone precipitation may not be a purification method of choice as it did not completely remove the impurities from the meat products and causing low visibility of protein bands. Proteins containing a high number of hydrophobic amino acids thoroughly characterized the proteins found soluble in the organic phases of different ratios of chloroform to methanol [12]. This has led to our second purification device of chloroform/methanol precipitation, it appeared to produce a better purity extract, where the protein bands can be visualized clearly and furthermore, the method is easier to perform than the TCA/acetone method and it is better in avoiding protein loss [13]. Therefore, chloroform/methanol purification method was applied in the subsequent analysis. It is known that once protein is precipitated, it may not be easily resolubilized in the solution form. In this study, we have observed the same phenomena where some of the TRIS extracted protein cannot be resolubilized in TRIS buffer; a modified buffer (TSE buffer) was used to increase the solubility of the proteins and shown to produce a better resolution of protein bands.

Besides testing on the cooked whole canned meat, we have tested the method on sausages and the result turned out to be satisfactory, where we obtained sharp and fine protein bands.

After an improved method was generated, this method is tested on chicken, pork and beef sausages. The sharp and distinct protein profiles were obtained from the three different sources of meat on the finished product, which can be used to differentiate the type of meat used in the food.

Conclusion

A method based on protein extraction, purification and separation was developed. The method was applied on the finished food product obtained from the market, where different meat gives different type of the animal tested produce different unique bands that can be distinguish each other.

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