DETERMINATION OF DNA QUALITY EXTRACTED FROM FOOD PRODUCTS

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Abstract

Deoxyribonucleic acid or DNA is the hereditary molecule of living things. Food products mostly contain ingredients originated from animals and plants. Food adulteration is the main concern in food industry. Food manufacturers might substitute raw materials with other cheap and abundant materials which is species substitution. With the help of DNA, scientists are able to identify the species used in the food products. The objective of this study are to determine the presence of DNA isolated from selected food products and to access the quality of the extracted DNA using gel electrophoresis. DNA were extracted using DNeasy™ Blood and Tissue kit (Qiagen, Germany) following manufacturer protocols with slight modifications. The extracted DNA's quality was then subsequently evaluated on 1.5% agarose gel. The food products chosen were chicken sausages, meat patty and chocolate confectioneries. Results obtained showed positive DNA bands in sausages and meat patty, but no bands were observed from chocolate confectioneries. Extracting DNA from a complex and highly processed foods was a very challenging task. Thus, it requires optimization and improvisation of the extraction procedures in order to achieve high quality DNA bands.

Keyword: DNA quality, food adulteration, DNA extraction, frozen food, chocolate

Introduction

Background of Study

Deoxyribonucleic acid is commonly known as DNA that act as a blueprint for life. Every living organism has its own DNA. DNA is a complex molecule that contains all the information essential to build and maintain an organism (Zhang & Hewitt, 1996). The isolation of DNA, either mitochondrial DNA or genomic DNA, is the first step in DNA-based species identification. Technology advancement allows more innovative approaches and tools, hence, improving the quality of DNA yield for identification process.

Problem Statement

Nowadays, certain food products somehow have involved in food adulteration. Some manufacturers are dishonest and, in some cases, presence of meat from other animal had been detected even though a particular product was labelled as chicken sausages (Schubbert *et al*., 2008). The way the samples being prepared for this study could affect the quality of DNA yield. Perhaps, by improving the method of DNA extraction from the selected food products, the quality of the DNA could be maintained.

Significance of the Study

The significance of this study is it delivers comprehensive details and knowledge on how samples preparation affects the quality of DNA yield in processed foods. By enhancing the sample preparation, the cost could probably be reduced without having to rely solely on

commercial extraction kits. Besides, the results from this research can be applied in certain organizations and even for consumers, especially in food adulteration and fraud cases. It is well known that nowadays, processed foods make up most of our daily food intake. As a matter of fact, most food manufacturers, use raw materials that are easily synthesized, abundantly available, and lowly priced to meet a particular demand (Sahilah *et al*., 2012), despite some traces from the raw materials have the possibility of inducing allergic reactions and other health problems in certain people.

Materials and Methods

Samples Preparation

Before proceeding to the extraction of DNA, all samples were homogenized using liquid nitrogen. The samples should be ground using mortar and pestle with presence of liquid nitrogen. It is important to finely grind the samples into powdery state, so that all the constituents in the samples are properly mixed.

DNA Extraction

The procedures used in this study was followed by the manufacturer protocols QIAGEN DNeasy® DNA extraction kit with slight modification. A 25mg of ground sample was added with 20µl of proteinase K and 200µl of ATL buffer (lysis buffer). The mixture was put in a water bath of 40-50°C and left overnight (Knebelsberger & Stoger, 2012). The next morning, buffer AW1 and AW2 were added (wash buffer). Next, a 200 µl AE buffer (elution buffer) was also added. The DNA elution would then be incubated at room temperature for 1 minute and centrifuged at 11000rpm for 3 minutes. The procedures were repeated using the selected food products (chicken sausages, meat patties and chocolate confectioneries) from different brands. The DNA extracted then was stored at 4 °C for later use.

Gel Electrophoresis to Assess DNA Quality

Gel electrophoresis was performed in order to assess the quality of the DNA obtained. The concentration of agarose gel used was 1.5% (w/v). 0.6 g of agarose powder were mixed with 40 mL of 1x TBE buffer in a conical flask. The mixture was transferred into a microwave for 1 minute until it was fully dissolved. The flask was taken out and left for cooling to 50-55°C. Upon cooled, 2 µL of GelStar® Nucleic acid stain was added. The flask was then gently swirled and poured on a gel tray. After an hour, the gel became solidified and the comb was removed. 1x TBE buffer was poured on top of the gel until fully immersed in an electrophoresis chamber. 10 µL of extracted DNA was then mixed with 2 µL of loading dye on a parafilm. The mixture was then transferred to the well on the gel at negative pole (cathode). The electrophoresis was run at 45V/50V/70V for at least 1 hour and a half. Then, the gel was photographed with gel documentation system.

Result and Discussion

Evaluating Quality of the DNA Yielded

Gel electrophoresis was used in this experiment to prove if there was any DNA trapped in it. The concentration of the agarose gel used in this study was 1.5% and this was determined and decided to prevent the gel from overheating and melting. Using the voltage of 45 - 50 Volts, the gel was run for 2 hours. This low voltage prevented the gel from overheating and melting, thus making the band resolution to improve (Barril, and Nates, 2012). The result obtained showed that there was DNA presence in all samples except from chocolate confectioneries. The quality of the isolated DNA was evaluated based on the intensity of the band on the agarose gel. The result showed variation in the intensity of the band.

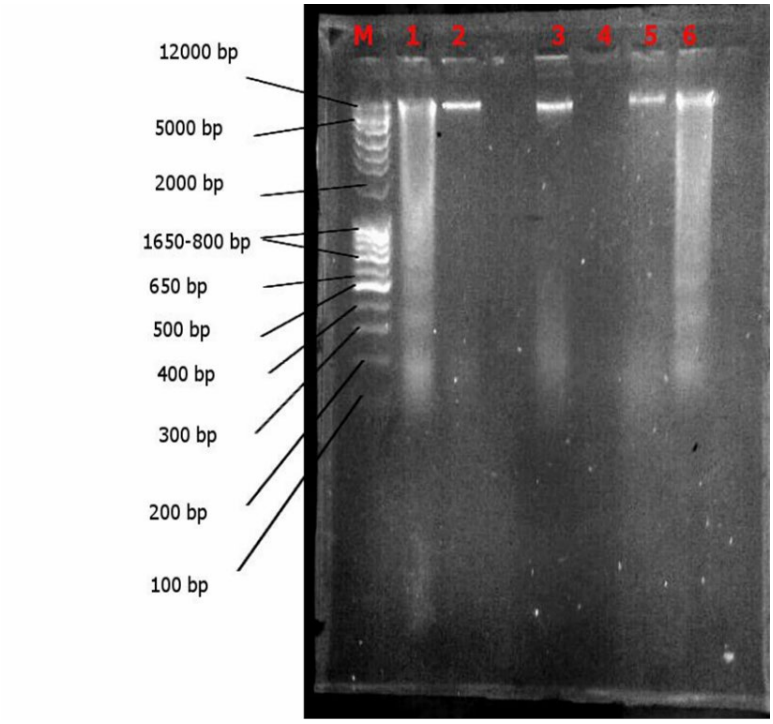

Figure 1 1.5% agarose gel electrophoresis of six frozen food samples using modified method. M: 1 kb DNA ladder, Lane 1: Purnama Beef patty, Lane 2: Azmi Meat balls, Lane 3: Ramly Beef patty, Lane 4: Crab Filaments, Lane 5: 1Malaysia Beef patty, Lane 6: Purnama Chicken patty

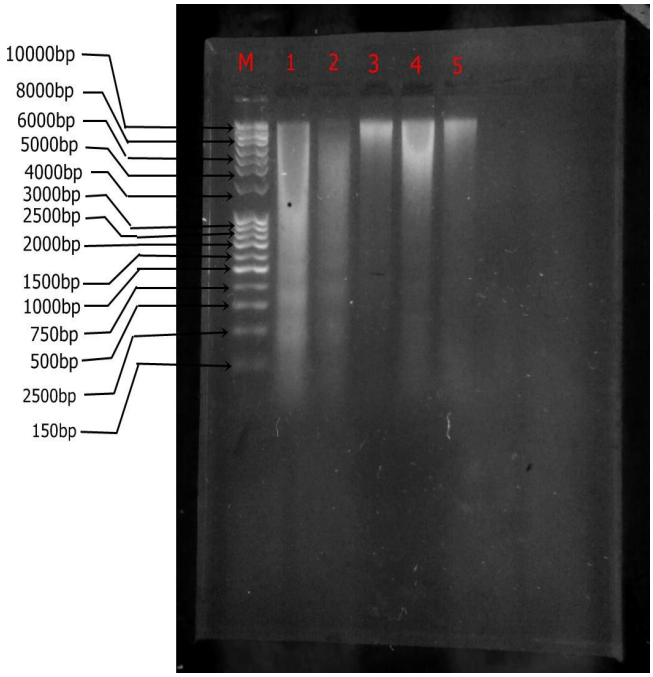

Figure 2 1.5% agarose gel electrophoresis of DNA extracted from five brands of chicken sausages. M: 1 kb DNA ladder, Lane 1: Easy, Lane 2: Jodi, Lane 3: Jimat Fiesta, Lane 4: Biffi, Lane 5: 1Malaysia.

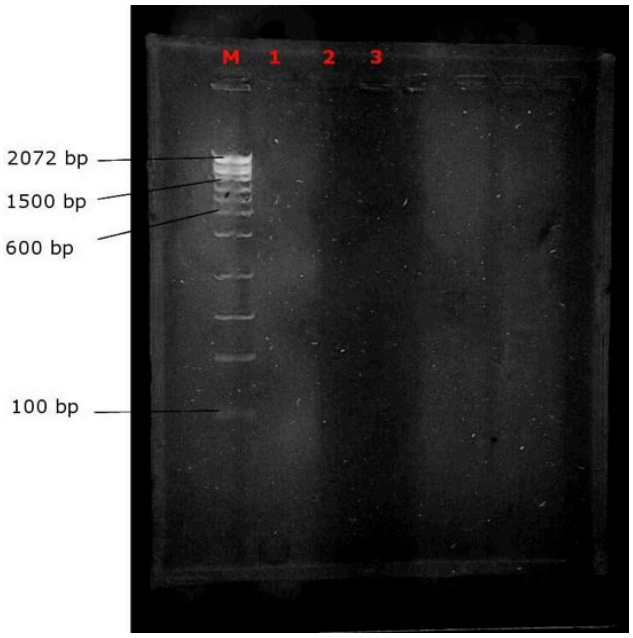

Figure 3 1.5% (w/v) gel electrophoresis of chocolate confectioneries. M: 100 bp DNA Ladder, Lane 1: Cadbury Black Forest Raisin Chocolate bar, Lane 2: Toblerone Chocolate Candy, Lane 3: Cadbury Dairy Milk Chocolate bar

Figure 1 shows the gel electrophoresis run at 45- 50 Volts for 1 hour and 45 minutes. All of these samples show positive presence of DNA, although perhaps the DNA obtained were probably degraded. The DNA obtained in each sample showed prominent and distinct bands (Azmi meat balls, Ramly beef patty and 1Malaysia beef patty) at approximately 10 000 base pair (bp) to 12 000 bp. It might suggest that the DNA obtained was not immensely sheared and suitable for amplification (Mafra et al., 2008). Meanwhile, **Figure 2** revealed that all DNA are heavily smeared with no intact band. **Figure 3** shows no bands was formed in each lane that was loaded with the elution obtained from the chocolate confectioneries samples. Enhanced and optimized procedures failed to retrieve the DNA in the chocolate confectioneries samples. No bands produced showed negative presence of DNA, even after several testing were performed. **Table 1** shows the simplified results.

Table 1 Summarization of The DNA Extraction

Sample (25 mg)	Extraction Method	Band produced
Purnama Chicken patty	DNeasy® Blood and Tissue Kit (QIAGEN,Germany)	[+-]
Purnama Beef patty	DNeasy® Blood and Tissue Kit (QIAGEN,Germany)	[+-]
Crab Filament	DNeasy® Blood and Tissue Kit (QIAGEN,Germany)	[--]
1Malaysia Beef patty	DNeasy® Blood and Tissue Kit (QIAGEN,Germany)	[++]
Azmi Meat ball	DNeasy® Blood and Tissue Kit (QIAGEN,Germany)	[++]
Ramly Beef patty	DNeasy® Blood and Tissue Kit (QIAGEN,Germany)	[++]
Easy chicken sausage	DNeasy® Blood and Tissue Kit (QIAGEN,Germany)	[+-]
Jodi chicken sausage	DNeasy® Blood and Tissue Kit (QIAGEN,Germany)	[-+]
Jimat Fiesta chicken sausage	DNeasy® Blood and Tissue Kit (QIAGEN,Germany)	[+-]
Biffi chicken sausage	DNeasy® Blood and Tissue Kit (QIAGEN,Germany)	[+-]
1Malaysia chicken sausage	DNeasy® Blood and Tissue Kit (QIAGEN,Germany)	[-+]
Cadbury Black Forest Raisin Chocolate bar	DNeasy® Blood and Tissue Kit (QIAGEN,Germany)	[--]
Toblerone candy	DNeasy® Blood and Tissue Kit (QIAGEN,Germany)	[--]
Cadbury Dairy Milk	DNeasy® Blood and Tissue Kit (QIAGEN,Germany)	[--]

The results on agarose gel were indicated in square parentheses; [+-]: showed strong band but smeared, [-+]: showed weak band but smeared, [--]: no bands, [++]: showed strong, distinct band.

Analysis of the Result

The different intensity of the band might be due to the degradation of the DNA in the sample itself. The storage temperature could also be the factor that determines the integrity of the extracted DNA. From the results obtained, samples that showed disintegrated quality of the DNA were the ones stored in the refrigerator at an unsuitable temperature. Repeated freezing and thawing might be the reason for the degradation and fragmentation of the DNA (Thomson *et al.*, 2009). As can be seen in the Figure 1, Figure 2 and Figure 3, the 1kb DNA ladder is clear and sharp. Thus, the smearing problem might not be related to the gel concentration or the 1X TBE buffer. The other possible factor that led to this problem is using too high or improper voltage while running the gel electrophoresis (Lewis, 2001). Too high a voltage might reduce resolution and could lead to band streaking for large DNA molecules. However, too low a voltage could even lead to broadening of band for small DNA fragments due to dispersion and diffusion (Magdeldin, 2012). Another factor that contributed to the smearing could also be due to the contamination in the DNA extracted such as the presence of RNA in the samples since no RNAase was added during the process of extracting. Nevertheless, loading the gel with too much DNA could also cause a problem (Garner, Revzin, Rickwood, & Hames, 1990). Besides that, protein contamination might affect the DNA quality itself too. When DNA was extracted from the samples which were originally from poultry, the protein frequently remained in the DNA solution. Protein is tightly bound to DNA, and complete removal of protein is not always possible. Furthermore, higher temperature for lysis could also be the reason for the degradation of the DNA (Chen et al., 2010). Similarly, DNA degradation might possibly have been affected by the exposure to the DNase contained in the water bath while incubating the sample during lysis.

After all, the samples used were already exposed to several treatments; it is possible that DNA constituents in the samples were already sheared to the point of no return. Certain manufacturers might utilize radiation sterilization of meat and meat products (Brennan, 2006). Some techniques such as high-pressure processing has already been used commercially to improve the microbiological quality in certain food products. Usually the technique is applied on products that utilized raw meats from livestock. Blanching is also one of the techniques in food processing, which is almost similar to pasteurization. Blanching causes cell death, other than physical, and metabolic chaos within the cells (Brennan, 2006). It might even affect the DNA constituents in food products, which could be the explanation for degraded DNA yield. The quality of the DNA extracted from food samples is generally influenced by some factors including the grade of damage such as the depurination of the DNA and the average fragment length of the extracted DNA (Elsanhoty, Ramadan, & Jany, 2011). The presence of contaminants such as nucleases might also be the cause of the degraded DNA. Thus, it is important for DNA isolation methods to provide sufficient removing of food residues, additives and preservative, which might interfere in the subsequent processes. Using the modified methodology that was found in the literature proves to yield better results than the manufacturer's procedures. Hence, the results obtained from the manufacturer's procedure are not shown in the result section. There is a need to modify the procedure accordingly since the kit used in this study specializes in animal tissues and blood. The genomic DNA extracted by using commercial kits was normally degraded and appeared on the gel as faint bands (Pirondini *et al*., 2010). The conventional methods such as CTAB and Tween based method usually make more stable DNA yield and hence, producing strong and distinct bands on gel electrophoresis (Pirondini *et al*., 2010). The extracted DNA that was used afterwards for the gel electrophoresis showed more definitive and distinct bands with less smearing. This proves that unregulated storage temperature would disrupt the DNA constituents and hence, degradation of the DNA would occur. Some of the samples might contain inhibitors that prevent the gel electrophoresis to run perfectly. These inhibitors should be eliminated and removed from the samples during the process of the isolation of the DNA. Lysis factor could also be one of the reasons. Failure to lyse the tissue membrane to access the DNA inside of the tissue sample is also one of the reasons on the negative DNA presence on the gel electrophoresis. The presence of carbohydrates in the samples might also affect the band migration on the gel (Mafra et al., 2008). Hence, it is important to improve the washing of the spin column in the DNA isolation procedures. Some traces of carbohydrates might be present on the filter column and was forced to elute together with the DNA during the elution step. Enhancing the procedures might actually not only limit one, but all steps. Unsuitable protocols and procedures could also be one of the reasons. Several researchers have compared many protocols for DNA isolation of foods. CTAB protocol has been proven to perform well in obtaining and retrieving the DNA in processed food samples such as chocolates and biscuits (Gryson, Messens & Dewettinck, 2004). CTAB protocol shows the best performance in isolating the DNA in highly processed food samples, when compared to other commercial kits, which explains the negative presence of DNA in the chocolate confectioneries.

Conclusion

To conclude, isolation of DNA especially in heavily processed food products requires several optimization and improvisation to the procedures. The DNA in the food product samples already underwent destructive process that affected its constituents and integrities. The methodology of retrieving fragmented DNA in the food samples should be enhanced and optimized along with the development of molecular biological technology. Obtaining the

intact DNA is the first step in the DNA based species identification in food products. Hence, it is important to optimize and improve the DNA isolation method to be on par with the food processing technologies and techniques.

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Conflict of interests

Author hereby declares that there is no conflict of interests with any organization or financial body for supporting this research.

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