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Impact of Meat Processing on DNA Quantity, Purity, and Integrity in Commercial Pork Products Assessed by TaqMan qPCR

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Abstract *- TaqMan qPCR analysis for detecting adulteration in processed meat products requires DNA with a high quantity, purity, and integrity. This study aims to evaluate the quantity, purity, and DNA integrity of various commercial pork products focusing on their ability to amplify the Cyt b gene. Fresh pork was used as a positive control, while five processed products - jerky, floss, meatball, canned corned, and smoked pork - were extracted for analysis. The DNA concentration and purity then measured, visualized using gel electrophoresis, and subjected to qPCR amplification. Statistical analysis of DNA concentration, purity, and ct value was carried out using one-way ANOVA followed by the Tukey post hoc test. The results showed significant differences in concentration, purity, and Ct values between all samples and positive controls ($P<0.05$). Meanwhile, the Tukey test showed that all samples and fresh pork concentration differed significantly ($P>0.05$) except between canned corned and fresh pork ($P<0.05$). The purity was significantly different between the positive controls with pork meatball and smoked pork ($P<0.05$), while the Ct value of all samples and positive controls were significantly different ($P<0.05$) except for fresh pork and jerky ($P>0.05$). All the commercially processed products experienced DNA fragmentation. Meat processing affects DNA concentration, purity, genomic integrity, and the amplification ability of qPCR.*

Keywords: *DNA, pork, processed meat products, qPCR*

INTRODUCTION

A brief identification of meat species in food products is essential to authenticate, support safety, and prevent food adulteration (Kang et al., 2020) by protecting consumer health, economy, and religion (Piskata et al., 2019). The adulteration of pork in processed meat products requires surveillance in Indonesia because most citizens are Muslim. The products are prone to counterfeiting practices because the shape cannot be physically recognized after processing. The products use three essential preservation technologies on an industrial scale: drying, preservation, and smoking (Seman et al., 2018). These processes involve heating during production, which results in protein denaturation. Hence, the protein-based species identification method is limited (Kim et al., 2016). The PCR technique is an option for species identification analysis because it is DNA-based and has higher thermal stability than protein (Zia et al., 2020). The success of the analysis using this technique requires DNA evaluation, including the quantity (Kang, 2019) and quality (Suadi et al., 2020).

The heating technique of raw or processed meat products affects the quantity, quality, and amplification ability of conventional PCR (Piskata et al., 2019) and Evagreen qPCR (Dinurrosifa et al., 2020; Rahayu et al., 2020). Furthermore, it significantly affects the quantity and purity, resulting in genomic DNA degradation (Piskata et al., 2019; Dinurrosifa et al., 2020; Rahayu et al., 2020). However, the fragments can still be amplified by PCR even though the intensity of DNA bands produced is weak (Piskata et al., 2019), and a high ct value is obtained (Rahayu et al., 2020). There is limited information on the use of Taqman qPCR in evaluating the effects of heating on processed meat products. The Taqman qPCR technique is more sensitive and specific than conventional PCR and qPCR dye-based (Ali et al., 2012).

This study aims to analyze the DNA extracted from several commercial pork processed products to evaluate their quantity, purity, and integrity on the amplifiability of the Cyt b gene in Taqman qPCR. It is hoped that this research can add information about the quality and quantity of DNA from processed meat products to decide the type of extraction kit, primer, and probe used to detect pork DNA fragments in other product.

MATERIALS AND METHODS

The study began with samples collection, DNA extraction, analysis of DNA purity and quantity, DNA integrity testing, and DNA amplification using Taqman qPCR. One way Anova was used for statistically analysis, followed by Tukey honestly significant difference (HSD) test.

1. Collection of samples

Samples used were five processed pork products: jerky, floss, meatballs, canned corned, and smoked pork. The jerky, shredded, and meatballs were obtained from the same brand, while others were not. Furthermore, fresh pork was used as a positive control. The samples and control were obtained from the online marketplace.

2. DNA extraction

The DNEasy Mericon food kit (Qiagen, Germany) was used to extract each sample and control. Each processed product and control were extracted in five replicates. Weigh 2 g product and add 25 µL proteinase K and 10 ml food lysis buffer. The solution was vortexed for 30 seconds to go incubated at 60°C for 30 minutes and shaking on mode 15 (Genie, USA). Furthermore, the mixture was cooled at room temperature (25-27°C) for 10 minutes before in the fridge at 8°C for 10 minutes. It was also centrifuged at 2500 G x for 10 minutes. A total of 700 µL of the clear top solution was transferred to a 2 mL centrifuge tube, then add 500 µL chloroform. It was vortexed for 30 seconds and carried centrifuged as 14,000 x G for 15 minutes. Transfer 350 µL clear top solution to a 2 mL tube, then add 350 µL PB buffer and vortexed for 30 seconds. It was next sent to a spin column and centrifuged as 14,800 x G for 3 minutes. Replaced the tube with a new one, add 700 µL buffer AW2 and centrifuged at 14,800 x G for 3 minutes. The column was transferred to a 2 mL tube and centrifuged at 14,800 x G for three minutes. The column move to a new 2 mL tube, and 100 µL buffer EB was added. The column was incubated for 5 minutes at room temperature (25-27°C) and as being centrifuged at 14,800 x G for 3 minutes. Furthermore, when the DNA solution isn't used immedietly DNA can be stored at -20°C.

3. Assessment of Quantity and Purity DNA

A UV spectrophotometer (Nanodrop one, Thermo Scientific) was applied to measured extracted DNA's concentration and purity. The measurement based on the methods described by Desjardin and Cocklin (Desjardins & Conklin, 2010). The ds DNA module measured 1.5 µL of DNA. The concentration measured at the 260 nm, and the purity was calculated based on the absorbance ratio at 260 nm and 280 nm.

4. Visualization of DNA integrity

DNA integrity testing was carried out by electrophoresis according to the method reported by Lee et al. (2012) with minor modification. The extracted DNA was electrophoresed on a 1% agarose gel of 1x TAE buffer and dyed with sybrsafe 1µg/100 mL at a constant voltage of 100 for 30 minutes. The resulting fragments were assesed by gel documentation (Biorad, USA), and the image lab software was used for image processing.

5. Amplification of Cyt b gene using Taqman qPCR

The pork Cyt b primer and probe used were designed by Tanabe et al. (2007). The sequences of the primer and probe are shown in Table 1. Both were obtained from Applied Biosystems, USA. The qPCR reaction volume was 25 µL, with 22.5 µL on qPCR reagent mix and the DNA template. The mixture contained 12.5 µL for 1x Taqman universal fast master mix, 0.75 µL in primer and reverse (10 µM each), 0.5 µL in probe (10 µM), and 8 µL nuclease-free water (Tanabe et al., 2007). All qPCR test were performed with a positive control and nuclease-free water as a non-template control (NTC). The qPCR was carried out in a 7500 Fast real-time PCR system (ABI) with the quick thermal cycling profile begin at 50°C for 2 minutes, denaturation at 95°C for 10 minutes, annealing at 95°C for 15 seconds followed by 45 cycles, and extension 60°C for 60 seconds. The 7500-software version 2.3 was used for data collection and processing.

Table 1. Sequences of primer and probe (Tanabe et al., 2007)

	Sequences
Primer forward	5'-CTTGCAAATCCTAACAGGCCTG-3'
Primer reverse	5'-CGTTTGCATGTAGATAGCGAATAAC-3'
Probe	5'-(FAM)-ACAGCTTTCTCATCAGTTAC-(NFQ)(MGB)-3'

6. Data analysis

They were statistically analyzed with the one-way ANOVA test and the Tukey honestly significant difference (HSD) post hoc test with a 95% confidence level using statistical products and service solutions (SPSS) version 23.

RESULT AND DISCUSSION

1. DNA quantity and purity

Table 2 shows the average DNA yield and purity. All processed meat product concentrations differed significantly, except for canned corned and fresh pork. The heating process conducted on commercially processed products resulted in a higher concentration than fresh pork because the process disrupted cell membranes, allow more to be released from the cells. The provision of non-meat additives to these products can be a source of DNA. However, differences in processing resulted in various effects on the concentration. Canned corned pork had the lowest average value of DNA concentration because its processing involves heat and pressure. This process changes the DNA's double strands into singles due to the breakdown of hydrogen bonds and damage to the heterocyclic DNA ring. Hence, the absorption of UV light at 260 nm decreases (Lee, 2017). Rokhim et al. (2021) and Rahayu et al. (2020) reported that the concentration of extracting DNA from canned corned beef was 13.41 ng/µL and 16.17 ng/µL, respectively.

Table 2. Results of DNA Processed Pork Products

Sample no	Sample type	DNA concentration	DNA purity	Ct value
1	Fresh pork	30.67 ± 4.73 ^{a}	1.91 ± 0.05 ^{b}	15.15 ± 0.34 ^{a}
2	Caned Cornet pork	23.73 ± 3.52 ^{a}	1.91 ± 0.03 ^{b}	19.15 ± 0.43 ^{d}
3	Pork jerky	44.86 ± 3.00 ^{b}	1.91 ± 0.02 ^{b}	15.46 ± 0.18 ^{a}
4	Pork floss	54.16 ± 1.69 ^{c}	1.86 ± 0.04 ^{ab}	21.30 ± 0.36 ^{e}
5	Pork meatballs	95.92 ± 5.05 ^{d}	1.83 ± 0.01 ^{a}	16.80 ± 0.15 ^{b}
6	Smoked pork	62.43 ± 5.89 ^{c}	1.82 ± 0.01 ^{a}	18.01 ± 0.17 ^{c}

Note: $^{a-e}$mean Tukey's test shows significant variation ($p<0.05$) for subscript value

Other than canned corned pork, commercial pork processed products have a higher average concentration. They are significantly different from fresh pork because the process used was not the same as canned corned and added more additives. Also, Meatballs had the highest average concentration value because they contain additional ingredients such as spices and tapioca flour as a source of more DNA than others that only contain herbs.

According to Table 2, the average purity showed variety. The results showed that the DNA was pure, indicating the successful removal of contaminating molecules and low contamination of protein and aromatic compounds (Zhao et al., 2018). The purity was determined by measuring the ratio absorbance at a wavelength of 260 nm and 280 nm (A260/A80). A prescence of aromatic rings on their constituent amino acids, such as tryptophan, tyrosine, and phenylalanine produced the protein absorbance at 280 nm, while the largest absorbance by nucleotides occurred at 260 nm (Purwanto 2014). The average absorption value at 280 nm was 0.33, 0.25, 0.47, 0.58, 1.05, and 0.69 for fresh, canned corned, pork jerky, floss, meatballs, and smoked pork, respectively.

The DNA purity of smoked and pork balls was lower than other samples because the UV light absorption at 280 nm for bacon and meatballs was more significant than fresh and processed products. The high absorption of UV rays is caused by aromatic compounds, such as polycyclic aromatic hydrocarbons produced during the smoking process (Racovita et al., 2020), while pork meatballs are caused by the presence of other aromatic compounds from their additives.

2. DNA integrity

For appropriate DNA isolation, the integrity DNA from genomes was seen and examined using electrophoresis. The integrity of positive control and processed products shown in Figure 1. In contrast to fresh pork, all the processed pork product's genomic DNA was broken apart. The DNA band showed a variety of size fragmentation, from more than 1 kb to 250 bp. Canned corned and floss had a heavy process of damaging their DNA. Therefore, their fragmentation pattern was similar, and their band hung down about 250 bp in size. Rahayu et al. (2020) showed an invisible band in canned cornet pork and canned corned beef, while Fakhry et al. (2021) showed fragmented DNA in canned sausage. The temperature processing resulted in the physical degradation or denaturation of DNA. The mechanism of destruction is based on depurination or deamination.

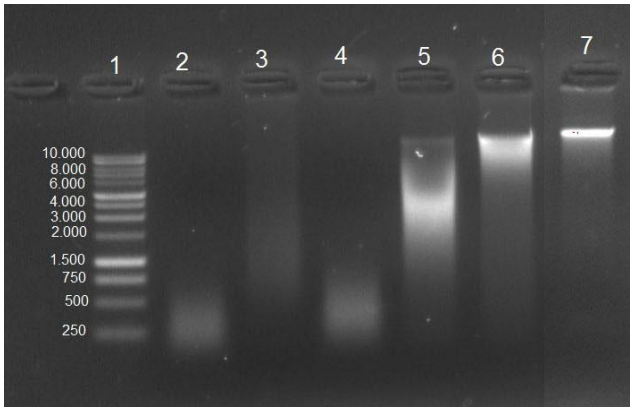

Figure 1. Total DNA electrophoresis: 1. DNA ladder 1 kb, 2. canned corned pork, 3. pork jerky, 4. pork floss, 5. pork meatballs, 6. smoked pork and 7. positive control

3. Amplification of Cyt b gene by TaqMan qPCR

Figure 2 showed that all commercially processed and fresh products amplified the Cyt b gene, while NTC did not. The Ct value obtained is shown in Table 2. There are significantly

different values for all processed products and positive control except for jerky and positive control. The amplification plot showed that the positive control and jerky curves coincided while the others were separated. The jerky processing may not injure DNA. As result, its Ct value did not differ substantially from that of fresh pork. The pork jerky is processed using semi-dried conditions at 70°C temperature (Kim and Kim, 2017). Therefore, the process does not delay PCR amplification due to degradation (Amaral et al., 2017). Although the results of electrophoresis analysis showed DNA smear bands, there was still a good target fragment size available for PCR amplification. The smear banding pattern indicates partial DNA degradation.

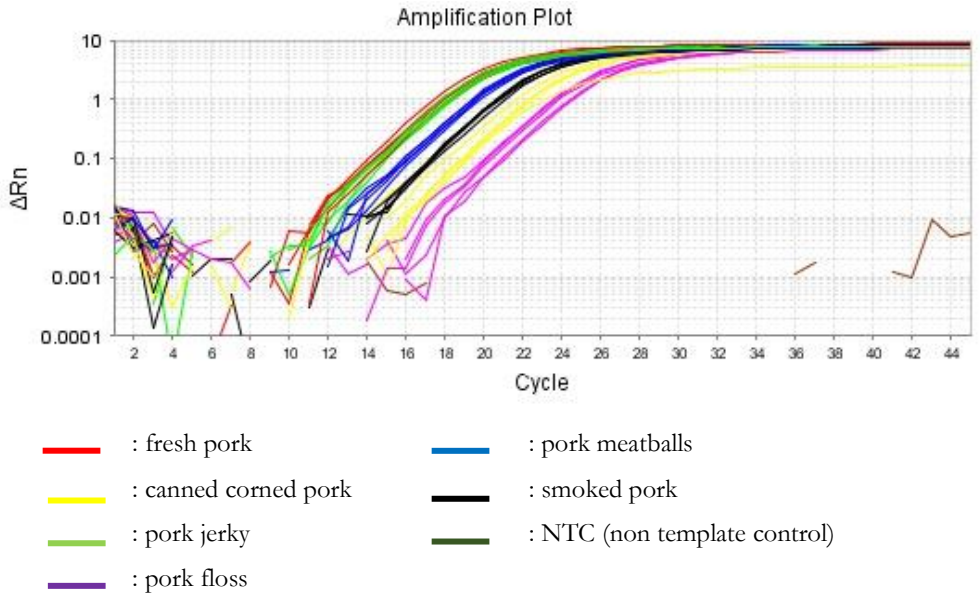

Figure 2. Amplification curves of TaqMan qPCR detection for pork

All processed products had significantly different Ct. It may lead to their differences in processing which causes damage to the fragments. The thermal process decreased the sensitivity owing to degradation with consequence difficulties for its amplification (Amaral et al., 2017). The pork jerky had the lowest Ct value, because the process does not cause damage to the DNA, so there is no delay in amplification. The floss using three different heating methods: boiling, drying, and frying (Melia & Lee, 2017) had the highest Ct value. The process causes DNA degradation and makes it difficult to amplify, so the Ct value increases (Gryson, 2010).

Meanwhile, the canned corned process involves heat and pressure, but their Ct value was lower than floss. It may cause the pork to floss less than in canned corned. Based on the labels, floss contain several ingredients such as spices as DNA sources. Therefore, amplification ability is the best indicator for DNA quality, although A260/280 is also important (Piskata et al., 2017).

CONCLUSION

Meat processing influences affects DNA amount, purity, and genomic integrity as well as amplifies the pork Cyt b gene in TaqMan qPCR. Therefore, further studies are needed to evaluate the DNA of pork content in processed meat products for label verification.

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