Diet Halalan toyyiban: Porcine blood plasms Detection in chicken meatball by Conventional polymerase chain reaction (PCR) analysis

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Abstract

The addition of blood plasm in meatballs become an issue and concerned by Muslim's consumers in Malaysia due to its uncertain Halal status on that product especially on non-Halal logo product certified by Department of Islamic Development Malaysia (JAKIM). Although the quantity of animal blood plasma consumption is low, its effect on the elasticity and structure of meatball are good. This study was conducted to detect the presence of chicken and porcine DNA in chicken meatballs by conventional polymerase chain reaction (PCR) assay targeted mitochondrial DNA (mtDNA) on chicken and short interspersed nuclear element (SINE) on porcine genome. Meatballs spiked with 1.0% (w/w) and 5.0% (w/w) porcine meat and gelatin, respectively, were prepared and heat-treated using five (n=5) cooking methods: boiling, pan-frying, roasting, microwaving and autoclaving. A pairs of mtDNA and SINE primers were targeted in short sequences using polymerase chain reaction (PCR) analysis, producing 129 and 161 bp amplicons, respectively. Electrophoresis analysis showed positive results for chicken and porcine DNA at 1.0% and 5.0% for both chicken meat and blood plasma for all of the different cooking techniques. In conclusion, in the presence study demonstrated the PCR analysis using species-specific primers was very useful for the detection of porcine DNA in heat-treated meatballs.

Keywords: Halal toyyiban; Blood plasm; chicken meatballs; polymerase chain reaction (PCR)

Introduction

Islam has emphasized its followers to choose wholesome, clean and Halal foods in their daily lives. Muslims nowadays are exposed to various kinds of ingredients and manufactured foods, arising from the advancement of science and technology which extend of their concerned about food ingredients and additives produced by food industries in their daily use.

In Malaysia, the chicken meatballs are typically prepared using chicken meat and referred as *bebola ayam*. It being added in Malaysia cuisine such as soup, curry, fried rice, and vegetables.

The potential of blood plasma being added into various meatballs are greater due to plasma protein is a useful ingredient which has been reported able to enhance the gel strength, high protein and iron content and used as fat substitute in fat-reduced meat products (Jack and Yun-Hwa 2012). Plasma protein derived from blood is prohibited for Muslim consumers whether it comes from halal animal or non halal animal (haram).

In the present study, we prepared chicken meatballs from a mixture of finely ground chicken meat and spiked with 1% and 5% (v/w) porcine blood plasma. Those chicken meatballs were heat-treated using five (n=5) cooking methods: boiling, pan-frying, roasting, microwaving and autoclaving. The DNA chicken meatballs which have been heat-treated will be extracted and subjected to PCR analysis using species-specific primers.

Materials and Methods

Samples for analysis

Different types of meatballs were prepared and spiked with 1.0% (v/w) and 5.0% (v/w) porcine blood plasm, respectively. A commercial Pig Genomic DNA (Novagen®, Germany) and extracted chicken's DNA from chicken meat were used as a positive control.

Meatball preparation and treatment

A total of two (2) types of meatballs with a different percentage of porcine blood plasm were prepared according to a formulation as listed in Table 1. Meatballs were made according to basic formulations, according to Azhana (2011). Finally, all of the meatballs were packed in Ziploc® plastic bags and stored in a freezer at -20°C until used.

Table 1 basic ingredients for meatball which added with meat or porcine gelatin.

Ingredients	**Percentages of porcine meat or blood plasm**	
	1.0% (w/w)	5.0% (w/w)
Minced chicken meat	69.0	65.0
Blood plasm	1.0 (v/w)	5.0 (v/w)
Shortening	5.0	5.0
Isolate soy protein (ISP)	4.5	4.5
Sodium triphosphate (STPP)	0.3	0.3
Potato starch flour	3.6	3.6
Black pepper	0.1	0.1
Salt	1.5	1.5
Sugar	2.0	2.0
Ice cubes	13.0	13.0

Meatballs spiked with 1.0% and 5.0% (v/w) porcine blood plasm were further treated with five (5) different cooking methods – boiling, pan-frying, roasting, microwaving and autoclaving –

based on a study by Arslan et al. (2006), with a slight modification in which the temperature and time were adjusted. Table 2 summarizes the methods for each treatment.

Table 2 Method used for meatball treatments

Heat treatments	Condition	Temperature (ºC)	Time (min)
Boiling	Boiling water with 2.0% (w/v) of salt	90-95	5
Pan frying	Vegetable oil	-	5
Roasting	Conventional oven	180	15
Microwaving	Medium level	-	5
Autoclaving	Temperature-resistant container, containing 250 ml of hot water and 2.0% (w/v) of salt	121	20

DNA extraction

All of the meatballs were minced, and a total of 300 mg of each type were transferred into a 2.0-ml sterile microcentrifuge tube. The DNA was extracted using a Qiagen Blood and Tissue Kit (Germany) according to the manufacturer's instructions and quantified using a MaestroNano® Spectrophotometer (MaestroGen, USA). The DNA was then stored at -20ºC until further analysis.

Oligonucleotide primers

The oligonucleotide primers targeting mitochondrial DNA (mtDNA) regions of cytochrome b (Aravindran, 2015) for chicken and oligonucleotide primers targeting nucleus DNA (nDNA) regions of short interspersed nucleus element (SINE) (Calvo et al., 2001) were used in the PCR assays. The sequences of those primers were cyt b (F), 5'-5'- CCT AAC TTG ATT CAC CTT CTC TCT GC -3' and cyt b (R), 5'- GAA GCT TAG GTT CAT GGT CAG GT -3'; and SINE (F), 5' – GGA TCC GGC ATT GCC GTT AG- 3'and SINE (R), 5' – GTC TTT TTT TGC CAT TTC TTG G- 3'. All of the mtDNA and nDNA primers were synthesized and supplied by First Base Laboratories Sdn. Bhd. (Selangor, MY).

PCR amplification

The PCR simplex amplification technique using the cytochrome b primers targeting mtDNA of 129 bp for chicken was conducted as described by Aravindran (2015). While for porcine DNA, method described by Calvo et al. (2001) was used to amplify 161-bp target gene of short interspersed nucleus element (SINE).

Results and Discussion

In the present study, we added the fresh blood plasm in the chicken meatballs. Two different concentration of blood plasm were applied; 1 % (v/w) and 5 % (v/w). Both concentration of chicken meatball were undergone 5 different treatments. As shown in Figure 1 and Figure 2, using *cty* b primers for chicken meat detection, both concentrations of chicken meatballs were able to detect the presence of chicken DNA. Similar observation was also shown by SINE primers (Data for 1 % of is not shown).

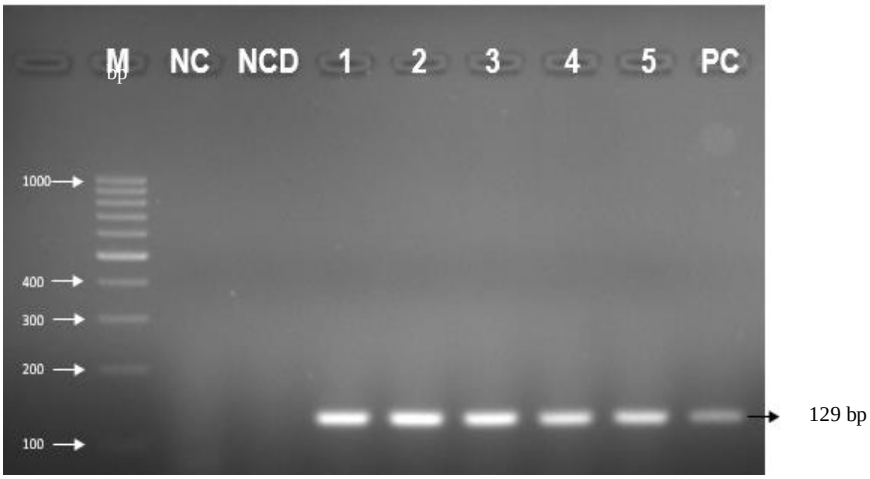

Figure 1 Amplicons of cytochrome *b* gene fragments (129 bp) on 5% (w/v) chicken meatball with different heat treated on 3% (w/v) agarose gel electrophoresis. M: Marker 100 bp DNA ladder, NC: Negative control, NCD: Negative control for DNA extraction, 1: Autoclave, 2: Microwave, 3: Boiling, 4: Roasting, 5: Frying, PC: Positive control (129 bp)

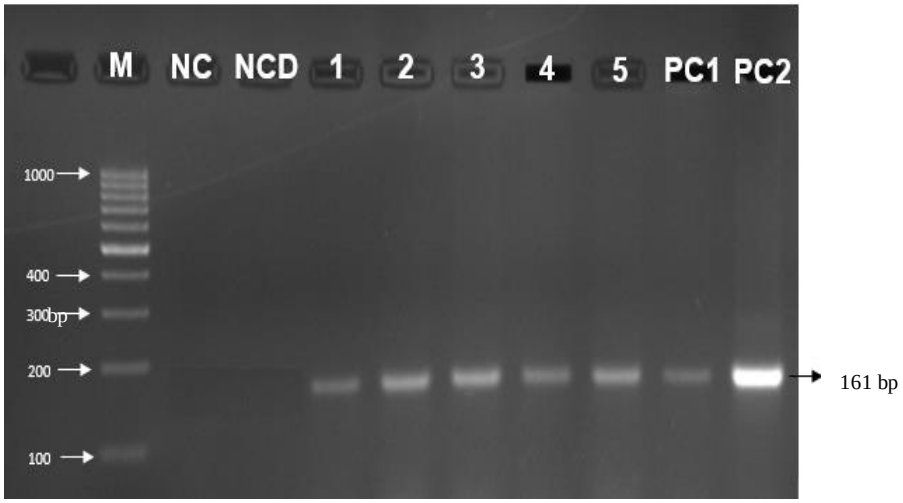

Figure 2. Amplicons of short interspersed nuclear element (SINE) gene fragments (161 bp) on 5% (w/v) of chicken meatballs added with 5% (w/v) porcine blood plasm on 3% (w/v) agarose gel electrophoresis. M: Marker 100 bp DNA ladder, NC: Negative control, NCD: Negative control for DNA extraction, 1: Autoclave, 2: Microwave, 3: Boiling, 4: Roasting, 5: Frying, PC1: Positive control, PC2: Positive control (161 bp)

When both primers were used as multiplex PCR, the SINE primers failed to produce amplicon in 1% chicken meatball concentration, but not for 5% concentration. Both primers were able to produce amplicons in 5% (v/w) meatballs and both bands were shown up in 3% (w/v) gel electrophoresis.

Conclusion

The SINE primers were not be able to detect porcine DNA in 1% (v/w) meatballs when these primers were multiplex PCR with *cyt* b primers for chicken. In the presentation study, it was demonstrated that the multiplex PCR analysis is useful to detect DNA from blood source in meatball with higher concentration (5 %).

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