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Species DNA Detection Using PGR Gene Genetic Markers in Chicken Nuggets

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***Abstract*— Species DNA detection using genetic markers of the PGR gene on chicken nuggets was carried out to see whether these genetic markers could be used to test species DNA detection in chicken nuggets food products. The test method used in this study is a real-time PCR test method using the SYBR green technique where the test results can be either Ct or Tm values which indicate amplification or detection. The results of DNA isolation showed that the concentration and purity of the isolated DNA were in the range of 35.10 ng/µL – 36.10ng/µL with an average of 35,488 ng/µL. As for the purity value measured at the A260/A280 wavelength, the results were obtained with a purity range between 2,110 – 2,250 with an average of 2,165. For the results of real-time PCR amplification, the Ct value of the chest sample was at 24.50, the Ct LOD value was at 21.20 and the Ct value of the positive control was 27.10. For the Tm value of the busty sample at 81.10, the Tm LOD value at 81.20 and the positive control Tm value at 82.10. In a conclusion, in this study, genetic markers of the PGR gene can be used to test specific DNA detection of chicken species in chicken nugget products.**

***Keywords*— Chicken, DNA, Nugget, PCR**

I. INTRODUCTION

Detection of species DNA in chicken meat processed food products is an analytical method that continues to develop according to advances in science and technology, where initially the test method only used endpoint or conventional PCR and then switched to real-time PCR. The challenge that then arises in the molecular analysis is how to provide specific and more varied test primers so that the choice of methods and primers used becomes more varied. With the many methods and varieties of primers used, it will certainly make the process of testing this product easier to do.

There are several common genetic markers that are often used to perform DNA detection tests for chicken species, including Co-1. [1-4], Cyt b [5,6] and D-Loop genetic markers [7]. These various genetic markers are of course primers developed from species DNA testing in research in the field of molecular biology, where the primers used to detect animal species are integrated to detect the presence of DNA in processed food products that use animal meat as a source of raw materials.

One of the genetic markers that can also be considered in carrying out DNA detection tests for chicken species in nugget processed food products is the progesterone receptor (PRG) gene. This gene in molecular studies is often carried out to show changes in progesterone signalling (through the PGR receptor) [8]. The progesterone receptor gene is a gene that is associated with the muscle mass transcription system so that studies of this gene can be used to see the productivity of chickens as pets that are used as a source of animal protein.

Therefore, this study was conducted to provide information regarding references to the use of other genetic markers other than Co1, Cyt b or D-Loop in detecting DNA of chicken species in food products so that in the future it can be used in similar research or in the authentication test of processed-based food products. chicken meat.

II. MATERIAL AND METHODS

A. Material

The materials used in this study were chicken nuggets, Proteinase K and DNA/RNA patho-gene Spin isolation kits.

B. Dna Isolation

DNA isolation was carried out by weighing 0.5 g of the sample and then placing it in a 2 ml centrifuge tube. Add 1 mL of lysis buffer and 20 µL of Proteinase K then incubated at 70 C for 2 hours. Remove the sample from the incubation then continue with a centrifuge for 10 minutes with a centrifuge rotation speed of 14,000 rpm. In the centrifuge tube, 2 layers of pellets and supernatant will be formed, take the supernatant by pipetting it and transfer it to the 2 mL centrifuge tube then discard the pellets in the tube. To the supernatant in the 2 mL centrifuge tube, 750 µL of chloroform was added then centrifuge for 20 minutes at a speed of 14,000 rpm, then

pipette the solution that was on the top layer into a new 2 mL centrifuge tube and added 750 µL of binding buffer VB and incubated for 2 minutes at room temperature. The vortex then puts all of the liquid in the 2 ml centrifuge tube gradually until it runs out into the spin column and centrifuge. After all the liquid runs out, enter and centrifuge, then add 500 µL RW1 wash buffer and centrifuge again for 1 minute with a rotation speed of 14,000 rpm. Add 700 µL of RW2 wash buffer then centrifuge again for 1 minute with a rotation speed of 14,000 rpm. Transfer the spin column into a new 1.5 mL centrifuge tube then add 70 µL of elution buffer (EB) then centrifuge with a rotation speed of 14,000 rpm for 1 minute. Discard the spin column and the remaining liquid in the 1.5 mL centrifuge tube is the isolated DNA that will be measured for purity and concentration before use.

C. Purity and Concentration Analysis

Purity and concentration analyzes were carried out using a nano photometer NP80 (IMPLEN), the method setting; Nucleic acid, dsDNA type, nano volume mode, 2 µL sample volume, nucleic acid factor 50.00, background correction 320 nm, air bubble recognition off, manual dilution factor 1.000

D. Primer Setup

The PGR primers used were designed from the NCBI site with the primary sequence F: GAA GTC AGA CAG GTG CCG AA, and the primer sequence R: CCA GCA CGC ATC AAA ACA CA with a primary sequence length of 114 bp.

E. Master Mix Setup

Pipette 10 µL of real Q plus 2x master mix, then add 1 µL of forward and reverse primers each and add 2 µL of PCR grade H2O, then input 7 µL of template DNA. Spin down the master mix and the DNA template that has been mixed into the PCR tube for 1 minute. After that, the sample is ready to continue with the PCR stages [(9)].

F. Real-Time PCR Setup

Real-time PCR settings use a two-step method: Pre-denaturation at 95 ^{o}C for 15 minutes for 1 cycles, denaturation at 95 ^{o}C for 30 seconds for 40 cycles, and annealing at 60 ^{o}C for 60 seconds for 40 cycles. After that, proceed with setting the melt curve analysis: ramp from 50 ^{o}C to 90 ^{o}C, hold for 90 seconds on the 1st step and hold for 5 seconds on next steps. Interpretation of Results [(9)].

G. Controls Used in Testing

The control used in this test is positive (control used as a reference in determining positive results); Negative control (the control used as a reference is to determine negative results and as a reference to see if there is contamination in a series of testing processes. NTC control (no template control) (control is made to see whether the master mix used is contaminated with target DNA or not) Extraction control (control made to see whether contamination occurs in the extraction process or not) As well as 2 types of control that are often used invalidation or verification processes, namely specificity control (control made to see whether the primer used is specific), this control is carried out by entering non-target DNA as a template, and control LOD (a control made to see the tool's detection ability on smaller target DNA and templates) LOD control is made by diluting the sample DNA 10 times from the DNA template used.

H. Data Analysis

Data analysis was carried out based on 2 main criteria which include: (1) Ct analysis (Cycle threshold), which is to see the Ct value of the sample (2) analysis of melting temperature (Tm), namely at what temperature the melt occurs.

III. RESULT AND DISCUSSION

A. Analysis of Isolated DNA

Analysis of the isolated DNA was carried out using a nano photometer as presented in (Table 1). From the table, it can be seen that the concentration values of the extracted samples were in the range of 35.10 ng/µL – 36.10ng/µL with an average of 35,488 ng/µL. As for the purity value measured at the A260/A280 wavelength, the results were obtained with a purity range between 2,110 – 2,250 with an average of 2,165.

Table 1. DNA Isolation Results Data

Sample	Nanophotometer Analysis	
	Concentration (ng/µL)	Purity (A260/A280)
1	35.10	2.210
2	35.20	2.195
3	36.10	2.110
4	35.20	2.150
5	35.70	2.140
6	35.70	2.130
7	35.50	2.135
8	35.40	2.250
Average	35.488	2.165

At the time of DNA isolation, proteinase K is an enzyme that has an important role. This enzyme plays a role in cell lysis so that when centrifuged the solid tissue and protein residues can settle to the bottom of the tube. Some extraction techniques also use CTAB (cetyltrimethylammonium bromide) which is a nonionic detergent to make the lysis step more efficient. included in the range of good isolated DNA [(10-13)].

B. Real-Time PCR Application Results

Based on the results of the analysis of real-time PCR data, the results are as shown in (Table 2). In the table, it can be seen that the average value of Ct and Tm for the test sample is the value of 8 repetitions of the research sample.

Table 2. Real-Time PCR Analysis Results

Treatment	Analysis	
	Ct	*Tm*
Sample	24.50	81.10
NTC Control	-	-
Negative Control	-	-
Other DNA Control	-	-
Extraction Control	-	-
LOD Control	21.20	81.20
Positive Control	27.10	82.10

In the table of research results (Table 2), in the sample, real-time PCR analysis showed amplified primers at Ct 24.50 and Tm 81.10. This shows that in the samples used in this study, the DNA of the chicken species was detected. The NTC control, extraction control and the negative control shows the undetermined value. This is a result that can be used to see the process of the stages of testing carried out to monitor whether there is DNA contamination when testing is carried out. For other DNA controls, namely the DNA of other species used in this study in the form of bovine DNA, showing undetermined values, these results can be used to see whether the primers used are only able to amplify chicken species or can amplify other species, so this control is often referred to as other DNA controls. For the LOD control and positive control, the results showed that the real-time PCR analysis was able to amplify the LOD control and positive control. Control LOD is used to see the ability of the test method used to detect small concentrations of the target DNA used for testing.

Two general methods are often used to perform real-time PCR analysis, namely the SYBR green method and the Probe method [(14)]. For the green SYBR analysis, Ct and Tm data will be used as the interpretation of the results, while the analysis using the probe will only use the Ct value as the interpretation of the results. The value of Tm in real-time PCR analysis can be influenced by the composition of the nucleotides C and G, while the value of Ct is influenced by the concentration of the DNA template used. The higher the concentration of template DNA used, the smaller the Ct value, and vice versa [(15-17)].

According to [(18),] the green SYBR method has a cheaper economic value when compared to other tests using the probe technique. This method is generally carried out with a 2 step cycling method, with Denaturation 95 ^{o}C for 45 seconds and Annealing / Extension 60 ^{o}C for 45 seconds..

IV. CONCLUSION

As a conclusion in this study, the specific DNA of chicken species was detected at a Ct value of 24.50 and a Tm value of 81.10, which means that the DNA of a chicken species was detected in a test sample of chicken nugget food products..

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