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Research Article

Detection of Species DNA in Chicken Meatball Products Using NGF Genes as Molecular Markers

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Abstract. Species DNA detection in chicken meatball products using the NGF gene as a molecular marker was carried out to see if the NGF gene could be used to test species DNA detection in chicken meatball processed food products. The test method used in this study is the SYBR green test method which is read using real-time PCR and the extraction technique used is the centrifuge column technique. The test results in this technique can be in the form of Ct (Cycling) and Tm (Melting Temperature) values. The results showed that the DNA isolation carried out showed that the concentration and purity of the isolated DNA were in the range of 51.100ng/µL – 52.300ng/µL with an average of 51.883ng/µL. As for the value of purity is the absorption value measured at wavelength A260/A280, the results are obtained in the range between 1.850 – 1.920 with an average of 1.880. The results of the real-time PCR analysis obtained the Ct value of the chest sample at 21.10, the Ct LOD value at 25.20 and the positive control Ct value at 20.30. For the Tm value of the busty sample at 78.20, the Tm LOD value at 78.80 and the positive control Tm value at 79.10. Based on the results of this study, it can be concluded that the genetic markers of the NGF gene can be used to test specific DNA detection of chicken species in processed chicken meatball products so that this test method can be used to detect species DNA.

1. INTRODUCTION

Food products made by meatballs are one type of food that is vulnerable to the misuse of food substitutes as raw materials. This type of processed food can be made from various types of meat, including beef, goat, chicken, fish and so on. The development of the world of testing species for processed products based on raw meat requires many variations of appropriate testing methods to carry out species DNA detection tests. One way that can be done to anticipate the development of testing techniques and test species variants in processed food products made from animal meat is with the right primer design so that species DNA detection tests can show a good level of sensitivity and specificity. One of the genes that want to be tested for its detection ability in detecting species DNA in

processed meatball products made from chicken is the NGF (Nerve Growth Factor) gene.

The NGF gene is a gene that has a role in the non-neural system, where this gene binds to tyrosine kinase A which is a member of neurotrophins (Bertaux *et al*., 2004). In chicken studies, in general, this gene can be used to see cell proliferation that occurs in the early stages of embryonic development (Manca *et al*., 2011).

Research on DNA detection of chicken species in meatball products was carried out by Sari *et al*. (2016), using genetic markers for the Cyt b gene. Species DNA research on food products is research that develops from species DNA research on native species, where research on chickens has used several common genetic markers in the form of the Co-1 gene. (Yacoub *et al*., 2015; Peng *et al*., 2019), Cyt b (Yacoub *et al*., 2013) and D-Loop genetic markers (Yacob and Fathi, 2013).

Based on this background, this research was conducted to provide additional information regarding the DNA testing technique of species of meatball processed chicken meat products so that it can be applied to similar research. After passing the validation/verification process first so that the method used can guarantee a more recognized test result.

2. METHODS

2.1. DNA Isolation

Isolation of DNA using a mericon food extraction kit [Qiagen] which was extracted with the Qiacube [Qaigen] automatic robotic system.

2.2. Isolated DNA Analysis

The isolated DNA was analyzed for purity and concentration using a nano photometer (Sophian, 2021; Sophian *et al*., 2021).

2.3. Primers

The primer used was the NGF gene designed from the NCBI site with a Forward primer sequence: AAA CAG CAA TGT GGT GCG TC, and a Reverse primer sequence: AAT CCG GCC ATT AGC ACA CA with a primary sequence length of 137 bp.

2.4. Master Mix Setup

The master mix used in this study is QuantiNova SYBR Green (Qiagen) whose use procedure is following the manual book in the kit.

2.5. Real-Time PCR Setup

Real-time PCR analysis using Rotor-Gene Q 5Plex with 2 steps cycling method system followed by Melt Curve analysis with the following settings: Denaturation 95^{o}C for 45 seconds and Annealing / Extension 60 oC for 45 seconds, setting melt curve method step one 72^{o}C for 90 seconds and step two 95^{o}C for 5 seconds.

2.6. Test Control Method

The test controls used are extraction control, NTC control (No Template Control, negative control, LOD control and positive control).

2.7. Data Analysis

Data analysis was carried out by conducting an average test of Ct and Tm values (Sophian *et al*., 2020; Sophian *et al*., 2021).

3. RESULT AND DISCUSSION

3.1. DNA Isolation

The DNA isolation steps in this study were carried out using a DNA extraction robot with the results as shown in (Table 1).

Table 1. DNA Isolation Results

Sample	Nanophotometer Analysis	
	Concentration (ng/µL)	Purity (A260/A280)
1	51.100	1.875
2	52.200	1.920
3	52.100	1.850
4	51.500	1.910
5	52.300	1.880
6	52.100	1.850
Average	51.883	1.880

One of the advantages of extraction techniques that use robotic or automatic extraction systems is that they are easier to use, making the extraction steps able to produce stable concentration and purity results. This can be seen in (Table 1).

According to Sophian (2021) and Sophian *et al*. (2021) good DNA isolation in food products is in the range of values from 1.7 to 2.2. These results were obtained in research conducted on processed food products of crab and salted fish. The difference in concentration and purity values when isolating DNA can be influenced by several things, such as weighing the number of different samples, the use of isolation stages and the use of a different volume of solvent from the standard method used without a verification and validation process.

In performing real-time PCR analysis, the value of isolated DNA has an important role in supporting the amplification process, but several studies have shown that DNA extraction values that are below the good concentration and purity values can still be detected (Kamau *et al*., 2013; Xu *et al*., 2015; Srisutham *et al*., 2017).

3.2. Real-Time PCR Analysis

The results of the real-time PCR analysis can be seen in (Table 2) where the data table shows the sample Ct value detected at 21.10, and the sample Tm value at 78.20. This value indicates that the test sample is detected to contain chicken species DNA. This result can be confirmed by looking at the positive control and LOD control, where the positive control was detected at Ct 20.30, the Tm value at 79.10, while the LOD control Ct value was detected at 25.20 with the Tm value at 78.80.

Table 2. Real-Time PCR Analysis Results

Treatment	Analysis	
	Ct	*Tm*
Sample	21.10	78.20
NTC Control	-	-
Negative Control	-	-
Other DNA Control	-	-
Extraction Control	-	-
Control Positive	20.30	79.10
Control LOD	25.20	78.80

In table 2 above, it can also be seen that the negative control, NTC, other DNA control and extraction control showed no detectable results. These results illustrate the use of the master mix. Extraction gaze and primary specificity used in the test are in good condition and are not contaminated or contamination occurs in the test process carried out.

In this study, the real-time method used is the green SYBR technique. This technique will produce two types of test data, namely the value of Ct and Tm. The Ct value is a value that is influenced by the amount of template concentration used, while the Tm value is influenced by the concentration of G and C content in the

primer and template used (WHO, 2016; Sophian *et al*., 2020; Sophian *et al*., 2021).

According to Cai *et al*. (2021), there are two types of real-time PCR methods, namely the probe method and the green SYBR method. When compared between these two methods, the green SYBR method has a cheaper economic value when compared to the probe method (Piknova *et al*., 2015).

4. CONCLUSION

Based on the research results, all samples detected NGF genes. It can be concluded that all samples used detected DNA of chicken species.

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