



Detection of *Listeria monocytogenes* in Frozen Meatballs Using Real-Time PCR

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Abstract — Detection of pathogenic bacteria *Listeria monocytogenes* in processed food products of frozen meatballs is one of the critical test parameters in product quality testing. The challenge in testing product quality is how to find a reliable and effective test method to generate test results data. With this research, it is hoped that it can provide information or references for similar research. The method used in this research is the enrichment method with a direct isolation technique and amplification using real-time PCR. Data analysis is based on the Ct (Cycle threshold) and Tm (Melt Curve) values. The results of the tested samples were detected at Ct 11.60 with a Tm value of 81.50. For NTC control not detected, this indicates that the master mix used is in good condition and no contamination occurred when testing the sample. The use of this NTC control plays an important role in monitoring the quality of the master mix and test run. For the LOD control and positive control, the results were detected, whereas in the LOD control, the Ct value was detected at 10.08 and the Tm value was at 81.30. for positive control, the value of Ct was detected at 10.85 and the value of Tm was at 82.0. Based on the results of the study, it can be concluded that the test method used can detect *Listeria monocytogenes* in processed meat products in the form of frozen meatballs.

Keywords — Food, *Listeria*, Meatballs, PCR, pathogen

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I. INTRODUCTION

Contamination of *Listeria monocytogenes* in food can cause "foodborne diseases" that is, diseases caused by ingestion of food contaminated with the pathogen. The disease caused by the bacterium *Listeria monocytogenes* is known as listeriosis [1]. The most common symptoms of listeriosis are diarrhoea, fever, headache, and myalgia. However, listeriosis can also cause severe illnesses such as meningitis and/or septicemia in adults, infection of the fetus and miscarriage in pregnant women, or neonatal infection [1][2][3]. By looking at the impact of *Listeria monocytogenes* in food products, a reliable and fast testing technique is one way that can be done to take preventive measures. Therefore, the *Listeria monocytogenes* testing technique began to develop from using conventional techniques to changing to molecular techniques [4].

Detection of *Listeria monocytogenes* in food products has been carried out on dairy products [5][6], fish-based snacks

[7] and meat products [8]. The development of testing techniques also gave birth to the detection technique of *Listeria monocytogenes*, which initially used conventional techniques and then developed using real-time PCR [9][10][4]. The detection test of pathogenic bacteria using the molecular method has many advantages when compared to the conventional method when viewed from the time of the test, where the average time required for testing is 50-52 hours with the PCR method carried out within 24-48 hours [4].

Based on the above background, this research was conducted to detect *Listeria monocytogenes* in processed meat products, namely frozen meatballs. The novelty aspect of this research is the testing technique that uses real-time PCR where this method is still minimal in information. With this research, it is hoped that it can provide information or references for similar research.

II. MATERIAL AND METHODS

A. Material

The materials used are frozen meatballs, NFW, Half Frazer Broth enrichment media, TSA, McFarland Standard, PCR kit using QuantiNova SYBR Green (Qiagen).

B. Sample Preparation

The sample consisted of 15 samples of frozen meat processed food spiked with positive control of *Listeria monocytogenes*. Spike was conducted to see the performance of the method used in detecting *Listeria monocytogenes*.

Weigh the sample weighing 10 g, then add 90 mL of half Frazer solution, after it is incubated for 24 hours at a temperature of 35-37 °C. After 24 hours, the sample was then scratched on TSA media and incubated again for 24 hours at a temperature of 35-37 °C. Colonies grown on TSA media are then ready to be confirmed using real-time PCR.

C. DNA Isolation Stage

This step was not carried out because it used a direct PCR technique. The results of isolation from selective media were then scratched on TSA media and incubated for 24 hours. The incubation results were then made a test suspension using NaCl and clouded equivalent to 1 Macfarland. The suspension is then used as a template. [11][12].

D. Real-Time PCR Setting Method

Amplification was carried out using qPCR (QIAGEN 5 Plex) with the following method: Denaturation for 45 seconds at 95 °C and Annealing/Extension for 45 seconds at 60 °C [4]. The primer sequences used consisted of forwarding (5'-CTA AAG CGC GAA TCT CCC TT-3'), and reverse (5'-CCA TTG TCT TGC GCG TTA AT 3').

E. Reaction Setup

The master mix used in this study found a total of 10 L consisting of 5 L master mix, 2 µL primer (1 µL forward and 1 µL reverse), 1 µL NFW and 2 µL DNA template. [4][13][11].

F. Positive Control

The positive control used was the pure culture of *Listeria monocytogenes* which had been enriched and streaked on enrichment media so that it was tilted. The colony was then taken one ode and made a test suspension using NaCl with turbidity according to the Macfarland standard.

G. NTC Control

NTC (No Template Control) is a master mix control consisting of a master mix combined with NFW. The total NTC control volume is 10 µL consisting of 5 µL master mix, 2 µL primary (1 L forward and 1 L reverse), and 3 µL NFW [14][13][15][16].

H. Data analysis

Interpretation of the results based on 2 criteria, namely the analysis of Ct (Cycle threshold) and (2) analysis of the melting temperature (Tm) [14] [13][16][17].

III. RESULT AND DISCUSSION

A. Observations on Enrichment Media

The results of observations on the half Frazer enriching medium showed that the solution turned cloudy. The observations on TSA showed white colonies. Turbidity in the enrichment media can be a reference or indicator to see the growth of bacteria in the sample.

B. Real-Time PCR Application Results

The results of the analysis as presented are shown in **Table 1**. In the table, it can be seen the average value of Ct and Tm of the test sample.

TABLE 1
SAMPLE TEST RESULT DATA USING REAL-TIME PCR

Treatment	Value	
	Ct	Tm
Sample	11.60	81.50
NTC control master mix	-	-
LOD Positive control	10.08	81.30
Positive Control	10.84	82.00

The results of the samples tested showed that the samples were detected at Ct 11.60 with a Tm value of 81.50. For NTC control not detected, this indicates that the master mix used is in good condition and no contamination occurred when testing the sample. The use of this NTC control plays an important role in monitoring the quality of the master mic and test run. Both types of controls used were detected positive with a Ct value of 10.08 and a Tm of 81.30 in the LOD control, while positive controls were detected at Ct 10.85 and a Tm value of 82.0.

C. Discussion

Detection of *Listeria monocytogenes* in frozen meatball products was carried out using the real-time PCR technique with the enrichment method, where in the early stages of the study, enrichment was carried out to optimize the growth of the target bacteria in the sample to be analyzed.

The analysis carried out in this study used the direct technique, namely the resulting sample that grew after the enrichment process was then made in suspension colonies, where the level of turbidity was equalized using the McFarland standard. The use of this equalization technique has also been carried out by [11], who in his research on the confirmation test of Salmonella bacteria, it was found that this

equalization method can be used as a reference in making DNA templates in the direct technique. This directive technique causes this research to not carry out the stages of measuring DNA quality which include concentration and purity. This direct technique has many advantages when compared to the isolation method with the extraction system, magnetic beads or boiling method.

In the direct technique, the DNA that is the target of amplification will be extracted when real-time PCR analysis is carried out, where at the denaturation stage, the denaturation temperature reaching 95 degrees will lyse the bacterial cell wall and the DNA contained in the cell will be denatured so that the DNA extraction process takes place at this stage. . Because using this direct technique, the potential for the inhibitor produced will be quite high due to the cell parts that have been destroyed and not used in the amplification process will become inhibitors in molecular analysis.

The test results using real-time PCR showed that the detected CT values were quite low in the range of 10.08-11.60. This shows that the concentration of the sample used as the template is large enough so that the detected Ct value is quite small. According to [4][12][13][14], in testing using real-time PCR, the value of Ct in the test results can be influenced by the volume of template concentration, while the value of Tm in the test results can be influenced by the content of the GC sequence.

In carrying out test analysis using direct techniques in real-time PCR analysis, it is necessary to consider the use of templates to be used, wherein the direct technique, the inhibitor potential of the template to be used is also high so that if the concentration of DNA in the target bacteria is small, the inhibitory potential of the target bacteria is low. templates can also be used as a source of investigation in the event of a failure in testing. Another thing that must also receive attention in conducting real-time PCR analysis is the potential for false positives. False positives can occur due to several factors, including the process of processing samples that are not accurate, potential inhibitors of DNA samples, and the reliability of the test method used. For this reason, this study uses controls to prevent unexpected results from the tests carried out. Research conducted by [18], showed the potential for false positives in performing real-time PCR analysis.

IV. CONCLUSION

From the results of the research conducted, information was obtained that the test method used was able to detect *Listeria monocytogenes* in the test sample. suggestions for further research is to conduct similar tests on other products so that information about the methods used can have a wide range of test matrices.

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