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Research Article

Analysis of Purity and Concentration of DNA Isolation Results on Chondroitin Samples

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Abstract. Analysis of the purity and concentration of isolated DNA is a way to see the quality of isolated DNA. This research was conducted as an initial stage to give. Information about research conducted in future research. The purpose of this research is to provide references and information regarding DNA isolation techniques in chondroitin samples so that they can be used in similar studies. The sample consisted of a sample of chondroitin which was weighed 50 mg and tested 10 times. The isolation method used in this study is the centrifuge column isolation method, while the purity and concentration analysis were analyzed using a nano photometer which was read at a wavelength of A260/A280. The value shown from the nano photometer was then analyzed statistically using the average test to determine the interpretation of the results from the data obtained. The results of DNA isolation obtained that the DNA concentration values tested were in the range of 39.10 - 54.70 with an average concentration value of 45.15. The value of the purity of the isolated DNA tested was in the range of 2.16 – 2.28 with an average purity value of 2.22. Based on the results of the DNA confirmation test of the isolation carried out using real-time PCR, it showed that the isolated sample was amplified at a value of Ct 36.43 while the positive control was amplified at Ct 32.49. Based on the research results, it was found that all samples tested showed good average values of DNA concentration and purity so that the results of the DNA isolation tested could be used as templates in real-time PCR analysis.

1. INTRODUCTION

Analysis of the purity and concentration of isolated DNA is a way to see the quality of isolated DNA. This research was conducted as an initial stage to give. Information about research conducted in future research. This is important to do because in molecular analysis using real-time PCR, the success of DNA isolation is the key that can determine the success of the research to be carried out.

For about 40 years, chondroitin sulfate together with glucosamine, which is a component of the joints, has been used as a remedy for osteoarthritis (Vangsness et al., 2009). In general, Chondroitin sulfate is

made from the cartilage (trachea) of cattle, pigs, and sharks (Alamsjah et al. 2014), Due to limited raw materials, in the last decades only derived from the trachea of pigs which is often used for several reasons, namely in cattle. there is mad cow disease that hit Europe (Mierendorff, 2006), while from aquatic biota, sharks are constrained by the presence of shark species that are included in the protected animal category (Lucifora et al., 2011).

The use of chondroitin made from pork cartilage makes this product unacceptable to the majority of Indonesians who are Muslim, causing the circulation of this product to be monitored. Therefore, several molecular DNA-based testing techniques are needed to monitor claims for raw materials for medicinal products or health supplements made from chondroitin.

So based on this background, this research was conducted to provide references and information regarding DNA isolation techniques in chondroitin samples, so that they can be used in similar studies.

2. METHODS

2.1. Materials

The materials used in this study were samples of health supplements made from chondroitin which were weighed 50 mg and tested for 10 repetitions, Super Scrip III Platinum One-Step qRT-PCR system (Invitrogen), and Qiamp Mini DNA extraction kit [Qiagen].

2.2. DNA Extraction

DNA isolation was carried out by weighing 50 mg of the sample then put into a 2 ml centrifuge tube and adding 200 μL of ATL buffer and 30 μL of proteinase K. Incubate at 70 ^{o}C for 60 minutes while mixing at 1400 rpm, then add 200 μL of AL buffer. and incubated again at 70^{o}C for 10 minutes while being mixed at 1400 rpm. Let stand at room temperature for 2-5 minutes then add 200 μL of 96% ethanol and vortex for 30 seconds. Transfer the entire solution in the 2 mL centrifuge tube into the spin column, then centrifuge for 1 minute at a speed of 14000 rpm, discard the collection tube and transfer the spin column into a new collection tube then add 650 μL of AW1 buffer, and centrifuge again for 1 minute at a speed of 14000 rpm, discard the collection tube and then transfer the spin column to a new collection tube, then add 650 μL of AW2 buffer and centrifuge again for 2 minutes at a speed of 14000 rpm. Discard the collection tube and transfer the spin column to a 1.5 centrifuge tube and add 100 μL of elution buffer AE, then centrifuge at 14000 rpm for 1 minute. Discard the spin column, and store the isolated DNA in the 1.5 centrifuge tube in the freezer at -20^{o}C for long time use or refrigerator temperature for immediate use (Qiagen 2018).

2.3. Analysis of Purity and Concentration of Isolated DNA

The isolated DNA was analyzed for purity and concentration using a nano photometer with a wavelength of A260/A280 (Sophian, 2021; Sophian et al, 2021; Sophian & Syukur 2021; Wulan et al 2021; Sutanta et al. 2022).

2.4. Confirmation Test of Isolated DNA using Real-Time PCR

A confirmation test of isolated DNA was carried out using Real-Time PCR. This step is carried out to ensure that the extracted DNA that has been isolated can be amplified so that the isolated target DNA can be confirmed for its presence.

Real-Time PCR Master Mix Setup

The composition of the master mix used in the Real-Time PCR analysis is presented in Table 1.

Table 1. Master Mix Setup

Component	Volume (µl)
SuperScript™ III RT/ Platinum *Taq*Mix	0.5
2x Reaction Mix	12.5
Primer-F 7,5 µM	0.5
Primer-R 7,5 µM	0.5
Probe 5 µM	0.25
ROX™ Reference	0.05
Magnesium Sulfate	1
Nuclease Free Water	4.7

Real-Time PCR Program

Real-time PCR setup method adapted to Super Scrip III Platinum One-Step qRT-PCR system manual kit (Invitrogen) (Thermo. 2016).

2.5. Data Analysis

Data analysis was carried out by testing the average value of the purity and concentration of DNA isolated from the results (Sophian 2021; Sophian et al 2021; Sophian & Syukur 2021).

3. RESULT AND DISCUSSION

3.1. DNA Isolation Results

The results of DNA isolation obtained DNA concentration values in the range of 39.10-54.70 with an average concentration value of 45.15. The purity value of isolated DNA was in the range of 2.16-2.28 with an average purity value of 2.22. Complete data as presented in Table 2.

Table 2. DNA Isolation Results

Number	Concentration (ng/ui)	Purity (A260/A280)
1	53.90	2.21
2	54.70	2.17
3	39.10	2.25
4	40.90	2.26
5	41.90	2.28
6	39.30	2.26
7	40.60	2.16
8	44.90	2.21
9	46.70	2.21
10	49.50	2.19
Average	45,15	2.22

A good value of DNA concentration and purity is if the concentration of isolated DNA has a value greater than 20 (ng/µl), while the purity that is read using a nano photometer at wavelength A260/A280 is in the range of 1.8-2.0 (Kriby 1990; Sambrook 1989). A different opinion was expressed by Eppendorf (2016), who stated that the optimum value of DNA purity that was read using a nano photometer which was read at wavelengths A260/A280 was in the range of 1.8-1.9, while RNA was in the range of 1.9-2.0.

In general, a DNA extraction process with an extraction system consists of three processes, namely cell lysis, purification, and precipitation. Each of these stages has its function. In the lysis stage, this process occurs with the help of proteinase K enzymes and Sodium Dodecyl Sulfate (SDS). At this lysis stage, SDS will lyse fats and proteins on the cell membrane so that the contents in the cell membrane come out. Because the cell wall has been destroyed and released the contents of the cell then this process is called lysis or the destruction of the cell wall to remove the DNA. Lysis is carried out by heating at a temperature of 70^{o}C while shaking or

shaking. The purpose of this heating process is to activate the proteinase K enzyme so that it can actively work for lysis (Renshaw. 2015).

The extraction system which generally uses Phenol-Chloroform works by using phenol as a binder for proteins, fats, and carbohydrates, which will then be separated from other macromolecules. At the time of centrifugation, macromolecules such as proteins and polysaccharides bound by Phenol and Chloroform, Isoamyl Alcohol will settle at the bottom of the tube, while DNA and water are in the top layer (Kado, et al. 1981). The way that can be done to separate and purify the DNA is by centrifuge column, where at the end of the washing process using alcohol so that the remaining salt and phenol contained in the sample will come out and leave DNA pellets. To withdraw DNA pellets from this spin column, you can use sterile distilled water or nucleotide-free water.

According to Matlock (2015), the low quality of DNA purity isolated could be due to the presence of residual phenol or other reagents used in the extraction process. Therefore, in carrying out the DNA extraction or isolation process, it is necessary to pay attention to each stage of the work that must be carried out according to the right procedure to avoid the failure of the DNA isolation process (Sophian et al 2021; Sophian 2021).

3.2. Confirmation Test Results

The results of DNA confirmation test results from the extraction are shown in Figure 1.

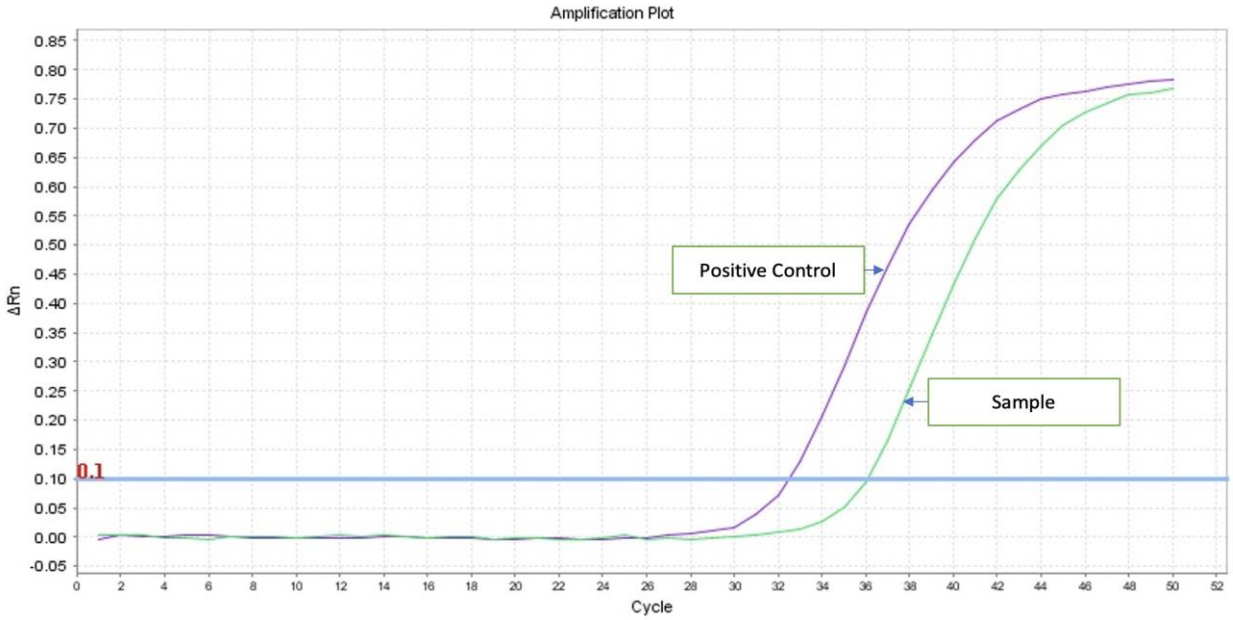

Figure 1. Confirmation Test Results using Real-Time PCR

Based on the results of the DNA confirmation test of the isolation carried out using real-time PCR, it showed that the isolated sample was amplified at a value of Ct 36.43 while the positive control was amplified at Ct 32.49. These results can be

used to confirm or confirm that the results of DNA isolation carried out can be amplified.

4. CONCLUSION

Based on the research results, all samples tested showed good average values of DNA concentration and purity so that they could be used as templates in real-time PCR analysis.

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