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# *Short Communication: Application of Double Wash Technique for Species DNA Isolation in Soft Capsule Shell Samples*

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ABSTRACT / ABSTRAK

Keberadaan komponen gelatin dapat diidentifikasi melalui lemak, protein maupun DNA. DNA dapat diperoleh melalui proses isolasi DNA yang merupakan proses pemisahan DNA dari komponen-komponen penyusun sel lainnya. Tahap pemurnian DNA merupakan hal terpenting dalam isolasi DNA karena pada tahapan tersebut dapat mengurangi seminimal mungkin jumlah kontaminan pada isolat DNA sehingga menentukan kemurnian dan konsentrasi dari isolat DNA yang didapatkan. Tujuan dari teknik ini adalah untuk menghasilkan isolat DNA yang rendah inhibitor atau berada pada rentang kemurnian 1.7-2.1. Metode ekstraksi yang digunakan pada penelitian ini menggunakan teknik pencucian ganda, teknik tersebut dimodifikasi dari tahapan uji yang sesuai dengan manual kit hanya saja pada teknik yang digunakan ini dilakukan modifikasi pada tahapan pencucian dengan masing-masing pencucian dilakukan sebanyak dua kali pada bagian cuci pertama dan cuci kedua sehingga total pencucian yang dilakukan sebanyak empat kali. Hasil isolat DNA selanjutnya dilakukan analisis kemurnian dan konsentrasi DNA yang didapatkan menggunakan nanophotometer, isolat DNA yang didapatkan berada pada kisaran 4.400 - 5.500 ng/µL dengan rata-rata sebesar 4.950 ng/µL. Sedangkan untuk nilai kemurnian yang diukur pada panjang gelombang $A_{260}/A_{280}$ diperoleh hasil dengan rentang kemurnian antara 1.760 - 1.840 dengan rata-rata sebesar 1.800. Kesimpulan dari penelitian ini adalah semua sampel yang diekstraksi menunjukkan hasil isolat DNA yang masuk dalam kategori DNA yang baik dan memenuhi persyaratan yang dibutuhkan dalam analisis molekuler.

*The presence of gelatin components can be identified through fat, protein and DNA. DNA can be obtained through the DNA isolation process which is the process of separating DNA from other components of the cell. The DNA purification stage is the most important thing in DNA isolation because at this stage it can reduce to a minimum the number of contaminants in DNA isolates to determine the purity and concentration of the DNA isolate obtained. The purpose of this technique is to produce DNA isolates that are low inhibitors or are in the purity range of 1.7-2.1. The extraction method used in this study used the double wash technique, the technique was modified from the test stage following the manual kit used, it's just that the technique used was modified at the washing stage with each washing done twice in the washing section. first and second washing so that a total of four items of washing were carried out. The results of the DNA isolates were then analyzed for the purity and concentration of DNA obtained using a nanophotometer, the DNA isolates obtained were in the range of 4,400 - 5,500 ng/µL with an*

*average of 4,950 ng/µL. As for the purity value measured at the wavelength $A_{260}/A_{280}$, the results were obtained with a purity range between 1,760 - 1,840 with an average of 1,800. This research concludes that all the extracted samples show the results of DNA isolates that fall into the category of good DNA and meet the requirements needed in molecular analysis.*

**Keywords:** DNA, Gelatine, Purification, Methode
**Kata Kunci:** DNA, Gelatin, Pemurnian, Metode

## 1. Introduction

Gelatin is a polypeptide compound obtained by partial hydrolysis of collagen extracted from connective tissue and animal bones (Zhang et al 2009). In the pharmaceutical industry, gelatin is used in the manufacture of soft and hard capsule shells, as a tablet binder, as a stabilizer for nutritional supplements, and as an emulsifier for pharmaceuticals in the form of liquids and pastes. Gelatin is still considered the best material to protect medicines from air, light, moisture and microbial contaminants (Sahilah et al 2012). Soft gelatin capsules (SGC), also referred to as softgels or soft elastic capsules (SEC), have gained popularity in delivering therapeutic compounds solubilized or suspended in nonaqueous vehicles. A softgel is a one piece, hermetically sealed soft gelatin shell containing a solution, a suspension, or a semisolid, referred to as fill formulation, fill material, or fill. Softgels offer many advantages over other conventional oral dosage forms, including improving swallowability, masking odors and unpleasant taste, protecting the encapsulated compound against oxygen and light, and able to readily dissolve in the gastric juices (Gullapali 2010).

The presence of gelatin components in a sample can be identified through the composition of fat, protein and DNA components (Fibriana et al 2012). The DNA of good quality is required to be extracted from the gelatin and gelatin-derived capsules. The effort of extracting DNA from these products has been very challenging because the negligible amount of DNA was expected to be highly degraded as it underwent extreme conditions such as exposure to elevated temperature, concentrated acidic and alkaline treatment and mechanical pressure along the production line (Mohamad *et al* 2015). DNA can be obtained through the DNA isolation process, which is the process of separating DNA from other cell components (Nooratiny et al 2013). This process involves the destruction or lysis of cell membranes, separation of DNA from cell proteins, and purification of DNA (Kheyrodin et al 2012). The DNA purification stage is the most important thing in DNA isolation because in this stage it can reduce the amount of contaminants in DNA isolates to a minimum so as to determine the purity and concentration of the DNA isolates obtained.

The biggest challenge in carrying out DNA extraction is how to isolate the DNA in the sample by removing the interfering inhibitors that sometimes participate in the elution of the isolated DNA. If the isolated DNA can produce DNA templates that have good purity and concentration, the detection test on this type of sample will certainly be easier to do. Based on this, this research was conducted to provide information about DNA extraction techniques on soft capsule shell samples with the double wash technique developed by the research team. The use of this method in similar research is expected to begin with optimization and validation/verification first.

## 2. Methodology

### 2.1 Materials

In this study, a softgel capsule was obtained from a local company. The sample used is a whole capsule and there is no special treatment for it. DNA isolation was carried out using the RADI PREP Serum kit and the Plasma DNA/RNA KIT (KH Medical) and modified with double wash technique for each washing stage.

### 2.2 DNA Isolation

Take 1-2 capsules to be extracted and then put into a 2 mL tube. Add 700 µL of KH buffer and 100 µL of proteinase K. The samples were then incubated at 70 °C for 60 minutes using a thermomixer. At this stage, it is important to monitor the capsule shell of the sample used, if, within 30 minutes of the incubation period the sample has not been destroyed, the incubation can be continued until the sample used is destroyed. The centrifuged sample will be divided into 2 phases, remove the top layer or supernatant and put it into a 2 mL tube that already contains 500 µL of 96% ethanol. The sample was then homogenized by vortexing for 2 seconds, after which the entire solution was transferred to the spin column and centrifuged at a speed of 13500 rpm for 2 minutes. If there is still residual solution, then this step needs to be repeated until the entire solution is used up. Washing stage with each washing done twice in the washing section. first and second washing so that a total of four items of washing were carried out (RADI PREP Serum and Plasma DNA/RNA Kit, KH Medical).

### 2.3 Analysis of Purity and Concentration of Isolated DNA

The results of DNA isolates then need to be analyzed for the purity and concentration of the DNA produced using a nano photometer (Thermo Scientific). To estimate the purity of nucleic acids, the absorbance ratio at 260 nm to 280 nm (ratio A260/A280) was used. Select the method used, namely Nucleic acid, dsDNA type, nano volume mode, 1-2 **µ**L sample volume, nucleic acid factor 50.00, background correction 320 nm, air bubble recognition off, manual dilution factor 1,000.

### 2.4 Data Analysis

The data obtained were then tested on average to see the average value of the concentration and purity of the isolated DNA.

## 3. Result and Discussion

Gelatin contains proteins, peptides or nucleases (Malik et al. 2016). In testing using molecular techniques, nucleases are specific proteins that directly affect the testing process using molecular techniques. This test technique works efficiently in humid conditions. Can degrade DNA by chopping which causes the amplification step to be inefficient (Saeed A and Rasool N, 2020), so that protein contaminants as inhibitors must be removed from DNA isolates. According to Sophian (2021), a DNA extract purity value above 2 indicates that the DNA extraction results still contain protein contamination, whereas if the purity results show a value less than 1.8, it indicates that the DNA extract still contains phenol residues and other solvent contaminants.

Analysis of concentration and purity was carried out using a nano photometer. From the table 1, it can be seen that the concentration values of the extracted samples were in the range of 4,400 ng/µL – 5,500 ng/µL with an average of 4,950 ng/µL. while the purity value measured at the wavelength A260/A280 obtained results with a purity range between 1,760 – 1,840 with an average of 1,800. The results of the measurement of the purity of DNA isolates showed that the results obtained from the extraction process using the kit and double wash technique had good purity values and met the requirements needed for molecular analysis.

Table 1. DNA Isolation Results Data

| Sample | Nanophotometer Analysis | |
|---|---|---|
| | Purity (A260/A280) | Concentration (ng/µL) |
| 1 | 1.840 | 4.400 |
| 2 | 1.780 | 4.900 |
| 3 | 1.820 | 5.100 |
| 4 | 1.800 | 5.000 |

| Sample | Nanophotometer Analysis | |
|---|---|---|
| | Purity (A260/A280) | Concentration (ng/µL) |
| 5 | 1.790 | 4.500 |
| 6 | 1.760 | 4.700 |
| 7 | 1.810 | 5.100 |
| 8 | 1.820 | 5.400 |
| 9 | 1.840 | 4.800 |
| 10 | 1.800 | 5.300 |
| 11 | 1.760 | 4.600 |
| 12 | 1.780 | 5.500 |
| **Average** | **1.800** | **4.950** |

If you look at the results of DNA isolation as presented in (table 1) above, it can be seen that the concentration of DNA produced is below 20 ng. According to Sophian et al (2021), a good concentration of DNA isolation results is obtained if it has a concentration value greater than 20 ng. This concentration value has a significant effect when the real-time PCR amplification process is carried out, where the Ct value in real-time PCR analysis is influenced by the concentration of the DNA template used (Sophian et al., 2021). Even though the isolation results show concentrations below 20 ng, these results can be continued to the PCR stage. this is because real-time PCR can carry templates from a concentration of 0.1-100 ng when amplified.

The double wash technique is a technique that is modified from the extraction stages following the manual kit but in the washing stage with each done twice in the first washing section and the second washing so that the total washing is done four times. The purpose of this technique is to produce DNA isolated with low inhibitors or in the purity range of 1.7-2.1. this is done so that the resulting isolated DNA has a better purity value (Matlock 2015).

In carrying out DNA extraction, lysis is a step that plays an important role in producing good DNA concentration and purity. At this stage proteinase K which is an enzyme that works in assisting the lysis process can only work well at an optimum temperature of 65-70 $^{o}$C. This enzyme works to eliminate interfering proteins by digesting them. When compared with methods that use chemicals, enzymes work quite effectively because they directly target amino acid bonds in protein lysis. Some studies using this method sometimes need to optimize the method before using it. Christensen (2012) has optimized the incubation period of proteinase K during DNA extraction lysis, which shows that at a temperature of 65 $^{o}$C, the lysis will work well when incubated for approximately 3 hours or more. If the incubation time is below 3 hours, the lysis process cannot take place completely. The cells have undergone lysis which is characterized by turbidity in the lysis solution. The lysis process is an important step in DNA extraction because at this stage the DNA trapped in the cell will be released so that at this stage it has a very important role (Held 2001; Sophian A. 2021).

In the DNA isolation process, the stage begins with the lysis process. This stage has a fairly important program, where the lysis process is a stage of removing DNA and inside the cell by damaging the cell wall using the proteinase K enzyme. This enzyme works by destroying the protein-making cells so that lysis occurs. According to Cristensen (2012), the proteinase K enzyme works requires time and certain incubation temperature conditions, where this enzyme will be active if incubated at a temperature of 65-70 $^{o}$C with an incubation duration of 3 hours. However, Sophian (2021) and Sophian et al (2021) have different opinions, which state that the proteinase K enzyme can work from 30 to 3 hours after incubation.

In the DNA isolation process, there are two types of common solutions used for elution, namely nucleotide-free water or tris HCl 1 mM EDTA. The elution buffer has a different volume of use between the types of kits used. The concentration of DNA isolation prose can also be done at this stage, where when doing elution, reducing the value of the elution buffer volume can concentrate the final concentration of isolated DNA. As a suggestion for the future, the double wash technique should be developed to produce purer DNA results when compared to the one-wash method. In the future, it is hoped that this method can be optimized and tested more comprehensively with various approaches so that it can become a standard method whose reliability can be justified.

## 4. Conclusion

Based on the data from the research and discussion carried out, it was concluded that the extracted samples showed the results of the DNA analysis produced were included in the good DNA category and met the requirements needed for molecular analysis.

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