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A característica da extração de agarose a partir de dimetil sulfeto e isopropanol

The characteristic of agarose extraction from dimethyl sulfide and isopropanol

La característica de la extracción de agarosa de sulfuro de dimetilo e isopropanol

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Resumo

Introdução. Este estudo teve como objetivo obter o produto de agarose com qualidade padrão de mercado que poderia ser aplicado para eletroforese. Métodos. O material utilizado no processo de extração da agarose foi a barra de ágar-ágar seca extraída com sulfeto de dimetil e embebida em solução de isopropanol com 85%, 90% e 95% de concentração de isopropanol. O parâmetro observado neste estudo consistiu em rendimento, teor de água, teor de sulfato, teor de cinzas, resistência do gel e gel de eletroforese. Resultados. A pontuação média do rendimento de agarose usando diferentes concentrações de isopropanol (IPA) foi de cerca de 46,76% - 57,53%, com o menor rendimento de 46,76% e o maior rendimento de 57,53%. Enquanto isso, o teor médio de água variou de 18,32% a 18,64%. O teor médio de cinzas foi de cerca de 0,15% - 0,16% e o teor médio de sulfato foi de 0,14% - 0,15%. A resistência média de um gel a 1% preparado em isopropanol foi de 1170 a 1183,33 gr / cm2. A melhor concentração de IPA para absorver o extrato de agarose foi na concentração de 95%, com característica padrão de mercado como 57,54% de rendimento, 18,35% de teor de água, 0,14% de teor de sulfato, 0,15% de teor de cinzas e 1183,33 de gel. A aplicação da

eletroforese em gel de agarose mostrou que as bandas do grande fragmento de DNA molecular não puderam se separar. Amostras de proteínas parcialmente hidrolisadas apresentaram maior teor de proteínas, cores mais claras e menor grau de desnaturação e melhores propriedades funcionais em comparação com os isolados tradicionais de proteínas.

Palavras-chave: Agarose; Dimetil sulfeto; Eletroforese; Gelatina; Isopropanol.

Abstract

Introduction. This study aimed to obtain agarose product with market standard quality which could be applied for electrophoresis. Methods. The material used in agarose extraction process was dried agar – agar bar extracted using dimethyl sulfide and soaked in isopropanol solution with 85%, 90%, and 95% of isopropanol concentration. The parameter which observed in this study was consisted of yield, water content, sulfate content, ash content, gel strength and electrophoresis gel. Results. The average score of agarose yield using different concentrations of isopropanol (IPA) was around 46.76% - 57.53% with the lowest yield of 46.76% and the highest yield of 57.53%. Meanwhile the average water content ranged from 18.32% to 18.64%. The average ash content was around 0.15% - 0.16% and the average sulfate content was 0.14% - 0.15%. The average strength of a 1% gel prepared in isopropanol was 1170 – 1183.33 gr/cm_2. Best IPA concentration to soak agarose extract was on 95% concentration, with market standard characteristic as 57.54% of yield, 18.35% of water content, 0.14% of sulfate content, 0.15% of ash content and 1183.33 of gel strength. Conclusions.The application of electrophoresis of agarose gel showed that the bands of large molecular DNA fragment could not separate. Partially hydrolyzed protein samples had higher protein content, lighter color, and lower degree of denaturation and better functional properties compared to the traditional protein isolates.

Keywords: Agarose; Dimethyl sulfide; Electrophoresis; Agar; Isopropanol.

Resumen

Introducción. Este estudio tuvo como objetivo obtener un producto de agarosa con calidad estándar de mercado que podría aplicarse para la electroforesis. Métodos El material utilizado en el proceso de extracción con agarosa fue agar seco - barra de agar extraída con sulfuro de dimetilo y empapada en solución de isopropanol con una concentración de isopropanol al 85%, 90% y 95%. El parámetro que se observó en este estudio consistió en rendimiento, contenido de agua, contenido de sulfato, contenido de cenizas, resistencia del gel y gel de electroforesis. Resultados El puntaje promedio de rendimiento de agarosa usando diferentes

concentraciones de isopropanol (IPA) fue de alrededor del 46.76% - 57.53% con el rendimiento más bajo del 46.76% y el rendimiento más alto del 57.53%. Mientras tanto, el contenido promedio de agua varió de 18.32% a 18.64%. El contenido promedio de cenizas fue de alrededor de 0.15% - 0.16% y el contenido promedio de sulfato fue de 0.14% - 0.15%. La resistencia media de un gel al 1% preparado en isopropanol fue de 1170 - 1183.33 gr / cm2. La mejor concentración de IPA para remojar el extracto de agarosa fue en una concentración del 95%, con una característica estándar del mercado como 57.54% de rendimiento, 18.35% de contenido de agua, 0.14% de contenido de sulfato, 0.15% de contenido de cenizas y 1183.33 de resistencia del gel. Conclusiones. La aplicación de electroforesis de gel de agarosa mostró que las bandas de fragmentos de ADN molecular grande no podían separarse. Las muestras de proteínas parcialmente hidrolizadas tenían un mayor contenido de proteínas, un color más claro y un menor grado de desnaturalización y mejores propiedades funcionales en comparación con los aislados de proteínas tradicionales.

Palabras clave: Agarosa; Sulfuro de dimetilo; Electroforesis; Gelatina; Isopropanol.

1. Introduction

Agar is the mixture of two polysaccharide molecules, namely agarose and agar pectin (Sasuga, Yamanashi, Nakayama, Ono, & Mikami, 2017). Agarose is a polysaccharide extracted from seaweed and comprises 1,3-linked-β-D-galactopyranose and 1,4-linked-3,6-anhydro-α-L-galactopyranose (Jeon, Athukorala, & Lee, 2005). Agarose also known as the main component of gelling agent, whereas agar pectin is contained with sulfate esters and remaining impurity elements from processing that may obstruct the agarose gel formation (Hui et al., 2005). Therefore, agar pectin needs to be removed to obtain pure agarose.

However, the quality of agarose which accepted in market is agarose with high purity level. A high purity level could be indicated through the component of sulfate and gel strength within the agarose itself. The quality would be better when agarose contained in lower or none amount of sulfate and had high gel strength and no inorganic elements inside. As Selby and Wyne said that a good quality of agarose is defined by the sulfate content within the agarose (Selby & Wyne, 1973). The market standard of agarose in public market is usually started from 0.1% - 0.35% of sulfate content and ≥ 500 gr/cm^2 of gel strength (Subaryono, Utomo, WIkanta, & Satriyana, 2017).

Some techniques have been done in purifying the agarose due to obtain a good quality agarose as well as the market standard. For instance, Jeon et al used dimethyl sulfide for

agarose extraction. Dimethyl sulfide is an effective solvent that able to split the agarose from agar pectin. It is also soluble in water which help the extraction process become easier (Jeon et al., 2005). Another technique is using Isopropanol solution. Subaryono et al stated that the quality of agarose powder can be fixed by soaking it into isopropanol solution (IPA) before turning it into powder (Subaryono et al., 2017). Agarose was extracted from the commercial agar-agar bar using dimethyl sulfide solution and the extract then soaked into isopropanol. The produced agarose powder then applied to agarose gel electrophoresis. Agarose gel electrophoresis has function to separate DNA fragments of varying sizes ranged from 100 bp to 25 kb (Lee, Costumbrado, Hsu, & Kim, 2012). Hence, this study aimed to obtain the agarose product with market standard quality which can be applied for electrophoresis application.

2. Methodology

Materials

The materials used was dried agar-agar bar (AA Brand) produced by Sumber Laut Agar-Agar Company in Surabaya. For extraction process, the chemical substances used were dimethyl sulfide (Merck), isopropanol, aquadest, and filter paper. While the chemical substances used for analysis were divided into two groups; 1) group 1 is analyzing the sulfate contain using HCl, $BaCl_2$, ethanol and aquadest, and 2) group 2 is analyzing electrophoresis using DNA maker, DNA product, TBE (Tris-Borate-EDTA) solvent, ethidium bromide and loading dye.

Agarose Extraction

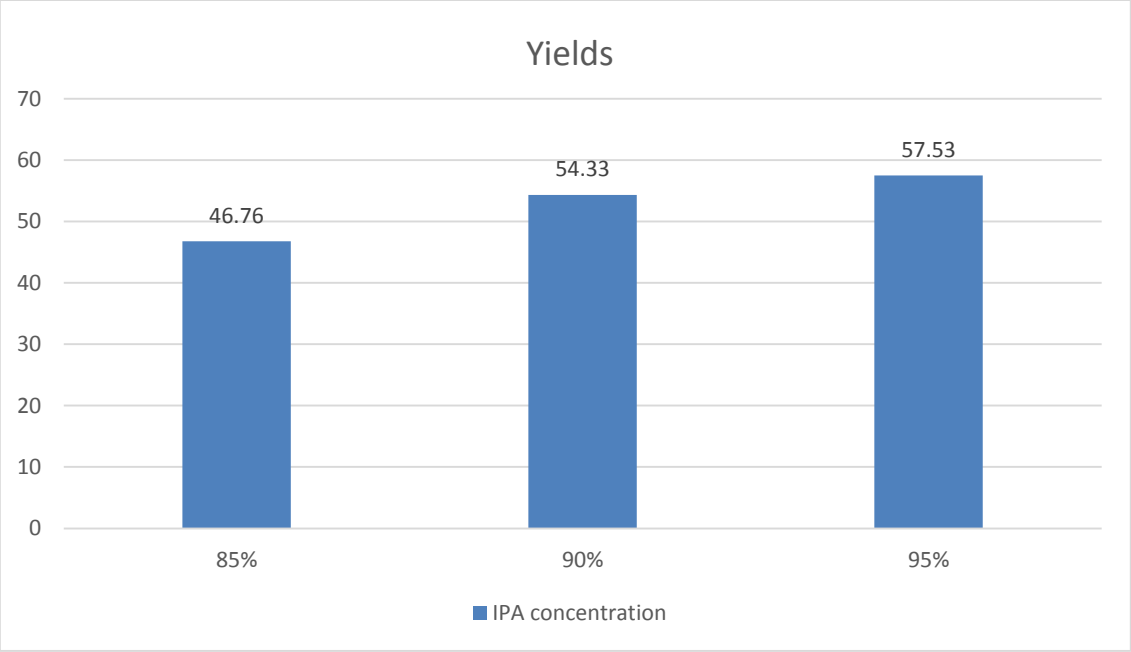
In this study, the agar extraction was conducted according to Jeon et al (Jeon et al., 2005). Agar-agar bar was first blended and weighed in 1 gr and poured into 100 ml of dimethyl sulfide. The solution was then stirred to dissolve agar-agar in dimethyl sulfide. The extraction was done using water bath shaker for 2 hours while stirring 450 rpm at 70^0 C. The separation between sediment and soluble substance was done by 20 minutes centrifugation at 4^0C at 300 rpm. The obtained filtrate was added with aquades as much as the filtrate volume, then kept for one night to form a gel. After the gel is formed, the gel was demolished and washed with aquades for several times to eliminate the dimethyl sulfide solution that remained within the agarose extract. Afterward, the agarose extract soaked in isopropanol solution with variation of concentration in 85%, 90% and 95% for an hour. This submersion was aimed to strengthen the gel strength since isopropanol was able to precipitate pure agarose before it getting powdered. After soaking the agarose extract, vacuum filter was done

to minimize the excess of remained water. The agarose then dried using freeze dryer and powdered and sifted in 60 mesh sizes. The observed parameter was consisted of yield, water content, sulfate content, ash content, gel strength of 1% agar-agar, and agarose gel electrophoresis. The application of agarose gel electrophoresis was using standard DNA (DNA marker) and DNA from Dokare banana leaves isolation. The data obtained was analysed in variant. If in case the result was different then Tukey's HSD (honestly, significance, difference) test was done due to identify the difference among the treatments. Tukey's HSD is a post-hoc test based on the range distribution. It helps to find out the differences of specific groups' mean compared to other groups (Stephanie, 2016).

3. Results

Yields

The average value of agarose yields was around 46.75% – 57.45%. While, the lowest yield was 46.76% and 57.53% for the highest yields. The average value of agarose yields for each treatments were shown in figure 1 below;



Source : own research

Figure 1. The average value of agarose yield

The result of variance analysis($\alpha > 0.05$) showed that there was significant difference between treatments toward the produced yields. Based on the further examination using Tukey's HSD test methodology, it could be seen that there was a significant influence in every levels based

on the treatments. For every 5% of IPA concentration enhancement, it able to increase the yield of agarose powder significantly. This occurred since higher IPA concentration could add the amount of hydroxyl group within the solution and it is able to precipitate the large amount of agarose (Salamah, Susanti, & Wikanta, 2005). The agarose submersion with 95% of IPA concentration had highest agarose powder yields, while the submersion in 85% of IPA concentration had the lowest agarose powder yields. Therefore, it can be concluded that the yields production will be bigger if the use of IPA concentration is also high.

Water Content

The average value of water content was around 18.35% – 18.64%. with the lowest water content for18.32% and the highest water content 18.64%. The result of variance analysis (α >0.05) showed that there was significant difference in every treatments toward produced water content of powdered agarose. Based on the advance testing using Tukey's HSD test, it showed that there was significant influence among IPA treatments; 85%, 90%, and 95% of IPA concentrationand vice versa. However, there was no significant difference between 90% and 95% of IPA concentration treatments. It happened because the solution of 85% IPA concentration was not able to draw the remained water yet within the agarose gel maximally. Hence, the agarose powder had higher water content after it is being dried.

Based on the analysis, higher IPA concentration tend to produce low water content due to its characteristic as a polar photic solution which functioned to pull the water. Similar to Zailanie et al who stated that higher isopropanol concentration make the amount of hydroxyl group higher thus it has powerful strength to bound the water (Zailanie, Susanto, & BW, 2001). Yani also claimed that the use of isopropanol can precipitate the alginate perfectly rather than using ethanol (Yani, 1998).

Sulfate Content

The average score of agarose powder's sulfate content was around 0.14 to 0.15%. The lowest sulfate content was 0.14% and the highest sulfate content was 0.15%. Variance analysis result (α >0.05) demonstrated that there was insignificant average differences among treatments toward sulfate content of produced agarose powder. It was suspected that the initial extraction result already showed low sulfate content. Hence in the submersion process, the sulfate content within the agarose was not decrease significantly. Based on the data, it could be seen that the agarose submersion with 95% of IPA concentration had the smallest average of sulfate content of agarose powder for 0.14%. This indicated that higher IPA concentration could increase the amount of hydroxyl group which able to pull out the remaining sulfate. A study done by Subaryono et al also used isopropanol solution in agarose extraction and got

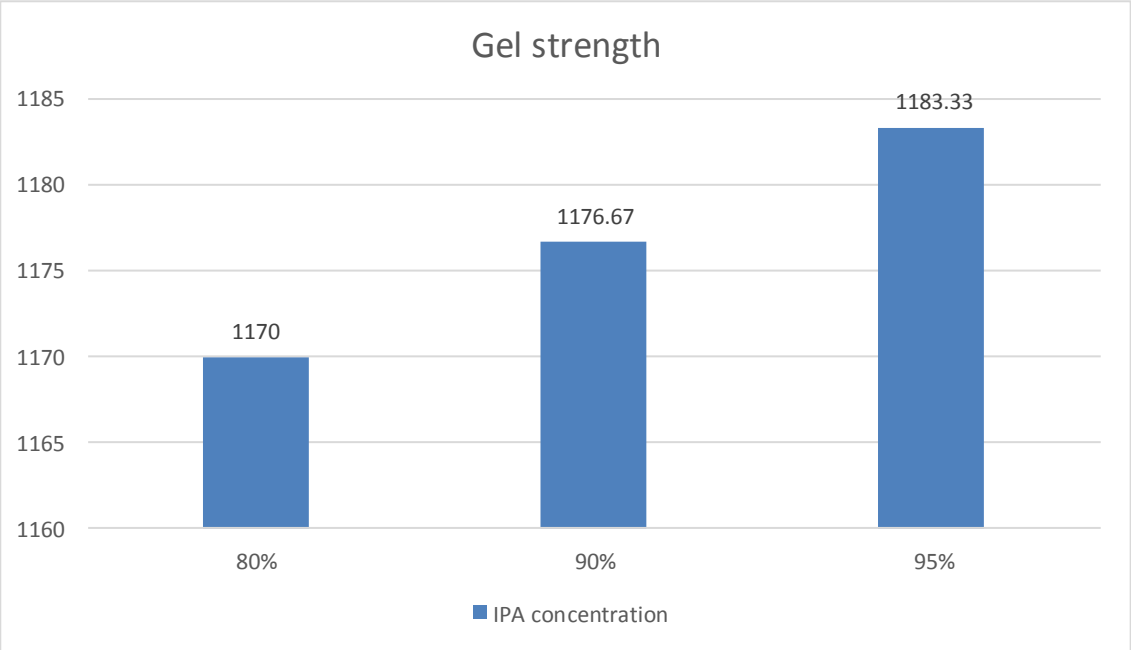
0.95% of agarose sulfate content (Subaryono et al., 2017). While, this study had 0.16% of agarose sulfate which already suitable with the quality of commercial agarose since the standard for agarose sulfate content in international market is ranged from ≤ 0.1% to 0.35%.

Ash Content

The total ash content within a product can be the indication of the huge number of mineral contained inside (Larry, Davidson, & Salminen, 1990). The average value of agarose powder ash content in this study was about 0.15 – 0.16%. The lowest ash content was 0.15% and the highest ash content was 0.16%. Variance analysis result (α 0.05) indicated insignificant average in every treatments toward ash content of produced agarose powder. Agarose submersion with 85% and 90% of IPA concentration produced the highest average of ash content. While, the agarose submersion with 95% of IPA concentration produced the smallest ash content of powdered agarose. This happened because the IPA solution in low concentration could not bind the remained inorganic elements within the agarose gel. Therefore, it can be concluded that higher IPA concentration tend to produce low ash content. Isopropanol was a solution that could precipitate agarose, thus the inorganic compound could be pulled out by isopropanol. The produced ash content in this study was lower than the previous studies. For instance, a study conducted by Salamah et al showed the agarose extraction from seaweed and obtained 4.02% of ash content (Salamah et al., 2005). The study written by Do and Oh was found agarose with 1.28% of ash content from gelatin extraction using cethyl pridinium chloride (CPC) (Do & Oh Songnam, 1999). Therefore, the agarose extract submersion in isopropanol solution could produce better result.

Gel Strength

The average score of gel strength in every 1% of agarose powder was around 1170 – 1183.33 gr/cm². The lowest gel strength was 1170 gr/cm² and 1183.33 gr/cm^2 for the highest gel strength score. The average score of agarose gel strength in each treatments was shown figure 2 below;



Source : own research

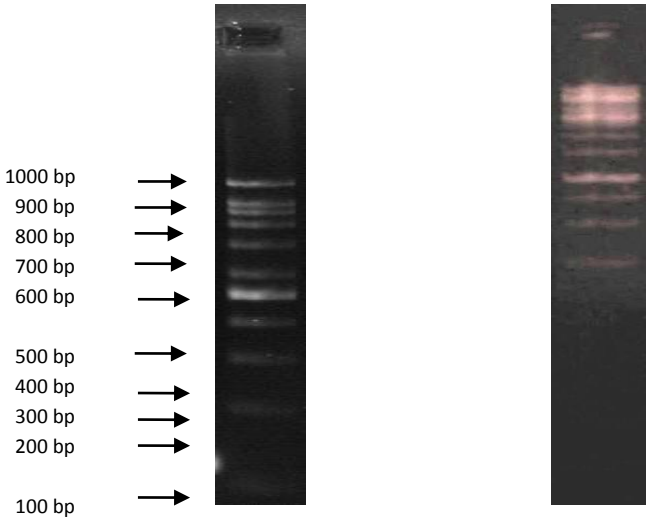
Figure 2. The average value of agarose gel strength

The variance analysis result (α 0.05) showed there was difference in significant average of each treatment toward gel strength of produced agarose powder. It showed that IPA submersion was more effective to purify agarose thus its gel strength was increased. The gel formation was occurred because hydrogen in 3.6-anhidro-L-galactose residue forced the molecule to form a helix structure. The replacement of L-galactose with 3.6-anhidro-L-galactose caused rigidityof helix structure and the gel was started to form (Glicksman, 1983). A study by Joen et al indicated that produced agarose with 1190 gr/cm² of gel strength was obtained from the gelatin extraction result using DMSO solution (Jeon et al., 2005). Subaryono et al found that agarose extraction using *cetyl piridium cloride* and *carrageenan* produced agarose with 118.14 gr/cm² of gel strength (Subaryono et al., 2017). From these studies, it could be concluded that by submersing in IPA solution, the agarose could be purified and increased the gel strength. The produced gel strength around 1170 – 1183.33 gr/cm² was already suitable with commercial agarose standard that was ≥ 500 gr/cm² (Salamah et al., 2005).

The application of agarose powder on electrophoresis

The application of agarose gel used in this study was electrophoresis zone and electrophoresis horizontal techniques since the agarose gel was located in electrophoresis tool horizontally

and functioned to analyse DNA. The electrophoresis used agarose gel and commercial agarose gel (Vivantis brand). The agarose gel had 18.35% of water content, 0.14% of sulfate content and 1183.33 gr/cm^{2}of gel strength. Meanwhile, the Vivantis agarose had characteristics such as 10% of water content, 0.15% of sulfate content, and 1200 gr/cm^2 of gel strength. The result of (1%) of agarose gel electrophoresis with DNA marker sample was illustrated in figure 3 below;



A DNA marker using commercial agarose (Vivantis)

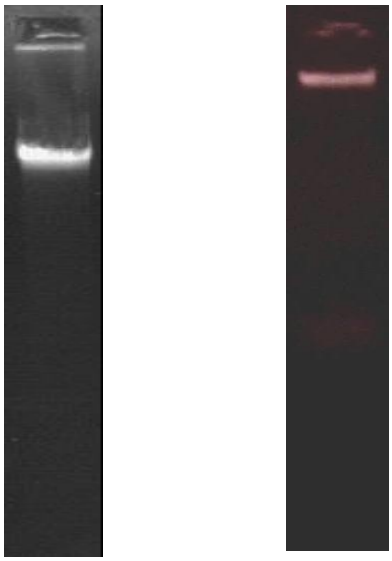
B DNA marker using agarose gel of this study

Source : own research

Figure 3. Electrophoresis result of DNA marker sample toward 1% agarose gel.

In figure 6 it could be seen that agarose used in this study was able to use to migrate the DNA marker molecule as illustrated in figure B. Compared to the commercial agarose in figure A, B figure was still could not yet separate the DNA fragments significantly. The agarose gel used in this study was not able yet to split the big size of DNA fragment due to the slow migration of big DNA. It was identified by the accumulation of DNA bands on the top was pile up. Meanwhile, the smaller molecule of DNA bands were able to separate. This probably happened because agarose used in this study had relatively high water content for 18.35%, while Vivantis agarose's water content was only 10%. Moreover, the gel strength of Vivantis agarose was higher than agarose used in this study. Electrophoresis migration was rated when

DNA moved and penetrated the agarose gel influenced by certain factors, especially the size of DNA molecule and agarose's concentration in gel (Fatchiyah, Arumingtyas, Widyarti, & Rahayu, 2011). A small DNA molecule will be easier to pass through the gel pores (Pratimi, 2017). The DNA molecule migrated through small pores and formed a solid agarose gel. In this case, if the agarose had high water content, then the concentration in gel will be smaller and make the pores position not assemble. This will affect the DNA molecule cannot well migrated and the DNA bands separation within the electrophoresis become unclear. The amount of agarose's gel strength also influenced the DNA movements in electrophoresis.if the gel strength was higher then the DNA would migrate perfectly and suitable with the molecule size. The second agarose gel application was electrophoresis using DNA sample of isolation result from Dokare banana leaf. The result of electrophoresis of (1%) agarose gel with the DNA sample from isolation of Dokare banana was illustrated in figure 4 below;



C	**D**
Dokare banana sample DNA using commercial agarose gel (Vivantis)	Dokare banana sample DNA using agarose of this study

Source : own research

Figure 4. Electrophoresis result of Dokare banana sample toward 1% agarose gel.

In figure 4 it could be seen that agarose gel resulted from this study was able to use to migrate the isolated DNA from Dokare banana leaves as shown in picture D above. The

electrophoresis with Vivantis agarose gel also showed the same results, namely the presence of one DNA band migrating. However, picture D still showed a deficiency when compared to picture C. The results of agarose gel electrophoresis showed that the color of the DNA fragment band was not bright (picture D) compared with the color of the band in Vivantis agarose gel (picture C). This was probably happened due to the fact that there were still inorganic elements in agarose resulted from this study. DNA resolution in electrophoresis will be inhibited if there was a high mineral content in agarose gel (Jeon et al., 2005). If DNA resolution was inhibited, the DNA bands' color became less bright during the documentation process.

4. Discussion

Best treatment determination

Best treatment determination was based on best agarose quality with qualifications such as high yield agarose, low water content, low sulfate content, low ash content and high gel strength. The data obtained in the second stage could be seen as follows:

- The highest yield was 57.45% obtained from treatment with 95% of IPA concentration.
- The lowest water content was 18.35% obtained from treatment with 95% of IPA concentration.
- The lowest sulfate content was 0.14% and obtained from treatment with 95% of IPA concentration.
- The lowest ash content was 0.15%. It was obtained from treatment with 95% of IPA concentration.
- The highest gel strengthwas 1183.33 gr/cm^2. It was obtained from treatment with 95% of IPA concentration.

In conclusion, the best treatment taken was treatment with 95% of IPA concentration which showed the best parameter.

5. Conclusion

The best technical IPA solution for soaking agarose extract before processed into powder was 95% concentration, with characteristics of agarose powder as the market quality standard, namely; 0.14% of sulfate content, 0.15% of ash content and 1183.33gr/cm^2 of gel strength. While the obtained yield was 57.45% and the water content was 18.35%. In the application of agarose gel electrophoresis indicated that the bands of large molecular DNA fragments still

could not be clearly separated based on the molecule size. The color of the band was less bright compared to the results of commercial agarose gel electrophoresis due to the high moisture and ash content.

For further research, It is needed a more appropriate methodology in doing the agarose extraction in order to reduce the amount of inorganic content in agarose powder. Therefore, a good characteristic of agarose could be obtained and make the application of agarose gel electrophoresis shows the same results as agarose gel commercial.

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