The effect of centrifugation speeds of 11,000 g and 13,000 g on small salivary protein profiles (less than 30 kDa)

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The effect of centrifugation speeds of 11,000 g and 13,000 g on small salivary protein profiles (less than 30 kDa)

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Abstract. Previous research suggested that centrifugation is not necessary to see proteins <30 kDa. There is no standard procedure for regulating the centrifugation speed to separate proteins <30 kDa. The aim of this study is too determine the effect of centrifugation speeds of 11,000 g and 13,000 g on separating salivary supernatant proteins <30 kDa. After being centrifuged at 11,000 g and 13,000 g, the salivary protein profile was tested using SDS-PAGE. The frequency of salivary supernatant protein occurrence (after being centrifuged at 11,000 g and 13,000 g) were 35 and 45 with ranges of 9–27 kDa and 8–18 kDa respectively. Compared to 11,000 g, the centrifugation speed of 13,000 g separates more proteins <30 kDa with a narrower range.

1. Introduction

Saliva contains a wide variety of proteins. Among these are many proteins with molecular masses below 30 kDa, which are useful for diagnostic purposes and being a blood sample substitute. Saliva proteins with small molecular masses include insulin (5kDa), histatin (3–4.5 kDa), lysozyme (16 kDa), and aprotinin (6 kDa) [1]. When detecting protein profiles in saliva, a method is needed to separate proteins from other ingredients in the saliva—such as bacteria and food debris [2]. A technique that can be used is centrifugation. By using centrifugation, the solid phase concentration of the sediment/pellet layer and the supernatant phase contains protein with a small molecular mass [3]. Based on Kriscahyani's research, it is known that, by using salivary centrifugation at 10,000 g, proteins can be separated by molecular mass greater than or equal to 30 kDa [4] Another study conducted by Francis *et al.* on protein precipitation for the centrifugation rate speed of 12,000 g reported a loss of salivary supernatant protein with a molecular mass less than 14 kDa [5]. This suggests that increased centrifugation rates have an effect on the protein molecule mass of saliva.

Centrifugation can lead to the deposition of proteins with large molecular masses [5]. The higher the rate of centrifugation, the more proteins with smaller molecular masses also come into sedation [3]. The smaller the molecular mass is, the greater the centrifugal force required for the occurrence of sedimentation [6]. However, to the researcher's knowledge, there is no standard centrifugation speed associated with effect on protein separation based on its molecular mass in the supernatant layer—especially for proteins with molecular masses less than 30 kDa. Based on previous research using a centrifugation rate of 10,000 g, proteins less than 30 kDa are difficult to identify [4]. The centrifugation rate of 12,000 g can help in identifying a protein of up to 14 kDa in the salivary supernatant [5]. From both studies, it appears that an increase from the speed of 10,000 g can help in identifying a molecular mass less than 30 kDa. Based on this, this study aimed to test the effectiveness

of centrifugation speeds of 11,000 g and 13,000 g in separating saliva protein molecules with a molecular mass less than 30 kDa.

2. Materials and Methods

This research was a laboratory experiment using saliva samples taken from 15 subjects (up to 3 ml for each person). Saliva samples were divided into two treatment groups, which were centrifuged at 11,000 g and 13,000 g. Centrifugation was carried out for 10 minutes at a temperature of 4 °C. The centrifugation tool used was "Sorval Legend RT" with the Microliter Rotor 7500 3332 (minimum radius of 5.9 cm and maximum radius of 8.7 cm).

For the centrifugation saliva supernatant, the total protein concentration was measured using the Bradford test. After the result of total protein concentration, the salivary supernatant was equated with a concentration with sterile phosphate-buffered saline (up to a total volume to 50 µl). As much as 24 µl of the supernatant was taken and mixed with 8 µl of the native buffer to be denatured with a thermos block at 100 °C for 5 min. After that, the sample was prepared to be identified by the SDS-PAGE technique. Electrophoresis was carried out for 60 minutes with a voltage of 150 V and a current of 80 mA. The polyacrylamide gel result of SDS-PAGE was analyzed with gel doc. The data of this research were then analyzed descriptively by looking at protein with a molecular mass <30 kDa.

3. Results and Discussion

3.1 Results

The gel doc showed the frequency of salivary protein after centrifugation at 11,000 g and 13,000 g (Table 1). The minimum and maximum molecular masses were obtained from the overall profiles of centrifuged saliva protein (Table 2) and the molecular mass of less than 30 kDa (Table 3) both from the centrifugation rate group of 11,000 g and 13,000 g. The assessment of salivary protein frequency more than or less than 30 kDa after centrifugation at 11,000 g and 13,000 g can be seen in Figures 1, 2 and 3. Figures 4 and 5 show the effect of centrifugation rates of 11,000 g and 13,000 g on the separation of salivary proteins. It was seen that there is a difference in frequency distribution pattern of saliva protein occurrence for molecular mass as a whole as well as for molecular mass less than 30 kDa.

Table 1. Frequency of protein occurrence after centrifugation

kDa	Centrifugation Speed 11,000 g	Centrifugation Speed 13,000 g
<30	35	45
≥30	46	66
Total	81	111

Table 2. Saliva protein molecular mass range after centrifugation

Protein Molecular Mass	Centrifugation Speed 11,000 g	Centrifugation Speed 13,000 g
Minimum Value	9.53 kDa	8.79 kDa
Maximum Value	333.05 kDa	360.83 kDa
Range	323.52 kDa	352.04 kDa

Table 3. Saliva protein molecular mass range <30 kda after centrifugation

Protein Molecular Mass	Centrifugation Speed 11,000 g	Centrifugation Speed 13,000 g
Minimum Value	9.53	8.79
Maximum Value	26.77	17.70
Range	17.24	8.91

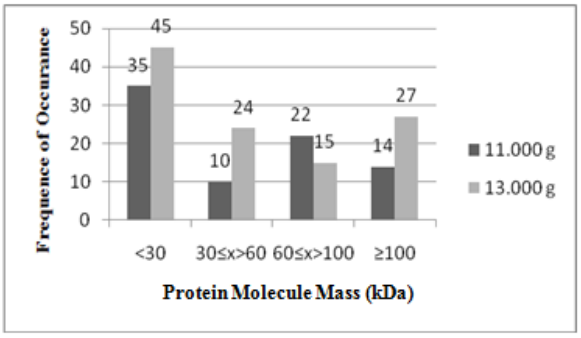

Figure 1. The frequency distribution of saliva protein based on molecular mass

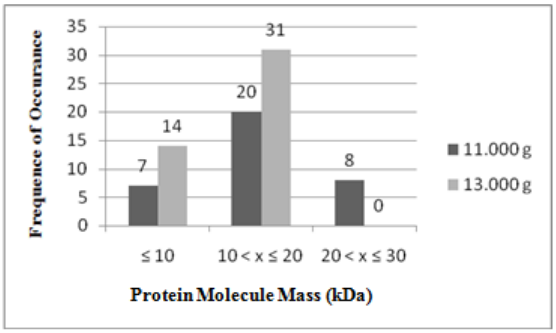

Figure 2. The frequency distribution of saliva protein with a molecular mass less than 30 kDa

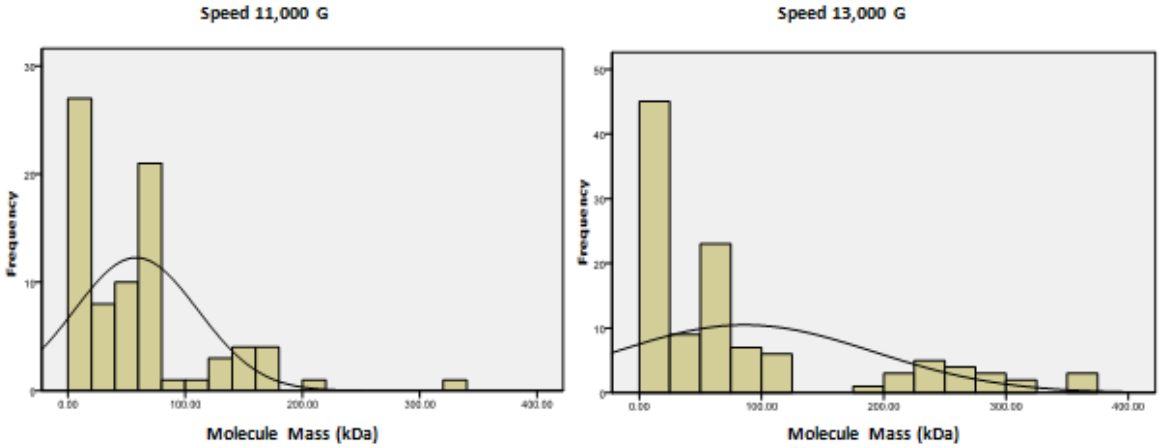

Figure 3. Frequency of saliva protein based on molecular mass. Description: (left) centrifugation rate of 11,000 g, (right) centrifugation rate of 13,000 g.

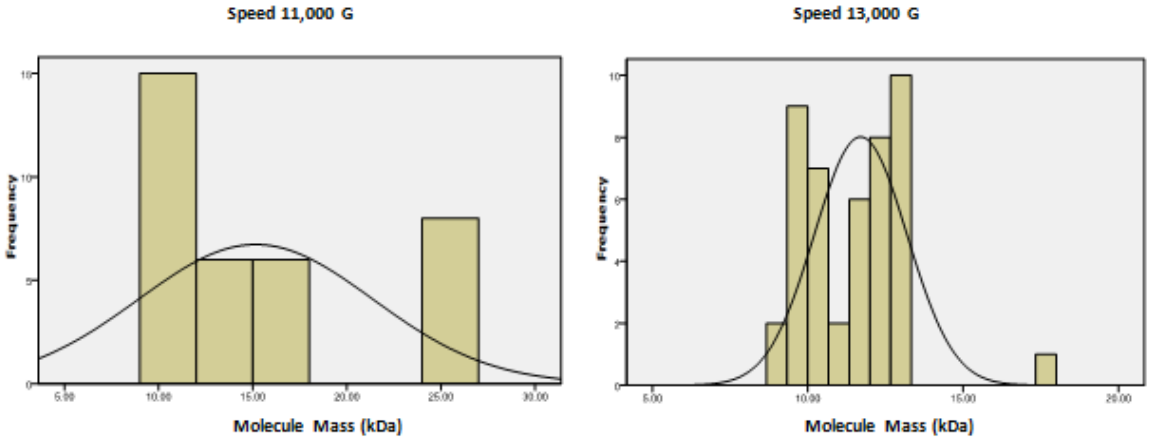

Figure 4. Frequency of saliva protein with a molecular mass less than 30 kDa. Description: (left) centrifugation rate of 11,000 g, (right) centrifugation rate of 13,000 g

3.2 Discussion

The results of this study indicate that the centrifugation rate of 13,000 g increases the frequency of protein occurrence more than the speed of 11,000 g. The same is true for separating proteins with

molecular masses less than 30 kDa, whose frequency is higher for a centrifugation speed of 13,000 g [4]. The results of this study showed that an increase in the centrifugation rate caused an increase of protein emergence with a molecular mass of less than 30 kDa (35 for 11,000 g and 45 for 13,000 g). The results of this study differ with centrifugation theory, which states that the faster the centrifugation, the lower the finding of emerging protein will be [7]. The explanation for the results of this study may be due to the high-speed centrifugation causing proteins with a low molecular mass of the same shape and size, which will bind chemically in the supernatant so it does not participate in sedation.

In this study, saliva protein with a molecular mass of less than 8 kDa could not be identified. It is known that some saliva proteins that can be used as a diagnostic fluid have a molecular mass of less than 8 kDa (i.e., insulin [5 kDa] and histatin [3-4.5 kDa]) [1]. Unidentified protein with a molecular mass of less than 8 kDa can be a result of a chemical bond between small protein molecules and large protein molecules [8]. As a result of the centrifugation of proteins with large molecular masses; it will first be sedimented [3]. This is done so that small molecules follow large molecules into sedimentation. The unidentified proteins less than 8 kDa correspond to the results of a study by Kriscahyani, which states that molecular masses below 9 kDa are not found in centrifuged saliva [4]. In accordance with centrifugation theory (an increased centrifugation rate leads to an increase in the frequency of proteins with small molecular masses), the frequency of proteins less than 9 kDa occurring in this study is higher than previous studies that used a speed of 10,000 g. In centrifugation theory, it is stated that the centrifugal force as well as the density of particles and solutions influence the movement of particles [9]. In addition, this study showed that, for saliva proteins less than 30 kDa, centrifugation at 13,000 g produces a higher frequency of protein emergence with a narrower range of molecular mass than11,000 g. In the previous study with a centrifugation of 10,000 g, the protein molecule mass ranged below 30 kDa [4]. Based on sedimentation theory, the greater the molecular size and centrifugal force, the faster the sedimentation particles will be [9]. Thus, it can be seen that with a higher speed, the molecular mass range value below 30 kDa gets smaller.

From the pattern of frequency distribution of protein emergence (described above), it can be seen the frequency of occurrence of centrifuged saliva at 11,000 g and 13,000 g. In the saliva centrifuged at 11,000 g, the frequency mode of occurrence is present in the molecular mass range from 9 kDa to 12 kDa. However, in the saliva centrifuged at 13,000 g, the frequency mode of occurrence is present in the molecular mass ranging from 12 kDa to 13 kDa. This shift in frequency mode of occurrence may be due to an increase in the centrifugation force employed. Thus, small molecules are more likely to participate in sedation because they are bound by large molecules. Based on previous study on salivary protein precipitation, the samples were centrifuged at a speed of 12,000 g and experienced molecular weight loss of less than 14 kDa [5]. In this study, at a speed of 11,000 g, protein loss occurred with a molecular mass of less than 9 kDa. For the speed of 13,000 g, the loss of protein occurred with a molecular mass of less than 8 kDa. The incompatibility of the pattern of decreasing the molecular mass of lost proteins based on the rate of centrifugation may be due to differences in the denaturation techniques. In the study of Francis *et al*. the supernatant was mixed with EDTA after centrifugation [5]. In this study, however, denaturation was obtained from the native buffer and heating to100 ºC for 5 min. Denaturation of proteins can occur in high temperatures, extreme pH, organic solvents, mechanical influences, detergents, or urea [9].

Various factors influence the detection of salivary proteins, including centrifugation techniques, SDS-PAGE, and salivary protein properties. In this study, preparative centrifugation was used. Other centrifugation techniques (such as differential centrifugation and density-gradient centrifugation) may be used to analyze saliva protein to obtain a cleaner supernatant [7,10]. The polyacrylamide concentration in the SDS-PAGE gel affects the quantity of separated molecular mass [11]. The greater the percentage of acrylamide, the smaller the molecular mass range that is separated. The acrylamide concentration in the SDS-PAGE gel of 12% has been known to separate the molecular mass of the proteins from 10 kDa to 200 kDa [11]. It may be necessary for larger acrylamide concentrations to obtain molecular masses below 8 kDa as well as to consider other factors. In addition, the SDS buffer

clarity affects the speed of the protein moving toward the anode. The weakness of this study is that the researchers did not identify the thickness of the protein bands, so identifying the protein can only be done through the presence or absence of protein based on molecular mass.

4. Conclusion

Compared with the centrifugation rate of 11,000 g, the centrifugation rate of 13,000 g yields a salivary protein pattern of less than 30 kDa with a narrower range of molecular mass. In addition, the rate of centrifugation of 13,000 g can separate more saliva proteins with molecular masses of less than 30 kDa compared to the speed of 11,000 g. Therefore, if we want to separate proteins with a molecular mass of less than 30 kDa in a more subtle way, they should be centrifuged at a speed above 11,000 g. Further studies of saliva protein analysis are needed on centrifugation duration adjustment and protein band thickness identification in SDS-PAGE results.

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