

## Effect of centrifugation at 7,000 g, 8,000 g, and 9,000 g on the salivary protein profile $\geq 30$ kDa

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**Abstract.** There are no established standard operational procedures of centrifugation for protein separation. Centrifugation at 10,000 g separates  $\geq 30$  kDa salivary proteins. The aim of this study is to determine the effect of centrifugation at 7,000 g, 8,000 g, and 9,000 g on the salivary protein profile and the frequency of  $\geq 30$  kDa salivary proteins. Post-centrifuged salivary supernatant was analyzed using SDS-PAGE. An increased centrifugation speed resulted in the decreased frequency of  $\geq 30$  kDa salivary protein emergence. Each centrifugation speed resulted in a different protein profile. Centrifugation at 7,000 g, 8,000 g, and 9,000 g influences the frequency of salivary protein emergence and protein profiles  $\geq 30$  kDa.

### 1. Introduction

Over the last few years, the awareness of saliva analysis as a beneficial diagnostic tool has increased. The superiority of saliva as a diagnostic media over blood test is that saliva collection is easy, non-invasive, and not painful. In addition, saliva sampling is safe for the operator and the patient; there is also a lower risk of infection [1]. Saliva can be used as a biomarker to detect the physiological condition of the body. Saliva contains various components that can be used to assess and monitor the health and status of a disease. It can also be used to determine drug response, hormones, and pollutants; it can detect bacteria, viruses, and systemic diseases [2]. Saliva consists of 99% water and various electrolytes (Na, K, Ca, Mg, bicarbonate, phosphate), proteins and nitrogen products [3]. One of the components that are contained in the saliva are proteins. The proteins contained in the saliva are proline-rich protein,  $\alpha$ -amylase, mucin, cystatin, serum albumin, and immunoglobulin [4]. Research by Bandhakavi et al. has identified 444 types of proteins in human saliva, and around one-fifth of the proteins are also found in the plasma [2]. Therefore, saliva can potentially act as a laboratory examination material, replacing blood in biological test samples.

It is necessary to standardize a saliva collection technique—especially when saliva is used as a research material. Saliva contains various components from different sources. Separating the specific protein from other components contained in the saliva can be resolved with the pre-treatment of saliva, such as collecting the samples in temperatures that can inhibit the metabolism or using centrifugation to eliminate bacteria and cellular packing [5]. Centrifugation is a process used to separate the particles or the concentrated material, such as the cell, subcellular organelle, viruses, and large molecules (including proteins and nucleic acids), to obtain pure samples of the entire particle or material. The basic theory of centrifugation is the effect of gravity on the particles in the suspension. Centrifugation will separate particles based on size, shape, and density into two phases: supernatant and pellet [6].



Based on the theory of centrifugation, greater centrifugation power is needed to restructure smaller cellular components [7].

The research by Kriscahyani (2011) stated that identifying proteins with the mass of molecules greater than or equal to 30 kDa required centrifugation [8]. In general, the centrifugation speed of 10,000 g is often used to separate material. Research by *Biochemistry Laboratory* reported that centrifugation at a speed of 8,000 g can identify  $\alpha$ -amylase with a 62 kDa molecule mass [9]. However, standard centrifugation speed settings are currently not known for proteins with molecule masses above 30 kDa. Research by Kilmer et al. (about protein electro blotting and mass of protein molecules) differentiated molecules into three groups: low (<25 kDa), medium (25–150 kDa), and high (>150 kDa). Among the various saliva proteins with molecule masses greater than or equal to 30 kDa, there is a functional protein (such as albumin [66 kDa]) that functions as a biomarker for diabetes mellitus [10]. C-reactive proteins (CRP) (115 kDa) as biomarker for inflammation [2] and IgA (150 kDa) as biomarker for HIV [11]. Therefore, research is still needed to find a relational pattern of centrifugation speed and the mass of the saliva's protein molecule before the standard procedure of material separation techniques on saliva proteins can be done. This research will examine the effect of a centrifugation speed less than 10,000 g on the separation of protein saliva with various molecular masses greater than or equal to 30 kDa.

## 2. Materials and Methods

Experimental laboratory research was carried out using samples of unstimulated saliva; these samples were obtained from 15 subjects from the Faculty of Dentistry at the University of Indonesia. The range of their age was 18–22 years. Samples of saliva were then divided into three treatment groups (centrifugation speeds of 7,000 g, 8,000 g, and 9,000 g) with 0.6 ml in each group. Samples of saliva were then centrifuged for ten minutes at a temperature of 4°C. The supernatant of the centrifugation result was seen in the proteins' total concentration through the Bradford test, and it was standardized to obtain the same total concentration of proteins. The determination of the saliva protein profile was carried out using SDS-PAGE to view the mass of protein molecules that were read through the Gel doc and identified mass of protein molecules saliva which has  $\geq 30$  kDa. The results were then analyzed descriptively.

## 3. Results and Discussion

### 3.1 Results

The frequency of the emergence of proteins with molecule masses <30 kDa and  $\geq 30$  kDa is identified in the results of supernatant centrifugation at 7,000 g, 8,000 g, and 9,000 g, which can be seen in table 1. The range, maximum, minimum, and mode values of the entire mass of protein molecules (which were identified in the results of supernatant centrifugation at 7,000 g, 8,000 g, and 9,000 g) can be seen in table 2. The range, maximum, minimum, and mass mode values of  $\geq 30$  kDa protein molecules (which were identified in the results of supernatant centrifugation at 7,000 g, 8,000 g, and 9,000 g) can be seen in table 3. The frequency of the occurrence of proteins was classified based on the mass of the molecule in the saliva supernatant into four classes according to Manuela Kriscahyani's research. The chance of occurrences of proteins with molecule masses  $\geq 30$  kDa in the supernatant after centrifugation at speeds of 7,000 g, 8,000 g, and 9,000 g can be seen in table 4.

**Table 1.** The frequency of the occurrences of proteins with molecule masses <30 kDa and  $\geq 30$  kDa

	7,000 g	8,000 g	9,000 g
<30 kDa	30	24	12
30 kDa $\geq$	61	59	43
Sum	91	83	55

**Table 2.** The value of the range, maximum, minimum, and mode overall masses, protein molecules that are identified in the results of supernatant centrifugation at 7,000 g, 8,000 g, and 9,000 g

	7,000 g	8,000 g	9,000 g
Range	278.53	279.04	210.07
Maximum (kDa)	288.26	288.26	219.61
Minimum (kDa)	9.73	9.22	9.54
Mode (kDa)	12.30(a)	65.03	54.12

(a) There is some mode value. Mode is displayed with the value (smallest kDa).

**Table 3.** The value of the range, maximum, minimum, and mode for  $\geq 30$  kDa protein mass molecules that were identified in the results of supernatant centrifugation at 7,000 g, 8,000 g, and 9,000 g

	7,000 g	8,000 g	9,000 g
Range	257.96	238.26	167.59
Maximum (kDa)	288.26	288.26	219.61
Minimum (kDa)	30.30	50.00	52.02
Mode (kDa)	63.50	65.03	54.12

**Table 4.** Chance of occurrence for proteins with molecule masses  $\geq 30$  kDa after being centrifuged at 7,000 g, 8,000 g, and 9,000 g

Speed of centrifugation	Mode frequency	Number of sample	Chance of occurrence
7,000 g	31	15	2.07
8,000 g	30	15	2
9,000 g	20	15	1.33

### 3.2 Discussion

The identification of the mass of the protein molecule in relation to the theory of centrifugation can be caused by the structures of the proteins, which are varied. Proteins also bind with each other, forming a long chain. Small-sized proteins can bind to proteins that are larger, making a complex bond. Centrifugation only separates proteins based on the nature of the physical, not based on the nature of its chemical composition [7]. Generally, the speed of centrifugation is 10,000 g to 14,000 g for 15–25 minutes to ensure that the maximum retention of the aggregated proteins can be separated. Other factors that may affect the appearance of the protein are the obtaining technique of saliva supernatant and the concentration of polyacrylamide gel [12]. In general, this research is influenced by the speed of centrifugation, protein structure, and the method. The saliva protein profile of  $\geq 30$  kDa in the supernatant centrifugation results (for 7,000 g, 8,000 g, and 9,000 g) in this research can be seen in figure 8. The increase in the centrifugation speed from 7,000 g to 9,000 g produces a decrease in the frequency of the proteins with molecule masses  $\geq 30$  kDa in the supernatant saliva. Increasing centrifugation from 7,000 g to 8,000 g causes the mass range of protein molecules  $\geq 30$  kDa in the supernatant to experience a narrowing value, which was mainly caused by the change in the minimum value. This change may occur because the small proteins near the minimum value bind with bigger proteins [13].

By increasing the speed of centrifugation from 8,000 g to 9,000 g, the mass of the protein molecules with a mass of  $\geq 30$  kDa experienced a narrowing value, which was mainly caused by the maximum value decreasing; the minimum value can be said to have not changed. The maximum value changes in accordance with the theory of centrifugation (which is: the higher the speed is, the larger

the protein molecules that will be deposited). The mode value is relatively unchanged, which seems to indicate that centrifugation at 7,000 g, 8,000 g, and 9,000 g is optimal for identifying medium-sized proteins (50–150 kDa) [14]. The overall results seem to show there is a narrowing range, decreasing the frequency of proteins with molecule masses  $\geq 30$  kDa by increasing the speed of centrifugation from 7,000 g to 9,000 g [15]. This means that increasing the speed of centrifugation is not able to separate the finer proteins with molecule masses  $\geq 30$  kDa.

#### 4. Conclusion

From the results, it can be concluded that increasing the speed of centrifugation from 7,000 g to 9,000 g decreases the frequency of the occurrence of saliva proteins with molecule masses  $\geq 30$  kDa. Increasing the centrifugation speed from 7,000 g to 8,000 g removes small proteins in the saliva from the supernatant, and increasing the centrifugation speed from 8,000 g to 9,000 g removes large proteins in the saliva from the supernatant. A centrifugation speed between 7,000 g and 9,000 g is optimal for the identification of the saliva proteins 25–150 kDa in the supernatant.

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