The Serum Separating Capacity of Ipomoea batatas Starch in Creatinine and Total Cholesterol Determination

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Abstract - Separator gels are composed of polymer gels contained in a tube which are used to separate serum from formed elements for various diagnostic tests. An alternative serum separator gel, 0.25 g/mL of *Ipomoea batatas*, was used in total cholesterol and creatinine test determination. Results show that *Ipomoea batatas* starch can be used as an alternative serum separator gel for the determination of creatinine and total cholesterol as all the samples in the alternative serum successfully separated the serum from the formed elements. Despite its ability to lower the cholesterol levels, *Ipomoea batatas* can be used as a serum separator gel for the total cholesterol determination as there is a stronger agreement [$\rho_c = 0.84$] in the total cholesterol levels of serum separator tubes and *Ipomoea batatas* starch serum separator tubes, as compared to the agreement [$\rho_c = 0.53$] in the creatinine levels.

Index Terms- *Ipomoea batatas*, Cholesterol Determination, Creatinine Determination, Starch, Serum Separator Gel

I. INTRODUCTION

Cardiovascular disease is the number one cause of death worldwide, with an estimated number of 17.5 million deaths in 2012, nearly 31% of deaths globally (World Health Organization, 2012). In addition to cardiovascular disease as one of the leading causes of death worldwide, kidney failure ranks 8th (Centers for Disease Control and Prevention, 2009). According to United States' Renal Data System's 2013 Annual Data Report, 10% of the country's population is suffering from Chronic Kidney Disease, and 9 out of 10 did not know they have it (CDC, 2011). Immediate determination of the amount of cholesterol and creatinine present in the blood is essential in the early diagnosis and treatment of kidney failure.

Separator gels are important in routine diagnostic tests which requires the use serum, which can not be obtained unless it is separated from the other components in the blood. Serum separator gels acts as a barrier that separates serum from the different cellular elements in the blood, when the blood is placed into tubes and centrifuged. These gels are commonly made from viscous liquid, tackifiers and fillers along with substances which act as gelling agents such as dibenzylidene sorbitol. It is important to note that the serum separator gel does not react with the serum and other components of the blood which may interfere with the different laboratory tests and results.

The researchers proposed *Ipomoea batatas* as alternative for commercial serum separator due to its cost and availability. *Ipomoea batatas*, a native root crop abundant in tropical countries like the Philippines (Reynoso, 2011), is easy to grow as it requires little attention and water for successful cultivation. Currently, researchers in United States of America, Japan, and China are researching on the different industrial uses of *Ipomoea batatas* (North Carolina State University, 2008).

II. OBJECTIVES

This research aims to produce an alternative serum separator gel through the use of *Ipomoea batatas* starch extract. Additionally, to determine if there is a significant agreement in the creatinine and total cholesterol determination of serum using serum separator tubes and 0.25 g/mL *Ipomoea batatas* starch gel.

III. METHODS

3.1 Selection of Subjects
For this study, ten subjects were randomly selected from the class of the researchers. Venous blood was extracted through the evacuated tube method in order to obtain two tubes containing three mL of blood from each subject. The blood samples (2) taken from each of the subjects would be allocated a separate commercial serum gel separator for one and the other the 0.25 g/mL alternative *Ipomoea batatas* starch gel. Correspondingly, the serum obtained from both samples of each subject would be tested for creatinine and cholesterol determination.

3.2 Experiment procedure
The preparation of the serum gel separator was done by extracting starch from *Ipomoea batatas*. These were cut into cubes and were placed inside a blender with distilled water. The mixture was then filtered using cheesecloth. It was squeezed until the filtrate turned clear and the solid residues were dry. The filtrate was placed in the refrigerator overnight to allow the starch to settle at the bottom of the container. The liquid part of the filtrate was removed to isolate the starch. After the extraction of starch, 7.5 g of starch was weighed using an analytical balance. Afterwards, 30 mL of normal saline solution (NSS) was used as a solvent and was added to the starch in order to obtain the concentration of 0.25 g/mL. NSS was used as a solvent because it does not lyse the red blood cells when the gel comes in contact with the cells during the blood extraction. The resulting mixture was then heated to $300°C$ on a hot plate until a gel formed. The temperature of the gel was

maintained at 70 to 75°C. The prepared gel was approximated to the level of serum separator gel and incorporated in each of the red top evacuated tubes. The tubes were centrifuged for the gel to settle at the bottom. The gel was allowed to cool for one day before it was used as a serum separator.

After preparing the serum separator, venous blood samples were taken from the subjects using evacuated tube method. The first step was to attach a two way needle into an adaptor. The tourniquet was then applied to the subject three to four inches above the site of puncture. The site was cleansed with 70% isopropyl alcohol. After letting the site dry, the vein was punctured by the two-way needle attached to the adaptor. The first tube, which was the red top tube containing the *Ipomoea batatas* extract, was pushed into the adaptor until three mL of blood has been collected. The same was done with the second tube which contained the commercial serum separator gel. After collecting the two samples, the tourniquet is loosened and the needle is withdrawn from the subject.

The total cholesterol of the samples was determined by means of enzymatic hydrolysis mediated by the enzymes cholesterol esterase, cholesterol oxidase, and peroxidase. Twenty-one cuvettes were prepared and labelled as blank (reagent blank), standard (200 mg/dL cholesterol standard), sample 1 (serum from commercial separator tube), and sample 2 (serum from 0.25 g/mL starch gel concentration). Using a serological pipet, an aliquot portion of 0.01 mL serum from commercial separator tube, 0.25 g/mL starch gel concentration, and 0.01 mL of standard solution was dispensed to their respective cuvettes. Then, 1.0 mL of the reagent was added to each of the cuvette. The cuvettes were covered with parafilm, mixed and incubated for ten minutes at 20 to 25°C. The absorbance of the standard and samples were measured against the reagent blank at a wavelength of 500 nm using the Biosystem BTS-310. Lastly, the concentrations of the samples were calculated using their corresponding absorbances.

The creatinine of the samples was determined by Jaffe reaction involving the reaction of creatinine with picric acid forming an orange-red colored creatinine-picrate complex. Twenty-one cuvettes were prepared and labelled as blank (air blank), standard (2 mg/dL creatinine standard), sample 1 (serum from commercial separator tube), and sample 2 (serum from 0.25 g/mL starch gel concentration) respectively. The reagent for creatinine determination was prepared by mixing one mL of picric acid with one mL of diluted sodium hydroxide following the 1:4 ratio. Using a serological pipet, an aliquot portion of 0.1 mL serum from commercial separator tube, 0.25 g/mL starch gel concentration, and 0.1 mL of standard solution was dispensed to their respective cuvettes. Then, 1.0 mL of the reagent was added to each of the cuvette except to the one labelled as blank (air blank). The cuvettes were covered with parafilm and mixed. After 30 seconds, the first absorbance of the standard and samples were read at wavelength of 520 nm using the Biosystem BTS-310, and exactly after two minutes, the second absorbance was read. Lastly, the concentrations of the samples were calculated using their corresponding absorbance. The serum obtained from the starch gel and the control were both subjected to the Jaffe reaction for creatinine determination. The creatinine concentrations were determined spectrophotometrically.

3.3 Statistical Analysis

Lin's concordance correlation coefficient, including its 95% confidence interval, was used to determine the level of agreement in the serum total cholesterol and creatinine determination using 0.25 g/mL *Ipomoea batatas* starch and the commercial serum separator tube. These were performed using MedCalc version for Windows, ver 15.11.0 (MedCalc Software, Ostend, Belgium). Concordance coefficients of <0.90 indicates poor agreement, while 0.90 to 0.95, 0.95 to 0.99 and >0.99 indicates moderate, substantial, and almost perfect concordance, respsectively (McBride, 2005).

IV. RESULTS AND DISCUSSION

Both the ten samples of the *Ipomoea batatas* starch gel with a concentration of 0.25 g/mL and the ten samples of the control have successfully separated the serum component from the formed elements and formed a barrier between the components of the blood.
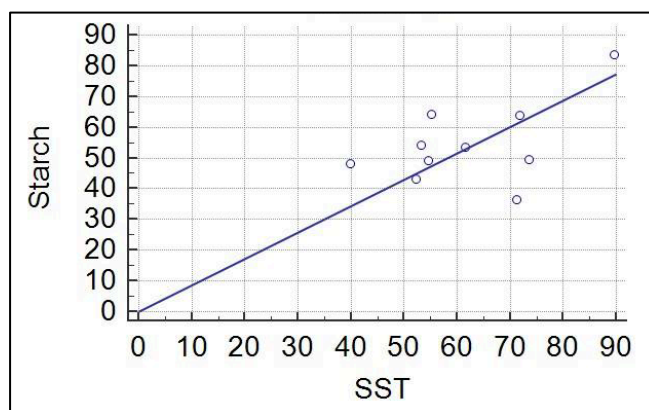

Figure 1. Scatterplot of creatinine concentration (in µmol/L) of SST and *Ipomoea batatas* starch gel

The concordance correlation coefficient of $\rho_c = 0.53$ [CI$_{95\%}$: -0.15 to 0.87], indicates a poor agreement between the creatinine levels of serum separator tubes and *Ipomoea batatas* starch serum separator tubes. See Figure 1.

The Lin's concordance coefficient for the total cholesterol is $\rho_c = 0.84$ [CI$_{95\%}$: 0.45 to 0.96], which likewise, indicate a poor agreement between the cholesterol levels of serum separator tubes and *Ipomoea batatas* starch serum separator tubes. See Figure 2.
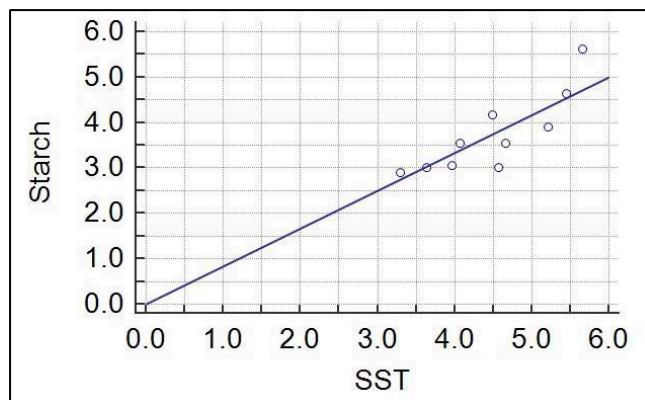

Figure 2. Scatterplot of total cholesterol concentration (in mmol/L) of SST and *Ipomoea batatas* starch gel

Results of the experiment were similar with Castro *et al.* (2015) where all samples of the 0.25 g/mL concentration of the *Ipomoea batatas* starch gel have successfully separated the serum from the formed elements and formed a barrier between these components of blood. But unlike in their study, where $\rho_c = 0.13$ in determining agreement in glucose concentrations, our study have shown that both creatinine and total cholesterol levels showed better agreement. Moreover, the results of this research were in accordance with Trinidad *et al.* (2013) where sweet potatoes were not able to lower serum total cholesterol. This coincided with the research of Allane (2015) wherein it was discussed that the lowering ability of starch inside the body was due to its binding with bile acids, causing increased excretion of cholesterol inside the body. In this research, wherein the starch was only added to the serum after venous collection, there was no fermentation of starch in the tube. Despite that results in our experiment did not reach, at least, the moderate agreement, coinciding with the other researches (Trinidad *et al.*, 2013; Allane, 2015) have shown that total cholesterol tests can be done using the *Ipomoea batatas* starch.

V. CONCLUSION AND RECOMMENDATIONS

Ipomoea batatas starch can be used as an alternative serum separator gel. Despite its ability to lower cholesterol levels, *Ipomoea batatas* can still be utilized as a serum separator gel for the total cholesterol determination, more than the creatinine determination. The results for creatinine however showed a possibility of interference by *Ipomoea batatas*. For future related research in the field of biological alternatives, the researchers suggest to determine other concentrations apart from the 0.25 g/mL that can serve as an optimum concentration where the gel would settle between the serum and blood cell components. The researchers also suggest to use the starch extract in other serum routine tests, like electrolytes because it has been known that there is a separate machine being used for electrolyte determination because the commercially available serum separator tubes can affect the electrolyte values. Future researchers can study whether the starch extract can be used in electrolyte determination or not. The use of a method other than the Jaffe reaction and cholesterol oxidase method may be used to test creatinine and cholesterol levels respectively. Also, to prevent the growth of molds, the researchers suggest that the proper preservation and shelf life of the starch extract be noted.

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