The Serum Separating Capacity of *Ipomoea batatas* Starch in the Routine Glucose Determination

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Abstract- Serum separator tube is composed of synthetic polymer gels designed to separate serum from the formed elements. In line with this, an alternative serum separator gel using 0.25 g/mL concentrated *Ipomoea batatas* starch extract was produced. Results show that all the samples in the alternative serum separator gel successfully separated the serum from the formed elements. However the serum blood glucose levels using the alternative serum separator gel showed no evidence of agreement [ρ_c = 0.133, $CI_{95\%}$: -0.23 to 0.46] when compared with commercial serum separator tube.

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

I. Introduction

The average human body is composed of 5 L of blood. The functions of the blood include: transportation of oxygen from lungs to tissues; transportation of glucose, proteins and fats; and movement of wastes to the liver and kidneys. Blood consists of formed elements, which is further subdivided into red blood cells (RBC), white blood cells (WBC) and platelets, and plasma, which provides coagulation enzymes that protect vessels from trauma and maintain the circulation (Rodak, Fritsma, & Keohane, 2012). Without an anticoagulant, the blood's clotting factors are activated to form a clot, which is encapsulated by the protein fibrinogen, and plasma is transformed into serum (Bishop, Fody, & Schoeff, 2013). Blood tests performed for clinical diagnostic and disease monitoring purposes commonly use serum or plasma. Electrolytes, enzymes, and hormones are important analytes of interest and assayed in serum and plasma. Thus, there is a need to separate serum or plasma from the formed elements and sustain a physical barrier between these blood components (Sun, Oh, Emerson, & Raghavan, 2011).

Serum separator tube (SST) is designed to separate serum from the formed elements. Typically, whole blood is drawn and transferred to SST that contains a separator gel. The separator gel is designed to have a density between that of the formed elements and serum. The tube is then centrifuged and the gel liquefies and settles in between the denser formed elements and the less dense serum by virtue of relative densities because of the thixotropic property of the gel. After centrifugation, the gel returns to its gel state, leaving a soft barrier (Sun *et al.*, 2011). Preferably, interaction with separator gels should not affect the laboratory results; however, effects on analyte concentrations were seen on several reports. Drug absorption to the gel may be influenced by specimen volume, storage time, temperature, and gel type. Materials that interfere with analytical assays may also be released from the separator gels (Bowen, Hortin, Csako, Otañez, & Remaley, 2010).

Starch, an insoluble polymer of glucose residues produced by the majority of higher plant species, is a major storage product of seeds and storage organs produced agriculturally and used for human consumption (as cited in Chen *et al*., 2015). Starch, as a polysaccharide, has applications which include use as thickeners, thixotropic agents, and flow and texture enhancers (Clasen & Kulicke, 2000).

Ipomoea batatas, typically known as sweet potato, is rich in carbohydrates, carotene, and polyphenolic antioxidants as well as a good source of vitamins A-C, calcium, iron, and phosphorus. In the Philippines, where it is commonly known as *kamote*, *Ipomoea batatas* is cultivated as a basic staple food. Promotion of *Ipomoea batatas* production and consumption is led by the Department of Agriculture, specifically the Bureau of Agricultural Research. Moreover, it is included as a priority among the root crops through the continued collaborative effort by the Philippine government. Although the use of *Ipomoea batatas* as ingredient in food products such as ketchup, jam, soy sauce, jellies, and other bakery and non-bakery products is increasing in popularity in the country, its industrial use is considerably superseded by China, Korea, and Vietnam, where it is being utilized for the production of chemical products, paper, paint, and ink, among others (Reynoso, 2011).

II. Objectives

This study aims to propose an alternative serum separator gel through the use of *Ipomoea batatas* (sweet potato) starch extract. Specifically, the research aims to determine if the serum is separated from the formed elements using 0.25 g/mL *Ipomoea batatas* starch gel; and to determine if there is a significant agreement in the glucose 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, the samples were obtained from five subjects through simple random sampling. Five different set-ups were used wherein each set-up consists of two tubes: control (commercial serum separator tube) and alternative *Ipomoea*

batatas starch gel with 0.25 g/mL concentration. The serum, which would be tested for glucose determination, would come from the venous blood extract using the syringe method of the abovementioned subjects.

3.2 Experiment procedure

The preparation of the separator gel was done by extracting the starch from *Ipomoea batatas (*sweet potato). First, the root crops were cut into cubes and placed inside the blender with distilled water. The resulting mixture was filtered using the cheesecloth. The solid residues were squeezed until dry and the filtrate was set aside in the plastic containers and refrigerated overnight to let the starch settle at the bottom. The liquid part of the filtrate was discarded to isolate the *Ipomoea batatas* starch.
Once the starch has been extracted, 7.5 g of the starch was weighed using the analytical balance. Afterwards, 30 mL of normal saline solution (NSS), which served as the solvent, was added to the weighed starch to obtain the concentration of 0.25 g/mL. NSS was used as the solvent since 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 heated at 300˚C on a hot plate until a gel formed. The temperature of the gel was carefully monitored and maintained at 70 to 75˚C. The prepared gel was approximated to the level of serum separator gel and incorporated in each of the plain red-top evacuated tubes. The tubes were centrifuged in order for the gel to settle at the bottom. The gel was allowed to cool down for at least one day before being used for serum separation.

Figure 1. Separated Serum After Centrifugation (left), and Glucose Determination using Glucose Oxidase Method (middle and right)

Syringe method was used as the blood collection method. Ten-milliliter syringes were used to avoid multiple punctures on the antecubital fossa of the subjects. The two tubes in each of the five set-ups were filled with 3 mL of blood from each subject: control (commercial serum separator tube) and alternative gel with 0.25 g/mL concentration. Since the serum was the sample examined for glucose determination, the collected blood was allowed to stand for 15 minutes in the control for it contains a clot activator and 30 minutes for the alternative gel for maximum clot formation. These tubes were centrifuged and the serum was separated.

The levels as to which the serum is separated in the 0.25 g/mL tubes were compared to that of the control qualitatively to determine the effectiveness of the alternative separator gels. The glucose concentrations of serum in each of the ten tubes were determined using the glucose oxidase method. From the twelve cuvettes, one was labeled properly with blank (blank reagent), one with standard (100 mg/dL glucose standard), five cuvettes with control and the corresponding seat numbers of the subjects (serum from commercial serum separator tube), and the last five cuvettes with starch and the corresponding seat numbers (serum from 0.25 g/mL starch gel concentration). Using the mechanical pipet, 0.01 mL aliquots of each of the serums and the standard solution were aspirated and dispensed on their respective cuvettes. Then, 1.0 mL of the reagent was added to each of the cuvettes with serum. The cuvettes were covered with parafilm, mixed, and incubated for 10 minutes at 20 to 25˚C. The absorbance and concentration of the standard and the samples were measured against the reagent blank within 60 minutes using the Primus Humalyzer Spectrophotometer. Only the glucose concentrations were used for the statistical analysis. See Figure 1.

3.3 Statistical Analysis

Pearson coefficient of concordance was used to determine if glucose determination of serum using serum separator tubes agree with 0.25 g/mL *Ipomoea batatas* starch. The statistical test used 5% level of significance with the aid of MedCalc version 14.10.2 (MedCalc Software, Ostend, Belgium).

IV. Results and Discussion

All the five samples of 0.25 g/mL concentration of alternative *Ipomoea batatas* starch gel have successfully separated the serum from the formed elements and formed a barrier between these components of blood, as well as all of the five samples of control (commercial serum separator tube).

The serums obtained from the control and the alternative *Ipomoea batatas* starch gel were subjected to glucose oxidase method. The glucose concentrations of the serums were measured spectrophotometrically. See Table 1.

Table 1. Glucose concentrations (in mg/dL) of SST (control) and Starch.

Samples	SST (control)	*Ipomoea batatas* Starch gel
1	2.32	3.50
2	0.53	2.61
3	0.45	2.20
4	3.10	2.61
5	1.05	3.02

Concordance coefficient is found to be 0.13 [$CI_{95\%}$: -0.23 to 0.46], indicating that there is no evidence of agreement between the glucose levels of commercial serum separator tubes (SST) and *Ipomoea batatas* starch serum separator tubes. See Figure 2.

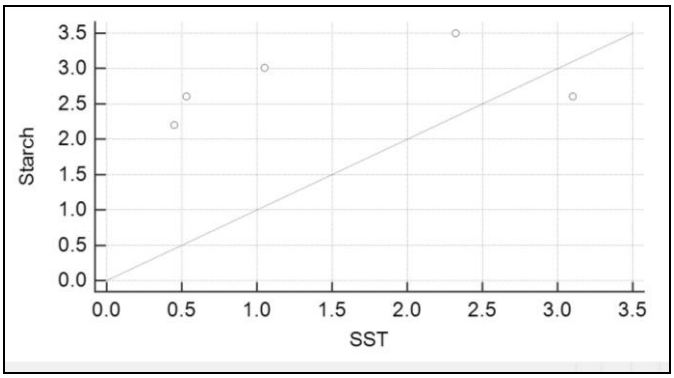

Figure 2. Scatterplot of Glucose Concentrations (in mg/dL) of Serum Using Serum Separator Tubes and *Ipomoea batatas* Starch

The results of this research were in contrast with the study conducted by Aldana *et al.* (2008) wherein it is stated that there is no significant difference in the mean glucose values of the serums collected from the different concentrations of *Solanum tuberosum* (potato) starch serum separator gels and the control. The results were also unparallel with the research conducted by Anico *et al.* (2014) wherein it is also stated that there is no significant difference in the glucose values of serums collected from the different concentrations of *Ipomoea batatas* starch serum separator gels and the commercial serum separator tube. It was also found that there is no significant difference in the mean potassium values of serums collected from the different concentrations of *Ipomoea batatas* starch serum separator gels, the positive control, and the negative control. However, 10 out of the 30 prepared *Ipomoea batatas* starch serum separator gels failed to separate the serum from the formed elements. Instead of direct heat, water bath was used as a source of heat. Homogenization of the starch mixture occurred inside the tubes instead of the beaker.

V. Conclusion and Recommendations

Ipomoea batatas starch gel was able to settle between the serum and the formed elements which makes it a potential alternative serum separator gel; however, there is no agreement between the blood glucose levels of the serum using the commercial serum separator tube and the alternative tube containing the 0.25 g/mL *Ipomoea batatas* starch gel.

For future researchers who would pursue this topic, the use of different concentrations other than 0.25 g/mL, to find the optimum concentration where the gel would settle between the serum and blood cell components is suggested. Furthermore, testing for the other serum routine tests, such as test for creatinine, total cholesterol, triglyceride, high density lipoprotein (HDL) and low density lipoprotein (LDL) may also be done. The use of a method other than glucose oxidase test may be used to test blood glucose levels. 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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