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Sweet Potato (*Ipomoea Batatas*) Starch Gel as a Serum Separator for Electrolyte Determination

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Abstract*—Tubes with separator gels comprised polymer gels that separate serum from formed elements for various diagnostic tests. However, previous studies mentioned that these tubes absorb analytes. Ipomoea batatas' amylopectin has the potential to make a gel. With this, the main objective of this study was to determine the capacity of *I. batatas* as a serum separator gel for electrolyte determination. This research utilized an experimental design. The researchers extracted starch from *I. batatas* to create an alternative gel and used seven rabbits as study subjects for separation. Different concentrations, such as 0.18g/mL, 0.20g/mL and 0.22g/mL, were used. Electrolytes tested are sodium, potassium, chloride and ionized calcium. The sample in the starch gel tube was subjected from 1 hour at 25-30ºC to 24 hours at 4ºC to determine its stability. The electrolyte testing was done in the Best Diagnostic Clinic in Tuguegarao City. The statistical method utilized was One-way ANOVA with Post Hoc Test (Tukey) to determine the significant difference and descriptive statistics. Results showed that *I. batatas* with a 0.18 g/mL concentration separated the serum from blood components among the three concentrations. A One-way ANOVA and the Tukey Post Hoc Test were employed, which shows that sodium and chloride in starch gel tube with 0.18g/mL were significantly different for 1 hour at 25-30C. However, only chloride in the starch gel tube significantly differed in 24 hours at 4C. A repeated-measures ANOVA showed a statistically significant difference between time effects. In conclusion, this shows that time has a significant effect on all the electrolytes. 1 hour at 25-30C significantly affects sodium and chloride while 24 hours at 4C significantly affect chloride only. Also, this study strongly suggests that having a specific procedure to control the temperature and viscosity of the gel and use another diluent electrolyte-free to avoid interference.

Keywords—Ipomoea batatas, starch, serum separator gel, electrolyte determination.

I. INTRODUCTION

Ipomoea batatas, commonly known as "sweet potato" is an important crop in numerous countries. It was ranked fifth among the most essential food crops in the tropics and seventh worldwide (Bach et al., 2021).

Sweet potato, *I. batatas L*. (Lam.), is an essential economic crop in many countries. In annual production, sweet potato ranks as the fifth most important food crop in the tropics and the seventh in the world after wheat, rice, maize, potato, barley, and cassava (Bach et al., 2021). Sweet potato fulfills several fundamental roles in the global food system, all of which have fundamental implications for meeting food requirements, reducing poverty, and increasing food security (El-Sheikha & Ray, 2017). Although *I. batatas* can be used to make ketchup, jam, soy sauce and jellies, its use in industrial area is more prevalent in countries such as China, Korea, and Vietnam, where it is also known to make chemical products and other things (Escobar-Puentes et al., 2022).

The starch, with its major biopolymer components, amylose and amylopectin molecules, is one of the main energy sources in the human diet, according to Ang et al. (2021). She stated that regular starches, such as sweet potato, contain 70-80% amylopectin and 20-30% amylose. The amylose content of sweet potato starches from the Philippines ranges from 29.4-32%, according to Moorthy et al. (2012). Yahia et al. (2016) also described the amylose and amylose pectin composition of *I. batatas*. Its amylose composition is mostly straight with few branches in contrast to amylopectin, which is extensively branched and has a greater molecular weight. She added that the former functions as a diluent and a swelling inhibitor and is in charge of starch retrogradation, whereas the latter, which makes up about 75% of the majority of native starches, is mostly in charge of starch gelatinization. According to their research, longer-chained starches form gels that are more viscous and stable than their shorter-chained counterparts. Ang et al. in 2021 provided a description of the rheological properties of starch, which are significantly modified by processing conditions, particularly high shear and temperature.

They discovered that waxy potato starch has shear-thickening properties after being heated to 120 degrees Celsius for 30 minutes in excess water while being continuously stirred at 300 rpm. Aggregation, sometimes referred to as micelle production, is a process that creates a shear thickening feature by utilizing hydrogen bonds. The shear thickening property of the polymers is also influenced by their electrostatic and hydrophobic interactions, according to Yahia et al. (2016).

The Philippines is known as one of the primary producers of food crops. One of the country's major crops is *I. batatas*, also known as sweet potato or kamote. According to Mwanga et al (2017), from 2016 to 2020, sweet potato production grew at an average annual rate of 0.8 %. During this year's production, it increased up to 138.68 thousand metrics. Previous studies used *I. batatas* as an alternative serum separator gel because of its composition- pectin. According to Zaidel et al. (2022), amylose and amylopectin act as thickening and gelling agents, which is a good factor for gelatinization.

Blood collection devices have the potential to introduce pre-analytical errors during laboratory testing due to their interactions with blood specimens (Lim, 2014). Serum separator gel is one of the additives used in laboratories. However, numerous reports have highlighted how components found in blood collection tubes can adversely affect serum stability. The exploration of substitutes has gained traction, especially in the medical field, because of increasing economic burdens. One of the avenues researchers are exploring involves identifying suitable types of starch and their concentrations. In a preceding study, a researcher proposed the use of *I. batatas* as a viable replacement for serum separator gel in assessing creatinine and total levels. This suggestion was rooted in its cost-effectiveness and wide availability (Galvez et al., 2015).

The introduction of serum separator tubes (SST) in the USA in 1976 has increased its importance in the field of the clinical laboratory (Sankaralingam et al., 2022). According to Castro et al. (2023), serum separator tube was made to separate serum from formed elements. The improvement of serum analyte stability in the primary tube and the altered need for aliquoting of the serum was markedly due to the development of blood collection tubes containing gel, forming a barrier after centrifugation. Although red-top tubes are used standardly for electrolyte determination, yellow-top tubes are commonly used in hospitals for faster turnaround times (Macugay et al., 2018). According to Iacovetti et al (2023), a gel-barrier tube is preferred when immediate results are needed, but a red-top tube is acceptable if centrifuged within 45 minutes.

In comparison, the serum gel separator tubes were centrifuged for 15 minutes at 3,500 rpm only, which is useful when many samples are needed to be tested (Macugay et al., 2018). Nevertheless, Macugay et al. (2018) also mentioned that components from blood collection tubes, such as stoppers, lubricants, surfactants, and separator gels, can lead into specimens and absorb analytes from a specimen. Electrolytes, enzymes, and hormones are essential analytes of interest and are 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 without interference (Sun et al., 2011). This study tested the capacity of *I. batatas* starch gel as a serum separator gel for electrolyte testing, specifically potassium, sodium, chloride, bicarbonate and calcium. With this, different concentrations of *I. batatas* were tested to determine the best concentration for separating blood components.

II. METHODS

The researchers employed a true experimental approach in this study. The extraction and gelatinization of *I. batatas* was held in the laboratories of University of Saint Louis (USL) Tuguegarao. The extraction of rabbits' blood was conducted at Dok Onat's Veterinary Clinic in Pengue Ruyu Diversion Road, Tuguegarao City, Cagayan Valley. Electrolyte levels were tested in Best Diagnostic Clinic located at Carig Sur, Tuguegarao City, Cagayan Valley. The study used five (5) rabbits (*Oryctolagus cuniculus*) to extract the serum specimen used in the experiments.

A. Gathering and preparation of I. batatas tubers

The researchers went to the Department of Agriculture for authentication of the sweet potato to be used. After which, the researchers asked a known local store around Tuguegarao City for the acquisition of fresh sweet potatoes. Then, the researchers bought sweet potatoes weighing five kilograms

B. Extraction of I. batatas starch

Preparation of *I. batatas* (sweet potato) separator gel begun with the extraction of its starch. The starch extraction was carried out using a modified method based on the starch extraction procedures described in the research conducted by Macugay et al. (2018). The fresh sweet potato was washed and left to dry naturally under normal room temperature conditions (around 25-30°C) for one day. The roots were then peeled and cut into smaller pieces, approximately 2 to 3 mm thick in the transverse direction, following the method described by Tortoe et al. (2017). A total of 200 grams of sliced sweet potato and 360 mL of water were placed in a blender and blended for 60 seconds. The resulting mixture's solid residues were thoroughly squeezed until dry, and the mixture was filtered using either cheesecloth or mesh nylon cloth. The filtered starch was allowed to settle for 6 hours, and the upper layer of water was carefully decanted. The wet starch was then left to dry for 24 hours under normal room temperature conditions, producing powdered starch.

C. Gelatinization of Extracted Starch

The researchers weighed 5.4g, 6g, and 6.6g (for three different concentrations that will be incorporated in the tube) of isolated *I. batatas* starch in the analytical balance. After that, 30mL of Normal Saline Solution (NSS) to get the mass concentration of 0.18g/mL, 0.20g/mL, and 0.22g/mL needed, respectively. NSS was the solvent of choice because it does not cause the rupture of red blood cells when it interacts with the gel during the process of blood extraction. Researchers used a temperature of 300℃ for 4 minutes in each concentration on a hot plate to convert the resulting mixture into a gel. The gel's temperature was closely monitored and kept for 70 and 75℃ with continuous stirring until it started to gelatinize as recommended by Galvez (2015). The prepared gel was incorporated into a red top tube. The researchers approximated

the amount of *I. batatas* starch gel to be placed in the red tube top. After this, it went through centrifugation for *I. batatas* gel to appropriately settle at the bottom of the tube. (Castro et al, 2023; Galvez et. al, 2015; Li et. al, 2015).

D. Blood Extraction

The process of extracting blood involved a team of two to three individuals, with one person responsible for restraining the rabbit, another for bleeding the rabbit, and a third person to handle the specimen transfer. The personnel followed the necessary safety precautions and wore appropriate personal protective equipment as mandated by their facility. The veterinarian used a razor to remove the rabbit's hair, and 70% isopropyl alcohol was applied to the rabbit's skin using a squirt bottle to prepare it for the procedure. For blood collection, the veterinarian utilized a winged needle set (21 gauges) and five 3mL syringe tubes. Additional materials such as gauze (3 inches by 3 inches), a sharps container, and a table or work surface to organize the materials were required for this technique.

Properly restraining the rabbit by trained personnel is vital for a successful extraction. The restrainer utilized their dominant hand to physically hold the rabbit by the loose skin at the back of its neck while placing their forearm along the rabbit's spine. The rabbit was then turned against the side of the restrainer, maintaining the hand placement on the neck scruff. The individual responsible for bleeding the rabbit positioned it in a lying-down position to administer anesthesia (Zoletil 50, a veterinary anesthetic for animals), which numbed specific areas to prevent pain and discomfort. Using a razor, the hair was removed by the bleeder from the thoracic inlet to the mandible on both sides of the neck. It was necessary to remove the hair in multiple directions to ensure clear visibility and accessibility of the external jugular vein.

To access the vein, it is essential for the rabbit's head, neck, and back to be aligned in a straight and perpendicular manner with respect to the bleeder. The bleeder utilized a cotton pad soaked in 70% isopropyl alcohol to cleanse the rabbit's skin in the neck area. By gripping the rabbit's wing, the bleeder inserted the butterfly needle at an upward angle with the bevel facing up. The bleeder adjusted and redirected the needle's angle as necessary to successfully puncture the vein. Once the vein was accessed, the bleeder filled the 3mL syringe tube and transferred the collected blood into specific tubes (yellow and red top tubes) with varying concentrations (0.18 g/mL, 0.20 g/mL, and 0.22 g/mL). This process was repeated five times. After the blood collection, the restrainer promptly applied pressure to the puncture site using a gauze pad to stop the bleeding. Following the procedure, the rabbit was returned to its cage and allowed to rest in a cool and dry area.

E. Electrolyte Testing

The researchers conducted a qualitative comparison between the serum separation levels achieved in the 0.18g/mL, 0.20g/mL, and 0.22g/mL tubes and a control sample to assess the efficacy of the alternative separator gels. The electrolyte concentrations of the serum in each tube were determined using the electrolyte panel method. Among the five tubes used, two were appropriately labeled as the positive control (serum from a commercial serum separator tube) and the negative control (serum from a red top tube). The remaining three tubes contained starch gel at concentrations of 0.18g/mL, 0.20g/mL, and 0.22g/mL, respectively, along with corresponding seat numbers. The serum samples in these tubes were subjected to different durations of time (1 hour and 24 hours) based on their respective starch gel concentrations. At the time of centrifugation, the gel tends to migrate downwards and settle between the separating gel and the tube wall. Gel has shear thinning property due to intramolecular force at 3,500 rpm for 15 minutes, which causes it to be less viscous during centrifugation allowing it to move in the direction directed by shearing force. The hydrophobic part of amylopectin prevents it to be combined with any solvent surrounding it. It precisely intercalated itself between blood components at the cellular level, and because of its thixotropy property as described previously, it regained its original consistency at the end of centrifugation, forming a solid barrier that isolates serum and red blood cells. The gel formed was biologically inert, and it served as a density gradient medium in a solid form rather than a filter. 0.01 mL aliquots of each serum were aspirated and dispensed onto their respective tubes using the mechanical pipet. The samples were measured using the Caretium Electrolyte Analyzer (MODEL X1 -931C). The test was performed at the Best Diagnostics Clinic, and only the electrolyte concentrations were considered for the statistical analysis.

F. Data Analysis

The mean of the electrolyte level subjected to each time and temperature were analyzed, computed, and presented using descriptive statistics with the statistical tools of mean, variance, and T-test. To determine whether there was a significant difference in the treatment, a One-way ANOVA was employed. The Tukey Post Hoc Test was then utilized to identify the where the significant differences between groups lie.

The Repeated One-way ANOVA was also used to determine the significant difference for the two-Time treatment groups. The data collected were presented in words and table form. The data were extracted to SPSS version 25.0 (IBM SPSS Statistics, 2019) for analysis to ensure accuracy.

G. Ethical Considerations

The researchers obtained an Animal Research Clearance from the DOH-Philippine Institute for Traditional and Alternative Health Care- Cagayan Valley Herbal Processing Plant with reference no: AR-2023-0183 before the conduct of the experimentation.

III. RESULTS AND DISCUSSION

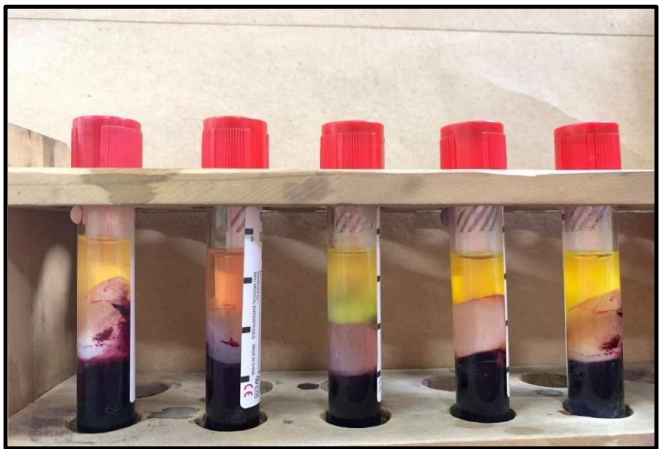

Fig. 1. Serum separation resulting from adding 0.18 g/mL *I. batatas* starch gel

Figure 2 shows that 5 out of 5 blood specimen drawn from different subjects contained in a tube with a 0.18 g/mL concentration of *I. batatas* starch gel consistently separated the serum from the red blood cell component.

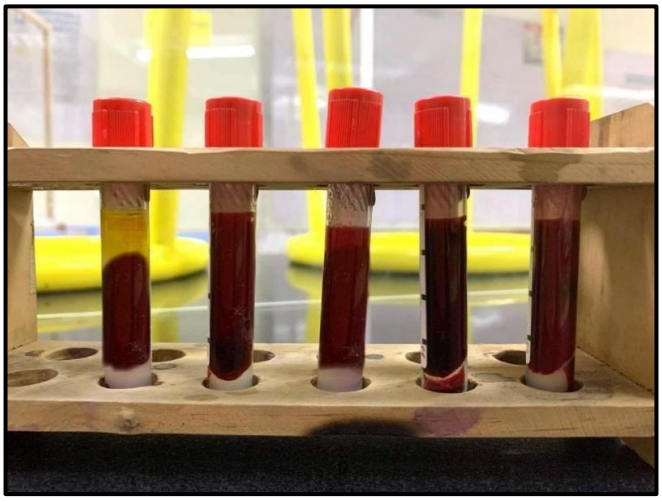

Fig. 2. Serum separation resulting from adding 0.20 g/mL *I. batatas* starch gel

Figure 3 shows that 5 out of 5 blood specimens drawn from different subjects contained in a tube with a 0.20 g/mL concentration of *I. batatas* starch gel were not able to separate the serum from the red blood cell component.

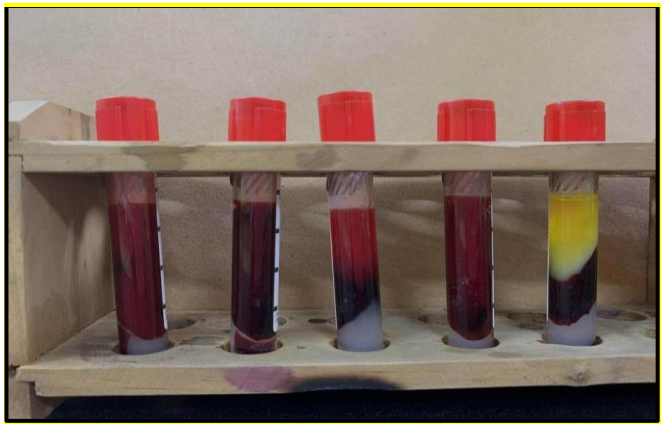

Fig. 3. Serum separation resulting from adding 0.22 g/mL *I. batatas* starch gel

Figure 4 shows that 5 out of 5 blood specimens drawn from different subjects contained in a tube with a 0.22 g/mL concentration of *I. batatas* starch gel were not able to separate the serum from the red blood cell component.

TABLE I. MEAN ELECTROLYTE LEVELS OF THE DIFFERENT SPECIMENS SUBJECTED TO EACH TREATMENT FOR 1 HOUR AT 25-30 ºC

Treatment	Electrolyte Levels (mmoL/L)			
	Sodium	**Potassium**	**Ionized Calcium**	**Chloride**
0.18g/mL *I. batatas* gel starcg	143	3.98	0.96	109.42
Positive control (Commercial Serum Separator Tube/ Yellow top tube)	139.50	3.99	1.71	104.58
Negative control (Plain/ Red top tube)	138.84	3.97	1.39	103.54

Table I shows that starch gel tube has the highest mean level of sodium and chloride followed by yellow top tube and red top tube at 1 hour at 25-30 ºC.

TABLE II. MEAN ELECTROLYTE LEVELS OF THE DIFFERENT SPECIMENS SUBJECTED TO EACH TREATMENT FOR 24 HOURS AT 4 ºC

Treatment	Electrolyte Levels (mmoL/L)			
	Sodium	**Potassium**	**Ionized Calcium**	**Chloride**
0.18g/mL *I. batatas* gel starch	145.58	4.55	0.95	109.92
Positive control (Commercial Serum Separator Tube/ Yellow top tube)	138.76	4.53	1.07	102.68
Negative control (Plain/ Red top tube)	139.98	5.36	1.01	104.44

In Table II, the starch gel tube has the highest mean level of sodium and chloride followed by red top tube and yellow top tube at 24 hours at 4 ºC. However, starch gel tube has the lowest

mean level of ionized calcium followed by red top tube and yellow top tube in both storage and time.

TABLE III. SIGNIFICANT DIFFERENCE IN THE ELECTROLYTE LEVELS GROUPED ACCFORDING TO TREATMENT AFTER 24 HOURS AT 4 ºC.

	Electrolytes	F-Value	p-Value	Decision
After 1 hour at 25-30 ºC	Sodium	10.365	0.002*	Reject Ho
	Potassium	3.491	0.064	Accept Ho
	Ionized Calcium	4.142	0.043	Accept Ho
	Chloride	.614	0.558	Accept Ho
After 24 hours at 4 ºC	Sodium	10.365	0.002*	Reject Ho
	Potassium	3.491	0.064	Accept Ho
	Ionized Calcium	4.142	0.043	Accept Ho
	Chloride	.614	0.558	Accept Ho
Repeated Measures (Time and Temperature)		8029.408b	<0.00*	Reject Ho

** SIGNIFICANT AT 0.01 LEVEL*

Table III shows a noteworthy distinction in the electrolyte levels when comparing the usage of Commercial serum separator gel and *I. batatas* starch serum separator gel for a duration of 1 hour at 25-30ºC. The difference is significant for both electrolyte sodium and chloride. With this, table 2a and 2b present the Post-Hoc Test Analysis of sodium and chloride levels between each tube. The table further shows a significant disparity in the levels of electrolytes when comparing the utilization of Commercial serum separator gel and *I. batatas* starch serum separator gel over a period of 24 hours at 4ºC.The difference is significant for the electrolyte sodium. With this, table 3a presents the Post-Hoc Test Analysis of sodium levels between each tube. The significant main effect of Time, indicated by a p-value of 0.000. This signifies a substantial disparity between the average values of the variables in the two-time treatment groups.

The results showed significantly higher sodium and chloride levels using starch gel tubes. According to Castro et al. (2015), diluent use must not lyse red blood cell and at the same time do not interfere to the level of electrolyte tested that is why in their study they use only potassium with a diluent Normal Saline Solution which may not contain potassium, only sodium and chloride. NSS is a crystalloid isotonic IV fluid containing water, sodium (154 mEq/L), and chloride (154 mEq/L). Its osmolality is 308 mOsm/L (Hoorn, 2016). With this, the use of NSS as a diluent in this study results as an interference to the levels of electrolytes tested, particularly sodium and chloride. Study of Macugay et al. (2015) mentioned that contents of the gel used may leak into the blood component where the analyte is also observed. Therefore, avoid using components that contain the analyte to be determined. Other electrolytes, such as calcium and potassium, were not significant. As for potassium determination, the findings obtained using different concentrations of *I. batatas* starch gel, along with the results of a one-way ANOVA, indicate that there is no notable difference of clinical significance. These results align with the findings of a previous study conducted by Castro et al. (2015). He stated that it might be due to time for the potassium to leak out from the blood cells and patient variability. The results of the study also show the stability of electrolytes from 1 hour at 25-30°C to 24 hours at 4°C, wherein the difference has significance for the red top tube (negative control), yellow top tube (positive control) and starch tube. For the red top tube and starch gel tube, most of the mean electrolyte levels increased except for ionized calcium when prolonged up to 24 hours at 4°C. For the yellow top tube, most of the mean level electrolytes decreased except for potassium when prolonged up to 24 hours at 4°C. The observed elevation in sodium and potassium levels in the red top tube aligns with the findings of Vijayasamundeeswari et al. (2017). Their study demonstrated that sodium levels remained stable for up to 48 hours in samples stored at temperatures ranging from 2 to 40°C, whereas changes were only observed after 24 hours in samples stored at refrigerated temperatures. In contrast, serum potassium levels exhibited significant fluctuations within the 12–24-hour timeframe at 2 to 40°C, whereas it took 36 hours for changes to occur under refrigerated conditions. The study stated that the increased potassium level is due to the prolonged contact with the red blood cells that may leak its potassium component. On the other hand, the increased chloride level differs from the study of Vijayasamundeeswari et al. (2017), the observed increase in chloride levels deviates from their study, which reported that chloride remained consistent with the baseline value for up to 24 hours at temperatures ranging from 2 to 40°C. This disparity might be attributed to patient variability, as mentioned by Castro et al. (2015). Similarly, Tanner et al. (2014) conducted a study involving 30 healthy adult volunteers, examining 35 analytes, and found that potassium stability was altered within 24 hours, while sodium levels remained stable for up to 24 hours. The observed decrease in ionized calcium levels is consistent with the findings of Tanner et al. (2014), who observed a decline in calcium levels in samples stored for 24 hours at both room temperature and refrigerated temperatures. The exact mechanism behind this phenomenon is not fully understood. One possible explanation could be the precipitation of calcium in the presence of significantly increased serum concentrations of phosphate (Tanner et al., 2014). On the other hand, the decreased level of sodium, chloride, and ionized calcium in the yellow top tube may be explained by the increased absorption of gel due to prolonged exposure as Castro et al. (2023) mentioned that analytes are subject to absorption, which is influenced by factors such as storage conditions, specimen volume, temperature, and the type of gel used. The unstable result of this electrolyte made the researchers find another alternative to separator gel (Macugay et al., 2015).

The increased level of sodium and chloride in the starch gel tube may be explained by the longer exposure of the serum to the gel containing the diluent NSS. On the other hand, the increased potassium may be explained by the increased time for the potassium to leak out from the blood cells, as stated by Castro et al. (2015). The decreased level of ionized calcium is the same as the explanation by Tanner (2014). However, its mechanism is also unclear for using starch gel. Also, changes in ionized calcium using starch gel are explained by patient variability, as Castro et al. (2015) stated.

Castro et al. (2015) stated that these significant levels of electrolytes are due to the time difference where the samples are subjected. This is the same with the present study, there is a significant difference between time subjects. The significant main effect of time is that the majority of the electrolyte increases as time is prolonged from 1 hour at 25C-3-C to 24 hours at 4C. However, the researchers were not able to discuss significant main effect of time since repeated measure ANOVA used in the data analysis do not show where the difference between groups lies as it is only an omnibus statistical test.

IV. Conclusion

Out of the various concentrations tested (0.18 g/mL, 0.20 g/mL, and 0.22 g/mL), only the 0.18 g/mL concentration was found to effectively separate the serum from the formed elements, indicating its potential as an alternative serum separator gel. Therefore, *I. batatas* starch gel, particularly 0.18g/mL concentration, can be used for potassium and ionized calcium determination since results show that there is no significant difference between the number of electrolyte levels. However, it cannot be used for sodium and chloride determination since results show a significant difference between the electrolyte sodium and chloride.

Furthermore, the accuracy of the samples was higher when they were analyzed within one hour at 25-30°C particularly for ionized calcium and potassium, as opposed to being stored for up to 24 hours at 4°C. This conclusion is supported by the significant increase observed in the main effect of time on the results.

V. Recommendations

Based on the findings and conclusions, the researchers strongly advise determining the optimum viscosity, temperature, time, and method to effectively yield a gel with a property that has the ability to separate serum from the formed elements. With this, the researchers strongly suggest using 300 degrees Celsius for 4 minutes and then adjusting the temperature from 70-75 degrees Celsius with continuous stirring until it starts to gelatinize. Minutes in stirring when adjusted from 70-75 C should be determined exactly by the future researchers because this is the factor that strongly affects the exact viscosity of the gel.

Researchers also recommend the utilization of other methods of extraction that will yield the most purified starch possible. Also, using other diluent other than NSS is recommended for sodium and chloride determination. With this, the diluent should not lyse red blood cells and, simultaneously, not affect the analyte level, especially for this electrolyte determination. Researchers also recommend using humans as a participant and controlling the patient's blood samples regarding sex, food intake, daily activities, and metabolism to avoid variation in the results of electrolyte determination. For future researchers pursuing this topic, testing other analytes found in serum using the starch serum separator gel is highly suggested. Furthermore, the determination of the coagulation properties of sweet potato starch and the application of a preservative of the starch serum separator gel to improve its shelf life may also be done.

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