Evaluation of *Manihot esculenta Crantz* (Cassava flour) as an alternative to Agarose Gel in Electrophoretic Lipoprotein Profiling in varying concentration

Abstract

This study explores the feasibility of using cassava flour (Manihot esculenta Crantz) as a sustainable alternative to agarose gel in electrophoretic lipoprotein profiling. Agarose gel, although widely used in molecular diagnostics, poses challenges in terms of availability and cost, particularly in resource-limited settings. Given the physicochemical properties of cassava starch—primarily its high amylose and amylopectin content—this study investigates its potential to replicate the gel matrix required for electrophoresis. The research utilized cassava flour at varying concentrations (8%, 10%, and 12%) to evaluate its performance in terms of gelation time, pH, clarity, and consistency. However, actual electrophoretic band data could not be collected due to practical limitations, but the results suggest that cassava flour gel possesses the essential characteristics to serve as a sustainable, low-cost substitute, indicating its potential as an alternative medium for electrophoretic applications. Future studies with further optimization are recommended to confirm and reach a definite conclusion.

Key words: cassava flour; agarose gel alternative; electrophoresis; sustainable laboratory materials; starch-based gel

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Introduction:-

Cassava flour (Manihot esculenta Crantz), also called tapioca, manioc, or yuca, is a perennial woody shrub with tuberous roots in the family Euphorbiaceae (Chisenga et al., 2019). It is a staple food and a common agricultural byproduct. Cassava flour is rich in starch and derived from cassava roots. The crop thrives on marginal sites and poor soils. It tolerates severe weather and drought (Ezui et al., 2018). Usually, low-income farmers in tropical and sub-tropical regions grow cassava (Onsay, E. A., 2021). In the Philippines, cassava (Manihot esculenta Crantz) is the most extensively produced root crop. The cassava industry in the Philippines is divided into three sections: food, starch, and dried chips for feed. Most cassava in the Philippines is used for food. However, much of the industry focuses on starch processing. Starch is the main component of cassava roots, accounting for up to 80% of the root's dry weight (Chisenga et al., 2019). Its flexible planting and harvesting means cassava is available all year. These agronomic traits make cassava a reliable crop for food security and many uses. Its broad applications in biotechnology, diagnostics, and the food industries make it a focus for researchers. Recent studies suggest that cassava flour may have unique physicochemical properties and be useful for specific biochemical applications, such as serving as a medium in electrophoretic separation.

Electrophoresis is a technique that separates molecules, such as amino acids and DNA, based on mass, charge, and size. The process uses gels with channels that act as paths for particle movement. Smaller particles travel faster, while larger particles move more slowly through the gel matrix (Cai, Y., 2020). A common electrophoresis medium is agarose gel, known for its ability to separate biomolecules. Understanding the properties of agarose gel provides a basis for comparing potential alternatives such as cassava flour.

Agarose gel is a linear polymer from red seaweed. It forms strong, thermoreversible gels and serves as a solidifying matrix for analyzing and purifying DNA fragments (Bagal-Kestwal et al., 2019). Agarose gel can resolve DNA

fragments that density gradient centrifugation cannot, which is why it is widely used in electrophoresis.

Although agarose gels are easy to cast and non-toxic for separating large and moderately sized DNA molecules over a wide range (Ume et al., 2022), they have limitations: they are not suitable for low molecular weight DNA, produce poor band resolution, and their high cost restricts molecular studies in resource-limited labs in developing countries. These challenges highlight the need to seek alternative gel matrices that may be more accessible and cost-effective, especially in developing settings.

Gelatinization is crucial for effective biomolecule separation and resolution in electrophoresis. Both cassava flour and agarose gel are polysaccharides that form gels. Since cassava flour contains starch that gelatinizes when heated and cooled (Bagal-Kestwal et al., 2019), researchers believe cassava flour (Manihot esculenta Crantz) may contain components similar to those in agarose gel. The gelling properties of both depend on pH, ionic strength, and other biopolymers (Abotbina, W., et al., 2022). Therefore, this study will assess whether cassava flour can substitute for agarose gel in lipoprotein profiling by electrophoresis and will evaluate how different cassava flour concentrations affect lipoprotein resolution and separation.

Research Design

This study used an experimental research design, relying on statistical analysis using One-Way ANOVA and visual observation of the utilization of Cassava flour (Manihot esculenta Crantz) as an alternative to agarose gel in electrophoretic profiling of lipoproteins. Moreover, in this study, Cassava flour (Manihot esculenta Crantz) was the experimental variable, evaluated at varying concentrations (8%, 10%, and 12%). Agarose gel served as a positive control, providing a baseline for comparison with cassava starch gel as an alternative. Moreover, this will aid in demonstrating that the cassava starch gel is effective in separating lipoproteins and producing the expected results. By using agarose gel as the positive control, the experiment can compare the separation, resolution, and reliability of lipoprotein profiles with those obtained with cassava flour gel. These controls helped in assessing whether cassava flour (Manihot esculenta Crantz) can be a feasible alternative to agarose gel for effective lipoprotein profiling in electrophoresis. The chosen participants for this study were 10 healthy 3rd Year Undergraduate Students of National University – Mall of Asia from the Medical Technology, Information Technology, and Marketing Departments. Since the sample size had been ascertained, the researchers used Convenient Sampling as a method to easily and equally select the respondents.

Participants and Sampling Technique

A prior examination was conducted to ensure a participant is healthy, including measuring body mass index, checking vital signs, and assessing current and past medications and medical history. For this experiment, each participant undergone 1 trial consisting of varying concentrations (8%, 10%, and 12%) and a positive (agarose gel) control, and contributed 5 ml of fasting venous whole blood. Participants were instructed to fast for 10–12 hours prior to sample collection to ensure accurate and reliable biochemical measurements. All blood samples were collected by the researchers under aseptic conditions. The blood samples were allowed to clot at room temperature for 30 minutes and then centrifuged at 3,000×g for 10 minutes to separate the serum. Following centrifugation, serum aliquots were transferred to cryotubes for gel electrophoresis.

The serum aliquots were safely stored and transported in coolers with ice packs to maintain the required temperature range of 2-8 degrees Celsius, in compliance with safety regulations and sample integrity. Hence, these samples remain viable for up to 24 hours after collection. One important factor in specimen management is temperature. The temperature during collection, transportation, and long-term storage can significantly affect the quality of the samples. Based on an article in the Journal of Proteome Research Laboratory (2009), the serum may be harmed by ice crystal formation if the formation of an ice pack containing a laboratory serum sample drops noticeably over 24 hours. This could harm the samples' proteins and other constituents, potentially impairing the precision of any further laboratory tests conducted on them. This is particularly true if the temperature falls below the serum's freezing point.

Comprehensive protocols will be followed to maintain sample integrity after collection and to minimize pre-analytical variability. Participant confidentiality was maintained throughout the process, in accordance with ethical research practices and institutional guidelines.

Data Gathering Procedure

Project Flow

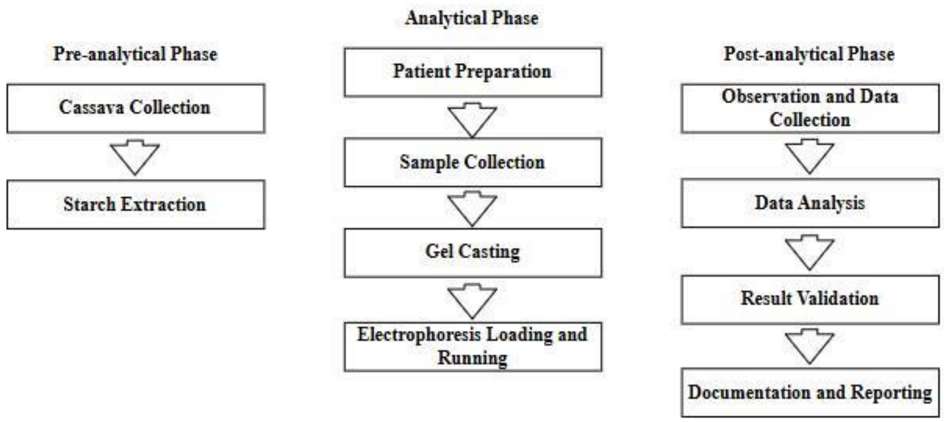


Figure 1. Project flow of the cassava flour (Manihot esculenta Crantz) substitution for Agarose gel in Electrophoresis from collection of cassava to document the results

Cassava Collection

The researchers purchased 10 kilograms (kg) of Fresh Cassava tubers (Manihot esculenta Crantz) from Pasig Mega Market, located on Market Ave. Corner Caruncho Ave., Pasig, Metro Manila, Philippines.

Starch Extraction

The cassava tubers were cleaned, peeled, and cut into 1-cm cubes, then mixed in a high-speed blender with water until no visible chunks remain. The mixture was poured into the strainer, and the excess water was removed by pressing it with a spoon. The cassava pulp that was not contained by the strainer was recovered and filtered through double-folded cheesecloth. The cassava dough was dried in the sunlight for 24 hours or until dry. After it had dried, it was crushed into a fine powder using a mortar and pestle. It dries an extra day for good measure. The cassava flour was used for further analysis.

Patient Preparation & Sample Collection

The study involved 10 participants, selected from 3rd-year undergraduate students in the Medical Technology, Information Technology, and Marketing Departments at National University – Mall of Asia. The selection of ten participants was based on the study’s methodological approach, which involved processing each sample to ensure accuracy and reproducibility of results. These participants must be in good health and must have fasted for 10 to 12 hours before blood sample collection. Prior to participation, they must complete a consent form and have the right to withdraw at any stage of the study. Blood samples were collected by the researchers using Serum Separator Tube (SST) and stored at 2 to 8°C after collection, prior to electrophoresis.

Preparation of 1 L of Tris-Glycine buffer from 10x stock

100 mL of 10x Tris-Glycine buffer was measured into a 1 L beaker. It was topped up to the 1 L mark with 900 mL of distilled water to obtain a working stock solution of 1x.

Preparation of 0.7% Agarose Gel (Control)

0.7 grams of Agarose powder were weighed and transferred into 50 mL of the stock solution. It swirled gently and microwaved for 30-60 seconds until the solution was clear. The solution was allowed to cool for about 3 minutes, and 10 µL of dye (CBB) was pipetted into the agarose solution. The solution was stirred gently and poured into the gel cast. A sixteen (16) well comb was inserted, and the gel was allowed to solidify and attain a firm texture.

Cassava flour Gel Preparation

A literature search found no clear protocol to suit our purpose. Hence, we used a novel protocol for making cassava starch gel by modifying and adjusting those previously described in our reference studies to achieve a cassava flour concentration suitable for forming a good electrophoresis gel. Based on our computation (Table 1.5), specific measurements of cassava flour were prepared to attain the varying concentrations. The amount of the modified cassava flour was weighed. 50 mL of Tris-glycine buffer was measured into a glass beaker. It was preheated to 300 °C and 250 °C for 8-20 minutes, respectively, until the solution was clear and well mixed. The solution was allowed to cool down for about 2 minutes, and 10 µL of dye (CBB) was pipetted into the solution and stirred gently to mix evenly. The cooled solution was poured into the gel cast. A sixteen (16) well comb was inserted, and the gel was allowed to solidify and attain a firm texture.

Determination of pH value

To determine the pH level of each sample, 4g, 5g, and 6g of Cassava flour were transferred into beakers containing 50 ml of 1x Tris-Glycine buffer. The solution was stirred thoroughly, then heated on a hot plate (250 °C) for 8-20 minutes, until the mixture became clear and well mixed. A pH meter was inserted immediately into the solution. The pH values of each sample were recorded.

Starch Clarity Determination

The clarity of the samples was determined by the Spectrophotometer Light Transmittance (%T) Method. The reconstituted starch solutions (1% w/w) were used to determine the clarity of starch (cassava flour and pure agarose) using the method for measuring light transmittance in starch solutions described by Craig et al. To determine the starch clarity, 4g, 5g, and 6g of cassava flour and 0.7g of agarose were weighed into conical flasks of varying capacities and dissolved in 100 ml of distilled water. Each solution was heated in a water bath at 90 °C for 50 minutes and allowed to cool to room temperature (the flasks were shaken at 1-minute intervals to prevent lump formation). The transmittance was measured at 610 nm using a spectrophotometer.

Electrophoresis Loading and Running

Place the solidified gel into the electrophoresis chamber. Then, add about 100 mL of the same solution used for gel preparation (1x Tris-glycine buffer) to each outer part of the chamber, filling it until the gel is fully submerged in the buffer. The samples were mixed with loading dye (CBB) to facilitate easier band monitoring. 5 microliters (5 µL) of each sample were pipetted into the wells. The

protein marker was thawed and prepared in the same manner. Run the gel at 120 V for 30-45 minutes. Monitor dye migration, stop when dye reaches ¾ of the gel. The gel was removed and viewed for band formation using a transilluminator.

Figure 2. An image of the Gel Electrophoresis System (Cleaver Scientific multiSUB Horizontal System)

Observation and Data Collection

For observation and data collection, quantitative analysis was performed using a densitometer that scans the stained lipoprotein bands and automatically calculates the relative concentrations of each fraction. This was done at the National University - Mall of Asia. This quantitative data is crucial for understanding the distribution and relative abundance of lipoprotein types, including high-density lipoproteins (HDL), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), and chylomicrons. In addition to quantitative data, a qualitative assessment was performed through visual inspection of the stained bands using a transilluminator. Simultaneously, the combination of densitometric analysis and visual inspection ensures a well-rounded evaluation of the samples.

Data Analysis Procedure

To determine the effectiveness of cassava flour gel as a substitute for agarose gel in lipoprotein electrophoresis, data was analyzed using One-Way ANOVA and descriptive evaluation. These methods were utilized to compare the characteristics differences of the two gel types, focusing on pH level, clarity, consistency, gelation time, and the resolution of lipoprotein bands.

In One-Way ANOVA, the data will be partitioned into two components: variation due to gel type (between-group variance) and variation due to random error (within-group variance). The F-statistics will be computed by dividing the between-group variance by the within-group variance. If the F-statisticsexceeds the significance level at $p < 0.05$, it indicates that one gel type performs better than the other. Meanwhile, descriptive evaluation will involve visual inspection to qualitatively assess the gels' physical properties and overall performance.

Ethical Considerations

In this study, the researchers aim to ensure adherence to ethical principles and best practices in the use of laboratory facilities and equipment to maintain the safety and well-being of all participants.

The process entails filling in the necessary ERC forms and having the research paper finalized and reviewed by the research adviser. All requirements have been fulfilled, the documents are presented to the NU-MOA ERC ethics committee. Upon review, the ethics committee sent an email to the researchers with any required revisions or notified them of approval.

Upon obtaining approval from the ethics committees, the researchers proceeded to recruit participants. The researchers obtained informed consent from all participants, clearly explaining the purpose, procedures, and potential risks and benefits of the study. Participants were informed that their participation is voluntary and that they may withdraw at any time without penalty. The researchers collected their personal information and took measures to protect its confidentiality. Participants' privacy was ensured, and their identities would not be revealed in the data collected. Sample collection was conducted by researchers with the assistance of a Medical Professional following strict biosafety measures to ensure the safety of participants, researchers, and professional practitioners. The researchers prioritized proper labeling, handling, and storage of samples, as well as double-checking, to prevent mix-ups or contamination. Disposal of biohazard materials will adhere to established waste management protocols.

In laboratory settings, the researchers followed proper laboratory protocol, including the use of appropriate personal protective equipment (PPE), such as gloves, masks, and lab coats, always. Laboratory guidelines were observed to maintain cleanliness, organization, and compliance with biosafety standards. Research findings were communicated with honesty and transparency, ensuring credibility and reliability. If data is to be shared with others, explicit consent will be obtained from participants. Importantly, researchers respect intellectual property rights by adhering to copyright laws, avoid plagiarism, and crediting original methods and findings appropriately.

Results and Discussion

Significant information and data that underwent statistical analysis were presented in this chapter. Thus, the ideas that have been constructed and inferred after the results were presented.

Starch Clarity and pH Level

Tables should be referenced in the text using the term "Table" The tables incorporated must adhere to the following specifications: they should be formatted with a font size of 8, centered, and created using the Microsoft Word table editor. Tables presented in the text mustn't be included as images; instead, they should be generated using the designated word processing software. The table title should be placed above the actual table. See the sample below for the table presentation.

Table 1. *Table showing the Clarity and pH level of Agarose gel and Cassava flour (Manihot esculenta Crantz) gel at varying concentratio*

Samples	Clarity (610nm)	pH
Agarose (Control)	100.00%	7.48
Cassava Flour (8%)	18.30%	7.70
Cassava Flour (10%)	16.9%	7.72
Cassava Flour (12%)	12.80%	7.76

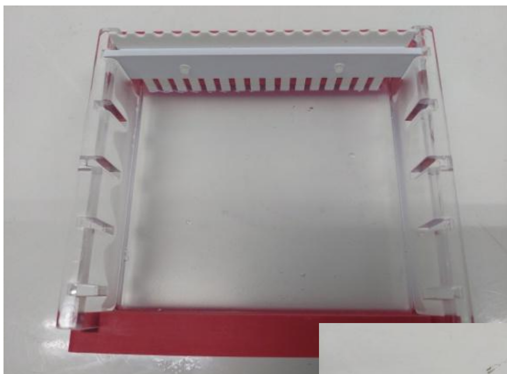
The findings showed that, compared to cassava flour at different concentrations ranging from 12.8% to 18.3% in clarity and 7.70 to 7.76 in pH, respectively, agarose, the standard control, had the highest clarity (100.00%) and the lowest pH (7.48).

Properties of Gels Formed from Agarose and Cassava Flour

The characteristics of gels made from cassava flour and agarose are displayed in Table 4.1.2. The findings showed that cassava flour (Manihot esculenta Crantz) gel at 8% to 12% concentration was unable to create a solid gel and had an opaque appearance. Depending on the quantity of agarose and cassava flour used, the gelling times (minutes) varied from 3 to 18 minutes and 12 to 20 minutes, respectively.

Table 2. *Table showing the physical properties of Agarose gel and Cassava flour (Manihot esculenta Crantz) gel at varying concentrations*

Samples	Concentration	Gelling Time	Gel Consistency	Clarity Appearance
Agarose (Control)	1%	100.00%	+++	Clear
Cassava Flour	8%	18.30%	+	Opaque
Cassava Flour	10%	16.9%	++	Opaque
Cassava Flour	12%	12.80%	++	Opaque



re3. *Agarose gel (Contr*

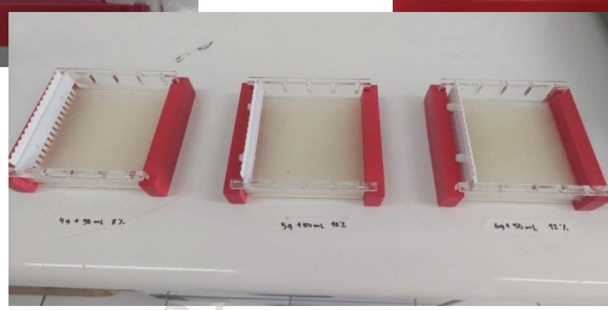


Figure4. *Cassava flour gel formation at varying concentrations.*

A. = 8% Cassava flour gel, B. = 10% Cassava flour gel, C. = 12% Cassava flour gel.

Gel Formation of Cassava Flour

Tables should be referenced in the text using the term "Table" The tables incorporated must adhere to the following specifications: they should be formatted with a font size of 8, centered, and created using the Microsoft Word table editor. Tables presented in the text mustn't be included as images; instead, they should be generated using the designated word processing software. The table title should be placed above the actual table. See the sample below for the table presentation.

Table 3. *Table showing the Gel consistency of Cassava flour (Manihot esculenta Crantz) gel at varying concentrations*

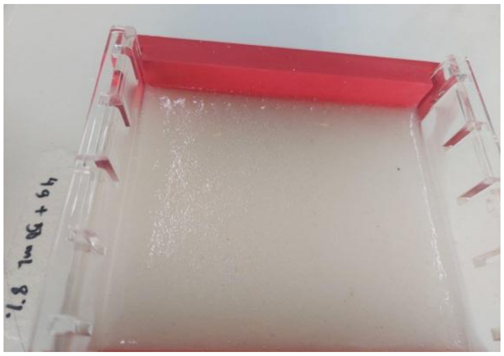
Samples	Concentrations		
Cassava Flour	**8%**	**10%**	**12%**
Lump Formation	+	+	+
Gel Formation	+	++	++

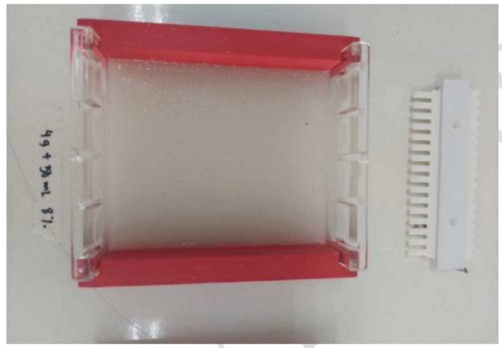
Lump Formation: (-) Neither gelled nor solidified, (+) Lumps were formed, Gel Formation:(-) Neither gelled nor solidified; (+) Soft gel, (++) Semi-solid gel, (+++) Solid gel

The results of cassava flour gel formation are displayed in Table 3 It showed that none of the concentrations (8%, 10%, and 12%) could produce a solid gel. However, they formed lumps. Soft gel was formed at 8%, while semi-solid gel was formed at 10% and semi-solid and sticky gel at 12%.

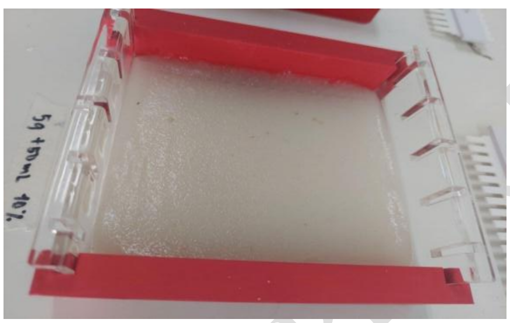
3.4 Cassava flour gel formation at varying concentrations. A. = 8% Cassava flour gel with visible formation of wells, B. = 10% Cassava flour gel with visible formation of wells, C. = 12% Cassava flour gel with visible formation of wells

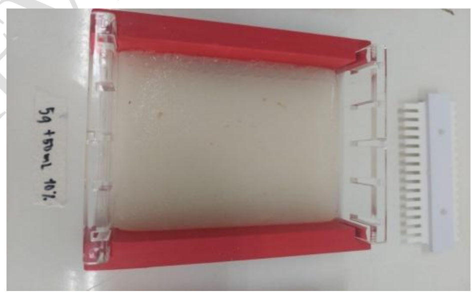
A



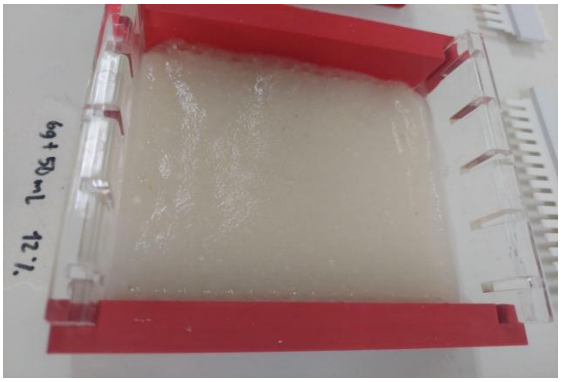


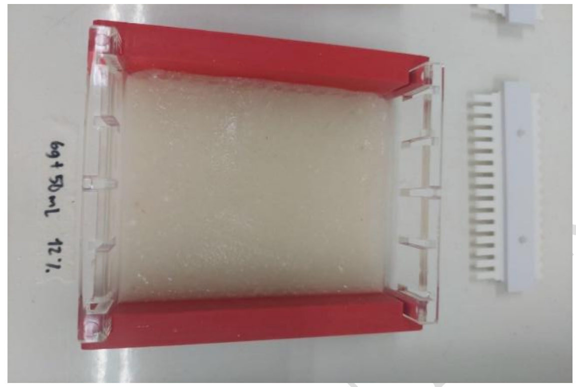
B





C





Electropherogram of Lipoprotein Bands on Agarose Gel and Cassava Flour Gel

During the experiment, the researchers faced difficulties. When the buffer was added to the gel chamber during electrophoresis loading, the cassava flour gel continued to disintegrate and move due to unstable gel consistency. This made it difficult to proceed with the experiment because gel stability is essential for lipoprotein profiling by electrophoresis. Due to the inability to continue with the experiment, neither agarose gel nor cassava flour gel was used in electrophoretic runs for lipoprotein profiling.

A. B. C.

Evaluation of Cassava Flour as a Potential Alternative to Agarose Gel in Electrophoresis Applications

Studies on cassava (Manihot esculenta Crantz) starch have revealed its content of two glucose-containing polymers, such as α-link large amylose and highly branched amylopectin (Ume et. al., 2022) compared to pure agarose, which is composed of agarobiose, a repeating disaccharide of D-galactose and 3,6-anhydro-L-galactopyranose units. Amylose's linear chains form a stable gel, while the branched amylopectin enhances porosity. Accordingly, a study on gel formation by starch established that starch granules swell, gelatinize, and hydrate easily when heated in water. (Chisenga, et. al., 2019).

The cassava flour (Manihot esculenta Crantz) we prepared was optimized at 8%, 10%, and 12%, while we were compelled to use 0.7% agarose gel for comparison. Due to the nature of our study, we needed a higher quantity of starch from cassava to ensure uniformity and stability of the gel; hence, varying concentrations were made. Results for cassava flour (Manihot esculenta Crantz) gel showed that it did not form a stable, solid gel under controlled conditions; instead, lumps formed (Table 4.1.3). These findings are consistent with the research of Liu, Y et al. (2019), which claims that several variables influence the course of starch gelatinization, with the main determinants being the starch source and amylose content, moisture level, and heating profile. Compared to regular starch, high-amylose starch is more challenging to gelatinize. A higher degree of starch gelatinization is typically the result of a higher water content and a higher heating temperature. However, the length of heating had no effect on the degree of starch gelatinization at low moisture content at high temperatures (100°C). According to the study by Gong, Yongqiang, et al. (2024), excessive heat shortened amylopectin molecules and decreased interactions between effective starch chains as the gelatinization temperature rose above the ideal level. This instability weakened the structural integrity and strength of the gel network. Similar findings were reported in a study on potato starch, in which excessively high temperatures compromised the gel structure (Torres, M., et al., 2018). Although temperatures above the gelatinization point generally promote gel formation, overheating can break starch chains, weakening the gel network.

Measurements of pH and clarity were also made, and the findings showed that the pH of cassava flour ranged from 7.70 to 7.76, and its clarity ranged from 12.8% to 18.3%. The standard control, agarose, had the lowest pH of 7.48 and the greatest amount of clarity, producing 100.00%. (Table 4.1.1). These findings correspond with Ume et al.'s (2022) investigation, which proposes that the high degree of contamination in the starch use, which would have decreased to minimal levels if highly purified starch had been used, could be the cause of the difference in clarity between composite starch and agarose. Furthermore, depending on the type of starch, an alkaline pH breaks hydrogen bonds and increases interactions between starch and water, often resulting in firmer, more stable gels. In contrast, acidic pH typically encourages starch hydrolysis, weakens gel structure, and can result in looser, softer gels with decreased hardness and elasticity. (Gong, Yongqiang, et al., 2024).

Due to certain difficulties, none of the gels were used in electrophoretic applications. When the buffer was added to the gel chamber during electrophoresis loading, the cassava flour gel continued to disintegrate and move. This made it difficult to proceed with the experiment because gel stability is essential for electrophoresis. This proves that our study's approach was unsuitable, as the cassava flour gel failed to solidify and remain stable for gel electrophoresis. The results also validate the findings of the report of Meyer, et. al. mentioned in the study of Ume, et. al (2022) that starch paste would produce a highly viscous liquid without addition of amylose; none of the starch concentrations used produced a strong and solid gel that was appropriate for gel electrophoresis; therefore, a small amount of agarose or agar-agar had to be blended with the starch paste before gelling in order to create a solid and stable gel. However, none of these were performed because they were outside the scope of the study’s procedures, as the researchers only employed freshly made, plain, and unblended cassava flour.

Evaluation of Cassava Flour as a Potential Alternative to Agarose Gel in Electrophoresis Applications

Table 5.*Table showing the interpretations of Agarose gel and Cassava flour (Manihot esculenta Crantz) gel at varying concentrations in Electrophoresis*

Samples	Clarity (610nm)	pH Level	Powder/Flour (g)	Gelation Time (Min)	Gel Consistency	Clarity Appearance	Remarks/ Interpretation
Agarose (Control)	100.00%	7.48	0.7g	10	+++	Clear	Ideal gel; serves as a standard reference for electrophoresis
Cassava Flour (8%)	18.30%	7.70	4g	20	+	Opaque	Weak gelation; unstable matrix, not suitable for electrophoresis
Cassava Flour (10%)	16.9%	7.72	5g	16	++	Opaque	Balanced consistency; porous and flexible, suitable for electrophoresis
Cassava Flour (12%)	12.80%	7.76	6g	7	++	Opaque	Rigid and less porous; may restrict molecular movement

Key: Conc = Starch Concentrations (%); Gtime = Gelling Time (Minutes). (-) Neither gelled nor solidified; (+) Soft gel, (++) Semi-solid gel, (+++) Solid gel

Table 5 presents the comparative results of varying cassava flour (Manihot esculenta Crantz) gel concentrations for clarity, pH, gelation time, and overall gel consistency. The purpose of this experiment is to identify the optimal cassava flour concentration as an alternative gel matrix for electrophoresis. Agarose was used as the control sample to establish standard gel characteristics, including clarity and firmness. By comparing the physical properties and formation behavior of each cassava gel, the study aims to determine which concentration yields the most stable, porous structure suitable for electrophoretic applications.

The results presented in Table 5 show the performance of cassava flour (Manihot esculenta Crantz) gels at varying concentrations and compare them with agarose, the standard gel used in electrophoresis. Among the tested cassava flour concentrations, the 10% solution demonstrated the most favorable characteristics. It exhibited a balanced gelation time (16 minutes) and semi-solid gel consistency (++), indicating moderate firmness and adequate porosity. These traits are important for electrophoresis, as the gel matrix must be firm enough to retain molecular samples while still allowing them to migrate through the pores efficiently (Lee et al., 2012; Green & Sambrook, 2019).

The 8% cassava flour gel produced an unstable, soft-gel structure that lacked uniformity, suggesting insufficient gelatinization due to its low starch concentration. Chisenga et al. (2019) emphasized that cassava starch gels require proper heating and concentration to form a continuous matrix network. In contrast, the 12% concentration yielded a semi-solid, sticky, viscous gel, reducing its porosity. This behavior aligns with the findings of Bagal-Kestwal et al. (2019), who explained that excessive polymer concentration leads to reduced gel pore size and limited molecular movement—making it less effective for electrophoresis applications.

Although agarose maintained superior clarity (100%) compared with cassava flour gels (12.8–18.3%), the cassava-based 10% gel showed promising physical properties and could be a potential substitute. Ume et al. (2022) demonstrated the feasibility of starch-based gels, such as cassava and sweet potato, as sustainable alternatives to agarose, particularly in resource-limited settings. These alternatives address both economic and environmental sustainability goals by promoting the use of locally available, biodegradable materials, in line with United Nations Sustainable Development Goal 12 on responsible consumption and production (United Nations, 2023).

Furthermore, the pH levels (7.7–7.76) of cassava gels were comparable to those of agarose (7.48), indicating their chemical compatibility with standard electrophoresis buffers (Serwer, 1983; Cleaver Scientific, 2020). This similarity implies that cassava flour gels can maintain stability during electrophoretic procedures without disrupting molecular charge balance. The findings also align with Cabral (2001), who explored cassava-based electrophoretic media for protein analysis and demonstrated that cassava gels could effectively separate biomolecules when optimized for structure and porosity.

Overall, the 10% cassava flour gel achieved the optimal balance of clarity, gelation time, and consistency, making it the optimal concentration for forming a stable, porous gel matrix. The result not only supports earlier research on the adaptability of cassava starch in electrophoretic separation (Ume et al., 2022; Ussif et al., 2020) but also contributes to the ongoing movement toward sustainable laboratory practices that minimize reliance on marine-derived agarose (Amina, 2019).

Comparing the Band Sharpness and Visibility of Lipoprotein Profiles Obtained

In addressing the third statement of the problem (SOP 3), which aimed to determine whether there are significant differences in the electrical band patterns of lipoproteins obtained using cassava flour gel and standard agarose gel, the present study faced certain limitations. Due to resource constraints, only a single experimental trial was conducted, and actual electrophoretic runs for lipoprotein profiling were not performed. Therefore, no direct band pattern data were collected for statistical comparison. However, the physical and chemical properties of the cassava flour gel obtained in this study provide valuable insight into its potential electrophoretic performance. The cassava gel, particularly at a 10% concentration, exhibited suitable pH (7.72), moderate clarity (16.9%), and semi-solid gel consistency (++)—properties that, according to Lee et al. (2012), are essential for efficient electrophoresis.

Conclusion

Cassava flour (Manihot esculenta Crantz), a staple food and byproduct, is rich in starch and has applications in biotechnology, diagnostics, and food industries, garnering significant attention in the research field. A recent study suggests that cassava flour (Manihot esculenta Crantz) may have unique physicochemical features, making it a viable option for specific biochemical applications, such as its potential use as a medium in electrophoretic separation.

In electrophoresis, gelatinization plays a crucial role in achieving effective separation and resolution of biomolecules. A significant portion of cassava flour is starch, which, when heated and chilled, may gelatinize to form a gel (Bagal-Kestwal et al., 2019). With this, the researchers believe that cassava flour (Manihot esculenta Crantz) contains a constituent similar to commercially available agarose gel. Accordingly, agarose gel and cassava flour are both made of polysaccharides that can form gels (Ume et al., 2022). Furthermore, variables such as pH, ionic strength, and the presence of other biopolymers affect the gelling properties of both agarose and cassava flour (Abotbina, W., et al., 2022). The progression of starch gelatinization is also affected by other parameters, with the main determinants being the starch source and amylose content, moisture content, and heating profile (Liu, Y., et. al., 2019).

Studies by Ume et al. (2022) and Ussif et al. (2020) further support that starch-based gels, including cassava and corn starch, can successfully separate biomolecules under optimized conditions. Given these findings, it can be inferred that the cassava flour gel, especially at its optimal concentration, may produce lipoprotein banding patterns comparable to agarose when subjected to electrophoresis.

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