# TITLE: ISOLATION OF POLYSACCHARIDE-PRODUCING MICROORGANISMS AND ITS EXTRACTION AND APPLICATIONS AS THICKENING AGENTS.

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## ABSTRACT-

Polysaccharides are high-molecular-weight carbohydrates that play a variety of structural and functional roles. Microbial polysaccharides are gaining popularity due to their natural origin, biodegradability, and versatility as thickening, stabilizing, and gelling agents. The study investigates the isolation and characterization of polysaccharide-producing microbes from sugar-rich sources such as fruits (apples, sapodilla, grapes), sweet potatoes (*Ipomoea batata*), and milk, and also its use as thickening agents.

Microorganisms were isolated by serial dilution method and spread plating on selective media, and mucoid colonies were examined for extracellular polysaccharide (EPS) synthesis. To increase yield, promising strains were cultivated under optimal pH, temperature, and carbon source conditions. Polysaccharides were precipitated with ethanol or acetone and then purified using dialysis or centrifugation.

The extracted polysaccharides were tested for viscosity and thickening ability using simple procedures such as drop-weight and flow-time tests. Isolates from sweet potatoes and milk produced substantial polysaccharide yields with promising thickening capabilities similar to commercial gums.

This study proposes a low-cost method for producing functional polysaccharides from microorganisms, thereby promoting the use of natural additives in line with clean-label and sustainability initiatives.

*Keywords: microbial polysaccharides, polysaccharide extraction, lactic acid substrate*, *bio- thickeners.*

## INTRODUCTION

Polysaccharides are commonly found in natural resources and have been studied for their bio-functions, such as immunomodulation, hypoglycaemic activity, hypolipidemic activity, Antitumor action, enhancing gastrointestinal function, and so on. Polysaccharides perform many functions and have vast bioactivities in life processes. Polysaccharides have attracted much attention due to their significant bioactivities, including hypoglycaemic activity, hypolipidemic activity, anti-cancer, antioxidant activity, immunity improving activity, antimicrobial activity etc. (Wang *et al*., 2018). They have enormous potential in the healthcare, food, and cosmetic industries due to their therapeutic properties and low toxicity.

Sweet potato (*Ipomoea batatas* L.) belongs to the Convolvulaceae family. It is the seventh major worldwide food crop after wheat, rice, corn, apple land, barley and cassava (Mohanraj, R. & Sivasankar, S., (2014). *Ipomoea batatas,* is an important root vegetable which is large, starchy, and sweet tasting (Purseglove JW, 1972; Woolfe J.A., 1992). This plant has many medicinal properties and is highly nutritious. Its fleshy root contains good amounts of carbohydrates, amino acids, vitamins and minerals. Sweet potato on fermentation with bacteria produce good amounts of exopolysaccharides. Exopolysaccharides (EPS) are also called capsular polysaccharides (CPS), or exo cellular polysaccharides (Vandamme et.al, 2002). They are of great interest in food and cosmetics industry. In food industries, EPS helps to improve the rheological and sensory properties of fermented products such as cheese and yogurt (Ismail, B., Nampoothiri, K.M., 2010) bread making, they improve the viscoelastic properties and volume of the dough, and increase the shelf-life of bread, while reducing the hardness of the bread crust (Kieran et.al 2018; Markus et.al 2020). Many different types of fruits can be used for the synthesis of Exopolysaccharides. Agro-wastes can also be used as less expensive carbon sources like milk whey (Yang and Silva 1995), citrus fruits (Roseiro Roseiro et al. 1992; Green et al. 1994; Bilanovic et al. 1994) olive mill wastewater (Lopez and Ramos-Cormenzana 1996), pineapple waste (Pyar et al. 2014), cabbage (Yang and Tseng 1988; Paulo *et al*. 2012), beet molasses (Roukas 1998), potato starch waste (Vidhyalakshmi *et al*. 2012), Sugarcane vinasse (Ventorino *et al*. 2019) for production of EPS.

This guideline outlines the extraction, separation, purification, and structural characterization of polysaccharides from natural sources. Because of their enormous structural variability, polysaccharide separation and purification methods differ from those used for other macromolecules such as proteins. However, achieving homogeneity is the first step in studying polysaccharide structure, pharmacology, and structure-activity connections (Lei Shi, 2016). Exopolysaccharides are prepared through extraction, separation, and purification. Water extraction yields crude polysaccharides. Separation and purification aim to enhance polysaccharide components and extract polysaccharide fractions (Tang *et. al*., 2020).

This study involves isolation of new polysaccharide producing bacteria from fermented fruit samples and sweet potatoes. Polysaccharides are highly useful biopolymers that have excellent structural diversity and functional adaptability. It has greater structural and functional qualities compared to synthetic materials. Polysaccharides are lengthy sequences of simple sugar molecules (monosaccharides) that produce complex carbohydrates. They are an important class of macromolecules that provide a variety of functions in living organisms, including energy storage and structural support. Polysaccharides are categorized into three categories based on their characteristics and natural appearance: structure, storage, and gel formation. Polysaccharides belong to a structurally varied class of macromolecules composed of monosaccharide residues linked together by glycosidic bonds.

Gel Polysaccharides: Mucopolysaccharides are sugar chains found in the body, including mucus and joint fluids. They are widely known as glycosaminoglycans. (Marta Izydorczyk, Steve W. Cui, Qi Wang, Dumitriu S. 2006, 2005). Mucopolysaccharides are structural molecules found in cartilage, bone, cornea, skin, blood vessel walls, and other connective tissues. They are carbohydrates, which contain amino sugars and uronic acids. Mucopolysaccharides often seen in urine include chondroitin-6-sulfate, heparin sulfate, and keratin sulfate. A

polysaccharide gel is a colloidal system with a continuous phase of polysaccharide and a dispersed liquid, such as water or organic compounds (e.g., ethanol, methanol, acetone). Gels have the density of liquids but have a more solid-like structure. A gel can have a liquid content of 80% or more. Agar is the most prevalent type of gel in polysaccharides. Gels can transition between fluid and solid states based on their mobility. This property is known as thixotropic. The process of creating a gel is known as gelation.

Polysaccharides originate from various sources, including bacteria, fungi, algae, and plants. Polysaccharides derived from algae and plants account for the majority of the global market. Biopolymers are extracted directly from biomass and can be converted into smaller molecules through chemical hydrolysis or fermentation. (Marta Izydorczyk *et.al*., 2006).

Microorganisms typically produce and accumulate polysaccharides after their development period. Microorganisms create polysaccharides in three types based on their position within the cell: There are three types of polysaccharides in cells: cytosolic polysaccharides, which provide carbon and energy; cell wall polysaccharides like peptidoglycans, rooftop acids, and lipopolysaccharides; and exopolysaccharides, which are exuded in the extracellular environment as capsules or biofilm. EPS are classified into two types: homopolysaccharides and heteropolysaccharides. Dextran and levan are examples of homopolysaccharides, which are made up of a single type of monosaccharide (Marta Izydorczyk *et al*., 2006). Plant polysaccharides include cellulose and starch, whereas animal polysaccharides include glycogen and chitin. Microbial polysaccharides are produced by bacteria and fungi, whereas semi-synthetic polysaccharides are modified copies of natural polysaccharides.

Plant polysaccharides:

- Cellulose: The most prevalent polysaccharide on earth, is the structural component of plant cell walls.
- Starch: It is a type of carbohydrate present in plants' seeds, fruits, and storage organs. Inulin is a fructose-containing storage polymer found in plants such as chicory and Jerusalem artichoke.
- Pectin: It is a polymer present in plant cell walls and used in culinary gels and jellies.
- Gums: Many polysaccharides like xanthum gum and Guar gum are used as thickening and stabilizing agents in food industries.
- Inulin: Fructose based polysaccharides in plants.

Animal polysaccharides:

- Glycogen: In mammals, glycogen is a storage carbohydrate present in the liver and muscle cells.
- Chitin: Chitin is a structural polymer found in arthropod exoskeletons and some fungi's cell walls.
- Hyaluronic Acid: Hyaluronic acid is a connective tissue component that helps to lubricate and hydrate the tissues.
- Heparin: Heparin is an anticoagulant found in both blood and mast cells.

- Chondroitin Sulphate: Chondroitin sulphate is a cartilage component that provides cushioning and flexibility.

**Microbial polysaccharides**:

Dextran: Dextran is a polysaccharide generated by bacteria and utilized in a variety of applications, including blood volume expanders.

Levan: Levan is a fructose-containing polysaccharide generated by bacteria.

Xanthan Gum: Xanthan Gum is a polymer generated by bacteria that is used to thicken and stabilize food and other products.

Gellan Gum: Gellan Gum is a polysaccharide generated by bacteria and used as a gelling agent.

Scleroglucan: Scleroglucan is a polysaccharide generated by fungi and used in a variety of applications, including medication administration.

## MATERIALS AND METHODS

**Materials for screening**: Screening of polysaccharides was done by taking various samples- Fresh fruits (apple, chickoo and grapes), Sweet potato and milk.

**Table no 1 - Media used for Isolation of Microorganisms from Fruit Samples**

| S. No. | Ingredients | Quantity |
|---|---|---|
| 1. | Peptone | 0.5g |
| 2. | Yeast extract | 0.5g |
| 3. | NaCl | 0.2g |
| 4. | KCl | 0.2g |
| 5. | $CaCl_2$ | 0.2g |
| 6. | Dextrose | 2.5g |
| 7. | Agar | 2 g |

| | | |
|---|---|---|
| 8. | Distilled water | 50 ml |

**Fermentation of fruit samples**

Fresh fruits like apples, chickoo (sapodilla) and grapes were washed thoroughly with distilled water to remove surface contaminants. The fruits were then cut into small pieces using sterile instruments and placed in clean, airtight containers with diluted soil samples in them and kept in a dark place (away from sunlight). The containers were kept at room temperature for two weeks to allow the natural fermentation.

**Screening methods**

1. The spread plate technique was used for screening polysaccharide-producing microorganisms, where the microbial samples were uniformly spread over the surface of agar plates containing a selective medium designed to support polysaccharide production.

2. 50 ml of distilled water was taken in a sterile container, 1.5 g of fermented fruit sample was added to it, and the mixture was kept for one hour to allow proper mixing and diffusion of microorganisms into the water.

3. Serial dilutions of the fermented fruits mixture was done.

4. 0.1 ml of mixture from different dilutions was taken and spread into the separate agar plates.

5. The plates were incubated for 48 hrs.

**FERMENTATION OF SWEET POTATOES**:

Similar process was used for Fermentation and Screening of microorganisms from sweet potato

**Media Preparation for Polysaccharide Production from milk**

| | **Media Composition** | **Quantity** |
|---|---|---|
| 1. | Fermented milk | 1.5 gm |
| 2. | Dextrose | Pinch |
| 3. | Distilled water | 50 ml |

**METHOD:**

Similar process was used for Fermentation and Screening of microorganisms from milk.

**Centrifugation of Polysaccharides from samples-**

1. The inoculated broth containing the bacterial growth was transferred into centrifuge tubes and kept for centrifugation at a speed of 8000 rpm for 15 min to separate the microbial cells from the liquid phase.

2. After the centrifugation, the clear supernatant (liquid) was carefully collected with the help of filter paper in the flask.

**Precipitation of Polysaccharides from different Samples-**

**From fermented fruit sample:**

1. The supernatant was transferred into the three flasks.
2. In one flask, 10 ml of supernatant (liquid) and 30 to 40 ml of chilled ethanol was added drop by drop into the flask.
3. In the second flask, 10 ml of supernatant (liquid) and 30 to 40 ml of chilled acetone were added drop by drop into the flask.
4. In the third flask, 20 ml of supernatant (liquid) and 50 ml of chilled calcium chloride solution was added.
5. The mixture was then kept at 4°C for several hours to enhance the precipitation. White precipitate was observed.

**Extraction Of Polysaccharides from Fermented Fruit Sample:**

1. After precipitation, the mixture was transferred into the centrifuged tube and kept in the centrifuge at 8000 rpm for 15 min to separate the microbial cells from the liquid phase.
2. After centrifugation, the pellet (solid part) was collected in the petri plates and the liquid part was discarded.
3. The pellet (solid part) then the pellet was kept for drying at room temperature, until all moisture had evaporated and a dry polysaccharide mass was obtained.
4. The dried polysaccharide was then scraped, weigh and stored in a clean, airtight container for further use in the applications.

Purification of polysaccharides:
Purification of polysaccharides is an important step after extraction, its main aim is to remove proteins, pigments, nucleic acids, and other impurities to get pure polysaccharides for further application.
Steps for purification of polysaccharides (Sevag method Thuy *et.al* (2018)-
1.The extracted powder was then subjected to protein removal (deproteinization) using the (chloroform :butanol in a 5:1 ratio.
2. Chloroform: butanol in a ratio 5:1 was added into the extracted powder and mixed thoroughly.
3. The mixture was then centrifuged and the aqueous upper layer containing the polysaccharides was collected.
4. Three volumes of cold ethanol was added to the aqueous upper layer.
4. Mix gently and keep at 4°C overnight.

5. A white or cloudy precipitate will form.
6. The process was repeated for further purification and pellet air dried.

**RESULT & DISCUSSION-**

Slimy mucoid colonies were observed on the agar plates prepared using serial dilutions from the fermented fruit samples (apples, grapes, chickoo). Screening plates containing mucoid colonies on agar indicates polysaccharide-producing microorganisms.

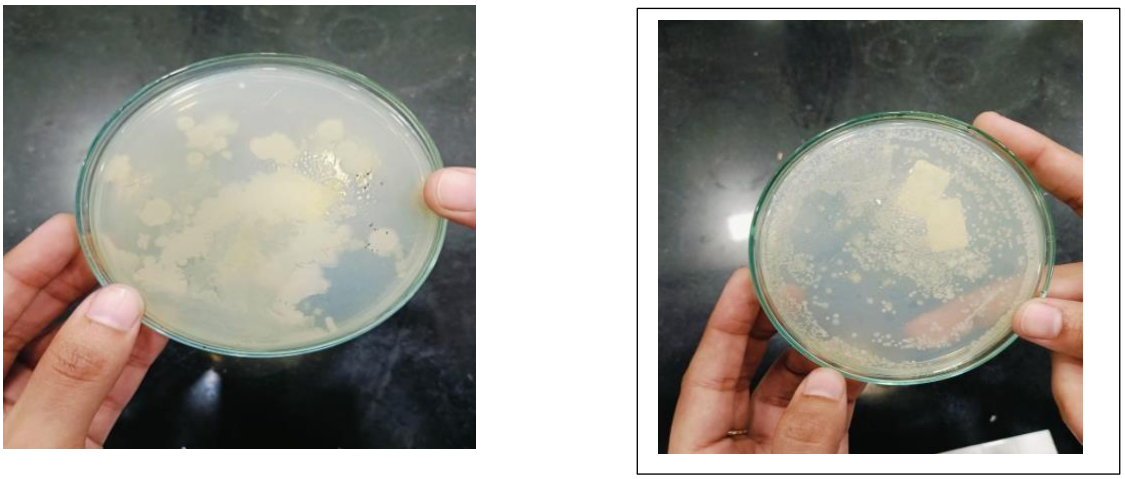


Fig. 1. Plates containing Polysaccharide producing microorganisms from fermented fruits

**From fermented sweet potato:**

Microbial colonies were observed on the agar plates prepared using serial dilutions from the fermented sweet potatoes samples. Screening plates showing the mucoid colonies on agar, indicating polysaccharide-producing microorganisms.

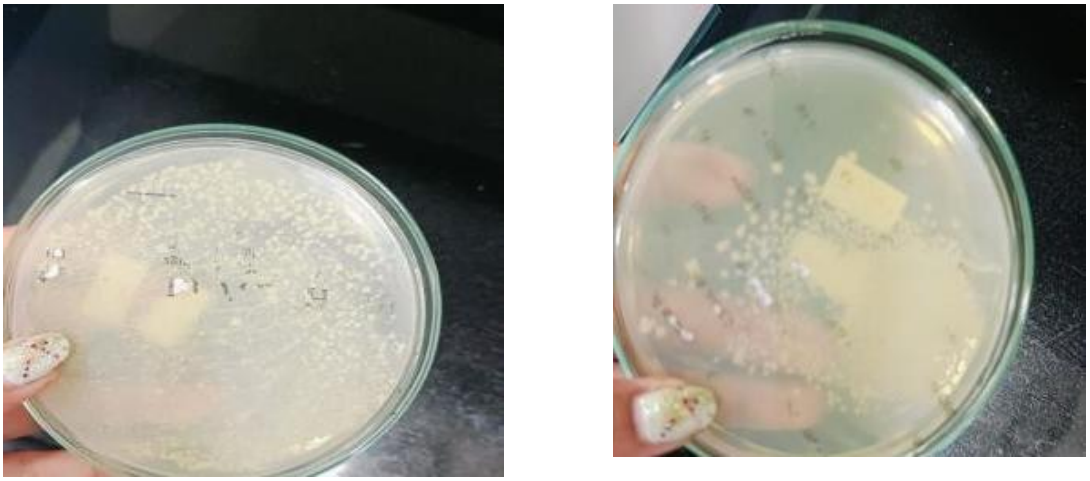


Fig. 2 Agar plates screening of Polysaccharide producing microorganisms from fermented sweet potatoes


Figure 3- Polysaccharide producing colonies on milk agar plate.

**Production:**

After the successful screening of polysaccharides-producing microorganisms on the agar plates, selected mucous colonies were inoculated into the broth for further production. The incubated broths were incubated at different temperatures for 3 to 5 days to promote microbial growth and polysaccharide secretion. During incubation, an increase in turbidity and viscosity of the broth was observed, indicating the microbial growth

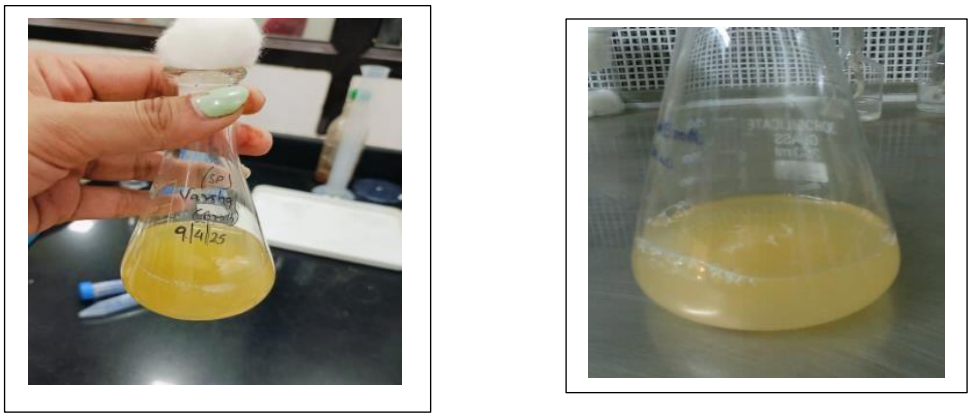
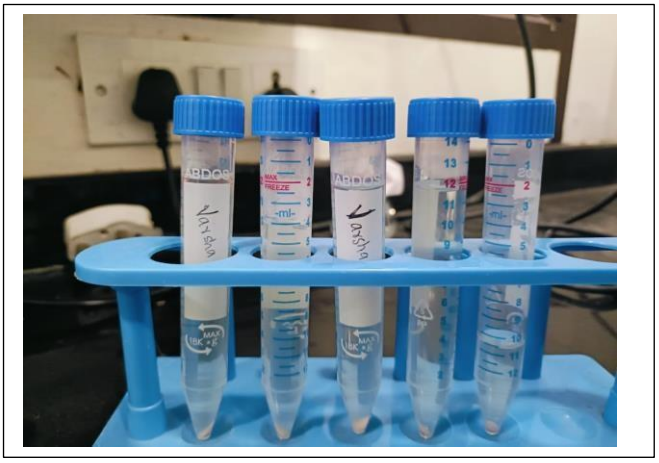


Fig 4- Inoculated broth from the screening plates of fermented fruit sample and sweet potatoes

Fig 5- Inoculated broth from the screening plates of milk producing microorganisms

**Centrifugation.**

The inoculated broth of fermented fruits, sweet potato and milk was then centrifuged. After the centrifugation, the supernatant (liquid) was collected and distributed into three flasks: two containing 10 ml and one containing 20 ml. Chilled ethanol, acetone, and calcium chloride ($CaCl_2$) were added in the flask, respectively. And they kept the flasks in the refrigerator. For 4 to 5 days for the precipitation of polysaccharides.



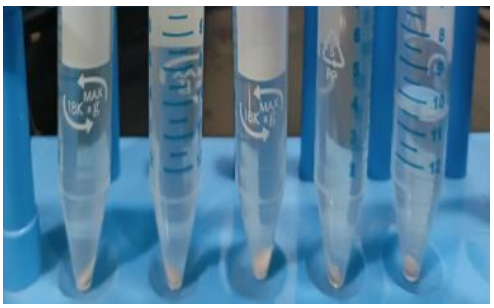


Fig 6- After centrifugation of the cultured broth.

**Precipitation of polysaccharide-**

The precipitation of polysaccharide was observed after the addition of chilled ethanol and acetone to the supernatant obtained post fermentation. A visible white to off-white precipitate began forming within a few minutes of ethanol addition, indicating the presence of polysaccharide compounds. The precipitate gradually increased in volume with the slow, dropwise addition of ethanol and acetone in a ratio 3:1 (ethanol: supernatant and acetone: supernatant) ratio was allowed to settle overnight or 2 to 4 days at 4 °C for complete precipitation.

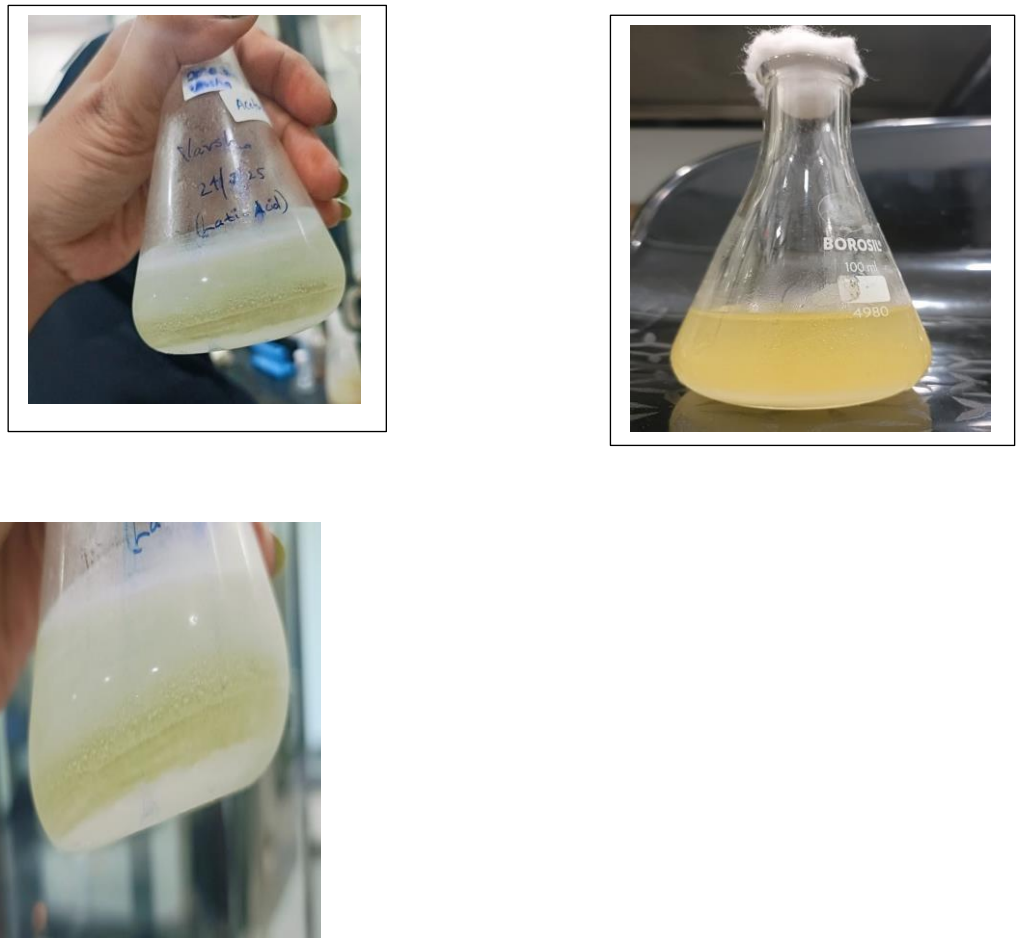


Fig 7- Precipitation of polysaccharide.

### 5.1 EXTRACTION OF POLYSACCHARIDE-

Various factors can influence polysaccharide extraction, including temperature, time, and the type of extraction method.

Fig 8. Extraction of polysaccharide from fermented fruits sample.

Fig 9. Extraction of polysaccharide from fermented sweet potatoes.


Fig 10. Extraction of polysaccharide from milk producing microorganisms

**5.2 APPLICATIONS**

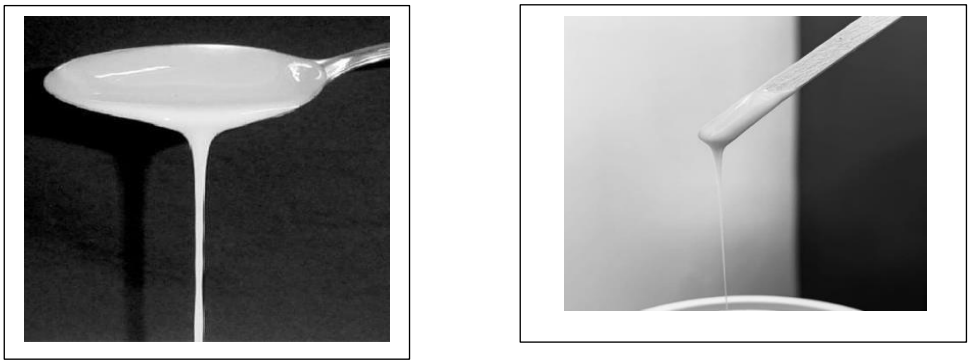


Fig 11. Showing the thickness of polysaccharides.

Fig 12- Jam preparation from orange extract using polysaccharides

Sugar-rich substrates apples, sapodillas, and grapes, as well as sweet potatoes milk were used for isolation of Polysaccharide producing colonies. These sources were chosen for their high sugar content, which promotes microbial growth and polysaccharide production. Following screening, numerous microbial colonies with mucoid or ropy features were identified. In the figures 1, 2 & 3, mucoid colonies can be clearly observed, indicating the presence of polysaccharide producing microorganisms from the fermented fruits sample, sweet potatoes and milk. After screening for polysaccharides producing microorganisms, enrichment and large-scale production was done in broth medium (Figure 4).

Extraction was performed by adding ethanol and acetone to the cell-free supernatant, resulting in the evident precipitate of polysaccharides (Xu Y *et.al,* 2019). Figures 8, 9 and 10 shows extraction of EPS from various samples. The extracted sample was then dried and used for further applications process. For removal of molecular contaminants, simpler methods such as alcohol precipitation and filtration were also used.

The industrial applications of EPS-producing LAB are gaining a growing interest because of their positive impact as thickening and structuring agents on rheology, texture and mouth-feel properties. When applied to food systems such as jam-making, the isolated polysaccharides demonstrated exceptional thickening characteristics. EPS are widely used as thickening, stabilizing, and gelling agents in the food industry, where they enhance the texture, appearance, and shelf-life of a diverse range of products, including dairy (Goh, Y. J., & Klaenhammer, T. R., 2014). LAB-derived EPS have also gained growing interest in other sectors (e.g., pharma, nutraceutical) due to their biocompatibility, non-toxicity and biodegradability features (Angelin J. & Kavitha M. (2020).

The extracted microbial polysaccharides were used in preparing jam using orange pulp as a fruit base. Figures 11 & 12 shows the thickness of Jam prepared from the extracted polysaccharides and from fruit pulp. The drop method was used to evaluate the thickness of the isolated polysaccharides. The polysaccharide acted as a natural thickening and stabilizing agent in place of synthetic pectin. After mixing the orange pulp, sugar, and microbial polysaccharide, the mixture was heated and allowed to cool. The final jam showed favorable consistency and spreadability. The texture was smooth, with moderate viscosity and no phase separation, indicating good gelling ability of the extracted polysaccharide.

Sweet potato and lactic acid had the highest polysaccharide yields of all the substrates studied, showing that they are effective fermentation substrates. This could be due to their higher carbohydrate content and improved microbial friendliness. This promotes the potential use of microbial polysaccharides in the food sector, particularly as natural and biodegradable additives. Overall, the results show the viability of manufacturing microbial polysaccharides from low-cost substrates. The isolated strains and polysaccharides show potential for industrial applications, particularly in eco-friendly food formulation and biotechnology.

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