Development and Characterisation of Sustainable Biodegradable Film from *Dioscorea alata* and *Beta vulgaris*

Dissertation submitted to Mahatma Gandhi University in partial fulfilment of the requirements for the degree of

Bachelor of vocational studies

B.Voc. food processing technology

by

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DECLARATION

We, **Ardra Santhosh (VB22FPT001), Aishwarya Sreelakshmi S (VB22FPT002),** and **K. Marwa Nasir (VB22FPT012),** hereby declare that this project entitled **"Development and Characterisation of Sustainable Biodegradable Film from *Dioscorea alata* and *beta vulgaris*"** is a Bonafide record of the project work done by us during the course study and that the report has not previously formed the basis for the award to us of any degree, diploma, fellowship, or other title from any other university or society.

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CERTIFICATE

This is to certify that the project report entitled **"Development and Characterisation of Sustainable Biodegradable Film from *Dioscorea alata* and *Beta vulgaris*"** is a Bonafide work in partial fulfilment of the requirement for the award of degree of B.Voc Food Processing Technology of St. Teresa's College (Autonomous), Ernakulam, under my supervision and guidance, and the report has not formed the basis for the award of any other degree, diploma, associateship, fellowship, or any other similar title to any candidate of any university.

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ABSTRACT

The growing environmental concerns associated with non-degradable plastics have led to an urgent need for the development of biodegradable alternatives. The present work investigates the development and characterization of a biodegradable film from *Dioscorea alata* (purple yam) and *Beta vulgaris* (beetroot).

Aim: The present work was undertaken to develop and characterize biodegradable film using sustainable raw materials, specifically *Dioscorea alata* and *Beta vulgaris* and chitosan. The goal is to evaluate the potential of film as a sustainable alternative to traditional plastic and its suitability for food packaging applications.

Methodology: The biodegradable film was produced by extracting, blending, and heat-treating the raw materials with chitosan and glycerol. The physicochemical properties of the film, such as density, thickness, water solubility, soil degradation, humidity, swelling properties, migration of molecules, and transparency, were evaluated. The film was

Result: The final product has a low density of 0.24 g/cm^3 and an average thickness of 0.2 mm, indicating its lightweight and flexible nature. The biodegradable film exhibited a high water solubility of 70%, a swelling index of 30%, and a water content of 14.29%. The film showed excellent biodegradability and revealed no leaching or degradation.

Conclusion: Successful utilization of *Dioscorea alata* and *Beta vulgaris* in the chitosan-based film. The biodegradable film is a sustainable alternative to conventional plastic materials, making it suitable for short-term food packaging and also contributing to zero-waste consumer practice.

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1. INTRODUCTION

The surge in global economic development—particularly within developing nations—has been accompanied by a growing sense of environmental responsibility (Skogen et al., 2018). However, urgent challenges such as climate change and biodiversity loss continue to demand sustainable innovations, especially in materials science. One of the most pressing environmental concerns today stems from the widespread use of synthetic plastics, which, while advantageous due to their durability, low thermal conductivity, and corrosion resistance, have contributed to long-lasting ecological degradation (Napper & Thompson, 2019).

Currently, plastics are integral to modern life, playing essential roles in packaging, agriculture, healthcare, and countless other sectors. Yet, global plastic production exceeded 3.59 million tons as of 2018 and continues to increase rapidly (Zhu & Wang, 2020). If this trend persists, it is projected that the plastic industry could consume up to 20% of the world's oil resources by 2050 (Shen et al., 2020a; 2020b). In response, governments, research institutions, and industries are pursuing sustainable alternatives to traditional petrochemical plastics.

Among these alternatives, **biodegradable films** have emerged as a promising solution. According to the International Union of Pure and Applied Chemistry (IUPAC), biodegradable films are materials derived from biomass or plant-based monomers that can be modified during processing. Though they account for just around 1% of global plastic production, they are expected to grow at an annual rate of over 30% by 2025 (Environment, 2021). Importantly, biodegradable films can be either **bio-based** or **biodegradable**, though these terms are not synonymous.

Unlike conventional plastics, which can persist in the environment for hundreds of years, **biodegradable plastics** can break down into carbon dioxide and water in as little as 20–45 days under proper environmental conditions, such as the presence of oxygen, moisture, and specific microbial populations (Mar. Environ. Res., 2016). Despite this potential, many biodegradable materials suffer from low mechanical strength, limiting their practical applications.

To address this limitation, researchers are turning to natural reinforcements such as **lignocellulosic fibers** and bio-derived additives like **chitosan**, which are both sustainable and biodegradable (Polymers, 2019). In recent studies, agricultural byproducts such as **purple yam (*Dioscorea alata)*** and **beetroot (*Beta vulgaris*)** have been investigated as functional components in biodegradable film production due to their high starch content, bioactive compounds, and antioxidant properties.

Purple yam, commonly cultivated in tropical and subtropical regions, is rich in dietary fiber, vitamins, and minerals, and is notable for its anthocyanin content, which provides antioxidant activity and potential health benefits (Tamaroh et al., 2021). It has been utilized in various research efforts to create starch-based biodegradable films. Beetroot, meanwhile, contributes betalains, phenolic compounds, and dietary nitrates, offering antioxidant, antimicrobial, and even anti-inflammatory benefits (Bangar et al., 2022; Pandita et al., 2020).

Complementing these components is **chitosan**, a deacetylated form of chitin known for its biodegradability and antimicrobial efficacy. While chitosan alone lacks ideal mechanical and water-resistant properties, it forms improved films when combined with starch-based ingredients (Mani et al., 2021).

This study proposes a novel biodegradable film composed of **purple yam starch**, **beetroot extract**, and **chitosan**—combining structural, functional, and antimicrobial properties. This natural composite aims to provide a sustainable and eco-conscious alternative to petroleum-based plastics, particularly in food packaging and agricultural applications, contributing meaningfully to global efforts toward environmental sustainability.

1.1 PURPLE YAM *(Dioscorea alata)*

Purple yam (*Dioscorea alata*), commonly known as ube, is a tuberous root vegetable widely grown in tropical and subtropical regions. Its distinctive purple hue comes from anthocyanins—natural antioxidants that have been associated with potential health benefits. This vibrant tuber is deeply rooted in the culinary traditions of many Asian and Latin American cultures, where it is often featured in a variety of dishes. Owing to its naturally sweet, nutty flavor, ube is a popular ingredient in desserts, pastries, and beverages. Beyond its appealing taste and color, purple yam is also a nutritious food source, offering high levels of dietary fiber, complex carbohydrates, and essential nutrients such as potassium, iron, and vitamin C.

Fig. 1: Purple yam

Aside from its nutrient-based health benefits, purple yam has attracted scientific attention for its potential therapeutic properties. Studies suggest it may offer antioxidant effects, support digestive health, and help regulate blood sugar levels. Its deep violet hue and versatility in preparation have made it a favored ingredient in both traditional and modern culinary practices around the world.

1.2 **Health Benefits of *Dioscorea alata* (Purple Yam)**

1. Purple yam contains a group of acylated anthocyanins that exhibit stronger antioxidant activity than their non-acylated counterparts, contributing to cellular protection and overall health. *(Moriya C. et al., 2015)*
2. In an experimental study using hyperlipidaemic hamsters, resistant starch extracted from purple yam was shown to enhance lipid profile and promote the growth of microbiota. *(Li Ti et al., 2019)*

   Research indicates that purple yam tubers contain steroidal saponins with immunomodulatory potential, suggesting their possible use in supporting immune function.
3. Investigations into Taiwanese purple yams have shown that their rich anthocyanin content may help combat several diseases, including liver disorders, hypertension, impairments, microbial infections, and diarrhea. *(Sujatha et al., 2015)*

1.3 Composition of Purple Yam

- **Carbohydrates:**

  Purple yam is primarily composed of carbohydrates, accounting for approximately 17.10–29.37%, making it a significant energy source. *(Tamaroh et al., 2021)*

- **Proteins:**

  It contains 1.29–3.00% protein, which supports tissue growth and repair. *(Tamaroh et al., 2021)*

- **Fats:**

  The fat content is very low, around 0.29%, making it a low-fat food option.

- **DietaryFiber:**

  Purple yam provides 6.70–11.62% dietary fiber, which aids digestion and supports gut health. *(Tamaroh et al., 2021)*

- **MoistureContent:**

  The moisture level ranges from 65.47–82.46%, reflecting a high water content. *(Tamaroh et al., 2021)*

- **Minerals:**

  This tuber is a rich source of several essential minerals:

  - Potassium: 224.54–483.21 mg/100 g
  - Calcium: 15.63–61.97 mg/100 g
  - Magnesium: 16.75–43.06 mg/100 g
  - Iron: 1.40–13.40 mg/100 g
  - Zinc: 0.43–2.83 mg/100 g
  - Phosphorus 329.37–699.91 mg/100 *(Tamaroh et al., 2021)*

- **Anthocyanins:**

  The tuber contains approximately 31 mg of anthocyanins per 100 g of dry matter, which contributes to its antioxidant potential. *(Tamaroh et al., 2021)*

1.4 SCIENTIFIC CLASSIFICATION

- **Kingdom:** Plantae
- **Order:** Dioscoreales
- **Family:** Dioscoreaceae
- **Genus:** *Dioscorea*
- **Species:** *Dioscorea alata*

1.5 PRODUCTION AND AREA

Purple Yam (*Dioscorea alata*)

Purple yam, scientifically referred to as *Dioscorea alata* and also known as water yam, is a prominent tuber crop cultivated throughout tropical and subtropical zones. Globally, yam production is estimated at approximately 52 million metric tons, covering over 5 million hectares across 57 countries. Notably, around 95% of this production is concentrated in West Africa (Anyanwu et al., 2015).

In the Philippines, *Dioscorea alata* production has fluctuated over time. Data from the Bureau of Agricultural Statistics shows a significant decline in production from 30,074 metric tons in 2006 to 15,799 metric tons by 2012. This drop is largely attributed to the plant's seasonality and the dormancy period of its tubers.

In India, this yam is cultivated across various regions, including Madhya Pradesh, the northeastern states, West Bengal, Bihar, Odisha, Uttar Pradesh, Kerala, Tamil Nadu, and Maharashtra. Its wide distribution across the country highlights its adaptability and agricultural importance (Sequeira et al., 2014).

1.6 BEETROOT (*Beta vulgaris*)

Beta vulgaris, commonly known as beetroot, belongs to the Chenopodiaceae family. The vibrant color of beetroot is due to pigments called betalains, which also serve as natural food colorants. Beetroot is nutritionally rich, containing essential components like proteins, carbohydrates, fiber, vitamins (such as B-complex and vitamin C), minerals, and various antioxidants, including flavonoids, coumarins, carotenoids, and triterpenes (Bangar et al., 2022).

Unlike sugar beets, beetroot contains lower sugar levels, making it suitable for culinary use in various food products. Nitrate present in beetroot is reduced to nitric oxide (NO) by the oral microbiome, which plays a role in improving muscle function and reducing fatigue (Wruss et al., 2015; Bailey et al., 2009; Hernandez et al., 2012; Larsen et al., 2006). However, excessive nitric oxide may lead to the formation of harmful metabolites, some of which may have carcinogenic properties (Habermeyer et al., 2015).

Additionally, beetroot offers several health benefits due to its antioxidant, anti-inflammatory, antibacterial, and antiviral properties. Regular consumption has been linked to positive effects on conditions such as diabetes, cardiovascular diseases, and even cancer (Pandita et al., 2020).

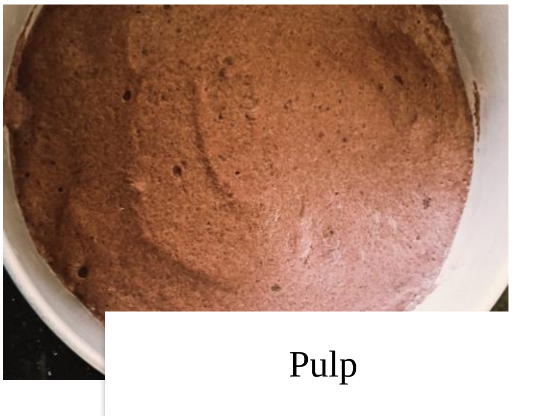


Fig. 2 Beetroot

1.7 SCIENTIFIC CLASSIFICATION

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Order: Caryophyllales

Family: Amaranthaceae

Genus: Beta

Species: Beta vulgaris

1.8 CHITOSAN

Chitosan is a deacetylated derivative of chitin. Chitosan is applied to manufacture biodegradable films for packaging, with their most frequent use being edible coatings to prolong the shelf life of fresh fruits and vegetables. Nevertheless, chitosan lacks good mechanical strength and is low in water resistance. Recently, composite films obtained by mixing chitosan with starch has exhibited better water vapor resistance and mechanical properties. Furthermore, chitosan exhibits good antimicrobial activity against several fungi, yeasts, and bacteria present in food; thus, it is a significant material for biodegradable food packaging, especially for shelf life extension of food products. (Mani et, al 2021)

2. LITERATURE REVIEW

2.1 Sustainability of biodegradable plastics: New problem or solution to solve the global plastic pollution?

The research paper published by Taofeeq D. Moshood et al. (2022) established that the use of plastic is contributing to an increase in environmental contaminants. Human health is at risk from plastic particles and other plastic-based contaminants that are present in our environment and food chain. The goal of biodegradable plastics, according to this viewpoint, is to make the planet greener and more sustainable while leaving less of an environmental impact. This evaluation should take into account the goals and priorities for creating a variety of biodegradable polymers over their whole life cycle. As long as adequate waste management practices, such as composting, are followed, biodegradable plastics can have qualities comparable to those of conventional plastics while also offering further advantages because of their reduced carbon dioxide impact on the environment. To lessen pollution and waste management problems, there is a growing need for affordable, environmentally friendly materials. Understanding biodegradable plastics manufacturing and applications research, product prospects, sustainability, sourcing, and ecological impact is the goal of this study. Recent years have seen a meteoric rise in corporate and academic interest in biodegradable polymers for sustainability. The triple bottom line—economic profit, social responsibility, and environmental protection—was employed by researchers to examine the sustainability of biodegradable polymers. A sustainable framework for enhancing the long-term viability of biodegradable plastics is also included in the study, along with the factors that affect their acceptance. A comprehensive yet straightforward theoretical design for biodegradable plastics is presented in this paper. A new direction for additional study and contribution to the field is offered by the research findings and upcoming projects.

2.2 Biodegradable plastic applications towards sustainability: A recent innovations in the green product

The research conducted by Fatimah Mahmud et al. (February 2022) examines how biodegradable plastics contribute to sustainability in a new, sustainable plastics economy where polymers serve their intended purpose without producing adverse externalities. Plastics that break down organically over time are known as biodegradable. A multidisciplinary strategy is a unique approach, with research conducted utilizing the triple bottom-line method across three distinct sustainability principles (social attitudes, environmental repercussions, and economic characteristics). Since the place of plastics in the plastics system would unavoidably be diminished if it could not be established for biodegradable plastics that give similar or improved material qualities in contrast to traditional plastics, biodegradable plastics became the first aim. Thus, this study aims to examine the many incentives that companies use to manufacture biodegradable plastic items and the elements that affect their long-term sustainability. The study concluded that the economic factor was the most significant, followed by the effects on the environment and social views. The study also addresses a sustainable framework for enhancing the long-term viability of biodegradable polymers and the factors that affect their acceptance. The results also evaluate the efficacy of the proposed framework, which comprises three sustainability levels and seventeen principles. The social dimension has nine, the economic dimension has eight, and the environmental dimension has seven. This study provides a thorough and effective method for assessing and identifying the best choices for the biodegradable plastics industry.

2.3 An overview of biodegradable packaging in food industry

Biodegradable food packaging is a significant development in sustainable food packaging, which also has apparent environmental benefits and a decreased reliance on traditional plastic products. The use of different biopolymers (e.g., chitin, chitosan, starch) has progressed significantly in the capability and function of biodegradable films for food preservation and antimicrobial use. We find the capability of these materials for barrier properties, as well as their possibility for reducing the overall environmental impact of the packaging industry, to be quite appealing for practical use. The biodegradable film and packaging industry has struggled with widespread adoption and further applications, mainly due to higher-than-desired production costs, a lack of consumer knowledge, and technology focused on improving durability and water resistance. Additionally, perhaps the most imposing challenge to the establishment of biodegradable packaging is its mechanical inefficiencies. Most biopolymers, for instance, chitosan, exhibit poor water resistance and mechanical strength and are therefore less desirable for certain packaging applications. To overcome this, scientists have examined composite formulations by blending biopolymers with starch and other natural polymers and found enhanced structural and barrier properties. In addition, biodegradable packaging materials must fulfill functional requirements, particularly oxygen and moisture barrier properties, to be effective in food preservation and quality, and this involves further improvements in areas of material science and manufacturing technologies.

An additional key characteristic of biodegradable packaging is its potential for environmental sustainability. Substituting petroleum-based plastics with biodegradable plastics decreases plastic pollution, carbon footprint, and landfill fill. However, large-scale biopolymers manufacture requires high agricultural resources, raising concerns related to land use and food security. These concerns can be mitigated with more efficient biopolymer manufacturing processes and alternative renewable resources such as agricultural residues and microbial fermentation. Moreover, regulations and incentives can be effective in motivating industries to transition to biodegradable packaging options. The future of biodegradable packaging will be driven by innovation in bio-based polymer technologies, advanced processing technologies, and consumer awareness. The demand for sustainable packaging solutions in the market will continue to rise, compelling manufacturers to make investments in research and development to increase material properties and cost-effectiveness. As we continue to move forward and further advancements in technology occur, biodegradable packaging can become an eventual universal solution throughout the packaging industry, paving the way for a sustainable and environmentally friendly future. The collaborative work of researchers, policymakers, and especially those embedded in industry will be necessary for the successful implementation of biodegradable material in international packaging systems.

2.4 Antioxidative Characteristics and Sensory Acceptability of Bread Substituted with Purple Yam (*Dioscorea alata L.)*

The growing interest in functional foods has driven studies on using antioxidant food ingredients like purple yam in staple foods. Purple yam is rich in anthocyanin and total phenol and is thus very active as an antioxidant. Previous research has pointed out the potential of tubers like *Dioscorea alata* to enhance the functional value of food products, with studies on similar tubers indicating that they improve the functional properties of bread without having a significant effect on consumer acceptance (Tamaroh & Sudrajat, 2021).

The work examined different substitution levels of purple yam flour (10%, 15%, 20%, and 30%) in wheat bread to evaluate their effect on anthocyanin content, total phenol content, antioxidant capacity, volume expansion, and sensory characteristics. Results showed that the incorporation of purple yam considerably boosted anthocyanin and phenol content without affecting desirable sensory qualities, rendering it a suitable replacement ingredient for use in functional bread production. In addition, other studies on other alternative flours, e.g., Chinese yam (*Dioscorea opposita*) and sweet potato (*Ipomoea batatas*), have also shown promise in enhancing the health attributes of bread without compromising consumer liking (Tamaroh & Sudrajat, 2021).

2.5 Performance and adaptability of two yam (*Dioscerea Spp)* varieties under Ifugao condition

Yam (*Dioscorea alata*) is a dominant food in most tropical and subtropical countries and is a prime component of world food security and its ability to grow under varying climatic regimes and soils (Coursey, 1967; Ayensu & Coursey, 1972). Estimated world yam production has been put at 52 million metric tons, concentrated in West Africa. However, Southeast Asian producers like the Philippines are also very important (FAO, 2004). The decrease in Philippine yam production from 30,074 metric tons in 2006 to 15,799 metric tons in 2012 indicates that yield becomes difficult to maintain even with growing domestic and export demand (BAS, 2004).

Research shows that yam prefers well-drained sandy loam soil and will grow best with a mean temperature of 25°C to 30°C (Onwueme, 1978). It is also commonly intercropped with vegetables and legumes for enhanced land efficiency (Nweke et al., 1991; Ramirez & Rodriguez, 1975). adaptable, with high yield potential, and with suitability for industrial food use (Nweke et al., 1991). Apart from its agronomic significance, Dioscorea alata is rich in bioactive compounds like alkaloids and saponins, which have therapeutic properties such as blood sugar regulation and antioxidant activity (Degras, 1993; PCHRD-DOST Bulletin, 2011). Exploring of greater yam (*Dioscorea alata L.*) genotypes through biochemical screening for better cultivation in south Gujarat zone of India

According to a study by Patel and others in 2019, the biochemical composition and genetic diversity of various genotypes of *Dioscorea alata* (greater yam) were analyzed in South Gujarat, India. The variation in the yam genotype results in high carbohydrate content; it ranges from 51.87% to 87.85%. Alata is comprised not only of valuable nutrients like carbohydrates but also crude fiber (1.10%–4.09%), crude fat (0.6%–2.32%), beta-carotene (0.97–1.88 µg/g), and anthocyanins (1.01–3.25 mg/g), all of which additionally contribute to the functional and nutritional properties of yam. In 2019, Patel and others studied the biochemical composition and genetic diversity of various genotypes of *Dioscorea alata* (Greater yam) in South Gujarat, India. High carbohydrate content was seen in different yam genotypes, ranging from 51.87% to 87.85%. The genotypes NGY-2 and NGY-3 indicated 96% genetic similarity, suggesting possible clonal propagation. This study provides useful information about *Dioscorea alata* cultivation, with an indication given to genotypes with great nutritional value and more adaptability to agricultural conditions in the region.

2.6 New acylated anthocyanins from purple yam and their antioxidant activity

The research paper published by Moriya et al. (2015) mainly concerns new acylated anthocyanin identification in purple yam (D. alata) and antioxidant property evaluation. The health benefits of anthocyanins are significant because they possess antioxidant activity. D. alata's beautiful deep purple color is due to the presence of anthocyanins. Purple yam is rich in D. alata, a fascinating source of natural antioxidants. The four new acylated anthocyanins, plantains D, E, F, and G, and four known anthocyanins were isolated and highlighted from the study. They used spectroscopic methods, including NMR and MS analyses, for their structural characterization. Yam enhances stability and antioxidant activity compared to non-acylated foods and may serve as a functional food. The new anthocyanins were also assessed against the oxygen radical absorbance capacity (ORAC) assay and the ferric reducing antioxidant power (FRAP) assay for their antioxidant capacity. The findings suggest that the recently detected anthocyanins, especially cyanidin and peonidin with free radical scavenging activities, have potent capabilities. Findings imply that eating purple yam is likely to provide vast health benefits, especially for diseases related to oxidative stress, such as heart disease and cancer. This study shows that D. alata can be a useful oxidative stress.

2.7 Anti-Inflammatory and Anticancer Activities of Taiwanese Purple-Fleshed Sweet Potatoes *(Ipomoea batatas L. Lam)* Extracts

The research conducted by Sugata et al. (2015) examines the anti-inflammatory and anticancer effects of extracts from purple-fleshed sweet potato (I*pomoea batatas L. Lam).* Purple sweet potatoes have high anthocyanin content, which is responsible for the antioxidant action. The present work deals with the "Tainung 73" cultivar, grown in Taiwan, which was extracted and processed by acidified ethanol to yield crude anthocyanin extracts. The biological activity of the extracts in this research was established, including their influence on cancer cell viability and inflammation.

The MTT cell viability assay confirmed that anthocyanin extracts were non-toxic to macrophage cells (RAW 264.7) and were potent anti-inflammatory compounds, inhibiting the production of nitric oxide (NO) and down-regulating the release of pro-inflammatory cytokines such as NF-κβ, TNF-α, and IL-6. An anticancer effect was noted with the capacity of the extracts to suppress the growth of breast (MCF-7), gastric (SNU-1), and colon (WiDr) cancer cells in a time- and concentration-dependent manner. The research also attested that the extracts triggered apoptosis in MCF-7 and SNU-1 cancer cells via intrinsic and extrinsic pathways. These observations indicate that extracts of purple-fleshed sweet potato can potentially be used in nutraceutical, pharmaceutical, and functional food companies.

2.8 Beetroot as a novel ingredient for its versatile food applications

Beta vulgaris, commonly known as beetroot, is a biennial plant in the Chenopodiaceae family. The color of beetroot comes from a class of water-soluble pigments known as betalains, which are classified as betacyanins (red-purple pigments) and betaxanthins (yellow pigments). Betalains contribute to the color of beetroot, but they also provide nutritional value; they have valuable antioxidant potential as well. Is also rich in proteins, carbohydrates, including sucrose dietary fiber, and vitamins, mainly B-complex vitamins and vitamin C. It also supplies minerals such as potassium, sodium, phosphorus, calcium, magnesium, copper, iron, zinc, and manganese. In addition, beetroot can accumulate bioactive phenolic compounds and flavonoids (e.g., astragalin, tiliroside, rhamnocitrin, kaempferol, and rhamnetin), coumarins, carotenoids, saponins, and inorganic nitrate (NO) A growing body of evidence suggests health benefits from beetroot consumption, including cardiovascular health, treatment of hypertension, glycemic regulation in diabetes, anticancer action, and protection against liver disease, including hepatic steatosis. The bioactive phytochemical profile of beetroot must be recognized, identifying it as a potentially significant source of nutraceutical compounds in creating functional foods. Its pigments can be used as a natural food colorant, while its nutritional and bioactive compounds enable the development of health-promoting functional food products.

2.9 Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Austria

Beta vulgaris L., or beetroot, is known for being a good source of various bioactive compounds, including betalains and inorganic nitrate. One study interestingly examined varied juices from seven native beetroot varieties sourced from Upper Austria, where minerals, betalains, oxalic acid, phenolic acids, and sugars were measured. The varieties studied had all been growing naturally in Upper Austria. However, important differences were observed between them, especially for nitrate, while other factors such as minerals and sugars appeared to be less variable. The concentration of total betalain was found to be between 0.8 and 1.3 g/L in the freshly harvested juice, almost all of which was comprised of 60% betacyanins and 40% betaxanthins, or 70–100% of the total phenolic compounds. A small percentage of the total hydroxycinnamic was also observed, comprising only up to 2.6% of total phenolics overall. In terms of nitrate concentrations, these varieties differed by as much as tenfold. Hverbye et al. (2014) noted that the sugar profile was remarkably similar among varieties, with an average of 7.7% total sugar content of 95%, which was determined to be sucrose. As for oxalic acid, concentrations did not vary much at all, with concentrations being about 0.3-0.5 g/L in fresh juice. The study then proceeded to measure nitrate concentrations in 16 commercial beetroot juices and 4 beetroot powders, as nitric oxide, or a metabolic product of nitrate, has been suggested to have various cardiovascular benefits (see Radshana et al., 2023). Within commercial product samples, there was considerable variation in nitrate concentrations, ranging from 0.01 to 2.4 g/L. In conclusion, these results demonstrate that beetroot varieties and commercial products differ compositionally, and it could be claimed that BEETROOT, especially nitrate, would be different from one another in terms of potential health benefits.

2.10 Beetroot

Beta vulgaris subsp. vulgaris L., also known as beetroot, is a root vegetable that belongs to the family Chenopodiaceae. Beetroot is ranked among the top ten vegetables with antioxidant activity due to its rich composition of bioactive compounds such as betalains, phenolic acids, saponins, alkaloids, steroids/triterpenes, catechins, and flavonoids. These antioxidants protect cells against oxidative stress incurred by reactive oxygen species (ROS) or free radicals, which can disrupt cell metabolism and cause damage to DNA structural integrity and the function of lipids and proteins. Apart from its antioxidant capacity, beetroot is a quality food supplement due to its content of nutrients, specifically minerals, vitamins such as the B complex and folic acid, and diverse bioactive compounds with various medicinal and therapeutic benefits. Beetroot has medicinal effects throughout the plant, to the extent that it is known to have analgesic, hepatoprotective, antioxidant, antimicrobial, anti-inflammatory, antimigraine, antihypertensive, antiviral, antihyperglycemic, anti-progestogenic, antiallergic, antithrombotic, and antitumorigenic effects. Lastly, beetroot has been shown to protect against neural degeneration and liver injury.

2.11 Hot air oven for Sterilization: Definition & Working Principle

A hot air oven marks the pivotal instrument for dry heat and sterilization and is majorly focused on the sterilization of instruments that cannot be exposed to moisture. In contrast to moist heat sterilization, which uses steam or boiling water, dry heat sterilization requires very high temperatures maintained over a long time to destroy microorganisms and bacterial spores. Therefore, hot air ovens are useful in sterilizing glassware, powders, oily materials, and metal equipment. Heating through dry heat involves conduction; the outer surfaces absorb heat, which is then transferred toward the center so that complete sterilization is accomplished. Usually, the process is carried out at 170 °C for 30 minutes, 160 °C for 60 minutes, or 150 °C for 150 minutes. The operating features of the hot air oven include mechanical and electrical components covered externally with stainless steel or aluminum for durability, fiberglass for insulation, and a chamber presenting adjustable shelves for the accommodation of objects of different shapes. The electrical system consists of heating elements, a thermostat for maintaining temperature, a timer, and a control panel that allows close tracking of the sterilization procedure.

Despite their merits, hot air ovens have certain limitations. Water is not needed for their operation, nor do they create any high pressure, making them safer than autoclaves; however, dry heat also implies that some organisms could survive. While the exhaustive sterilizing process can be said to be working in these laboratories, much depends on the temperature distribution, length of time for which heat is applied, and the actual characteristics of the materials under sterilization. With advances in the development of heating elements and insulating materials, hot air ovens have had improved performance; however, research in energy efficiency and technological improvements to use hot air ovens in other fields of interest needs to be enhanced. Innovations in hot air oven technology could thus become a strong link in the chain for a safer and more effective method of sterilization as laboratories and healthcare sectors become increasingly concerned about sterilization.

2.12 Properties of food ingredients during processing in a domestic mixer grinder and subsequent storage: A review

Research has focused on the changes wrought in the physical, chemical, and nutritional properties of these ingredients concerning processing, particularly domestic mixer-grinding. A few spices are ground to enhance taste, but excessive heat developed during grinding and storage affects the quality. Similarly, batters are forced into the global culinary repertoire in such a way that grinding enhances flavor extraction and nutrient availability. However, the end storage might produce rancidity, discoloration, and unsubtle seasoning. With lentil-and-rice batters, grinding provides a spectrum for fermentation, whereas storage disrupts the acidification of the batter, impacting nutrient composition, taste, and texture. The aim is to give bread and chickpea flour different subsequent food applications. However, such high-speed grinding must inevitably generate heat, which could result in starch being modified for the better or the worse. Long-term storage could cause discoloration, thereby lessening the suitability for certain preparations.

The analysis of Indian households concerns the practice of grinding food ingredients, including spices, purees, pastes, batters, chickpea flour, and rice flour, impacting their sensory and nutritional profiles. Such understanding may guide consumers in adopting grinding protocols that are safe for taste and nutrients. The authors suggest how grinding techniques could be optimized to improve food quality while limiting the negative aspects. Suresh and Varadharaj categorized the areas most deserving of further study, among them the effect of high-speed grinding on ingredient properties, including fair performance that causes adverse effects. Here studies may be carried out on the nutritional benefits of freshly ground ingredients, thus enhancing the understanding of the role of grinding in food quality.

2.13 Development of biodegradable films based on purple yam starch/chitosan for food application

Biodegradable polymeric films provide a sustainable packaging option and extend food shelf life. Reducing the usage of plastic packaging and using biodegradable materials from renewable resources support the packaging industry's current expectation of environmental preservation. Because they are abundant, inexpensive, biocompatible, non-toxic, and renewable, biopolymers are utilized as amatrix in a variety of industries (et al., 2015). Natural polymers, including starches and others, have already been researched as edible thin layers or biodegradable packaging to cover and prolong the shelf life of food items.
The alkaline deacetylation of chitin yields chitosan, a linear natural biopolymer. Chitosan has been utilized extensively as an edible film to coat foods because of its antimicrobial action, superior mechanical qualities, and biocompatibility. These films were divided into two categories based on their thickness: edible thin films or coatings (less than 30 μm) and films and blends.

2.14 Edible Wheat Gluten Films: Influence of the Main Process Variables on Film Properties using Response Surface Methodology

Edible films and coatings have special appeal to the food packaging industry due to their potential to enhance food quality and shelf life and offer biodegradable alternatives to synthetic packaging. Of the numerous biopolymers studied for film development, proteins and, more notably, wheat gluten, have been of interest due to their superior film-forming ability and cohesion.

Gontard, Guilbert, and Cuq (1992) conducted an extensive investigation on the manufacture of edible wheat gluten films, and the role of principal processing factors was emphasized in the functional properties of these. Applying Response Surface Methodology (RSM), researchers have deeply explored the effects of concentration , ethanol concentration, and casting solution pH on the characteristics of the films, such as opacity, solubility, water vapor permeability, puncture strength, deformation, and viscoelasticity.

The research established that ethanol and pH exhibited strongly interactive effects on the barrier and optical properties of the films. For instance, water solubility and opacity of the films were minimized to the lowest when ethanol concentration and pH were both at optimal values within specified ranges (32.5–45% ethanol, pH 2–4), and less soluble and more transparent films were formed. Water vapor permeability was also minimized at 20% ethanol and pH 6, and this suggests that increased protein unfolding and homogeneity under these conditions led to increased barrier properties.

Conversely, mechanical properties like puncture resistance and elasticity were greatly influenced by gluten concentration and pH. The films were mechanically strongest at 12.5% gluten concentration and pH 5, which confirms the significance of protein network establishment during drying. These findings suggest that protein concentration and solution acidity influence not only the physical structure of the films but also their functionality as food wrappers or coatings.

The study reveals that wheat gluten is a good and versatile material for the manufacture of edible films. Moreover, the application of RSM was effective in identifying the optimal conditions of film formation, and therefore, the method is helpful for the design of films with the required functional specifications.

The article gives a basis for how formulation conditions influence gluten film properties and offers avenues for future research, such as the addition of plasticizers, crosslinking agents, or hydrophobic agents in order to improve moisture barrier and mechanical strength

2.15 Comparison of Mechanical and Physicochemical Characteristics of Potato Starch and Different Sources

With growing concern for environment-related issues of synthetic plastics, commercial production of biodegradable films from renewable biomass has entered prime time. Biodegradable films from starch and proteins such as gelatin are a promising, biodegradable alternative to conventional petroleum-based plastics. Their performance, however, is greatly dependent on the source and nature of the raw material composition.

Recent research has investigated the employment of gelatin-starch mixtures as biodegradable films. (Fakhouri et al. 2012) contrasted the impact of varying plasticizers on mechanical performance, where tensile strength and flexibility depend on the type of plasticizer, protein, and starch content. Other studies, including (Fakhouri et al. (2013), compared processing techniques, in which casting yielded stronger and less permeable films compared to pressing or blowing techniques. Likewise, Podshivalov et al. 2017) alluded to the significance of plasticizers such as glycerol, which enhance flexibility but also serve as compatibilizers between starch and protein phases.

While recognizing all these advances, a glaring lack of comparison studies that would examine the effect of the gelatin source—i.e.,piscine (fish), porcine (pig), or bovine (cow)—on the resultant biodegradable properties was evident. Filling that gap, (Mroczkowska et al. 2021) conducted systematic studies on starch-based biodegradable films that are formulated with these three different sources of gelatins.

Their study brought forth the following major findings:

Fish-based gelatin biodegradable films had lowest water solubility and highest tensile strength (8.1 MPa), comparable to the strength of low-density polyethylene (LDPE).Surface roughness was greater in piscine gelatine-based biodegradable films, which might be for use where maximum ink adhesion for labeling is needed.
Opacity and color measurements indicated that piscine-based biodegradable films were less opaque and lighter in color, both of which are desirable traits in clear packaging.
Fourier-Transform Infrared (FTIR) spectroscopy revealed identical chemical bonds among all the samples, but (RP-HPLC analysis) revealed structural differences in the gelatins that explained the disparity in mechanical and physical properties.

This research brings into focus the primary significance of the source of biopolymer in dictating the performance properties of biodegradable materials. In particular, fish gelatine proved to be a superior alternative with better mechanical properties and water resistance without any loss of biodegradability.

(Mroczkowska et al.'s 2021) work offers useful insights into simplifying biodegradable formulations and adds to further work towards commercialization of sustainable packaging materials

2.16 Biodegradable Plastics from Mango Seed Starch for Sustainable Food Packaging—Effect of Citric Acid and Fillers

Manufacture of starch biodegradable films has been extensively explored as a bio-based alternative for conventional plastics. Indigenous starch films are, nevertheless, mostly plagued by low water resistance, inadequate mechanical strength, and high permeability, markedly limiting their application. In attempts to mitigate the deficiencies, novel research has, therefore, been focused on adding fillers, plasticizers, and cross-linking agents to the starch matrices with the aim of improving their mechanical and functional performance.

The starch-filler-plasticizer-cross-linker interactions within the structure were confirmed by FTIR spectroscopy. The hydrophilic properties of the biodegradable films were significantly increased. Particularly, the addition of CA and fillers led to a substantial decrease in the water uptake of the biodegradable films. For example, biodegradable films with CMC showed a 70% decrease in water uptake with 20% CA, whereas biodegradable films with CS showed only 12.5% water uptake. The modified biodegradable films had low water vapor permeability (WVP), ranging from 2.16×10^{-7} to 6.29×10^{-7} g day^{-1} m^{-1} Pa^{-1}, which is considered low enough for packaging. Addition of CA was the most effective in improving water resistance of the biodegradable films and reducing WVP by preventing their hydrophilic groups from being contacted with moisture and forming more regular

Mechanical properties were improved with the addition of CS and NCS fillers. This enhancement happens due to a more substantial hydrogen bonding of the NH_3^+ group from chitosan and the OH^- group of starch, which brings about biodegradable films with excellent cohesion.

In addition to the physical strength, the biodegradable films were also endowed with antimicrobial properties, which are crucial for packaging food items as they need to be safe and clean. The polymer also had good biodegradability, suggesting it is more eco-friendly compared to regular plastics.

In summary, the modified starch biodegradable films have improved water uptake, mechanical properties, antimicrobial properties, and biodegradability that make them best suited for food packaging applications.

3. MATERIALS AND METHODS

The materials and methodology used in the present investigation, entitled **"Development and Characterisation of Sustainable Biodegradable Film from *dioscorea alata* and *beta vulgaris*,"** are as follows:

3.1. Raw Materials Used:

- ***Dioscorea alata*** (Purple Yam)
- ***Beta vulgaris*** (Beetroot)
- **Chitosan**
- **Glycerol**
- **Water**

3.2. Equipment and Instruments:

- **Hot Air Oven**
- **Mixer Grinder**
- **Weighing balance**

3.2.1 Hot Air Oven:

The hot air oven is a critical tool for dry heat sterilization, particularly for items that are heat-stable and cannot tolerate moisture. This method relies on elevated temperatures to eradicate bacteria and bacterial spores through conduction, wherein heat is transferred from the exterior to the core of the material. Unlike moist heat sterilization, which uses water or steam, dry heat sterilization is a slower process but is essential for sterilizing materials like surgical dressings, rubber, and plastic.

Standard operating parameters include:

- **170°C for 30 minutes**
- **160°C for 60 minutes**
- **150°C for 150 minutes**

The timing of sterilization is crucial to ensure optimal availability and functionality of the equipment, particularly in a high-throughput environment (Saif Aldeen Saad Obayes, 2018).

Fig. 3 Hot Air Oven

3.2.2 Mixer Grinder:

Mixer grinders serve as indispensable tools in both culinary and industrial applications. In food preparation, they facilitate the smooth integration of ingredients, whether for baking, whipping, or kneading dough. Their efficiency ensures consistency and uniformity in food processing, significantly reducing time and effort.

Beyond the kitchen, mixer grinders are pivotal in the music and audio industries. Sound engineers rely on them to blend multiple audio sources and control key parameters such as volume, tone, and effects, making them essential for both studio and live performance settings.

Fig.4 Mixer Grinder

3.2.3 Weighing balance:

Analytical balances are precise instruments that are used to quantify small weights with great accuracy. The most important features are:

- Draft shield: A plastic cover over the measuring pan to exclude air currents and dust.
- Precise measurement: Measurable to measure masses as light as 0.1 mg with the assistance of special ventilating systems.
- Regulation of temperature: The sample must be kept at room temperature to prevent air currents due to convection to get a proper reading.

These features give precise and reproducible measurements in most scientific uses.

Fig.5 Analytical Balance

3.3. Sample Procurement:

Purple yam (***Dioscorea alata)*** was sourced from private agricultural lands in Kottayam, thoroughly washed, peeled, and measured for weight. Beetroot (***Beta vulgaris***) was procured from a local market, where it underwent the same preparation process. Chitosan and glycerol, serving as key additives and plasticizers, were obtained from a laboratory in the Ernakulam district. These raw materials were carefully selected for their unique properties in the preparation of the biodegradable film.

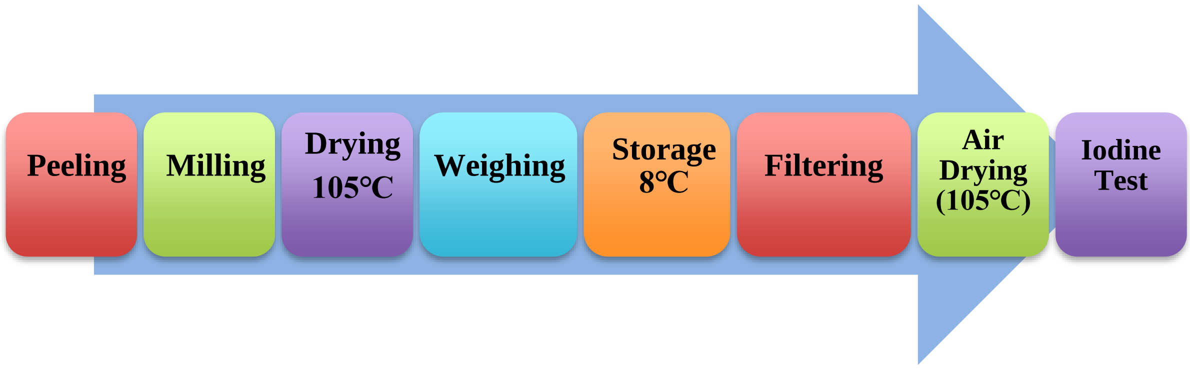
3.3.1 PREPARATION OF PURPLE YAM POWDER

The purple yam was first cleaned, peeled, and washed thoroughly, and then cut into small pieces. The yam pieces were subsequently milled into a fine paste using a grinder. The paste was then dried in a hot air oven at 105°C for 3 hours. The resulting dried mass was ground into a fine powder using a grinder. The powdered purple yam was stored at 8°C for future use in solution preparation.

Flow Diagram. 1 Preparation of Purple yam Powder

3.3.2 PREPARATION OF BEETROOT POWDER

The beetroot was first cleaned, peeled, and washed thoroughly, and then cut into small pieces. The beetroot pieces were subsequently milled into a fine paste using a grinder. The paste was then dried in a hot air oven at 105°C for 2 hours. The resulting dried mass was ground into a fine powder using a grinder. The powdered purple yam was stored at 8°C for future use in solution preparation.

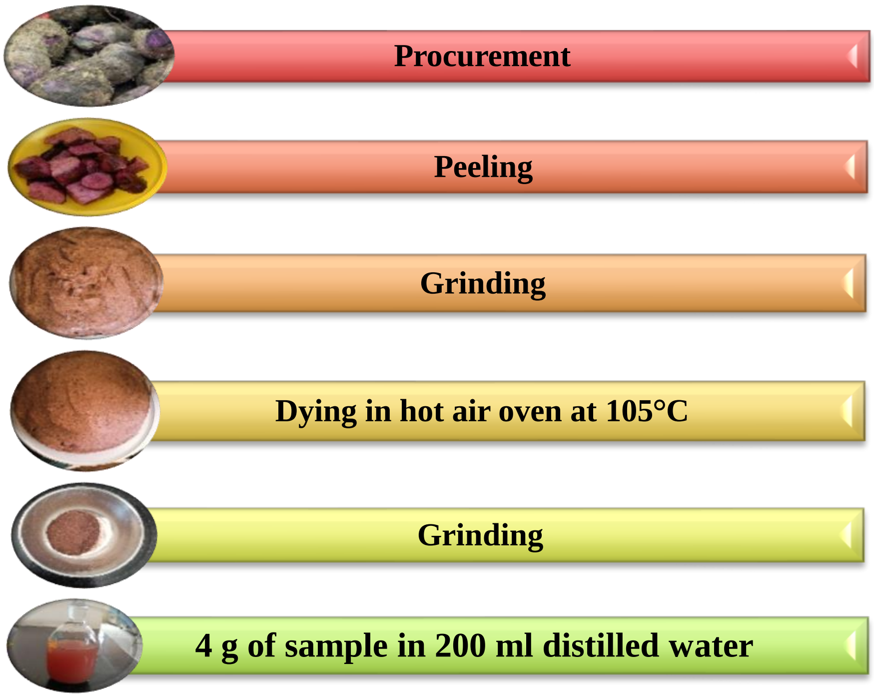


Flow Diagram. 2 Preparation of Beetroot Powder

3.3.3 PREPARATION OF PURPLE YAM SOLUTION

To prepare the purple yam solution, 4 grams of powdered purple yam were accurately measured using a magnetic analytical balance and transferred to a beaker. Subsequently, 200 millilitres of distilled water was added, and the mixture was heated in a hot air oven at 73°C for 30 minutes.

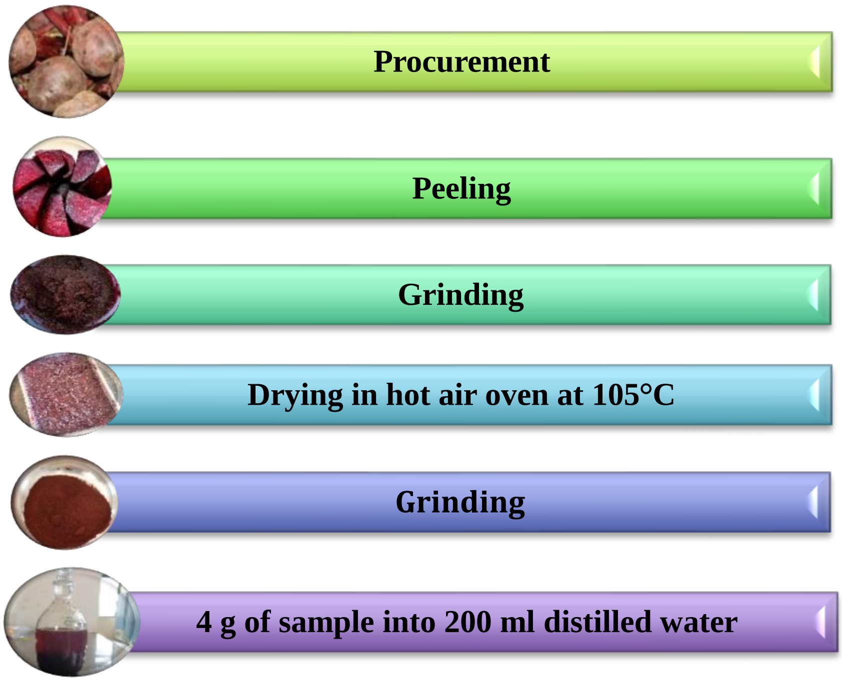
Fig. 6 Purple yam solution



Flow Diagram. 3 Preparation of Purple yam solution

3.3.4 PREPARATION OF BEETROOT SOLUTION

To prepare the beetroot solution, 4 grams of powdered beetroot was accurately measured using a magnetic analytical balance and transferred to a beaker. Subsequently, 200 millilitres of distilled water was added, and the mixture was heated in a hot air oven at 73°C for 30 minutes.

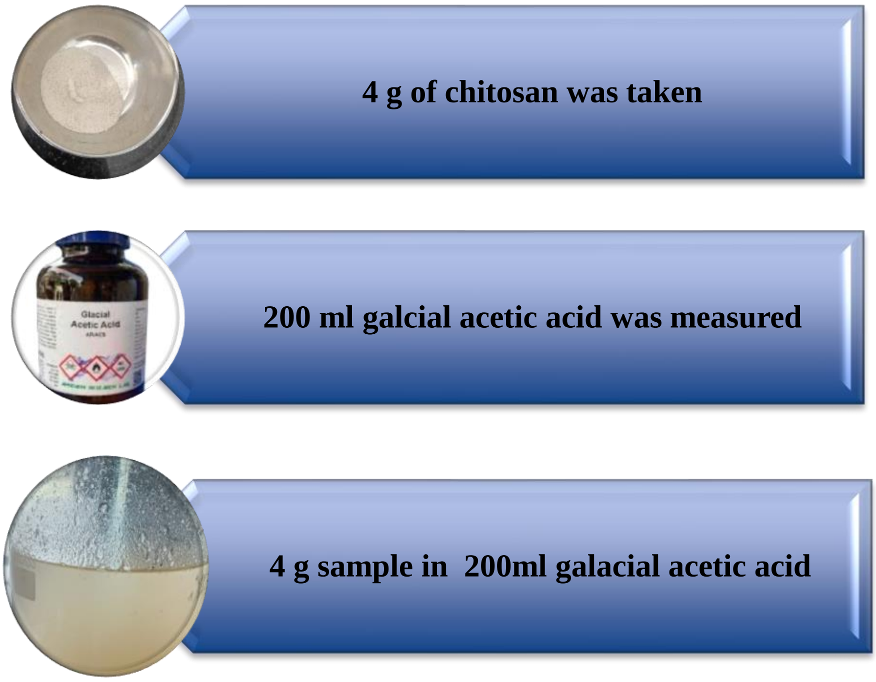


Flow Diagram. 4 Preparation of Beetroot Solution

Fig. 7 Beetroot Solution

3.3.5 PREPARATION OF CHITOSAN SOLUTION

To prepare the chitosan solution, 4 grams of chitosan was accurately measured using a magnetic analytical balance and transferred to a beaker. Subsequently, 200 milliliters of glacial acetic acid was measured and added. After thorough mixing, the solution was heated in a hot air oven at 40°C for up to 30 minutes, with stirring for 5 minutes in between, until a homogeneous mixture was obtained.

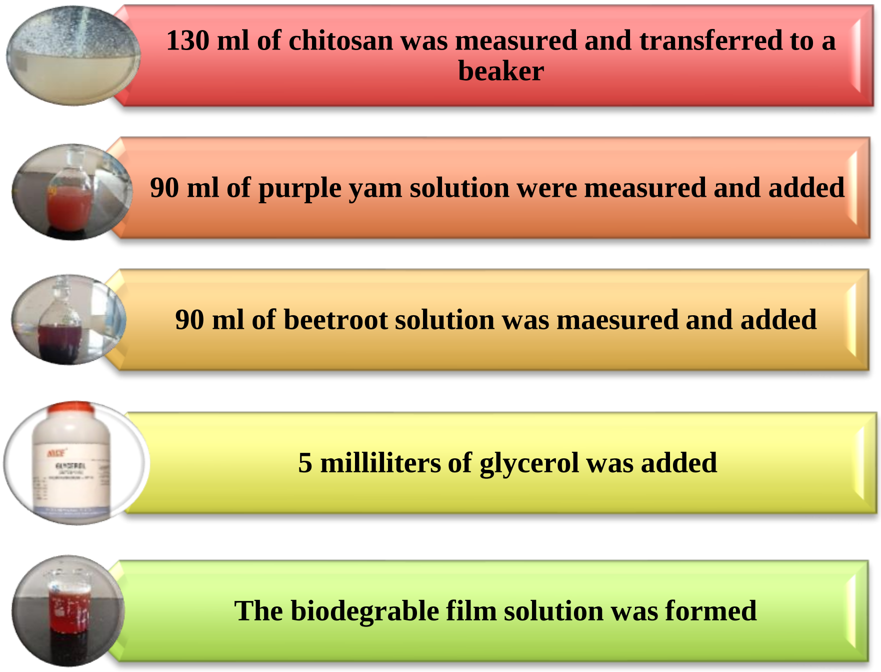


Flow Diagram.5 Preparation of Chitosan Solution

Fig. 8 Chitosan Solution

3.3.6 PREPARATION OF BIODEGRADABLE FILM SOLUTION

To prepare the biodegradable solution, 130 milliliters of chitosan was measured and transferred to a beaker. Subsequently, 90 milliliters of beetroot solution and 90 milliliters of purple yam solution were measured and added to the same beaker. Then, 5 milliliters of glycerol was added, and the mixture was stirred well.



Flow Diagram. 6 Preparation of Biodegradable Film Solution

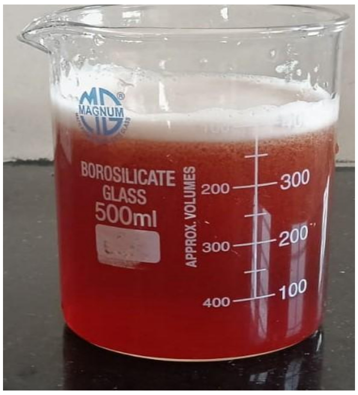
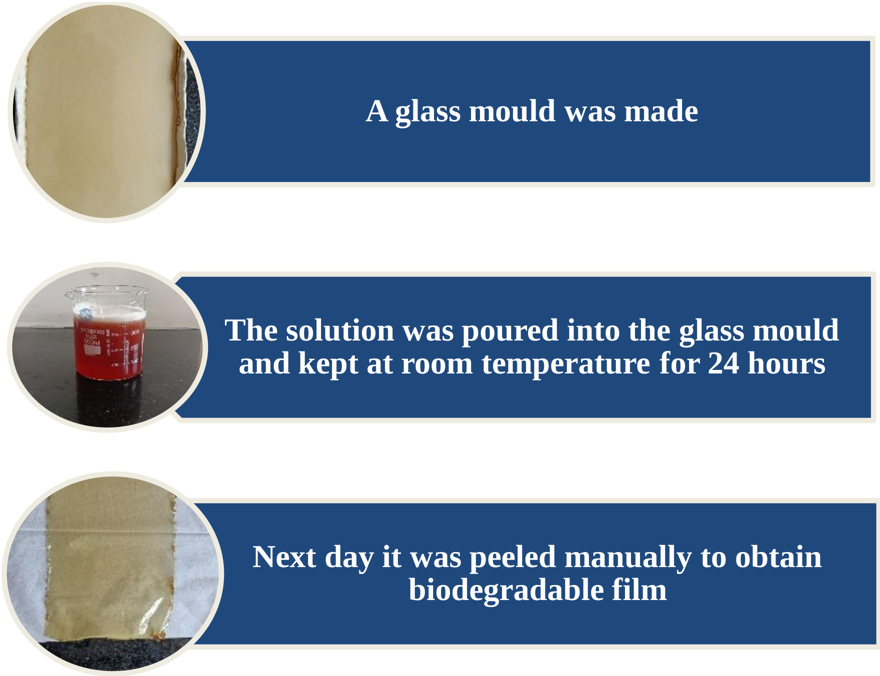


Fig. 9 Biodegradable Film Solution

3.3.7 CASTING OF BIODEGRADABLE FILM SOLUTION

The prepared biodegradable solution was cast on a piece of glass and kept at room temperature for 24 hours. Next day it was peeled manually to obtain the biodegradable film.



Flow Diagram. 7 Casting

Fig. 10 Biodegradable Film

3.4 Characterisation of Biodegradable Film

3.4.1 Density Measurement

The density of the synthesized biodegradable films was determined using the ASTM standard ASTM D792-91. A biodegradable film sample of size 2×2 cm was cut, and its mass was measured accurately using an analytical balance. The density (d) was then calculated using the following equation:

$$\text{Density (d)} = \frac{Mass}{Volume}$$

Where:

- Mass - Weight of the biodegradable film samples measured on an analytical balance.

- Volume - Dimensions of the biodegradable film .

3.4.2 Thickness Measurement

- **Procedure**:

  The thickness of the biodegradable films was measured at multiple points using a **digital micrometer** or **caliper** to ensure uniformity. The average thickness was calculated from the measurements taken at different locations on the film.

Gouveia, R. M., & Gama, M. (2015). Thermal degradation of starch-based biodegradable films. *Carbohydrate Polymers*, *122*, 231–237

3.4.3 Soil degradation test

Biodegradable film samples were cut into 2×2 cm^2 pieces and weighed accurately. Each sample was then placed in a soil-filled pot, embedded to a depth of 2 cm, and left exposed to the ambient conditions. Over a period of two weeks, the samples were examined daily to assess their degradation. At each interval, the samples were removed, weighed again, and the percentage mass reduction was calculated. The degradation rate was determined using the following formula:

$$\text{Mass reduction } (\%) = \frac{\textit{final mass} - \textit{inital mass}}{\textit{initial mass}} \times 100$$

3.4.4 Solubility Test

Solubility tests were performed to evaluate the behavior of the synthesized biodegradable films in various solvents. Samples were immersed in different solvents, including ammonia, acetic acid, acetone, methanol, sulfuric acid, and ethanol. After a specified immersion period, the solubility and physical changes—such as variations in color, shape, size, and weight gain—were carefully observed and recorded.

This method was adapted from the work of Sudha Joseph *et al.* on biodegradable plastics derived from mango seed starch, where the effects of citric acid and fillers were studied for sustainable food packaging applications

3.4.5 Organic Solvents Solubility Test

Organic solvents solubility of the biodegradable film sampless was determined using a modified version of the method proposed by Gontard *et al.* (1992). Circular disc samples of 2 cm diameter were first dried in an oven at 105 °C for 24 hours. The dried samples were then weighed to obtain the initial weight (Wi).

Each sample was then immersed in 50 mL of organic solvents (ethanol) and stirred at 120 rpm for 24 hours to promote moderate dispersion. After the immersion period, the samples were removed, oven-dried again at 105 °C for another 24 hours, and weighed to obtain the final weight (Wf).

The percentage of weight loss, which reflects the water solubility, was calculated using the following equation:

$$\text{Water Solubility (\%)} = \left(\frac{W_i - W_f}{W_i} \right) \times 100$$

3.4.6 Water Content

The water content of the biodegradable film samples was determined using a gravimetric method. Circular disc samples (2 cm in diameter) were initially weighed to record the wet weight (Ww). The samples were then dried in an oven at 105 °C for 24 hours to remove all moisture and subsequently weighed again to obtain the dry weight (Wd).

The water content was calculated based on the weight loss during drying and expressed as a percentage using the following formula:

$$\text{Water Content (\%)} = \left(\frac{W_w - W_d}{W_w} \right) \times 100$$

3.4.7 Swelling Test

The swelling behavior of the biodegradable film sampless was assessed following a gravimetric method, as described by Arrieta *et al.* (2013) with slight modifications. Circular disc samples (2 cm in diameter) were first oven-dried at 105 °C for 24 hours to remove all moisture, and their initial dry weight (Wd) was recorded.

The dried samples were then immersed in 50 mL of deionized water at room temperature for 24 hours. After immersion, the samples were removed, gently blotted to eliminate surface moisture, and weighed to determine the swollen weight (Ws).

The swelling index was calculated using the following equation:

$$\text{Swelling Index (SI)} = \frac{W_s - W_d}{W_d} \times 100$$

Where:

- **Ws** = Swollen weight of the sample after immersion (g)
- **Wd** = Dry weight of the sample before immersion (g)

This test provides insight into the hydrophilic nature and water absorption capacity of the biodegradable film samples, which are important parameters for packaging and environmental degradation performance.

3.4.8 Humidity Test

The humidity absorption capacity of the biodegradable film samples was evaluated to determine their sensitivity to atmospheric moisture. This test was conducted following the method described by Sanyang *et al.* (2016), with slight modifications.

Circular disc samples (2 cm in diameter) were first oven-dried at 105 °C for 24 hours to eliminate residual moisture and weighed to record the initial dry weight (Wd). The samples were then placed in a desiccator containing a saturated solution of sodium chloride (NaCl), which maintains a relative humidity (RH) of approximately 75% at room temperature for 7 days.

After the exposure period, the samples were removed and weighed to obtain the final weight (Wh). The percentage of moisture absorbed due to humidity was calculated using the following equation:

$$\text{Humidity Absorption (\%)} = \left(\frac{W_h - W_d}{W_d}\right) \times 100$$

This test provides insight into the biodegradable film's stability under humid storage or application conditions.

3.4.9. Migration test

The overall migration of the biodegradable film samples was assessed to determine their safety for food contact applications. The method was adapted from the European Union regulation (EU No. 10/2011) on plastic materials intended to come into contact with food.

Circular disc samples (2 cm in diameter) were weighed and then immersed in 50 mL of a food simulant—10% ethanol (for aqueous foods), 3% acetic acid (for acidic foods), and 95% ethanol (for fatty foods). The samples were stored at 40 °C for 10 days.

After the exposure period, the samples were removed, dried, and weighed again. The total migration was expressed as the percentage of weight loss due to the release of substances into the simulant. The formula used was

Where:

- Wi= Initial dry weight of the sample
- Wf = Final dry weight after migration test

This test helps assess the potential leaching of substances from the biodegradable film into food, an important parameter in evaluating material safety.

3.4.10 Transparency (Light Transmittance) Test

Objective:
The transparency test is used to evaluate how much light passes through the biodegradable film , which is important for applications like food packaging, where the visual appearance of the product is essential.

Method:

1. **Sample Preparation**:
   - Cut the biodegradable film into square or circular samples (typically around 2 cm × 2 cm or 2 cm in diameter).
2. **Observation Setup**:
   - Place the sample on a **white background** (e.g., a sheet of white paper or a lightbox) under a consistent light source.
3. **Visual Assessment**:
   - Observe how much light passes through the film.
   - Compare the film to a **clear control** sample (e.g., a clear plastic or glass sheet).
   - Evaluate the **degree of transparency** by noting the clarity of the film. Films that are more transparent will allow more light to pass through, whereas opaque films will block light.

3.4.11 APPLICATION OF BIODEGRADABLE FILM ON APPLE

Objective:
To assess the effectiveness of the biodegradable film made from *Dioscorea alata* (purple yam) and *Beta vulgaris* (beetroot) as a protective coating for apples, aimed at enhancing shelf life, preventing moisture loss, and reducing spoilage.

Materials Needed:

- Biodegradable film (prepared from purple yam, beetroot, chitosan, glycerol.

- Fresh apples (washed and dried)
- Scissors or a cutting tool
- Measuring scale (optional for weight consistency)
- Storage bags or containers for apple storage

Procedure:

To apply the biodegradable film to apples, the fruit was first washed and dried. Then, the film was cut into appropriate sizes to fully cover each apple, and the apples were wrapped with pre-formed sheets of the film, ensuring complete coverage. After applying the film, the apples were allowed to dry at room temperature for 24 hours. The coated apples were then stored in a cool, dry place or refrigerated to observe moisture retention and spoilage prevention over time. This process evaluated the biodegradable film 's effectiveness in extending the shelf life of apples by providing a biodegradable, moisture-retentive barrier

Fig. 11 Application of Film on Apple

4. RESULT AND DISCUSSION

4.1 Density measurement

Parameter	**Details**
Sample dimension	2x2x2 cm
Mass (weight)	1.9 g
Volume	8 cm^3
Density	0.24 g/cm^3

The density of the biodegradable produced from purple yam and beetroot was calculated to be 0.24 g/cm^3. The low density implies that the film is extremely light in weight and airy, which can be due to the natural starch content and fibrous nature of the raw materials. The finding implies a possibility for use in applications where biodegradable and low-density packaging materials are needed.

4.2 Thickness Measurement

Parameter	**Details**
Sample Thickness	0.2 mm

The **average thickness of 0.2 mm** for the biodegradable film is characteristic of materials intended for lightweight and flexible applications. The use of a **digital vernier caliper** ensured accurate measurements, and the consistency across different points suggests that the film was produced with uniformity.

With a thickness of **0.2 mm**, the film achieves an optimal balance between **mechanical strength** for handling and **flexibility** for diverse uses. This thickness is well-suited for **biodegradable packaging** and **disposable wraps**, where the material must be light enough to reduce weight while still being durable enough to fulfill its intended purpose.

4.3 Soil degradation test

Parameter	**Details**
Initial weight	0.40
Final observation	Fully degraded after 7 days
Degradation %	100%

The biodegradable film produced from purple yam and beetroot demonstrated **excellent biodegradability**, fully disintegrating within **7 days** under natural soil conditions. The high biodegradability of the film was established when it fully disintegrated within a day. This is an extremely desirable attribute for disposable and environmentally friendly packaging materials. Being very degradable also suggests that the material is highly reactive towards microbes and the environment.

4.4 Solubility test

Parameter	**Details**
Initial weight	0.30g
Final weight	0.09g
Dissolved amount	0.21g
solubility%	70%

This test revealed 70% water solubility, very high solubility. This may be because of water-soluble additives or polymers in the formulation. This is a desirable type of behavior for flushable, compostable, or rapid-dissolving product application. It precludes its application in humid or water contact-probable uses, however. Crosslinking agents or hydrophobic coatings may be added for more universal application to reduce solubility.

4.5 Organic Solvent Solubility Test

Parameter	**Details**
Initial weight	0.5g
Final weight	0.06g
Dissolved amount	0.27g
solubility%	88%

The 88% solubility indicates that the biodegradable film has a high tendency to dissolve or disperse in the organic solvent. This could suggest that the film is sensitive to solvent action, and the solubility behavior can be important for applications where the material might be exposed to solvents or similar chemicals.

4.6 Water Content

Parameter	Details
Initial weight	6.3
Final weight	5.4

The water content of **14.29%** suggests that a significant portion of the biodegradable film material is made up of moisture. This is common for biodegradable film s, as the raw materials, such as starches and polymers, tend to retain moisture following the fabrication process. The moisture content is a crucial factor as it affects the material's **mechanical properties**, including its **flexibility**, **tensile strength**, and **rate of degradation**.

4.7 Swelling test

Parameter	Details
Initial weight	0.80g
Final weight	1.04g
Weight gain	0.24g
Swelling %	30%

After 24 hours of soaking in water, the specimen absorbed 30% by weight, which is evidence of water absorption at high levels. The swelling renders the material hydrophilic in nature, which may consist of polar functional groups and be able to participate in hydrogen bonding with water molecules. Such a property is beneficial for applications in biomedical fields and controlled releases but not useful for wet storage or humid environments. Water absorption leading to size change can be detrimental to the structural integrity and uniformity of performance.

4.8 Humidity test

Parameter	Details
Initial weight	6.13g
Final weight	5.99g
Moisture loss	0.14g
Loss %	2.28%

Humidity test was conducted by heating the sample to 105°C for 80 minutes. The obtained 2.28% weight loss is the moisture content in the original material. Low moisture content signifies that the film is hygroscopic to a minor degree. While relatively low, the residual moisture will impact mechanical properties such as flexibility or brittleness and influence the shelf life. For use where water content is critical, additional drying or moisture-proof packaging will be required.

4.9 Migration test

Parameters	**Details**
Soaking liquids	Distilled water + acetic acid
Soaking time	43 minutes
Surface area tested	40.75cm² (total)
Observation	No degradation or leaching

Migration test was performed to mimic exposure to acidic and neutral liquid environments. Distilled water covered the samples for approximately 43 minutes and in solutions of acetic acid for approximately 43 minutes. There was no indication of coloration or material degradation. This demonstrates very high chemical resistance to migration. This is a very important property for food packaging materials, particularly where acidic ingredients are present. The lack of leaching is a sign of low risk for contamination, and this is a testament to the possibility of use under regulation in food-contact applications.

$$M = \frac{(WF - WI) \times 1000}{A \times t}$$

Where:

- **M** = Migration (mg/dm² or mg/kg)
- **WF** = Final weight of the simulant after the test (g or kg)
- **WI** = Initial weight of the simulant before the test (g or kg)
- **A** = Surface area of the plastic material exposed to the simulant (dm²)
- **t** = Test time (usually in hours or days)
- **1000** = Conversion factor to scale from grams to milligrams if necessary

4.10 Transparency (Light Transmittance) Test

Parameters	**Details**
Sample size	2x2 cm
Appearance	Yellowish-orange colour, rough texture

Transparency	Somewhat translucent, not entirely transparent.
Observation	Darker spots or imperfections visible within the sample

The bioplastic sheet looks relatively translucent, as light can pass through it to a certain extent. But it is not completely transparent because it has a rough surface and dark spots or blemishes.

4.11 APPLICATION OF BIODEGRADABLE FILM ON APPLE

The biodegradable film successfully helped retain moisture and prevent spoilage for up to one day. After 24 hours, the apples showed no signs of spoilage, indicating effective short-term protection. However, spoilage began to occur after this period, suggesting that the film’s moisture retention is limited. This may be due to its hydrophilic nature and permeability. To improve long-term preservation, further modifications, such as enhancing water resistance, will be necessary. These findings show the film's potential for short-term use, with further development needed for extended applications.

5. CONCLUSION

This study highlights the significant potential of sustainable biodegradable film derived from *Dioscorea alata* (purple yam) and *Beta vulgaris* (beetroot) as viable alternatives to conventional plastics. The resulting biodegradable film exhibited a low density of 0.24g per cm^3 and an average thickness of 0.2mm, confirming its lightweight and flexible nature. It demonstrated outstanding biodegradability, achieving 100% degradation within 7 days in natural soil conditions, and showed a high water solubility of 70% and organic solvent solubility of 88%, attributed to its hydrophilic components. The water content was measured at 14.29%, while the swelling behavior showed a 30% weight gain after 24 hours, reflecting strong water absorption capabilities. Moisture loss during the humidity test was relatively low at 2.28%, indicating minor hygroscopic behavior. The migration test revealed no leaching or degradation when exposed to distilled water and acetic acid, emphasizing the film's excellent chemical resistance and safety for food contact applications. Additionally, the film was somewhat translucent, with a yellowish-orange colour and minor surface imperfections. Application on apple slices showed that the film effectively retained moisture and prevented spoilage up to 24 hours. With its combination of biodegradability, solubility, flexibility, and environmental compatibility, this biodegradable film offers a promising, eco-friendly solution for short-term packaging. The development of such biodegradable materials plays a crucial role in reducing plastic pollution and promoting sustainable packaging solutions.

Further studies: Recommended to enhance the mechanical strength and improve water resistance through incorporating hydrophobic materials. Exploring the integration of bioactive compounds could also open new opportunities, particularly in food and health care centers.

6. REFERENCES

1. Alkadhim, S. A. S. (2018). Hot air oven for sterilization: Definition & working principle. Available at SSRN 3340325.

2. Anyanwu, C. F., & Ildefonso, R. L. (2015). Performance and adaptability of two yam (Dioscorea spp) varieties under Ifugao condition International Journal of Advanced Research, 3(7), 110–116.

3. Bourtoom, T., & Chinnan, M. S. (2008). Preparation and properties of edible starch-based films: A review. Food Research International, 41(9), 1229–1247.

4. da Costa, J. C. M., Miki, K. S. L., da Silva Ramos, A., & Teixeira-Costa, B. E. (2020). Development of biodegradable films based on purple yam starch/chitosan for food application. Heliyon, 6(4).

5. Gontard, N., Guilbert, S., & CUQ, J. L. (1992). Edible wheat gluten films: influence of the main process variables on film properties using response surface methodology. Journal of food science, 57(1), 190-195.

6. Joseph, S., Hegde, A. R., Gopalakrishnan, V., Yallappa, S., Nadzri, N. I. M., Joseph, K., & Meenakshi, K. (2024). Biodegradable Plastics from Mango Seed Starch for Sustainable Food Packaging—Effect of Citric Acid and Fillers. ChemistrySelect, 9(22), e202401312.

7. Margasahayam, A., & Balraj, Y. (2018). Properties of food ingredients during processing in a domestic mixer grinder and subsequent storage: A review. Journal of Food Process Engineering, 41(4), e12677.

8. Moriya C, Hosoya T, Agawa S, Sugiyama Y, Kozone I, Shin-Ya K, Terahara N, Kumazawa S. New acylated anthocyanins from purple yam and their antioxidant activity. Biosci Biotechnol Biochem. 2015

9. Moshood, T. D., Nawanir, G., Mahmud, F., Mohamad, F., Ahmad, M. H., & AbdulGhani, A. (2022). Sustainability of biodegradable plastics: New problem or solution to solve the global plastic pollution?. Current Research in Green and Sustainable Chemistry, 5, 100273.

10. Moshood, T. D., Nawanir, G., Mahmud, F., Mohamad, F., Ahmad, M. H., & AbdulGhani, A. (2022). Biodegradable plastic applications towards sustainability: A recent innovation in the green product. Cleaner Engineering and Technology, 6, 100404.

11. Mroczkowska, M., Culliton, D., Germaine, K., & Neves, A. (2021). Comparison of Mechanical and Physicochemical Characteristics of Potato Starch and Gelatine Blend Biodegradable films Made with Gelatines from Different Sources. Clean Technologies, 3(2), 424-436.

12. Pandita, D., Pandita, A., Pamuru, R. R., & Nayik, G. A. (2020). Beetroot. Antioxidants in vegetables and nuts-properties and health benefits, 45-74.

13. Patel KS, Karmakar N, Desai KD, Narwade AV, Chakravarty G, Debnath MK. Exploring of greater yam (Dioscorea alata L.) genotypes through biochemical screening for better cultivation in south Gujarat zone of India. Physiol Mol Biol Plants. 2019.

14. Punia Bangar, S., Singh, A., Chaudhary, V., Sharma, N., & Lorenzo, J. M. (2023). Beetroot as a novel ingredient for its versatile food applications. Critical Reviews in Food Science and Nutrition, 63(26), 8403-8427.

15. Shaikh, S., Yaqoob, M., & Aggarwal, P. (2021). An overview of biodegradable packaging in food industry. Current research in food science, 4, 503-520.

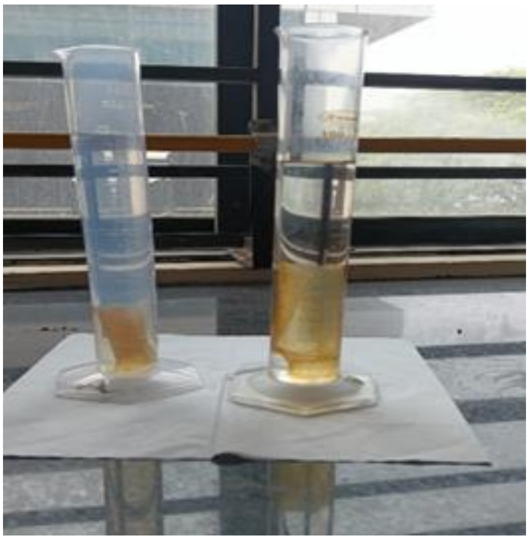
16. Sugata M, Lin CY, Shih YC. Anti-Inflammatory and Anticancer Activities of Taiwanese Purple-Fleshed Sweet Potatoes (Ipomoea batatas L. Lam) Extracts. Biomed Res Int. 2015

17. Tamaroh S, Sudrajat A. Antioxidative Characteristics and Sensory Acceptability of Bread Substituted with Purple Yam (Dioscorea alata L.). Int J Food Sci. 2021 Jul 14;2021:5586316.

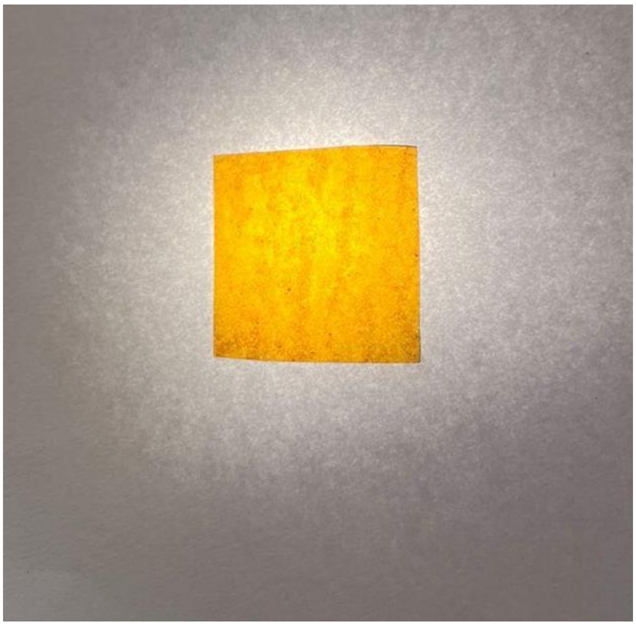
18. Wruss, J., Waldenberger, G., Huemer, S., Uygun, P., Lanzerstorfer, P., Müller, U,& Weghuber, J. (2015). Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Upper Austria. Journal of Food Composition and Analysis, 42, 46-55

7. APPENDIX

Migration Test



Transparency Test



Final Product

APPLICATION OF BIODEGRABLE FILM ON APPLE (After 24 Hours)