

Original Research Article

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Sustainable Utilisation of Agro Waste: Fruit Peel Waste as a Novel Media Formulation for Growth of Economically Important Fungi

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ABSTRACT

Commercial fungal culture media, such as potato dextrose agar and Sabouraud's dextrose agar, are expensive, whereas fruit peels are discarded in large quantities as environmentally harmful waste. This study aimed to evaluate fruit peel waste as a low-cost alternative medium for growing economically important fungi. Peels of pineapple, watermelon, lemon, sweet lime, yellow banana, red banana, pomegranate and papaya were collected from local vendors and juice sellers in Chennai. They were shade-dried, powdered and prepared as broths. *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Mucor*, *Penicillium*, and *Rhizopus stolonifer* were isolated from air and soil using open plate and serial dilution techniques and identified by colony morphology and lactophenol cotton blue staining. *Saccharomyces cerevisiae* was isolated from commercial dry yeast, and *Candida albicans* was a clinical isolate. Standardized inocula were added to the broths and incubated for 5–7 days. Growth was assessed visually against a Sabouraud's broth positive control and an uninoculated negative control. Most molds grew in nearly all peel media. Pineapple peel broth supported the greatest number of molds, giving the best growth of *A. niger*, *Penicillium*, *Rhizopus* and *Mucor*. Lemon and sweet lime peels also supported good growth, and sweet lime was best for *A. fumigatus*, *S. cerevisiae* and *C. albicans*. Papaya peel did not support *A. niger*, and yeast growth was generally limited across media. Fruit peel waste can serve as an inexpensive, readily available, and eco-friendly substrate for fungal cultivation. However, as the assessment was qualitative, further formulation and quantitative validation are required to formulate a fruit peel waste medium for the cultivation of economically important fungi.

Keywords

Fruit peel waste, alternative culture media, fungal growth, inexpensive, low-cost media Actinomycetes.

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Introduction

Globally, India is among the leading producers and exporters of fruits, ranking second after China in fruit production (APEDA, 2024; Keerthika *et al.*, 2024). After consumption, the peels of fruits are a nuisance to the environment as a type of solid waste. Most of the fruit peels are either dumped or incinerated after the main part of fruits are used (Kumar *et al.*, 2020). There is an urgency to mitigate this sort of action, because at the foremost, these fruits and vegetable peels are more useful for either agricultural purposes or for other different research activities in the coming future (Kumar *et al.*, 2020). The unused fruit peels can be used in different microbiological media preparation labs and also in the pharmaceutical industries to some extent (Kumar *et al.*, 2020). Therefore, the random disposal of those fruit peels without knowing the eclectic scope of its usage should be minimized.

It is anticipated that discarded fruit and waste material can be utilized for further industrial processes, such as fermentation and bioactive component extraction (Kumar *et al.*, 2020). Numerous studies have been conducted on the utilization of waste obtained from fruits and vegetables, dairy, and meat industries. In this regard, several efforts have been made to utilize pineapple waste obtained from different sources. Waste from pineapple canneries has been used as a substrate for bromelain, organic acids, and ethanol, as it is a potential source of sugars, vitamins, and growth factors (Ketnawa, Chaiwut and Rawdkuen, 2011; Fissore *et al.*, 2023). Several studies have been conducted over the past decades to explore the possibility of using these wastes.

Standard fungal cultivation typically relies on commercial formulations, such as potato dextrose agar (PDA), Sabouraud's dextrose agar (SDA), Rose Bengal agar (RBA), and corn meal agar (CMA), all of which carry a substantial cost burden for routine laboratory use (Anbu *et al.*, 2017). Basically, every fungus requires carbon, nitrogen, and an energy source to grow and survive. The utilization of agricultural waste as a substrate for fungal cultures for the production of value-added products has been reported in previous studies (Kumar *et al.*, 2020). Given their nutrient composition, fruit peel waste is a plausible candidate for formulating a low-cost alternative to these commercial media (Anbu *et al.*, 2017; Nirmala, Lochana and Sevvel, 2024). An added advantage is that such media are unlikely to support the full range of microorganisms that commercial

media do, which may help limit unwanted contamination in culture work. While fruit peel waste has already been valorized for a range of high-value products, its suitability as a fungal growth medium remains largely unexamined, prior to more recent studies (Anbu *et al.*, 2017). Building on this gap in the literature, the present study set out to formulate an economical culture medium for some economically important fungal cultures, that is, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Rhizopus stolonifer*, *Mucor*, *Penicillium* and *Candida albicans* and *Saccharomyces cerevisiae* using fruit peels waste as raw material.

Recent research has focused on finding alternatives to gelling agents of media, agar in particular and media in general, because of its exorbitant price (Sanchez-Cardozo *et al.*, 2019). In this study, the scope of the usage of fruit peels as a novel medium for the growth of some fungi was tested on a laboratory scale over a short time. Thus, this study does not guarantee its direct usage in any microbiology or pharmaceutical industries. The main hindrance behind the direct use of fruit peels as the media for the growth of fungi in any microbiology or pharmaceutical laboratory is the presence of phytochemicals such as tannins, alkaloids, flavonoids, steroids, essential oils, and minerals present in fruit peels (Oladele, Joy and Okosodo, 2019). These oils and phytochemicals may act as an inhibiting agent and may cause hindrance in the metabolism and growth process. Thus, scientific and systematic synthesis of fungal growth media on a large scale using fruit peels while maintaining all necessary prerequisites is a matter of urgency in the current scenario.

Materials and Methods

Materials Used

Fruit peels of all fruits were obtained from local fruit vendors and juice sellers, which were discarded as waste.

Sample

Fungal samples: *Penicillium*, *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, and *Mucor* were isolated from soil and air using serial dilution and open plate techniques and identified by lactophenol cotton blue staining. Meanwhile, *Saccharomyces cerevisiae* was isolated from commercial samples, *Candida albicans* was a clinical isolate, and was identified using Gram staining.

Raw materials: Common fruits peels such as red banana, yellow banana, pineapple, pomegranate, papaya, and watermelon were collected from local fruit vendors, and sweet lime and lemon fruit peels were collected from juice sellers.

Site of the study: The intended work on the usage of its peels as novel media for fungal growth was carried out in the microbiology lab of JBAS College for Women, Chennai. The laboratory was equipped with all the necessary materials and equipment required for this study.

Research method: The method for this study was qualitative analysis of growth of fungi in fruit peel media. This study was based on the culture method.

Fruit collection and peel waste preparation

The first step of the experiment involved the preparation of a dry powder from fruit peels. For this, the fruit peels were thoroughly washed. After washing, the peeled part of the fruit, that is, the outermost layer of the fruit, was air-dried at room temperature for 10 days to remove the moisture. Fruit peels might get discolored and even lose their important minerals and vitamins during direct sun drying; therefore, the peels were best dried in the shade. The dried peels were then powdered using a mechanical grinder and sieved to obtain a powdery form. The powder was stored in a polythene bag at room temperature.

Isolation and identification of fungi

One of the most important aspects of this experiment was to isolate and identify the fungi required for further experimentation on fruit peel media. First, fungi (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Mucor*, *Penicillium*, and *Rhizopus stolonifer*) were isolated from air and soil using the open plate and serial dilution techniques. In contrast, *Saccharomyces cerevisiae* was obtained from commercially available dry yeast balls, and *Candida albicans* was a clinical isolate.

The potato dextrose agar (PDA) plates were prepared in the lab and were plated aseptically. Some plates were then opened near the toilet site in the college premises. All the plates were left open for approximately 15 minutes. Lastly, the plates were incubated at room temperature for 7-10 days. To acquire a proper and well-grown colony, fungi were isolated from soil samples by serial dilution. Different soil samples from different

places on the college premises were collected. One gram of the soil sample was then serially diluted to 10^{-5} 0.1 mL of the sample was pipetted and spread on PDA media plates. The plates were incubated for 5-7 days at 28°C. On the other hand, *Saccharomyces cerevisiae* was directly isolated from yeast ball samples. The dried yeast was bought from the market and was used as the substrate for isolating yeast through it. The dried yeast sample was crushed into a fine powder, and 1 gram of the powdered sample was serially diluted to 10^{-5} . Successive dilutions were made, and 0.1 ml of samples was pipetted from different diluted tubes and spread on SDA agar plates. Finally, the plates were incubated at 28°C for 7-10 days.

Another pivotal and crucial stage of this experiment was the identification of the desired fungi. Fungi viz. All the fungal molds were identified by colony morphological characteristics and microscopic examination using Lactophenol Cotton Blue (LPCB) staining technique. The selected fungi were placed onto the stain with the help of sterile forceps, needles, and teased properly. After that, the selected fungi was placed onto the stain with the help of sterile forceps, needles and teased properly. The fungal isolates were covered by cover-slip and observed under a 40x objective lens. Meanwhile, *Saccharomyces cerevisiae* was observed under microscope by Gram's staining. *Candida albicans* was identified using Gram staining. *Candida albicans* was identified by Gram's staining technique. A wet mount of the fungal sample was made and observed directly under the microscope. Based on the mycelial feature, fruiting body and structure the selected fungal colonies were identified.

Maintenance of fungal isolates

After identifying the fungi, the fungal colonies were subcultured on freshly prepared PDA and SDA agar plates. For this, fungal isolates which were selected to observe under microscope were selected as the base culture for sub-culturing it as a new colony. Fungal isolates were pricked with the help of an inoculating loop, and the loop was streaked gently over the fresh media plates aseptically. Lastly, the media plates with the isolates were incubated at room temperature for 5 days.

Inoculum preparation

The suspension of 4 days old fungal molds was used to study the qualitative and quantitative growth analysis.

They were prepared in saline solution (0.85 % sodium chloride). The fungal cultures were inoculated into 50 ml of saline and incubated at room temperature for 5 hours. Saline solution with lower concentration helps fungi in their metabolism and growth process. Similarly, saline solution helps maintain and balance the osmotic pressure of the growth medium.

Preparation of fruit peel waste media

Approximately 4.0 grams of dried fruit peel powder was added to 100 ml of distilled water and sterilized by autoclaving at 121°lbs/pressure for 15 minutes. After sterilization, the fruit peel waste broths were cooled, and 1 ml of fungal inoculums was transferred to them.

Qualitative analysis of fungal growth

The inoculum-added broths were incubated at room temperature for 5–7 days. The presence or absence of fungal growth in the fruit peel media was visually observed every day, and the results were recorded daily. Yeast colonies were incubated at 37 degree celsius for 2 days and then identified.

Results and Discussion

The effect of eight different fruit peel media viz., pineapple, papaya, yellow banana, sweet lime, lemon, green banana and pomegranate on the qualitative growth of *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Mucor*, *Rhizopus stolonifer*, *Candida albicans* and *Saccharomyces cerevisiae* were studied and the results were given in Table 1. It was observed that the *Aspergillus niger* growth was recorded in the medium which contains pineapple, pomegranate, yellow banana and green banana. *Aspergillus niger* growth was not recorded in the medium containing papaya. *Rhizopus stolonifer* growth was noticed in the medium that contains pineapple, green banana, yellow banana and pomegranate. *Rhizopus stolonifer* growth was not recorded in the medium containing papaya and orange. However, the *Saccharomyces cerevisiae* growth was recorded in the medium which contains papaya, pineapple, orange and green banana. *Saccharomyces cerevisiae* growth was not recorded in the medium containing yellow banana and pomegranate. Positive control was Sabouraud's dextrose broth with inoculated fungi for each of the batch and negative control was uninoculated sterilized fruit peel medium for each batch.

Aspergillus fumigatus grew well in sweet lime peel broth followed by lemon. *Penicillium* grew best in Pineapple fruit peel broth, *Mucor* grew best in Pineapple. *Candida albicans* had grown best in sweet lime. Other fruit peels did not support the growth of *Candida albicans*

Tropical and subtropical fruits processing have considerably higher ratios of by-products than the temperate fruits. Fruit by-products are not exceptions and they consist of the residual pulp, peels, stem and leaves. The increasing production of fruit processed items, results in massive waste generations. This is mainly due to selection and elimination of components unsuitable for human consumption. Besides, rough handling of fruits and exposure to adverse environmental conditions during transportation and storage can cause up to 55 % of product waste. These wastes are usually prone to microbial spoilage thus limiting further exploitation. Further, drying, storage and shipment of these wastes is cost effective and hence efficient, inexpensive and eco-friendly utilization is becoming more and more necessary. (Nunes *et al.*, 2009)

The commercially available media used for the cultivation of fungi are generally very costly. Routine practical experiments require a large amount of media on a regular basis for streak plate, pour plate and spread plate experiments. Availability of low cost media providing rich in nutrients is much warranted. The search for alternative, cheap media for use in laboratory agents for routine microbiological experiments is going on. Recent research has been focused on finding alternatives to gelling agents of media, agar in particular, and media, in general, because of its exorbitant price (Ravimannan *et al.*, 2014)

Waste utilization in fruits and vegetable processing industries is one of the important and challenging jobs around the world. It is anticipated that the discarded fruits as well as its waste materials could be utilized for further industrial purposes viz. fermentation, extraction of bioactive components, extraction of functional ingredients etc. Fruit waste contains many reusable substances of high value and novel scientific technological methods are applied, valuable products from fruit wastes could be obtained. In this regard, cheap substrates, such as fruit peel wastes have promising prospects. Thus, environmentally polluting by-products could be converted into products with a higher economic value than the main product.

Fruit peel wastes may meet these requirements and work as a fungal growth medium and can replace expensive media in the market. This will add a benefit of minimal contamination in the cultures as it does not meet the needs of every microbe. Fruit peel waste has been exploited for the production of many high value products but its potential as a fungal growth medium has never been reported. The aim of the current study was to design a cost effective and efficient medium for fungal cultures, that is, *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Mucor* and *Saccharomyces cerevisiae* and *Candida albicans* using fruit peel wastes as raw material.

Fruit peels were obtained from fruit vendors and juice sellers. Then, they were subsequently washed and peeled, and the peels were dried under shade. The shade dried material was then powdered using a blender/mixer into a fine powder. According to (Gyawali, 2013) immediate drying prevents microbial fermentation and degradation of metabolites. In addition, protection from direct sunlight is essential to minimize chemical reactions induced by ultraviolet rays. The sample was ground to a powdery form using a mechanical blender. Fungal samples were isolated from air, soil, and active dry yeast balls. *Aspergillus niger* and *Rhizopus stolonifer* were isolated from air using the open plate technique, and *Aspergillus flavus*, *Aspergillus fumigatus*, and *Mucor* were isolated from soil using the serial dilution technique on PDA media plates. Similarly, *Saccharomyces cerevisiae* was isolated from commercially available samples using Saboraud's dextrose agar media. *Candida albicans* was obtained as a clinical isolate. Subculture of fungal colonies was performed after identifying the suspected fungal colony by lactophenol cotton blue staining. A loopful of subcultured fungal colonies was incubated in 0.85% saline solution at room temperature. Finally, the saline solution was poured into a flask containing autoclaved and sterile fruit peel solution (peel powder in distilled water). Qualitative growth analysis of respective fungi in different fruit peel media were made. (P Saranraj and S Anbu. 2017).

Growth was observed in almost all the fruit peel media for most of the fungal molds. However, the growth of yeast was less in most of the fruit peel media, suggesting the need for better definition of the media formulation.

Agricultural waste materials support the good growth of fungi. Microbiological studies depend on the ability to grow and maintain microorganisms under laboratory

conditions by providing suitable culture media that offer favorable conditions (Domsch and Anderson 1980). The nutrients in the wastes included protein, carbohydrate and minerals. Protein constitutes a significant portion of microbial cells and thus is necessary for the growth of microorganisms (Prescott and Harley 2002). The protein content of the formulated media must have ensured a good supply of nitrogen while the carbohydrate content served as an additional carbon source both of which are essential for good fungal growth. The mineral content of the wastes in the formulated media was probably useful for some aspects of the fungi's metabolism. Although, all organisms for their life processes require moisture (water) and fungi in particular require water for extracellular digestion of nutrients (Pelczar *et al.*, 1993), the moisture content of each of the samples has negligible or no effect on the growth of fungi tested because they were grown in the media containing water. Most fruit peels are discarded as waste after the inner fleshy portions have been eaten. It is vital that peels be removed from most fruits before eating; and more importantly before using them in fruit juice industries to prevent contamination. Processing of fruits into juices reduces and prevents wastage when fruits are in season. (Oladiji *et al.*, 2010).

Fruit waste contains many reusable substances of high value. The wastes from canneries have high exploitation potential with encouraging future. Furthermore, dietary fibers and phenolic antioxidants could be used as an impending nutraceutical resource, capable of offering significant low-cost nutritional dietary supplements for low-income communities.

The booming market of functional food has created a mammoth vista for utilization of natural resources. If novel scientific and technological methods are applied, valuable products from fruit wastes could be obtained. In this regard, cheap substrates, such as pineapple wastes have promising prospects. Thus, environmentally polluting by-products could be converted into products with a higher economic value than the main product. (P Saranraj and S Anbu. 2017).

In conclusion, this present study has revealed that the fruit peel waste materials contain minerals and nutrients that can meet the nutritional requirements of industrially important fungi. Thus, they can be utilized as alternative materials in the formulation of culture media for the in vitro cultivation of fungi for industrial and research purposes.

Table.1 Qualitative growth analysis of fungi in fruit peel waste media

	<i>A. niger</i>	<i>A. flavus</i>	<i>A. fumigatus</i>	<i>Penicillin</i>	<i>Rhizopus</i>	<i>Mucor</i>	<i>Candida albicans</i>	<i>Saccharomyces cerevisiae</i>
Papaya	-	+	+	+	+	-	-	-
Pineapple	++	+	-	++	++	+	-	-
Yellow banana	+	-	-	-	+	-	-	-
Sweet lime	+	+	++	+	+	+	+	+
Watermelon	+	+	-	-	+	-	-	-
Lemon	++	++	++	++	++	+	-	+
Red banana	+	-	+	-	+	-	-	-
Pomegranate	+	-	+	-	+	-	-	-

++- indicates good positive growth +- indicates moderate positive growth – indicates no growth

Table.2 Showing fruit peels that supported good growth for specific fungi/yeast

<i>A.niger</i>	<i>A.flavus</i>	<i>A.fumigatus</i>	<i>Rhizopus</i>	<i>Penicillin</i>	<i>Mucor</i>	<i>Saccharomyces</i>	<i>Candida</i>
Pineapple lemon, sweet lime yellow banana watermelon red banana pomegranate	Lemon, pineapple papaya sweet lime watermelon	Sweet Lime lemon pomegranate papaya Red banana	Pineapple lemon, papaya yellow banana sweet lime watermelon Red banana pomegranate	Pineapple lemon papaya sweet lime	Pineapple sweet lime lemon	Sweet Lime lemon	Sweet Lime

Growth of Fungi on Different Fruit Peel Broth



Aspergillus niger growing on
Pineapple fruit peel broth



Aspergillus flavus Growing On Lemon Fruit Peel
Broth



***Aspergillus Fumigatus* Growing On Sweet Lime Fruit Peel Broth**



***Rhizopus* Growing on Pineapple Fruit Peel Broth**

An important advantage of the fruit peels used in formulating the various media is that it is readily available in large quantities in India. Readily available fruits like pineapple, pomegranate, water melon, lemon, sweet lime, green banana, yellow banana and papaya can be taken as the base for formulating cost effective and useful fungal media.

The effect of eight different fruit peel media viz., pineapple, papaya, yellow banana, sweet lime, lemon, green banana and pomegranate on the qualitative growth of *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Mucor*, *Rhizopus stolonifer*, *Candida albicans* and *Saccharomyces cerevisiae* were studied.

It was observed that the *Aspergillus niger* growth was recorded in the medium which contains pineapple, pomegranate, yellow banana and green banana. *Aspergillus niger* growth was not recorded in the medium containing papaya. *Rhizopus stolonifer* growth was noticed in the medium that contains pineapple, green banana, yellow banana and pomegranate.

Rhizopus stolonifer growth was not recorded in the medium containing papaya and orange. However, the *Saccharomyces cerevisiae* growth was recorded in the medium which contains papaya, pineapple, orange and green banana.

Saccharomyces cerevisiae growth was not recorded in the medium containing yellow banana and pomegranate.

Aspergillus fumigatus grew well in sweet lime peel broth followed by lemon. *Penicillium* grew best in Pineapple fruit peel broth, *Mucor* grew best in Pineapple.

Candida albicans had grown best in sweet lime. Other fruit peels did not support the growth of *Candida albicans*. Positive control was Sabouraud's dextrose broth with inoculated fungi for each of the batch and negative control was uninoculated sterilized fruit peel medium for each batch.

Of all the fruit peels used, pineapple fruit peel broth was found to support the growth of most of the fungal molds, followed by sweet lime, lemon, pomegranate, red banana, yellow banana and water melon fruit peel broth supported the least growth of the fungal molds.

Sweet lime and lemon fruit peel broth supported the qualitative radial growth of both the yeasts, *Saccharomyces* and *Candida albicans* whereas none of the other fruit peels supported the growth of yeasts.

This study has shown that fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Mucor*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae* and *Candida albicans* can be grown in fruit peels also.

Therefore in conclusion, this study helps to mitigate the cost burden for producing fungal growth media. In solving the problem of the shortage of culture media for laboratory practicals, the result of this research will go a

long way in ameliorating this problem. Further research is still needed in the application of modern tools and methods in the study of fungal physiology as this will assist in manipulation of waste materials into usable forms.

Author Contributions

Anu Swedha Ananthan: Conceived the original idea and designed the model and wrote the manuscript. N. Dency Shirley: Methodology, validation, formal analysis, investigation. D. Dharani Shree: Methodology, validation, formal analysis, investigation. A. Farhana Ansari: Methodology, validation, formal analysis, investigation. M. Gayathri: Methodology, validation, formal analysis, investigation

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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