Biotechnological Valorization of Cashew Apple Waste for the Production of Oyster Mushroom (*Pleurotus ostreatus*)

A Dissertation for

GBT 651- Dissertation

Credits: 16

Submitted in partial fulfilment of Master's Degree

M.Sc. in Biotechnology

by

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Date: 8th April 2024

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "Biotechnological Valorization of Cashew Apple Waste for the Production of Oyster Mushroom (*Pleurotus ostreatus*) is based on the results of investigations carried out by me in the School of Biological Sciences and Biotechnology at Goa University under the Supervision of Dr. Meghanath Prabhu and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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PREFACE

In today's world, the pursuit of sustainable practices and innovative solutions is more crucial than ever. With environmental concerns mounting and resources dwindling, finding ways to repurpose waste into valuable products has become imperative. This project aims to address this challenge by proposing an innovative approach to utilize cashew apple waste as a substrate for mushroom cultivation. Cashew production is a significant industry in many regions, particularly in tropical areas. However, along with the desirable cashew nut, a substantial amount of cashew apple waste is generated during processing. Traditionally, this waste is disposed of, leading to environmental degradation and economic loss.

Recognizing the untapped potential of cashew apple waste, this project proposes a novel method to valorize this byproduct. By harnessing its organic matter and nutrients, cashew apple waste can serve as an ideal substrate for cultivating mushrooms. By repurposing cashew apple waste into a nutritious food source, this project not only contributes to reducing environmental pollution but also creates new opportunities for economic development and sustainable agriculture. Through collaboration and innovation, we endeavor to turn what was once considered waste into a valuable asset for both the environment and society.

ACKNOWLEDGEMENT

Firstly, I'm deeply grateful to Dr. Meghanath Prabhu, my guide, for his unwavering support, and invaluable guidance. His encouragement and wisdom not only shaped this dissertation but also enriched my academic journey in profound ways. Thank you for being an exceptional mentor.

I'd like to extend my sincere gratitude to Prof. Bernard F. Rodrigues, the Dean of the School of Biological Sciences and Biotechnology, Goa University and to Prof. Savita Kerkar, Former Dean, School of Biological Sciences and Biotechnology, for providing the necessary facilities for carrying out this research work.

I express my heartfelt gratitude to, Dr. Milind Naik, the Program Director of Microbiology for his assistance in my dissertation.

I'm thankful to the Biotechnology faculty, comprising Sr. Prof. Sanjeev Ghadi, Dr. Sanika Samant, Ms. Dviti Mapari, Dr. Samantha Fernandes D'mello, Ms. Snesha Bhomkar and Ms. Snigdha Mayenkar, for sharing their wisdom and expertise throughout my Masters degree. I am indebted to fellow research scholars, particularly Ms. Diksha and Ms. Hetika for their invaluable contributions to the study.

Special thanks to our non-teaching staff- Mr. Samir, Mr. Ashish, Ms. Jaya, Ms. Sandya , Mr. Serrao ,Mr. Popkar, Mrs.. Sanjana and Mr. Surendra from microbiology discipline for their invaluable assistance in meeting project requirements. I also thank Dr. Nitin S. Sawant, the

Program Director of Zoology, for granting access to the muffle furnace in their esteemed laboratory, and lab assistant Mr. Madukar for his assistance with it.

My deepest appreciation goes to my family for their unwavering support, encouragement, and love.

Lastly, I extend my gratitude to my friends Alister, Ambika, Ankit, Apurva, Carissa, Hari, Jeeya, Joleta, Kaushiki, Malvika, Manasi, Mariya, Melisha, Nabila, Nikhil, Poojan, Prajyoti, Rahul, Samradni, Sainil, Siddharth, Sushant, Swati, Tanmay, Tanvi, Tejas, Tushar, Vaishnavi, Varisha, Vedha, Vishwam for their continuous support and motivation.

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Abbreviations

Abbreviation	Full form
CAW	Cashew apple waste
TS	Total solid
VS	Volatile Solid
PDA	Potato dextrose agar
ml	Millilitre
L	Litre
g	Grams

CHAPTER 1.1: INTRODUCTION

Cashew apple (*Anacardium occidentale*) is a valuable tropical crop primarily cultivated for its nut, which holds a significant position in the global market. However, the cashew apple, a byproduct of cashew nut processing, is often overlooked and underutilized, despite its rich nutritional profile and potential economic value. This paper explores the concept of valorization as a means to enhance the market horizon of cashew apple products, thereby improving the livelihoods of cashew farmers and processors. (Runjala and Kella, 2017). Initially introduced to India by Portuguese sailors in the sixteenth century, the cashew tree has since become widespread in various tropical regions, including West Africa, Vietnam, Brazil, and Indonesia. While the cashew nut garners attention for its economic importance, the cashew apple, after the juice is extracted, often goes to waste, contributing to environmental concerns and missed economic opportunities. Valorization, in the context of cashew apple waste, involves preserving or enhancing its value through innovative processing techniques and product development. By recognizing the nutritional richness of cashew apple, which includes high levels of vitamin C, carotenoids, flavonoids, anacardic acid, tannins, vitamins, minerals, and dietary fibers, valorization aims to transform this overlooked byproduct into a valuable resource.(Das and Arora 2017).Moreover, valorization holds promise for sustainable agricultural practices, as cashew trees are known for their ability to thrive in diverse soil conditions, including poor and drought-prone areas. By maximizing the utilization of cashew apple waste, valorization contributes to reducing food waste and improving overall agricultural productivity.

Feni production involves using cashew apples. The process begins by crushing the cashew apples to extract the juice. This juice is then fermented and distilled to produce Feni, a popular spirit in Goa, India. However, during this process, a significant amount of cashew apple waste

is generated, including the fibrous residue after juice extraction and the discarded fruit pulp after fermentation.

This waste can pose environmental challenges if not managed properly, but it also has potential for various applications such as composting, animal feed, or even biofuel production. My aim to valorized cashew apple waste by using it as a substrate to grow mushroom.

CHAPTER 1.2: BACKGROUND

The background for the valorization of cashew apple waste for cultivating mushrooms as a substrate lies in the intersection of agricultural waste management, sustainable practices, and economic opportunities. Cashew apple waste, generated in large quantities during cashew nut processing, has historically been considered a nuisance and often discarded, contributing to environmental pollution in cashew-producing regions. However, with increasing awareness of the need for waste reduction and resource optimization, researchers and entrepreneurs have recognized the valuable potential of cashew apple waste as a substrate for mushroom cultivation. Cashew apple waste is rich in carbohydrates, nitrogen, and other nutrients, making it an ideal medium for mycelium colonization and mushroom growth. By utilizing cashew apple waste as a substrate, stakeholders aim to address several pressing issues simultaneously: reducing environmental pollution by diverting waste from landfills, creating economic opportunities for farmers and entrepreneurs through the production and sale of mushrooms, and promoting sustainable agricultural practices by turning waste into a valuable resource. This background underscores the importance of innovation and collaboration in leveraging agricultural waste streams for both environmental and economic benefits.

1.3: Aims And Objectives

Aim:

Valorization of cashew apple waste for mushroom cultivation.

Objectives:

- Characterization of cashew apple waste
- Cultivation of mushroom using cashew apple waste as a substrate

1.4: Hypothesis

The hypothesis of this research is Cashew apple waste is potential and efficient substrate for cultivation of Mushroom. Utilizing cashew apple waste as a substrate for mushroom cultivation will result in comparable or superior mushroom yields and quality compared to traditional substrates, due to the rich nutrient content and favorable physical properties of cashew apple waste. This hypothesis is based on the premise that cashew apple waste provides an ample source of carbohydrates, cellulose and other essential nutrients necessary for mycelium and mushroom growth. Additionally, the fibrous nature of cashew apple waste may create an optimal environment for mycelial proliferation and fruiting body formation. By harnessing the inherent qualities of cashew apple waste, we anticipate that mushroom cultivation on this substrate will not only be environmentally sustainable but also economically viable, providing a promising solution for both waste management and agricultural diversification in cashew-producing regions.

1.5: Scope

The valorization of cashew apple waste for mushroom cultivation presents a promising avenue for sustainable agriculture and waste management. Cashew apple waste, often discarded after cashew nut harvests, possesses significant nutrient content, including carbohydrates and nitrogen, making it an ideal substrate for mushroom growth. Through proper processing, such as grinding and sterilization, the waste can be transformed into a suitable medium for mycelium colonization and mushroom cultivation. This will not only reduces environmental pollution by diverting agricultural waste from landfills but will also create economic opportunities for farmers and entrepreneurs in cashew-growing regions. Additionally, the cultivation of mushrooms using cashew apple waste can lead to the production of value-added products like mushroom powder or extracts, further enhancing its economic potential. Continued research and development in this field could drive innovation, expanding the scope and profitability of utilizing cashew apple waste for mushroom cultivation, ultimately contributing to sustainable agricultural practices and economic development.

CHAPTER 2: LITERATURE REVIEW

Botany of cashew apple

Anacardiaceae family, produces a drupe fruit characterized by a kidney-shaped nut encased in a grayish hard shell. The nut is externally attached to an enlarged structure known as the cashew apple, often mistaken for the fruit itself. However, the cashew apple is not a true fruit, lacking distinct layers like exocarp, mesocarp, and endocarp. Instead, it originates from the thalamus, also referred to as the receptacle or pedicel, located outside the ovary. The plump thalamus connects to the pedicel at the dorsal side and the stylar end of the true fruit, with an apex groove. Cashew apples develop alongside the nut and can grow to an average size of 11 x 5 cm, much larger than the nut itself. Mature cashew apples are round or cylindrical, sometimes with a slight depression, resembling a pyriform hypocarp. As they mature, the initially firm, green, and immature cashew apples become soft and juicy, with outer skin coloration ranging from red, orange, to yellow depending on the variety (Fig. 1) (Preethi et al.2021).

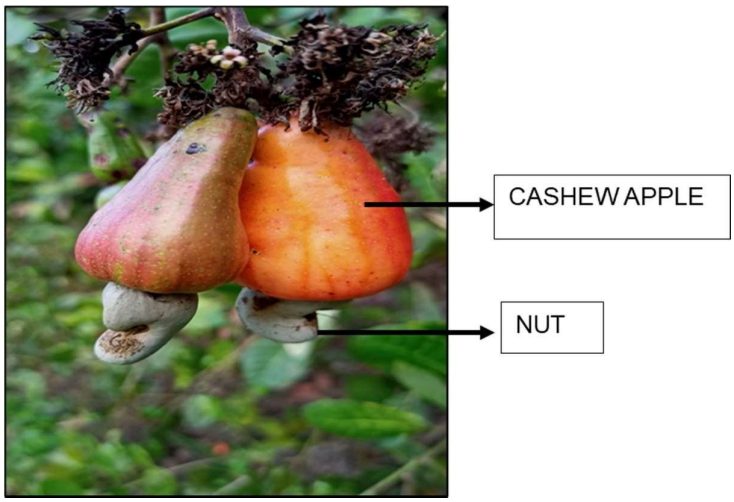

Fig 1. Image of Cashew apple (*Anacardium occidentale*)

Cashew Apple production in India and Goa

Cashew apple production holds significant importance in India, where it is considered one of the cash crops contributing to the agricultural economy. The cashew industry plays a vital role in both rural livelihoods and the overall economy due to its multifaceted utilization, including the processing of cashew nuts and the production of juices, jams, and other by-products from the cashew apple. Over the past decade, there has been a notable trend in cashew apple production in India, indicating fluctuations in output levels. However, the general trajectory suggests a stable or slightly increasing trend in total production. In India In India, complete cashew apple creation was around 56 lakh MT (Million metric tons).

In the state of Goa specifically, cashew apple production accounted for approximately 2.32 lakh MT, contributing significantly to the local economy. Despite variations in production levels, Goa remains a notable contributor to the overall cashew industry in India. (Kannan et al., 2021)

Problems In waste Management

Cashew apple waste management poses significant challenges due to the substantial amount of by-product generated during cashew nut processing and Feni production. Studies indicate that a considerable portion of cashew apple waste is produced annually, contributing to environmental concerns and economic losses if not properly managed (Sundaram et al., 2019; Raj et al., 2020).

The volume of cashew apple waste is exacerbated by the seasonal nature of cashew nut processing and Feni production leading to periodic surges in waste generation, particularly during peak harvesting seasons. Improper handling and disposal of this waste can lead to environmental pollution, soil degradation, and the proliferation of disease vectors (Olumide et al., 2018). Efficient management strategies for cashew apple waste are crucial to mitigate

these challenges. Literature suggests various approaches for handling cashew apple waste, including composting, anaerobic digestion, and utilization for bioenergy production (Ghosh et al., 2017; Adeleke et al., 2021). Furthermore, the lack of awareness among stakeholders regarding the potential value of cashew apple waste exacerbates mismanagement issues. Studies emphasize the need for interdisciplinary research and stakeholder collaboration to develop sustainable solutions for cashew apple waste management (Nwokoro et al., 2021).

In conclusion, the effective management of cashew apple waste is essential to address environmental concerns and maximize the potential value of this by-product. However, challenges such as seasonal fluctuations, inadequate infrastructure, and limited awareness hinder the implementation of sustainable waste management practices in the cashew industry. Future research should focus on developing innovative technologies and policies to enhance the utilization and valorization of cashew apple waste while minimizing its environmental impact.

Environmental Impact:

Studies highlight the significant environmental consequences of improper cashew apple waste disposal. Sundaram et al. (2019) emphasize the risk of soil degradation due to the high organic content of cashew apple waste, which can alter soil pH and nutrient levels. Furthermore, Olumide et al. (2018) discuss the potential for water pollution when waste is disposed of in aquatic environments, leading to oxygen depletion and eutrophication. Additionally, Mishra et al. (2019) underscores the contribution of cashew apple waste to greenhouse gas emissions, particularly methane, in landfills.

Health and Hygiene Concerns:

The literature emphasizes various health and hygiene risks associated with inadequate management of cashew apple waste. Foul odors from decomposing waste can impact

community well-being and quality of life (Sundaram et al., 2019). Moreover, Raj et al. (2020) the role of waste as a breeding ground for disease vectors such as flies and mosquitoes, increasing the risk of vector-borne illnesses. Respiratory issues may also arise from exposure to airborne pathogens and particulate matter (Ghosh et al., 2017).

Disease Spread Control:

Effective disease control measures are essential to mitigate the health risks associated with cashew apple waste. Adeleke et al. (2021) advocate for proper handling and disposal practices to minimize disease transmission. Composting and anaerobic digestion are proposed as viable treatment options to reduce pathogen load and mitigate health risks (Nwokoro et al., 2021).

Furthermore, vector control strategies, including insecticide spraying and habitat modification, can help prevent the spread of diseases associated with waste (Raj et al., 2020). Public awareness campaigns are also crucial for promoting behavior change and improving sanitation standards in cashew-producing regions (Mishra et al., 2019).

Products from fermented cashew apple juice

Fermented cashew apple juice offers a diverse range of products, each with unique characteristics and applications. Cashew wine, a traditional alcoholic beverage, is produced through natural fermentation of cashew apple juice, resulting in a product with fruity aromas and a slightly acidic taste reminiscent of apple cider (Berger et al., 2019). Cashew vinegar, obtained through a secondary fermentation process, possesses a tangy flavor profile with subtle sweetness, making it suitable for culinary uses such as dressings and marinades (Gomes et al., 2017). Cashew fruit juice, extracted directly from cashew apples, provides a sweet and tart flavor, rich in vitamins, minerals, and antioxidants, offering potential health benefits such as immune support and skin health (Nascimento et al., 2018). Cashew fruit jam, made by cooking cashew fruit pulp with sugar, retains the vibrant flavor of the fruit and is

utilized in various culinary applications, including as a topping for toast or filling for pastries (Cortez et al., 2019). Lastly, cashew fruit sorbet, a frozen dessert made from cashew fruit puree, offers a refreshing and creamy texture with a pronounced fruity flavor, appealing to consumers seeking dairy-free and vegan-friendly options (Bezerra et al., 2020).

Research on the valorization of cashew apple at International and National

Research on the valorization of cashew apple is conducted at various levels, including international, national, and local levels, aiming to maximize the utilization of this byproduct and minimize environmental impact. At the international level, studies have focused on exploring innovative methods for converting cashew apple into value-added products. For example, research has investigated the extraction of bioactive compounds such as phenolics, flavonoids, and antioxidants from cashew apple for use in functional foods and nutraceuticals (de Morais et al., 2019). Additionally, international collaborations have examined the feasibility of biofuel production from cashew apple waste through processes such as fermentation and anaerobic digestion, contributing to sustainable energy solutions (de Oliveira et al., 2020).

At the national level, research efforts have centered on developing cost-effective and scalable technologies for valorizing cashew apple waste within specific geographic regions. Studies have explored the production of cashew apple-based alcoholic beverages such as wine and cider, leveraging traditional fermentation techniques alongside modern process optimization strategies (Kulkarni et al., 2018). Furthermore, national research initiatives have investigated the potential of cashew apple waste as a feedstock for biogas production, utilizing anaerobic digestion to generate renewable energy while reducing organic waste (Kumar et al., 2017).

Feni production in goa

The production process of Feni from cashew apple is deeply rooted in traditional practices in Goa, India, and has garnered significant attention in the literature due to its cultural and economic significance.

Cashew Apple Harvesting and Processing:

The production process typically begins with the harvesting of ripe cashew apples from cashew orchards. The apples are then transported to processing units where they undergo cleaning and sorting to remove any debris or impurities (Faria et al., 2018). Subsequently, the cashew apples are crushed or pulped to extract the juice, which serves as the primary raw material for feni production (Rodrigues et al., 2018).

Fermentation:

Fermentation is a crucial step in Feni production, during which natural or commercial yeast strains convert sugars present in the cashew apple juice into ethanol and other volatile compounds. The fermentation process typically takes place in open containers or earthen pots, allowing for microbial colonization and the development of unique flavor profiles (Faria et al., 2018). Fermentation conditions, including temperature, pH, and duration, play a critical role in shaping the sensory characteristics of the final product (Santos et al., 2017).

Distillation:

Following fermentation, the fermented cashew apple juice undergoes distillation to concentrate the alcohol content and refine the flavor. Traditional pot stills, known as "copper alembics," are commonly used for distillation, imparting distinct character and aroma to the Feni (Gonçalves et al., 2016). The distillation process typically involves multiple stages to

separate alcohol from water and other impurities, resulting in the production of both "Urak," an intermediate spirit, and Feni (Rodrigues et al., 2018).

CHAPTER 3: METHODOLOGY

3.1 Sampling of Cashew apple waste (CAW):

Cashew apple waste (*CAW*), left after extraction of juice, was collected from a local cashew distillery unit at Shiroda, Goa India. It was brought to the laboratory and stored at 4℃ in airtight container until further use, but not longer than one week, as CAW is rich in sugar, it usually changes its properties due to microbial activity which are naturally present on the fruit.

Fig 2: Process of cashew apple waste production. A- Collected cashew apples from the cashew farm, B- Nuts separated from cashew apples at the distillery unit and C- Cashew apple waste left after the extraction of juice from cashew apple.

3.2 Estimation of total solid (TS) and volatile solid (VS):

The crucibles were first washed with tap water and then dried in the oven to eliminate any moisture. After drying, the weight of the empty crucible was measured (W1). Next, 2g cashew apple waste was added to the crucible, and the combined weight was measured (W2). The crucible with the cashew apple waste was then placed in the oven at 105°C overnight. After drying in the oven, the crucible was cooled in a desiccator, and its weight was measured again (W3). Subsequently, the crucible was subjected to incineration in a furnace for two hours at 550°C, followed by overnight cooling up to 80°C in furnace and further cooling to room

temperature in a, desiccator, and the final weight was measured (W4). Weight of CAW is (v). Using these values, the total solid, volatile solid, ash, and moisture content were estimated using following equations. Additionally, the VS/TS ratio was also calculated.

$$\textbf{Moisture content: } \frac{(W2) - (W3)}{V} X\ 100$$

$$\textbf{Total solid content : } \frac{(W3) - (W1)}{V} X\ 100$$

$$\textbf{Volatile solid : } \frac{(W3) - (W4)}{V} X\ 100$$

$$\textbf{Ash content : } \frac{(W4) - (W1)}{V} X\ 100$$

3.3. Physio-chemical characterization of CAW:

The CAW was dried at 105°C until it reached complete dryness, ensuring a consistent weight was achieved. Subsequently, it was pulverized into a fine powder using a blender.

3.3.1 Estimation of pH:

One gram of cashew apple powder was suspended in 10 ml distilled water, and vortexed. pH of this suspension was analysed using himadzu ATX224 pH meter.

3.3.2 Estimation of Chemical Oxygen Demand (COD):

Cashew apple waste powder (CAW) (2.0 mg) was weighed and transferred to a clean test tube containing 2 ml distilled water. Subsequently, 1.2 ml of dichromate digestion reagent was added, followed by the addition of 2.8 ml of sulfuric acid (H_2SO_4) reagent. The resultant mixture was subjected to a digestion process for a duration of 2 hours at a temperature of 148°C. Following the digestion period, the sample was allowed to cool down to room temperature. Once cooled, the absorbance of the digested sample was measured at a wavelength of 600 nm using a spectrophotometer. The Calibration process was followed as per procedure mentioned under APHA 2005-5200D (Closed Reflux Colorimetric Method).

3.3.3 Estimation of Phosphorus:

Cashew apple waste powder (CAW) (3.5 mg) was weighed and transferred to a clean test tube containing 3.5 ml distilled water followed by the prompt addition of 1 ml of freshly prepared mixed reagent. Then, 0.5 ml of distilled water were added to reach a final volume of 5 ml. The resulting solution was immediately analysed using spectrophotometry, with absorbance measurements taken at a wavelength of 450 nm within 20 minutes of adding the mentioned chemicals (appendix).

3.4 Mushroom spawn preparation

The healthy oyster mushroom (*Pleurotus ostreatus*) were brought from market vendor and brought to the laboratory. It was surface sterilized and then cut using a sterile scalpel. A piece of tissue measuring 0.5 cm from the centre was taken under sterile conditions. This tissue was placed on Potato Dextrose Agar (PDA) and incubated until mushroom mycelial growth was observed .

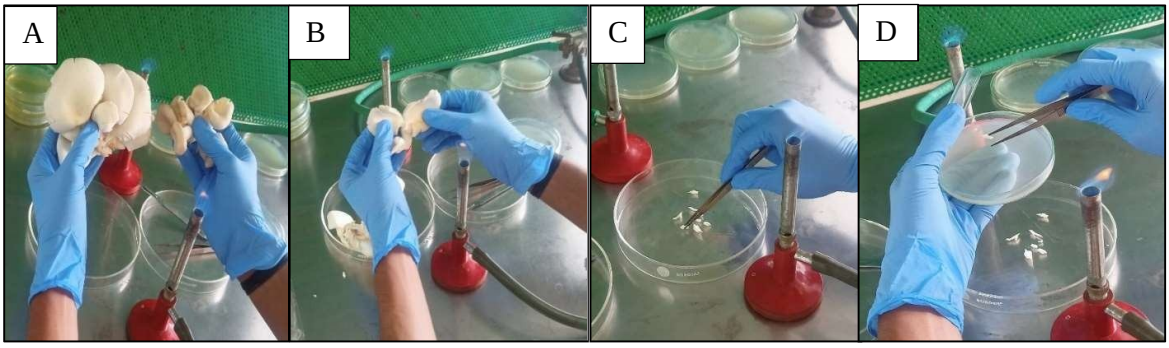

Fig. 3: Process of mushroom spawn preparation. A-Healthy oyster mushroom, B-Diced using scalpel, C-0.5cm tissue was taken, D-Tissue was placed on PDA agar.

Healthy and clean cereal grains were carefully selected for the cultivation process. These grains underwent boiling in water for a duration of 15-20 minutes to ensure proper sterilization. Excess water was then drained from the grains using a sieve, followed by drying in shade for a period of 4 hours. A mixture of CaCO3 (0.5%) was combined with the grains. Subsequently, 300 grams of these treated grains were filled into sterile bottles and autoclaved for a minimum of 1 hour. Once sterilized, the growing mushroom mycelium was inoculated onto the grains. The inoculated bottles were then placed in an incubator at room temperature and kept in the dark for a duration of 20-25 days (Thakur; Rathod et al., 2021).

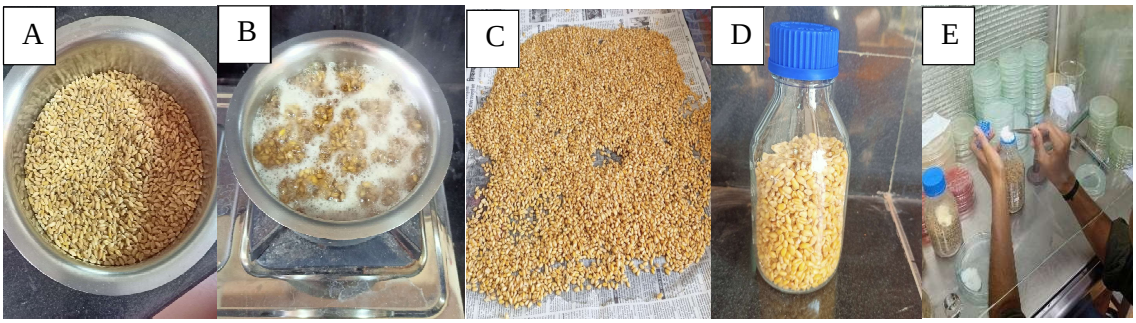

Fig 4: Mushroom spawn preparation. A- Health cereal Grains, B-Boiled grains, C- Excess water removed, D- Autoclaved grains after boiling and E- mycelium grown on plate was inoculated.

3.5 Preparation of CAW substrate for mushroom cultivation:

Following setup were made in duplicates for checking efficacy of CAW as a substrate for mushroom cultivation.

Table 1. Substrate setup description

Mushroom cultivation set up no.	Set up description
1	Rice straw (100%) (positive control).
2	Rice straw (50%) + cashew apple waste (50%).
3	Cashew apple waste (100%).

These setups were prepared in following manner:

Rice straw 100%

The rice straw was chopped into 1- to 2-inch long pieces. It was then soaked in water overnight. After soaking, excess water was drained, ensuring that the paddy straws remained moist. Once drained, the moist paddy straws were packed into autoclave bags. The substrate was autoclaved at 121°C for 1 hour. After autoclaving, the substrate was spread on a clean plastic mat to dry. The substrate moisture was maintained at 65% before spawning.

Rice straw 50% + cashew apple waste 50%

The substrate composition consisted of Rice straw (50%) and cashew apple waste (50%). This mixture was mixed and soaked in water. After soaking, excess water was drained,

ensuring that the substrate remained moist. Once drained, the moist substrate was packed into autoclave bags. The substrate was then autoclaved at 121°C for 1 hour. After autoclaving, the substrate was spread on a clean plastic mat to dry. The substrate moisture was maintained at 65% before spawning.

Cashew apple waste 100%

The substrate consisted solely of cashew apple waste. The cashew apple waste was washed in water. After washing, excess water was drained, ensuring that the substrate remained moist. Once drained, the moist cashew apple waste was packed into autoclave bags. The substrate was then autoclaved at 121°C for 1 hour. After autoclaving, the substrate was spread on a clean plastic mat to dry. The substrate moisture was maintained at 65% before spawning.

3.6 Mushroom cultivation (Filling of different substrates in bags, incubation and harvesting)

The autoclaved moist substrates of control (Rice straw 100%), Rice straw (50%) + cashew apple waste (50%) and Cashew apple waste (100%) were filled into plastic bags as layer by layer. First layer of substrate (2 inches thick in height), was added at the bottom, it was pressed firmly. This layer was inoculated with spawn at the periphery of the bag. The bags were further layered in similar fashion with three more. The height of each further layer (form bottom to top) was as follows: 4 inches, 4 inches, and 2 inches). Small holes were generated randomly on outside of the bag with a sterile pin, for aeration (Fig: 5). The bags were then incubated in the dark for 20-25 days for spawn running. After 20-25 days, when the mycelia were properly grown (observed visually), the bags were transferred to a well-ventilated area

(Kadhila; Mubiana et al., 2008). Mushroom formed after 23 days were allowed to grow till, they were matured. The mushrooms were harvested, fresh weight was taken and then they were dried and powdered for further analysis.

Fig 5: Plastic bags filled with substrates for mushroom cultivation. A- Rice straw (100%) (control), B- Rice straw (50%) + cashew apple waste (50%) and C- Cashew apple waste (100%).

3.7 Estimation of Protein and Carbohydrates content in mushroom

3.7.1 Estimation of Protein in Mushrooms

Mushroom powder (10 mg) was weighed and transferred into 2 ml tubes. Then, 1 ml of NaOH (0.25N) was added to each tube along with 6-8 beads. The tube was subjected to for 4 cycles bead beating (Beadbug mini homogenizer D1030), of 30 seconds each at for 2800rpm. Subsequently, the tubes were centrifuged at 3000 rpm for 10 minutes at a temperature of 4°C, and the supernatant was collected in separate tube.

For protein estimation, one ml of the supernatant was taken in tube, followed by the addition of 3 ml of Bradford reagent. The tubes were then incubated for 10 minutes at room

temperature. Protein concentration was determined spectrophotometrically at 660 nm. A standard solution of 1 mg/ml of Bovine Serum Albumin (BSA) was used for calibration. The total protein content was expressed in terms of mg/ml. (Bradford et al., 1976)

3.7.2 Estimation of Carbohydrates in Mushrooms

Carbohydrate estimation was conducted using Antrone's test, following the protocol outlined by (G. Ludwig et al. in 1956). Working standards ranging from 0.1 mg/ml to 1 mg/ml were prepared from a stock solution with a concentration of 1 mg/ml. Next, 10 mg of mushroom powder was weighed and added to 1 ml of distilled water, thoroughly mixed by vortexing. Subsequently, 4 ml of Anthrone reagent was added to each tube. The tubes were then placed in a water bath set to 100 degrees Celsius for 10 minutes for incubation. Afterward, they were allowed to cool to room temperature, and the absorbance was measured at 660 nm using a spectrophotometer (Niemi et al., 2024).

CHAPTER 4: ANALYSIS AND CONCLUSION

4.3.2) Estimation of total solid (TS) and volatile solid (VS):

The moisture content in the cashew apple waste was 80.28%, TS was 19.70%, VS was 16.74%, ash 2.96% and VS/TS ratio was 0.85%. The VS/TS ratio from cashew apple waste 0.85% indicates that the cashew apple waste is rich in organic content and highly biodegradable (Nathalie et al.2023).

Table 2. Results of CAW for moisture, (TS), ash and (VS) content.

Sample	Moisture (%)	TS (%)	Ash (%)	VS (%)	VS/TS (%)
CAW	80.28	19.705	2.965	16.74	0.85

4.4.1) Physico-chemical characterization of CAW

Estimation of pH

The pH of CAW was found to be 4.84. Acidic values of CAW indicate that it is a good biomass substrate for the growth for oyster mushroom. Such acidic pH values for rice straw substrate used for mushroom cultivation was reported as 5-6 in literature (Sobita; Nabin et al.2023).

4.4.2 Estimation of Chemical Oxygen Demand (COD):

Upon COD estimation using APHA 2005-5200D (closed reflux method) COD was found out to be 353.6 g oxygen/g of dry CAW. This high COD value indicates that CAW is rich in organic content. (Appendix)

4.4.3 Estimation of phosphorus

Phosphorus estimation by APHA phosphate method was found to be 0.85 g/g of dry CAW.(Appendix).

Total solids (TS) and volatile solids (VS) serve as critical indicators of the organic composition and decomposition potential within substrates. In our study, the average TS content of cashew apple waste measured at 19.70%, alongside an average VS content of 16.74%, signifies a notable organic fraction within the waste. This organic content presents a promising reservoir of nutrients for mushroom growth. The Chemical Oxygen Demand (COD) value obtained for the cashew apple waste was 353.6 g oxygen/g of dry CAW. COD reflects the quantity of oxygen required for the oxidation of organic compounds present in the sample. The relatively high COD value suggests a substantial organic load, indicating the potential for microbial activity and decomposition processes. These processes are essential for the release of nutrients during mushroom cultivation. Phosphorus emerges as a pivotal nutrient influencing fungal growth. The phosphorus content in the cashew apple waste samples was determined to be 0.65mg/L. This presence of phosphorus in the waste highlights its potential contribution to the substrate's nutritional profile. Consequently, it may lead to enhanced mushroom growth. Phosphorus can accelerate the growth of mycelium and increase the production of mushrooms (Sianturi et al., 2021).

4.5 Mushroom spawn preparation

After inoculation of mycelia on PDA plate growth of fungus was observed after 4 days (Fig: 6) this mycelium upon inoculation in sterile wheat grains, spawn growth was observed after incubation of 25 days in dark at room temperature.

Fig 6: Growth of mushroom mycelia on PDA plate.

Fig 7: Spawn ready for mushroom cultivation

4.6 Mushroom cultivation

After the inoculation of spawn in the substrate a good spawn growth was observed as seen in (Fig. 8 D, E, and F). By the end of day 14, in rice straw 50% + CAW 50%, 100% CAW as well as in the control 100% rice straw.

At the end of 20 days mushrooms started sprouting out of the bag and fully matured healthy oyster mushroom were seen by day 22 in all the experimental setup (Fig.10 g, h and i).

During the spawn running fungal contamination was observed in setup 2, (having 50% rice straw + 50% CAW) and setup 3 (100% CAW). In-spite of the fungal contamination, healthy mushroom growth was seen in setup having CAW.

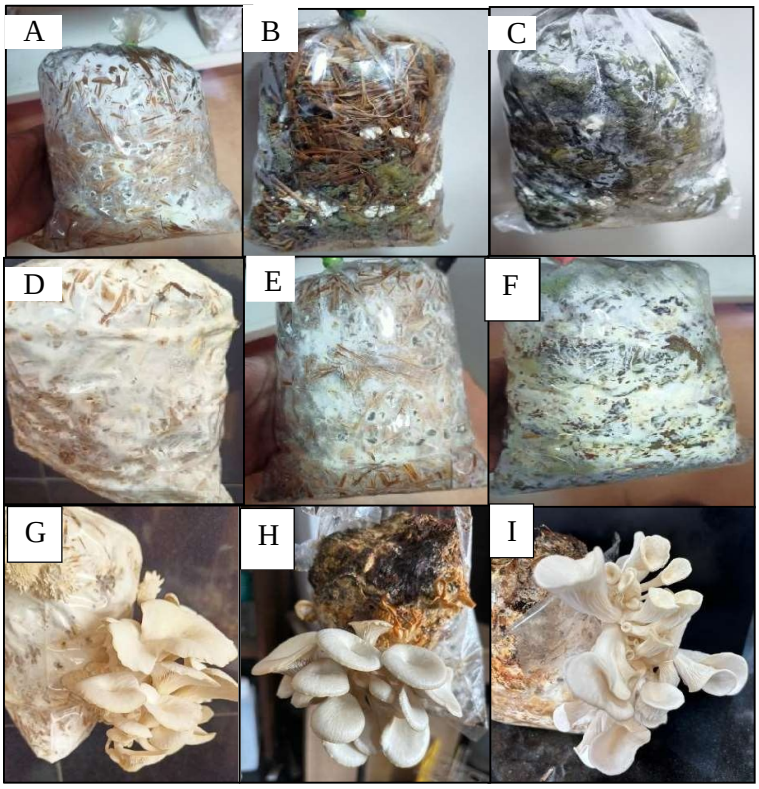

Fig. 8: Mushroom spawn running from day 0 to day 22. A-Day 1 of rice straw (100%) (control), B-Day 1 of rice straw (50%) + cashew apple waste (50%), C-Day 1 of cashew apple waste (100%), D-Day 14 of rice straw (100%) (control), E-Day 14 of rice straw (50%) + cashew apple waste (50%), F-Day 14 of cashew apple waste (100%), G-Day 22 of rice straw (100%) (control), H-Day 22 of rice straw (50%) + cashew apple waste (50%) and I-Day 22 of cashew apple waste (100%).

7 **Estimation of Protein and Carbohydrates content in mushroom**

The protein estimation by Bradford method in Setup 1, Setup 2 and Setup 3 was found to be 10.4±0.23%,10.8±0.36 and 11.2±0.29 respectively.

Similarly, carbohydrate estimation by Anthrone method, in Setup 1, Setup 2 and Setup 3 was found to be 28.5±0.61,29.5±0.22 and 30.5±0.36 respectively Table 3

Table 3: Protein and carbohydrate content in mushroom in different substrates

Sr.no	Substrate	Protein%	Carbohydrate %
1	Control (rice straw 100%)	10.4±0.23	28.5±0.61
2	50% Rice + 50% CAW	10.8±0.36	29.5±0.22
3	100% CAW	11.2±0.29	30.5±0.36

Healthy mushroom growth was seen even with 100% CAW, suggesting that CAW is very good substrate for cultivation of mushrooms. This support and proves the hypothesis of this study. Further, the results of mushroom cultivation using CAW indicated a progressive increase in both protein and carbohydrate content with the incorporation of CAW into the substrates. Specifically, the substrate containing 100% CAW exhibited the highest levels of protein (11.2%) and carbohydrates (30.5%), followed by the 50% rice and 50% CAW blend, compared to positive control (Table 3). These findings suggest that CAW not only support mushroom cultivation, but also holds potential as a valuable substrate component for enhancing the nutritional value of mushrooms. Furthermore, the utilization of CAW presents an environmentally sustainable approach to waste management and offers economic benefits by converting waste into a resource for mushroom cultivation. In accordance with Effiong et al. (2024), the literature suggests that oyster mushrooms grown on rice substrate exhibited a protein content of 17.06%. However, our study revealed a slightly lower protein content of approximately 11.2%. This variance could be attributed to several factors, including variations in environmental conditions, substrate composition, and perhaps even genetic differences in the mushroom strains used. Such differences highlight the need for comprehensive analysis and validation of findings across different experimental setups. Similarly, when it comes to carbohydrate content, the literature reported a higher percentage

(43.4%) compared to our findings. Again, this could stem from discrepancies in experimental methodologies, substrate composition, or even the stage at which the mushrooms were harvested. It's worth noting that carbohydrates play a crucial role in the growth and development of mushrooms, serving as a primary energy source. Thus, understanding and optimizing carbohydrate content are essential for maximizing mushroom yields. Where ever the Cashew apple waste is available in limited quantity it can be blended with the primary substrates such as rice straw, paddy straw etc. depending on the availability. Wherever it is available in bulk quantity it can be directly valorized instead of wasting cashew apple waste and throwing the waste in environment thus it can reduce resource consumption and production cost, thereby making mushroom cultivation more sustainable and economically viable.

Conclusion

In conclusion, based on extensive research, utilizing cashew apple waste for mushroom cultivation proves highly beneficial. The high chemical oxygen demand (COD) in cashew apple waste indicates its rich organic content, providing an ideal substrate for mushroom growth. Additionally, its favorable pH levels create a conducive environment for mushroom mycelium colonization and fruiting body development. Moreover, the waste contains significant phosphorus content, essential for mushroom growth and yield. The study also demonstrates that cashew apple waste cultivated excellent growth of mushrooms, further highlighting its efficacy as a substrate. Therefore, harnessing cashew apple waste for mushroom cultivation not only presents an eco-friendly solution for waste management but also promises a sustainable and nutrient-rich medium for mushroom production.

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Appendix:

1.Estimation of Chemical Oxygen Demand (COD).

Note-:

• For 1 L of Digestion reagent take oven dried 10.216g of Potassium Dichromate, 33.3g $HgSO_4$ in 167ml of H_2SO_4

• H204 reagent: 1% silver sulphate (1g/100ml)

Using the equation of the line y = 0.0004x the COD of CAW was found out to be 707.5 mg/l.

Table 1. Estimation of Chemical Oxygen Demand (COD).

Date	sample	Absorbance (1)	Absorbance (2)	Average	COD
21-03-2024	1	0.28	0.286	0.283	707.5 mg/l

COD was found out using Microsoft excel

Sr no	content	cod	od
1	standard	0	0
2		100	0.045
3		200	0.0855

4		400	0.164
5		600	0.2535
6		800	0.332
7		1000	0.4005
8	CAW	707.5	0.283

2. Estimation of Phosphorus by APHA Method

Phosphorus reagents

Note: The mixed reagent was prepared as follows:

For a total volume of 100 ml, the reagent comprised 40 ml of reagent A, 30 ml of reagent B, 25 ml of concentrated sulfuric acid, and 5 ml of distilled water. Preparation of reagent A involved dissolving 62.5 grams of Ammonium molybdate in 1 litre of distilled water, while reagent B was prepared by dissolving 4.1666 g of Ammonium Vanadate in 1 litre of distilled water.

Sr no	Vol of stock	Vol of d	Total volume	Conc (mg/ml)
1	0	5	5	0
2	0.1	4.9	5	0.0032
3	0.2	4.8	5	0.0064
4	0.3	4.7	5	0.0096

5	0.4	4.6	5	0.0128
6	0.5	4.5	5	0.016
7	0.6	4.4	5	0.0192
8	0.4	4.3	5	0.0224
9	0.3	4.2	5	0.0256
10	0.2	4.1	5	0.0288
11	1	4	5	0.0322

Sr.no	Volume diluted stock sample b	Volume of mixed reagent	Distilled water	Total volume (ml)	Concentration (mg/ml)	OD at 540 nm
1	3.5	1	0.5	5	0	0
2	3.5	1	0.5	5	0.00224	0.085
3	3.5	1	0.5	5	0.00448	0.177
4	3.5	1	0.5	5	0.00672	0.297
5	3.5	1	0.5	5	0.00896	0.372
6	3.5	1	0.5	5	0.0112	0.459
7	3.5	1	0.5	5	0.01344	0.572
8	3.5	1	0.5	5	0.01568	0.691

9	3.5	1	0.5	5	0.01792	0.751
10	3.5	1	0.5	5	0.02016	0.871
11	3.5	1	0.5	5	0.0224	0.943

Using the regresion of the line y = 42.894x - 0.006 , phosphorus was found out to be 0.65 mg/l.

Table 2. Estimation of phosphorus.

Date	Absorbance (1)	Absorbance (2)	Average	Phosphorous
21-03-2024	0.023	0.021	0.022	0.65 mg/l

3.Estimation of protien in mushroom.

Concentration mg/mL	O.D (595nm)
0	0
0.02	0.035
0.04	0.108

0.06	0.18
0.08	0.238
0.1	0.284
Control	0.777
50% Rice + 50% CAW	0.818
100% CAW	0.841

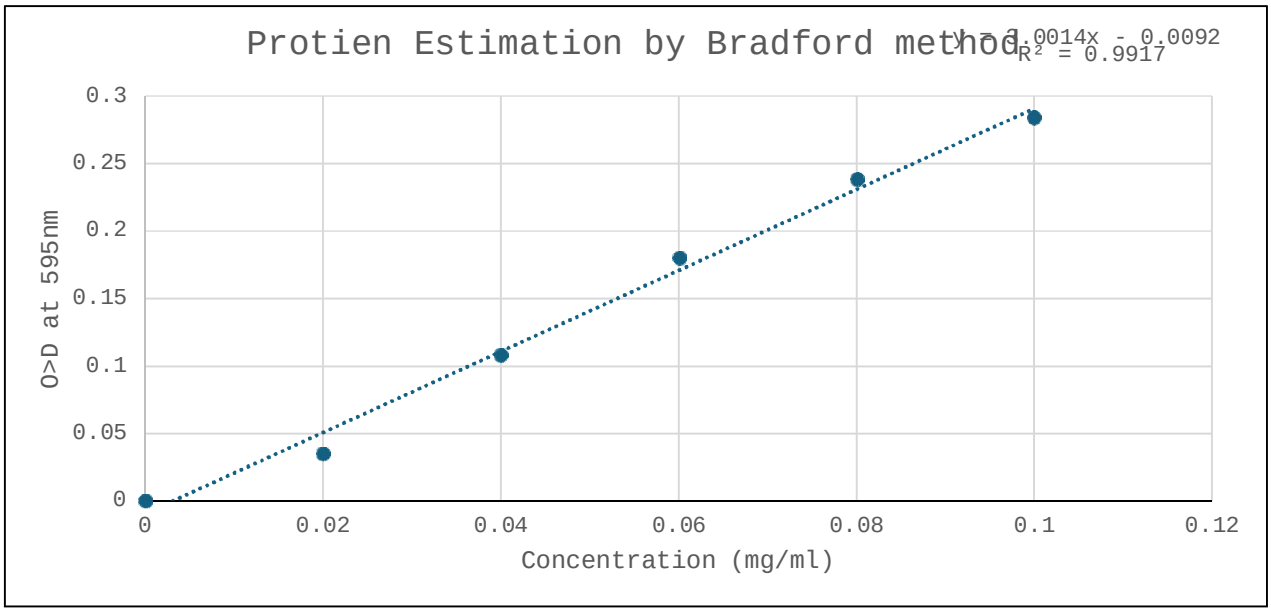

Fig 1: Standard curve graph for protein.

4. Estimation of Carbohydrates in Mushroom.

Table 3. Standard for Carbohydrates.

Conc in mg/ml	Absorbance 660
0	0
0.1	0.08
0.2	0.202
0.3	0.304
0.4	0.417
0.5	0.524
0.6	0.614
0.7	0.705
0.8	0.789
0.9	0.887
1	1.03
control	0.585
50% Rice + 50% CAW	0.605
100% CAW	0.622

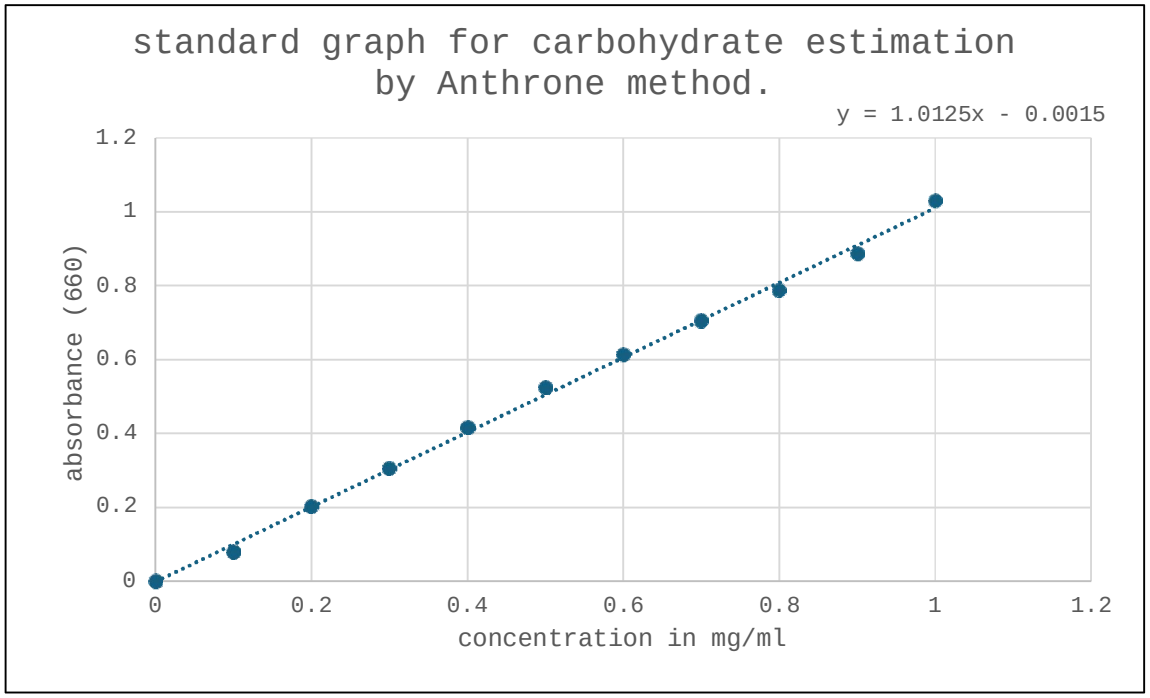

Fig 2:- Standard curve graph for carbohydrate.