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PRODUCTION OF ETHANOL FROM SAPODILLA

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ABSTRACT
This project investigates the production of ethanol from sapodilla (chikoo) fruit through a comprehensive methodology encompassing pretreatment, fermentation, and distillation processes. The sapodilla fruit is initially ground into a slurry, followed by acid hydrolysis under varying concentrations of sulfuric acid, temperatures, and durations to optimize the release of fermentable sugars. After hydrolysis, the slurry undergoes pH adjustment and sterilization to prepare for fermentation. Saccharomyces cerevisiae yeast is then introduced into the slurry, and the mixture is incubated for 15 days. Post-fermentation, the solution is filtered and subjected to a two-stage distillation process to isolate ethanol. The first distillation yields 60 ml of distillate from 150 ml of fermented solution, and a second distillation further refines the ethanol to 86% purity, yielding 25 ml of ethanol. Material balance calculations indicate that approximately 10 to 12 kg of sapodilla is required to produce 1 liter of ethanol. This study highlights the potential of sapodilla as a novel feedstock for ethanol production, offering a sustainable and efficient biofuel source while providing insights into the broader field of renewable energy and waste management.

Keywords:
Ethanol Production, Sapodilla (Chikoo), Acid Hydrolysis, Fermentation, Saccharomyces cerevisiae, Biofuel Feedstock.

I. Introduction
The rising global demand for renewable and sustainable energy sources has led to a surge in research focused on alternative biofuels. Ethanol, a renewable fuel that reduces greenhouse gas emissions and dependency on fossil fuels, has gained significant traction as a biofuel. Traditionally, ethanol production has relied on crops such as corn and sugarcane, but these sources present challenges like high production costs, competition with food supplies, and regional limitations. Exploring unconventional, locally available biomass feedstocks is crucial for improving sustainability and economic feasibility in biofuel production.
Sapodilla (Manilkara zapota), commonly known as chikoo, is a tropical fruit rich in fermentable sugars and widely cultivated in various regions. Despite its abundance, sapodilla remains an underutilized resource in biofuel production. The surplus or substandard fruits, which often go to waste, offer a promising raw material for ethanol production. By utilizing sapodilla, the biofuel industry can both add value to this agricultural product and address waste management concerns, making it a highly viable feedstock for ethanol production.
This project explores the production of ethanol from sapodilla fruit through a detailed methodology that includes pretreatment, fermentation, and distillation processes. By optimizing these stages, the

study aims to achieve high yields and ethanol purity. The findings offer insights into the feasibility of using sapodilla as a sustainable feedstock, contributing to the diversification of biofuel sources and advancing sustainable energy solutions. This research has the potential to influence future industrial applications in the field of biofuels, further promoting the use of locally available biomass for renewable energy.

II. Literature

The dependency on traditional energy sources has led to significant environmental challenges and the depletion of fossil fuel reserves, creating a global shift toward renewable energy, especially biofuels like bioethanol. Bioethanol production through microbial fermentation presents a promising approach to converting waste biomass into sustainable energy. This review discusses key liquid biofuels such as biodiesel and bioethanol, detailing the biochemical processes involved in ethanol generation from various carbon-based sources. It also explores different fermentation techniques and the use of substrates, focusing on lignocellulosic biomass pretreatment and non-aseptic production environments.

Innovative fermentation methods, such as cell recycle and vacuum fermentation, provide cost-efficient solutions for continuous ethanol production. These methods, particularly when using molasses as a substrate, can reduce capital investment by up to 71% compared to traditional batch fermentation. Despite molasses accounting for over 75% of production costs, utilizing yeast by-products can reduce net ethanol production expenses to approximately 80-82 cents per gallon for both cell recycle and vacuum fermentation processes.

The transition towards non-traditional energy sources has increased the focus on biomass as a dependable feedstock for ethanol production. This review highlights the potential of feedstocks like sugar beets, corn, wheat, and sugarcane for ethanol fermentation. Biomass usage for clean energy is anticipated to grow by 35% in the EU by 2030. Advances in biotechnology have enhanced microorganisms, particularly *Saccharomyces cerevisiae*, to ferment a wider range of sugars, improving ethanol production from diverse sources like agricultural, industrial, and municipal waste.

Continuous fermentation and liquid-liquid extraction methods for ethanol production have also seen significant development. A pilot plant operating with feed glucose concentrations of 10-45.8% (w/w) demonstrated that liquid-liquid extraction, combined with recycled fermented broth, effectively removes ethanol. Using ndodecanol and immobilized yeast helped prevent emulsification, with by-products like glycerol not affecting ethanol output. The plant achieved a 78% reduction in the volume of aqueous purge at higher glucose concentrations, making the process more efficient compared to lower glucose levels.

Ethanol fermentation technologies using sugar and starch feedstocks are critically reviewed, highlighting key areas often overlooked. While *Zymomonas mobilis* has better ethanol yield and productivity than *Saccharomyces cerevisiae* due to its unique metabolic pathway, its narrow substrate range and ineligibility as animal feed limit its broader application. Continuous fermentation models and the effects of high-gravity fermentation still require further investigation to enhance industrial ethanol production. Immobilizing yeast cells has proven to be both economically and technically challenging, though flocculation-based self-immobilization offers a more feasible alternative.

III. Methodology.

The methodology involves processing sapodilla fruit into a slurry, followed by acid hydrolysis to break down complex sugars. After pH adjustment and sterilization, *Saccharomyces cerevisiae* yeast is introduced for fermentation over 15 days. The fermented solution is then filtered and distilled in two steps to isolate ethanol, achieving an 86% purity. Material balance calculations indicate the need for 10-12 kg of sapodilla to produce 1 liter of ethanol.

2.1 Pretreatment

The pretreatment process begins with selecting ripe sapodilla fruits, which are washed, deseeded, and ground into a fine slurry. Acid hydrolysis is then performed using various concentrations of sulfuric acid (0% to 3% v/v) at temperatures ranging from 60°C to 110°C for periods of 3 to 48 hours to break down polysaccharides into fermentable sugars. After hydrolysis, the slurry's pH is adjusted to a range of 5.0 to 5.5 using sodium hydroxide, creating optimal conditions for fermentation. Finally, the slurry is sterilized through autoclaving at 121°C for 15 minutes to eliminate any unwanted microorganisms before fermentation.

2.2 Slurry Treatment

The slurry preparation process begins with selecting fresh, ripe sapodilla fruits, while damaged or spoiled ones are discarded. The chosen fruits are thoroughly washed to remove any dirt or impurities, then deseeded. The cleaned fruits are ground into a fine pulp using a mechanical grinder, resulting in a sapodilla slurry, which forms the base material for the subsequent hydrolysis and fermentation processes. This slurry serves as the primary substrate for ethanol production.

Fig 1. Sapodilla Slurry

2.3 Acid Hydrolysis

The acid hydrolysis process aims to break down the polysaccharides in the sapodilla slurry into fermentable monosaccharides. This is achieved by mixing the slurry with sulfuric acid (H_2SO_4) solutions of varying concentrations (0%, 0.5%, 1%, 2%, and 3% v/v). The acidified slurry is then heated at different temperatures ranging from 60°C to 110°C for periods of 3 to 48 hours, depending on the experiment's requirements. After hydrolysis, the slurry is cooled to room temperature, preparing it for pH adjustment and further processing. This step is critical for optimizing sugar release, which enhances the efficiency of the fermentation process.

2.4 Ph Adjustment

After acid hydrolysis, the pH adjustment step is crucial for creating an optimal environment for yeast fermentation. Initially, the pH of the hydrolyzed sapodilla slurry is measured using a digital pH meter. To bring the pH to an ideal range of 5.0-5.5, which is suitable for the growth and activity of *Saccharomyces cerevisiae*, 1 M NaOH (sodium hydroxide) is added to the slurry. Once the desired pH is achieved, the slurry is ready for sterilization and fermentation. This adjustment ensures that the yeast can efficiently ferment the sugars into ethanol.

2.5 Sterilization

The sterilization process is essential to eliminate any unwanted microorganisms that could interfere with fermentation. After pH adjustment, the hydrolyzed sapodilla slurry is transferred into sterilizable flasks. These flasks are covered with cotton wool, wrapped in aluminum foil, and placed in an autoclave. The slurry is sterilized by autoclaving at 121°C for 15 minutes, ensuring that all contaminants are removed. Once sterilized, the flasks are allowed to cool to room temperature,

preparing the slurry for the yeast fermentation process. This step ensures a clean environment for optimal ethanol production.

2.6 Fermentation

The fermentation process begins with the activation of *Saccharomyces cerevisiae* yeast, which is prepared by mixing 4 g of dry yeast with a nutrient-rich medium, including glucose, peptone, urea, and magnesium sulfate. The yeast mixture is incubated at 30°C for 24 hours. After activation, the sterilized sapodilla slurry is combined with the yeast solution in a 750 ml to 250 ml ratio. The combined mixture is sealed in a fermentation vessel and left to ferment at ambient temperatures (25-30°C) for 15 days. During this time, the yeast converts the fermentable sugars in the sapodilla slurry into ethanol. After fermentation, the solution is filtered to remove particulates and prepare it for distillation.

Fig 2. Yeast Activation

2.7 Distillation

The distillation process is used to purify ethanol from the fermented sapodilla solution. Initially, 150 ml of the filtered fermented solution is heated in a distillation apparatus to 78°C, the boiling point of ethanol, for about one hour. This first round of distillation yields 60 ml of a distillate containing a mixture of ethanol and water. The distillate is then subjected to a second distillation under the same conditions to further concentrate the ethanol. This second distillation produces 25 ml of ethanol with an 86% purity. This process ensures the ethanol is sufficiently refined for biofuel or other applications.

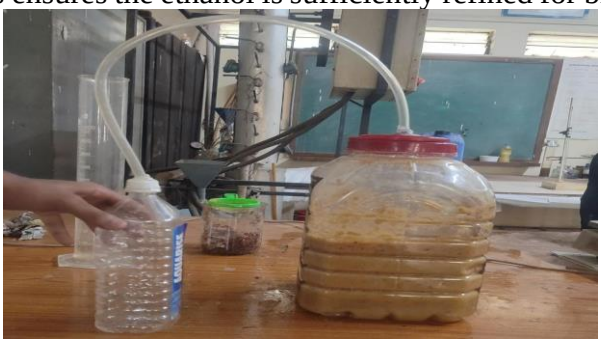


Fig 3. Process setup.

2.8 Material Balance

Material balance calculations are essential for assessing the efficiency and output of the ethanol production process. For raw materials, it was found that 1 to 1.5 kg of sapodilla fruit is needed to generate 1 liter of slurry. This slurry is then mixed in a 750 ml to 250 ml ratio with an activated yeast solution, creating the optimal environment for fermentation.

In terms of output, after the fermentation process is completed, 150 ml of the fermented solution produces 21 ml of ethanol after two distillation rounds. To scale this up, producing 1 liter of ethanol requires approximately 7 to 8 liters of fermented solution, which corresponds to about 10 to 12 kg of sapodilla fruit.

IV. Result and discussion

4.1 Material Balance and Ethanol Yield

Preliminary tests indicated that 1 to 1.5 kg of sapodilla was sufficient to create 1 litre of slurry. By combining this slurry with 250 ml of yeast solution, a fermentable mixture was created. After a 15-day fermentation, the mixture was filtered to remove any solids, yielding a clear liquid ready for distillation. In the first distillation, heating 150 ml of the fermented solution to 78°C produced 60 ml of distillate, primarily a combination of ethanol and water. A second distillation further purified the ethanol, resulting in 25 ml of distillate with an ethanol concentration of 86%, translating to 21 ml of pure ethanol. Scaling up, it was determined that to produce 1 liter of ethanol, 7 to 8 liters of fermented solution would be required, corresponding to 10 to 12 kg of sapodilla fruit. These findings validate the material balance calculations and show the scalability and feasibility of sapodilla-based ethanol production.

4.2 Optimization and Hydrolysis Condition.

The hydrolysis stage played a crucial role in breaking down the polysaccharides in the sapodilla slurry to release fermentable monosaccharides. A range of sulfuric acid concentrations, hydrolysis temperatures, and times were tested to find the optimal combination for maximizing sugar yield. The most effective conditions were found to be a sulfuric acid concentration of 2% v/v, a temperature of 90°C, and a hydrolysis duration of 24 hours. These parameters yielded the highest amount of fermentable sugars, significantly improving the subsequent fermentation process.

4.3 Fermentation Efficiency

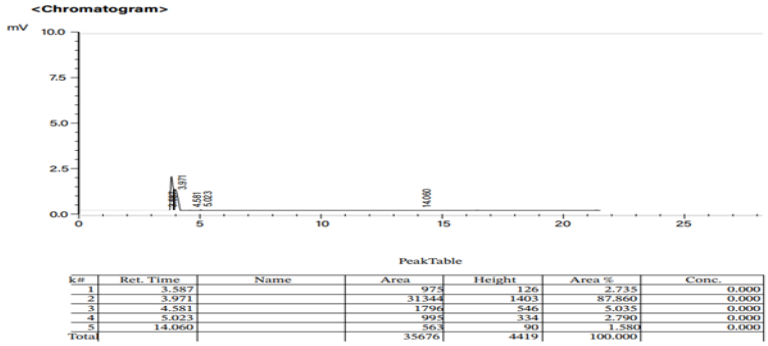
Fermentation, carried out over 15 days at ambient temperatures ranging between 25-30°C, successfully converted the monosaccharides into ethanol. The yeast Saccharomyces cerevisiae, which had been activated in a nutrient-rich medium, demonstrated strong fermentation activity. The filtration process, which employed cloth and filter paper, effectively removed particulates, ensuring a clear fermented solution and improving the efficiency of the distillation process.

A two-stage distillation method was used to purify the ethanol produced. The first distillation step concentrated the ethanol, and the second step further refined it, resulting in a final ethanol concentration of 86%. This level of purity was considered satisfactory for biofuel applications, though the process showed potential for further optimization to increase ethanol concentration.

4.4 Overall Process Efficiency

The methodology developed in this project demonstrated that sapodilla fruit can serve as a viable feedstock for ethanol production. With 10 to 12 kg of sapodilla fruit yielding approximately 1 liter of ethanol, the process achieved a good balance between material input and ethanol output. These findings indicate that sapodilla is a promising alternative to traditional feedstocks for ethanol production, offering a sustainable and economically viable option. The project proved the feasibility and efficiency of using sapodilla fruit for ethanol production. By optimizing hydrolysis and fermentation conditions and employing a two-step distillation process, high-purity ethanol was

obtained. This research contributes important insights into biofuel production and positions sapodilla as a potential feedstock for sustainable energy solutions.



PeakTable

k#	Ret. Time	Name	Area	Height	Area %	Conc.
1	3.587		975	126	2.735	0.000
2	3.971		31344	1403	87.860	0.000
3	4.581		1796	546	5.035	0.000
4	5.023		995	334	2.790	0.000
5	14.060		563	90	1.580	0.000
Tota			35676	4419	100.000	

Fig 4. Gas Chromatography Results

V. Conclusion

This project on ethanol production from sapodilla (chikoo) fruit has shown that this lesser-known tropical fruit can serve as an efficient and viable biofuel feedstock. By employing a comprehensive approach that includes pretreatment, fermentation, and distillation, the study successfully optimized conditions for each stage to maximize ethanol yield and purity. It was determined that 10 to 12 kg of sapodilla fruit can produce 1 liter of ethanol, with a concentration of 86% achieved after a two-step distillation process.

The conversion of sapodilla into fermentable sugars through acid hydrolysis, followed by fermentation using Saccharomyces cerevisiae and efficient distillation, highlights the potential of this fruit as a sustainable and cost-effective alternative to conventional biofuel sources. This study not only enhances the value of sapodilla as an agricultural resource but also promotes the diversification of biofuel feedstocks, contributing to sustainable energy efforts and providing a practical waste management solution. The findings open doors for further research and potential industrial applications, emphasizing the significance of exploring local biomass for renewable energy production.

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