DOI: https://dx.doi.org/10.4314/gjpas.v30i1.5

GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 30, 2024: 45-49
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ASSESSMENT OF THE YIELD OF MICROBIAL FERMENTED RIPE AND UNRIPE PAWPAW FOR BIOETHANOL PRODUCTION

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(Received 27 June 2023; Revision Accepted 15 August 2023)

ABSTRACT

The production of ethanol from *Carcia papaya* (pawpaw) fruit pulp using dried active baker's yeast strain (*Saccharomyces cerevisiae*) and isolated yeast from ripe pawpaw pulp was investigated using standard methods. Papaya fruits (ripe and unripe) were collected, washed, and peeled. The pulps were then collected and blended aseptically. Cleaned sterile cheese-cloth was used to sieve the two fruit pulps and the extracts fermented separately with the two yeast broth cultures. The effects of yeast concentration, duration of fermentation, pH, temperature, and different yeast supplements as they relate to the optimization of ethanol production were determined. The fermented pawpaw fruit pulps produced an ethanol content of 8.21% and 4.26% (v/v) for ripe and unripe pawpaw fruit, respectively. Physical and chemical properties of the pawpaw pulp showed that the initial pH of 4.6 was obtained before the start of fermentation with a reducing sugar value of 6.92±0.02. The results of this work show that the rate of alcohol production through fermentation of pawpaw fruit pulp by isolated yeast strain (*Saccharomyces* spp) increases with fermentation time and peaks at 72 hours. It is also increased with yeast concentration at the temperature of 30°C. An optimum pH of 4.6 was found to be ideal for the fermentation.

KEYWORDS: Fermentation, *Saccharomyces cerevisiae*, Ethanol, Pawpaw

INTRODUCTION

Pawpaw botanically known as *Carica papaya* is one of the fruits commonly used as food and medicine in Nigeria. It is eaten as fresh fruit or as a dessert (Desmond, 2015). The unripe matured pawpaw fruit is used for the production of papain by the making of incisions on the back of the fruits to get the latex for papain production (Akin-Osmaiye *et al.,* 2005). Ethanol production by fermentation faces competition with ethanol production from petroleum-based products as feedstocks. With the increasing cost of these petrochemical feedstocks, the fermentation of ethanol is bound to receive more attention (Ahmeh *et al.,* 2008). Secondly, the use of renewable materials would be more economical, since they are cheaper and easily available. Here, attempt was made to process the fruit waste into alcohol, which will have useful industrial applications. The increase in the use and need for ethanol as a universal energy source has triggered worldwide investigation, not only concerning high-yielding alcohol production yeast strains but also to cheaper available raw materials.

Fermentation results from the action of yeast (*Saccharomyces cerevisiae*) which provides the enzyme zymase that converts the sugar present in the fruit to produce alcohol, carbon dioxide, and other by-products. Nitrogenous compounds are essential for the growth and development of the yeast (*Saccharomyces cerevisiae*) in the fermentation process and they generally influence the percentage yield of the alcohol (Tse *et al.,* 2021). Pawpaw (*Carica papaya*) fill it is believed to principally contain inverted sugar with only traces of sucrose being present (Xu *et al.,* 2015). Ogbonna and Asiegbu (2010) reported the fermentation of pawpaw fill it for alcohol production to determine the effect of yeast fermentation on the nutrient contents of this fill it. However, they did not report the alcohol yield after the fermentation. Production of ethanol from *Carica papaya* agro waste with respect to the effect of saccharification has been studied and reported by Akin-Osanaiye *et al.* (2005).

In the aspect of Ethanol production investigations have been carried out over the years but for this study, fully ripe and unripe pawpaw (*Carica papaya*) were selected for the production of ethanol using baker's

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dry yeast (*Saccharomyces cerevisiae*) and yeast normal flora isolated from the substrate (pawpaw). In the fermentation process, ethanol can be produced with various substrates including sugarcane molasses which is the main raw material for ethanol production. Currently, the continuous depletion of non-renewable resources of fuels and chemicals has promoted the research and development of different alternatives for the replacement of fossil resources as the feedstock of fuels and chemicals (Jambo *et al.,* 2016). At present, one of the most important biofuels in the current economy, is bioethanol, contributing to 65% of the total biofuels production (Akin-Osanaiye *et al.,* 2008). The production of bioethanol is an attractive alternative because it would be produced using indigenous and native raw materials, therefore, the socioeconomic impact mainly in developing countries would be measured by the economic incomes and increase the quality of life of small and middle farmers. *Saccharomyces cerevisiae* is a "Sugar-fungus" and includes a group of strains of baker's yeast, and brewer's yeast responsible for producing our favorite carb-heavy treats: bread and alcohol. They work by feeding on sugars and converting that food into carbon dioxide (and alcohol given enough time) giving our baked foods that soft, air structure we love and our beer that bubbly nature (Pisat *et al.,* 2009). Brazil is supplying most of the world's ethanol (Malakar *et al.,* 2020). Bioethanol is ethanol produced through microbial fermentation of carbohydrates from plants or algae e.g. corn, sugarcane, wheat, lignocellulosic biomass (Slininger *et al.,* 2016).

AIM AND OBJECTIVES

This research was aimed at producing ethanol from ripe and unripe samples of *Carica papaya* using yeast (*Saccharomyces cerevisiae*) isolated from the fruit and also dry yeast to determine the sample with the highest ability for alcohol production. The specific objectives of this research are to:

1. isolate and characterize yeast isolates from ripe pawpaw samples
2. produce ethanol from both ripe and unripe pawpaw using yeast isolates
3. determine the percentage of alcohol present in both ripe and unripe bioethanol produced and;
4. compare the ethanol concentration of ripe and unripe *Carica papaya*.

MATERIALS AND METHODS.

Collection and preparation of fruit samples.

Ripe and unripe pawpaw fruits were purchased from Watt market in Calabar, Cross River State of Nigeria. The fruits were transported in a clean new cellophane bag to the Department of Microbiology Laboratory, University of Calabar for analysis.

Isolation of yeast from ripe pawpaw samples.

Using Sabouraud Dextrose Agar (SDA) medium, yeasts were isolated separately from collected fruit pulps as described by Bhadra *et al.* (2007). Yeast malt (YM) agar was used for sub-culturing and maintenance of isolated strains at 28°C. Yeast isolates were identified based on the morphological and physiological studies as described by Olowonibi, (2017), and species were identified by reference (Nwachukwu *et al.,* 2006). Yeast isolates were identified based on standard biochemical tests like carbohydrate fermentation, carbohydrate catabolism, triple sugar iron agar, catalase, and urease tests.

Fruit pulp extract preparation and fermentation.

Papaya pulps were prepared from freshly collected papaya fruits. The ripped fruits were weighed, and blended aseptically using sterile blender. Clean sterile cheesecloth was used to sieve the fruit pulps, which were then distributed into 500 ml conical flasks (each 100g/100 ml) in four different experimental sets with the addition of supplements. The pH of the media was adjusted to 5.0 and closed using a stopper with non-absorbent cotton wool and aluminum foil to ensure they were air-tight to provide anaerobic conditions. These were pasteurized by boiling in a water bath 64 ^{o}C for 15 min and were cooled to inoculate 1% of the Yeast strain for ethanol production. After inoculation, the flasks were tightly covered with aluminum foil, which would restrict but not eliminate gas exchange. Then flasks were incubated at 30 ± 0.5^{o}C for three days. At the end of fermentation, the alcohol was recovered by simple distillation using a laboratory distillation unit.

Analytical Methods

On the third day, the fermented samples were estimated for ethanol and sugar concentrations spectrophotometrically. Ethanol was determined with oxidation with acid dichromate solution and absorbance was measured at 660 nm. The total reducing sugar content was analyzed by optical density measurement at 520 nm by dinitrosalicylic (DNS) method (Miller *et al.,* 2012).

RESULTS

Cultural and morphological characteristics of the yeast isolates.

The cultural and morphological characteristics of yeast isolates are presented in Table 1. Based on the colony and microscopic morphology, two yeast isolates were identified to belong to the genera Saccharomyces.

Biochemical characterization and analysis of yeast isolates from papaya fruit pulps is presented in Table 2. The result showed that all the isolates were positive for catalase, fermentation of carbohydrates, and triple sugar iron agar tests and negative for urease, starch hydrolysis, and carbohydrate metabolism.

Results of physical and chemical properties of the pawpaw fruit pulp.

Table 3 presents the results of the physical and chemical properties of the pawpaw fruit pulp. The result showed that the initial pH was 4.6 pre-fermentation with a reducing sugar value of 6.92±0.02 and alcohol content of 2.86±1.27

Ethanol yield by isolated yeast strains

Table 4 presents the result of the ethanol yield by the isolated yeast strain and barker's yeast used respectively. The percentage of ethanol yield was significantly ($p < 0.05$) higher using the isolated yeast on ripe papaya pulp than the backer's yeast isolate on both pulps of ripe and unripe papaya after 72 hrs. The highest amount of ethanol yield was recorded when isolated yeast strain A from papaya was used for fermentation after 72 hrs.

Table 1: Morphological characterization of the yeasts isolates

Characteristics	Yeast isolate A	Yeast isolate B
Surface	Smooth	Smooth
Shape	Spherical/oval	Round/oval
Color	Creamy white	Creamy white
Pseudo mycelium	Present	Present
True mycelium	Absent	Absent

Table 2: Biochemical characteristics of the yeasts isolates

Biochemical tests	Yeast isolate A	Yeast isolate B
Catalase	Positive	Positive
Urease	Negative	Negative
Carbohydrate test	Negative	Negative
Fermentation of sugar	Positive	Positive
Starch hydrolysis	Negative	Negative
Triple sugar iron (TSI)	Positive	Negative

Table 3: Physical and chemical properties of the pawpaw fruit pulp

S/N	Parameters	Content
1	Moisture content (g/100g)	81.45±1.22
2	pH	4.6
3	Reducing sugar (g/100 g)	6.92±0.02
4	Alcohol (%v/v)	2.86±1.27
5	Percentage purity of alcohol (%)	91.34± 0.09

Values are means of triplicate readings ± SEM

Table 4: Amount of ethanol produced by isolated and backer's yeast using ripe and unripe pawpaw

Substrate	Ethanol yield (%)		
	Yeast isolate A	Yeast isolate B	Backer's yeast
Ripe papaya fruit pulp	8.21 ± 1.21	6.47 ± 0.04	2. 11 ± 0.07
Unripe papaya fruit pulp	4.26 ± 1.21	3.16 ± 0.11	1.84 ± 0.14

Values are means of triplicate readings ± SEM

DISCUSSION

Over the past few decades, there has been an increase in the demand for ethanol production not only for its use as feedstock for chemical manufacturing but also as a potential alternative source of liquid fuel for automobiles (Ameh *et al.,* 2008). In the present study, yeast strains were isolated from ripe and unripe papaya pulps on Yeast Malt (YM) agar medium, and 4 isolates were obtained. Previous studies have shown that yeast is commonly associated with sugar-rich samples such as leaves, flowers, sweet fruits, tree exudates, grains, roots, insects, animal dung, and soil (Miller *et al.,* 2012; Huang *et al.,* 2015). Due to readily available carbohydrates in synthetic media, it produced more amount of ethanol with both cultures. Apart from synthetic medium, the rate of ethanol production was higher in papaya pulp than in grape. The results of this work have shown that papaya agro waste could serve as raw material for the production of alcohol. The present investigation has revealed the processing of wastes and more production of ethanol by *S. cerevisiae* was in agreement with the reports of Gunasekaran, and Chandraraj, 2009, Akin-Osanaiye *et al.,* 2008 and Obire *et al.,* 2008 where they observed an increase in ethanol production using *S. cerevisiae*. The production of ethanol from papaya fruit is more than the grape pulp (Bhushan *et al.,* 2013; Sharma *et al.,* 2007; Pramanik and Rao, 2005). The result of this study indicated that yeasts, isolated from papaya fruit pulps showed a good fermentation process with agri-waste products. The result of this study agrees with what was reported by Ekumankama *et al.* (1997) and Akin-Osanaiye *et al.* (2008). The pH recorded is within the earlier reported value of 4.5-5.0 (Athms and Flynn, 2012; Monis and Sarad, 1990). In the region outside the optimum pH, the cells of *Saccharomyces cerevisiae* are less tolerant to the environment and hence less active and less efficient in substrate utilization (Ekumankama *et al.* 1997; Akin-Osmaiye *et al.,* 2005). Generally speaking, irrespective of the pH, the optimum fermentation period was observed to be 72 hours.

CONCLUSION AND RECOMMENDATIONS

Due to higher amounts of sugars in ripe papaya pulp, ethanol yield was observed to be higher. Ethanol can be used as a renewable energy source; thus, it can be produced from different agro-wastes successfully. As the global demand for energy increases, bioethanol produced from renewable feedstocks is a valuable and eco-friendly alternative to non-renewable fuels. However, with growing concerns over the global food supply, lignocellulosic non-edible biomass (second-generation bioethanol) and algal sources (third-generation bioethanol) are increasingly attractive feedstocks for bioethanol production. Fermentation efficiency and bioethanol yields are dependent on the feedstock, cultivar, and organisms used. Biotic microbial contamination and abiotic factors like nutrient, trace metal, and vitamin deficiencies must also be addressed to ensure optimum fermentation rate.

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