BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, October 2014. Vol. **11**(Spl. Edn. 1), p. 187-192

Production of Ethanol from Papaya Waste

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doi: http://dx.doi.org/10.13005/bbra/1409

(Received: 15 August 2014; accepted: 10 October 2014)

A study was carried out on yeast fermentation of carica papaya (pawpaw) agricultural waste using driedactive baker's yeast and brewer's yeast strain (Sacchromyces cerevisiae). The pawpaw considered asan agricultural waste was the tapped ripe pawpaw fruit harvested after the tapping of papain. Effect ofdifferent yeast strains on the percentage yield of ethanol was investigated. The effects of yeastconcentration, saccharification and different nutrient supplements as they relate to the optimization ofthe ethanol yield were also carried out. The fermented pawpaw yielded ethanol contents of 3.83 to5.19% (v/v). The reducing sugar in the pawpaw was determined before and after saccharification. The value recorded was 7.6 to 13.6 g/100g. Brewer's yeast gave a higher ethanol yield than baker's yeast. Saccharification for 48 h coupled with nutrient supplements significantly increased the ethanol yield

Key words: Paw-paw agro waste, baker's yeast, brewer's yeast, fermentation, pH, temperature, saccharification.

Bioethanol as a Sustainable Fuel

The conversion of wood or agricultural residue to ethanol and other industrial chemicals is an attractive option for utilizing all major components of biomass to produce a liquid automotive fuel and for environmental remedies.Fruit fermentation results from the action of yeast (Sacchromyces 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 yeast (Sacchromyces cerevisiae) in the fermentation process and they generally influence the percentage yield of the alcohol (Sun Y., *et al.*, 2002).Production of ethanol from Carica papaya agro waste with respect to the effect of saccharification have been studied and reported by Oner, *et al.*,(2005). Large amount of renewable biomass are available for conversion to liquid fuel ethanol. Ethyl alcohol can be made of three main class of abundant, renewable feed starch sources. Likewise, Sugarcane Industrial sites, agricultural low cost fruits, vegetables, starch materials cellulosic materials, agricultural by products of starch industries, such as potato pulp, tapioca pulp, papaya fruit pulp from raw starch hydrolate are attractive resources for economical production of ethanol.An efficient method for conversion of biomass into fuel is by ethanol production because ethanol is an economical as well as environmentally friendly fuel. Ethanol has the advantages of being renewable, cleaner burning and produces no greenhouse gases (Altintas *et al.*, 2002).

Mechanism

Glucose is broken down to form pyruvate

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in most organisms via the glycolytic pathway and this pyruvate can result in the production of ethanol under anaerobic conditions. Ethanol is one of the bioenergy sources with high efficiency and low environmental impact.Worldwide production of ethanol is approximately 51,000 million liters. Fuel encompassed 73% of production ethanol, while beverage and industrial ethanol constitute 17% and 10% respectively (Talebnia F *et al.*, 2008). As fuel enhancer, ethanol has some advantages. Cardona *et al.*, (2007) reported that "As an additive (ethanol), serves as a fuel volume extender, an oxygenate, and an octane enhancer. When blended with gasoline, ethanol increases the octane rating and the oxygen content of the fuel, which results in more complete combustion and reduction in exhaust emission such as carbon monoxide and unburned hydrocarbons."The use of these sugars for producing bioethanol leads to opportunities for farmers by increasing demand for their products, resulting in a boost in rural economics (Olfert *et al*., 2007).

Aim and objective

To study the effect of substrate concentration on ethanol production using Saccharomyces cerevisiae from papaya waste.

To study the effect of pH concentration on ethanol production using Saccharomyces cerevisiae.

To study the effect of Temperature concentration on ethanol production using Saccharomyces cerevisiae.

To study the effect of Time on ethanol production using Saccharomyces cerevisiae.

To study the productivity of ethanol from papaya waste.

To study the effect of Reducing sugar concentration on ethanol production using Saccharomyces cerevisiae.

Phytochemical analysis of papaya waste by Gas chromatography & Mass spectroscopy.

MATERIALS AND METHODS

Micro organism

Saccharomyces cerevisiae.For inoculum preparation,S. cerevisiae is grown in medium composed of 20g/L of glucose(Domini Sugar ,Domino Foods Inc., Yankers,NY),6g/L of yeast extract,0.3g/L of $CaCl.2H_2O$, 4g/L of $(NH_4)_2SO_2$, 1g/L of $MgSO_4.7H_2O$, 1.5g/L KH_2PO_4 at 30^0C for 24H. In order to maintain viability, the culture is stored at 4^0C and sub-cultured biweekly, whereas stockcultures are kept in 20% glycerol at -80^0C for long term storage.

Maintenance of the Cultures

Fungal cultures used for micro biological pre-treatment are maintained on potato dextrose agar. The Yeast used for Fermentation is maintained on MGYP medium (Wickerham's media).

Preparation of Samples

Pawpaw fruits weighing 5.85+0.21kg are thoroughly washed with distilled water before peeling using a sterile knife. The peels are from fruits are collected in sterile plastic containers already labelled for fruit. The weights of the pawpaw fruits after peeling are also recorded as 4.89 +0.93Kg. The peeled fruits of pawpaw are each blended separately using a sterilized automatic juice blender.Clean sterile cheese-cloth is used to sieve the juice from the pulp of each fruit. This is collected in three different sterile plastic containers from where measured pawpaw-251ml) are dispended unto three sets of sterile eight (8) conical flask already labelled according to the dates for each sample analysis. Conical flask is stopper with non-absorbent cotton wool and aluminium foil to ensure they are air-tight as to provide an anaerobic condition. This is repeated for the second and third replication. The pulp of the fruits collected on cheese-cloth is then transferred into three different sterile plastic containers already labelled for each fruits this is repeated for the second and third replication.

Determination of pH of the fruit juice

Samples of the fermented juice of pawpaw are collected at 3 days intervals for pH determination. 10ml of each sample are collected and their pH is determined using a pH meter. Duplicate readings are taken for each sample and the average results are recorded and ethanol production is also recorded.

Analytical methods for bio-ethanol production

Determination of alcohol production from fermented papaya fruit juice using the soxhlet extraction methodcarried out

Preparation of papaya fruit extract

Fermentation

Fermentation is carried out on the sample by using two different yeasts baker's and brewers.

Sample 100g,Distelled water 100ml, pH 4.5, Incubation period 3days at 30^0C, Simultaneous saccharification and fermentation is also carried out for the period of 72hrs.

Analytical methods

The pH of the syrup is determined and adjusted to 4.5 with 0.5 M NaOH. Saacharification is also carried out on the sample using Yeast for the period of 72h and the total reducing sugar is determined using DNS method. The values are determined after 24h, 48h and 72h of saccharification.

RESULTS AND DISCUSSION

The values of the reducing sugars obtained from the papaya in this study are shown in Table 1. The fresh pawpaw has the lowest value (7.6g/100g) while the value significantly increased to 13.6g/100g after 48h of saccharification. The ethanol yield when brewer's yeast is used is significantly higher than when baker's yeast is used. The percentage yield is increased when the concentration of the yeast is increased to 3.56 x 10 6 yeast cell/10ml and 10% respectively. This observation is consistent with the report of Morris and Saradd (1990), which indicated that the amount of yeast influenced ethanol production.Table 2 recorded the alcohol yield from the simultaneous saccharification and fermentation of the pawpaw. It is noted that the percentage yield of the ethanol increased significantly ($P< 0.05$) after 24hr saccharification in both yeast strains. Also when different treatments are carried out in the pawpaw (Table 6). The highest ethanol yield is recorded when brewer's yeast is used for the fermentation after 48hr of saccharification with different yeast nutrient supplements.The ethanol yield increased significantly to a value of 5.19% (v/v). The results of this work have shown that pawpaw could serve as raw material for the production of biofuel, alcohol. The findings of this work recommended that alcohol can be produced from pawpaw obtained as papain by-product to maximize profit evident that the fruit waste is very high in carbohydrate, which includes crude fibre and total reducing sugar present in the pawpaw fruit waste. This high carbohydrate content shows that the pawpaw fruit waste be a good source for ethanol production.The pH of the substance (4.3) is lower than the recommended pH range of 4.5-5.0 for optimum ethanol yield (Morris and Sarad, 1990; Adams and Flynn, 1982). The reducing sugar assay of the pawpaw waste gave 7.6g/100g of the sample which shows that not a carbohydrate in the sample is available as fermentable sugar. The

Table 1. Effect of saccharification on the amount of reducing Sugar in the pawpaw

Time (hr)	Amount of reducing sugar (g/100G)
T(0)	7.7
T(24)	8.6
T(48)	13.8
T(72)	9.5
T(96)	8.0

Table 2. Effect of saccharification and simultaneous fermentation on the percentage ethanol yield of the pawpaw

Time (hr)	Ethanol yield (%)	
	Baker's yeast	Brewer's yeast
0	4.5	4.6
24	4.8	5.4
48	5.5	5.8
72	4.9	4.5

Table 3. Effect of different pH on the ethanol production

pH	Ethanol production g/l
5	16.10
6	16.2
7	15.7
8	12.0
9	10.1

Table 4. Effect of different time periods on the ethanol production

Time (hrs)	Ethanol production g/l
10	6.5
20	7.0
30	12.5
40	14.0
50	15.4

Table 5. Effect of different Temperature on the ethanol production

Temperature	Ethanol production g/l
30	16
35	15.8
40	14
45	12
50	7.4

Table 6. Effect of different treatments of the pawpaw on the ethanol yield (v/v)

Temperature	Ethanol production g/l
A	5.4
B	6.8
C	5.0
D	5.2
E	7.0
F	5.6
G	6.3
H	6.0

A = Substrate + Brewer's yeast (3.56 x 106 yeast cell) without nutrient supplement.
B = Substrate + Brewer's yeast (3.56 x 106 yeast cell) + nutrient supplement listed in Table 2.
C = Substrate + 10g Baker's yeast, without nutrient supplements.
D = Substrate + 10g Baker's yeast + nutrient supplement.
E = Substrate + yeast (mixed culture) at 0 hour + Brewer's yeast + nutrient supplement.
F = Substrate +10g Baker's yeast + Aspergillus (1.09 x 106 yeast cell/5 ml).
G = Substrate + yeast (1.09 x 106 yeast cell/5ml) + Brewer's yeast (3.56 x 106 yeast cell/10ml) after 48h of saccharification.
H = Substrate + Yeast (1.09 x 106yeast cell) + 10g Baker's yeast after 48h of saccharification

ethanol yield obtained from the substrate when fermented at it initial pH and room temperature (27Â±3Â^{0}C) different duration of fermentation and different yeast concentrations. From these results, it is observed that ethanol can be produced from the pawpaw waste even at its initial pH 4.3 and that there was no significant difference (pâ%¥0.05) even when the pH was adjusted to 4.5. Also, the yield was increased when the concentration of the yeast was increased.The results recorded from the determination of the optimum condition for the production of maximum ethanol from these results. It was observed that the optimum pH for alcohol production using pawpaw waste 4.5. The result in this study agrees with what was reported by Ekumankama *et al.* (1997). This pH recorded is within the earlier reported value of 4.5-5.5 (Adams and Flynn,1982; Morris 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 utilisation (Ekumankama *et al*., 1997). Generally, irrespective of their pH, optimum fermentation period was 72 hours. It was also observed that there was increase in percentage alcohol yield with increase in yeast concentration up to the 10% (10 g/100g). This observation agrees with the report of Adams and Flynn (1982) that the yeast concentration in the fermentation should be between 5-10% of the total volume of the substrate.It is interesting to note that when some nutrient supplements were used, the ethanol yield was generally improved. It is equally observed that significant increase can only be achieved when these nutrient supplements are combined (combined[a]). Meanwhile, the yields recorded were

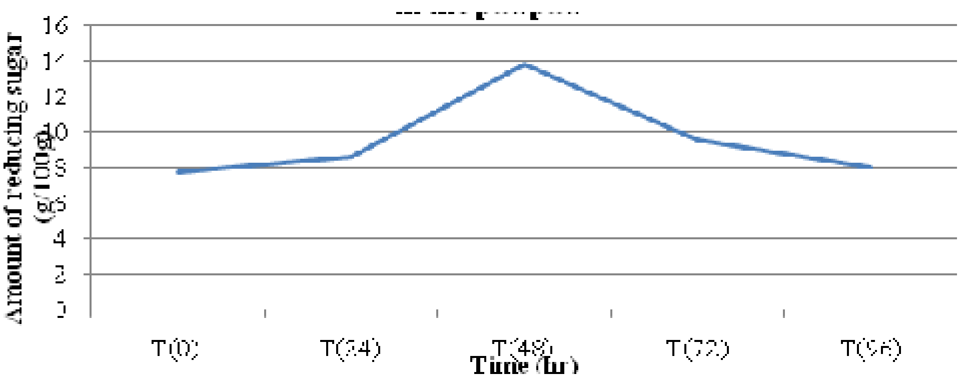

Fig. 1. Effect of saccharification on the amount of reducing Sugar in the pawpaw

combined[b] and combined[c] were used where significantly ($p<0.05$) increased than the one obtained from combined[a]. This may be as a result of the glucose and peptone added to these two combined nutrients and therefore the Saccharomyces cerevisiae prefers glucose for its utilisation to ethanol than the substrate. However, several workers have added glucose or sucrose to

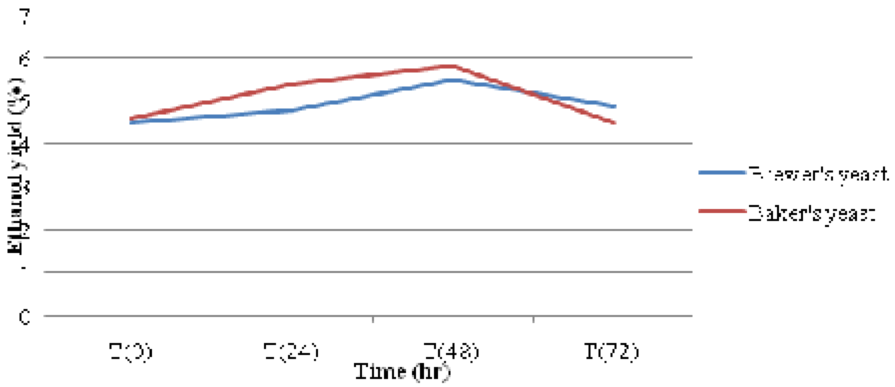

Fig. 2. Effect of saccharification and simultaneous fermentation on the percentage ethanol yield of the pawpaw

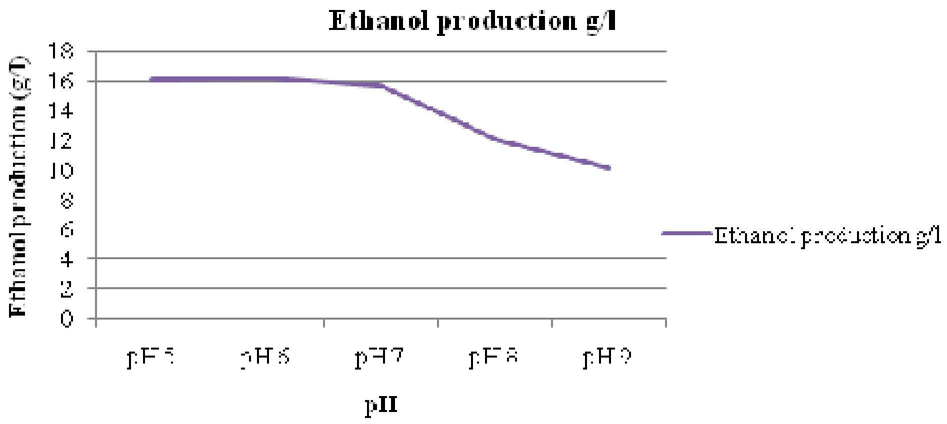

Fig. 3.

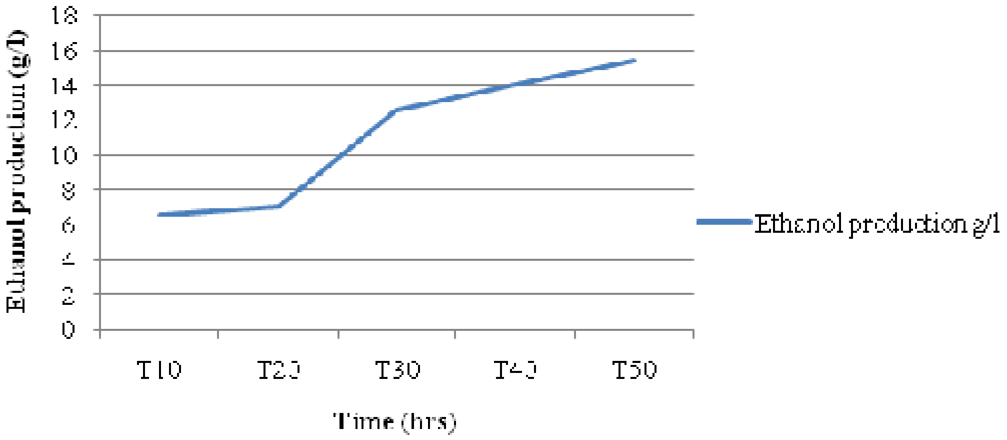

Fig. 4. Effect pf different time periods on the ethanol production

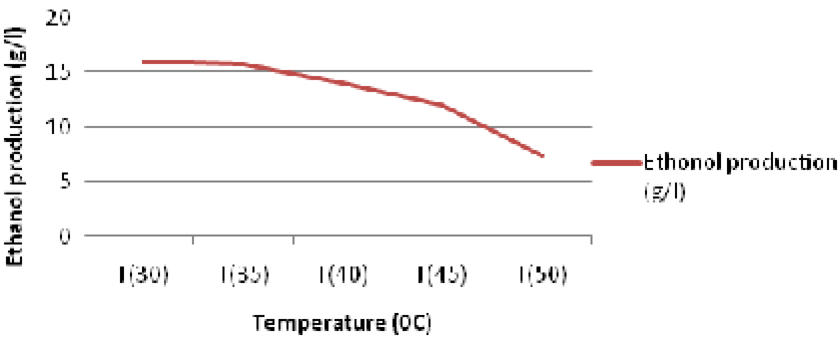

Fig. 5. Effect of different temperature on the ethanol production

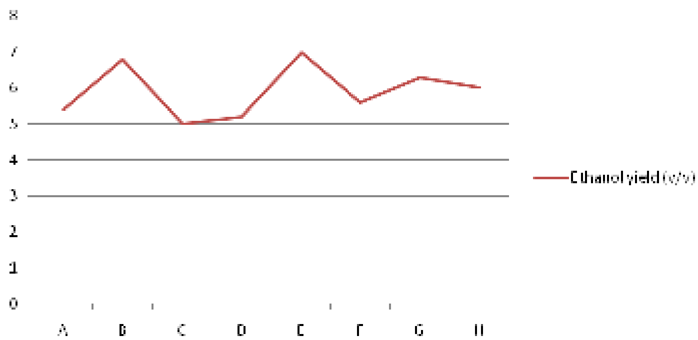

Fig. 6. Effect of different treatments of the pawpaw on the ethanol yield (v/v)

their substrate to improve the ethanol yield from such substrates (Gilson and Thomas, 1995; De *et al.*, 1999).

CONCLUSION

The result of this study show that the rate of alcohol production through fermentation of pawpaw wastes by bawker's yeast (*Sacchromyces cerevisiae*) increases with fermentation time and peaked at 72h. It is also increased with yeast concentration at the temperature of 30^{0}C. The optimum pH for fermentation is 4.5. The findings of this work suggest that alcohol can be produced from pawpaw waste obtained as papain by-product.To be concluded that the ethanol produced in this process can be scaled up industrially after detailed statistical studies and suggest that papaya waste can be used for various purposes like bio fuels and many therapeutic applications like antimicrobial, hepatoprotective, antidiabetic and antioxidant activity.

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