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Ethanol Production from *Carica papaya* (Pawpaw) Fruit Waste

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Abstract: The production of ethanol from *Carica papaya* (pawpaw) agricultural waste, using dried active baker's yeast strain (*Sacchromyces cerevisiae*) was investigated. The pawpaw fruit considered to be an agricultural waste was the tapped ripe pawpaw fruit harvested after the tapping of papain. The proximate composition, pH and the reducing sugar of the pawpaw fruit were determined. Effects of yeast concentration, duration of fermentation, pH, temperature and different yeast supplements as they relate to the optimization of the ethanol production were investigated. The fermented pawpaw fruit waste produced ethanol contents 2.82-6.60% (v/v). Proximate analyses of the dry fruit showed that pawpaw waste contain 90.82 g/100 g carbohydrate, 2.60 g/100 g lipid, 1.63 g/100 g crude protein, 4.95 g/100 g ash. The results of this work show that the rate of alcohol production through fermentation of pawpaw fruit waste by baker's yeast (*Sacchromyces cerevisiae*) increases with fermentation time and peaked at 72 h. It is also increased with yeast concentration at the temperature of 30°C. The optimum pH for fermentation is 4.5.

Key words: Pawpaw waste, baker's yeast, fermentation, pH, temperature, proximate, nutrient supplements

INTRODUCTION

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 remediation (Saddler, 1993). The increase in the use and need for ethanol as a universal energy source has stimulated worldwide investigation, not only with respect to high yielding alcohol production yeast strains, but also to cheaper available raw materials.

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 (Nzelibe *et al*., 2001).

Pawpaw (*Carica papaya*) fruit is believed to principally contain invert sugar with only traces of sucrose being present (Greenway, 1953). Ogbonna and Asiegbu (1990) reported the fermentation of pawpaw fruit for alcohol production to determine the effect of yeast fermentation on the nutrient contents of this fruit. However they do not report the alcohol yield after the fermentation. Production of ethanol from *Carica papaya* agro waste with respect to the effect of saccharification have been studied and reported by Akin-Osanaiye *et al*. (2005).

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Large quantities of pawpaw fruit wastes are available from plantations cultivated for papain production and their disposal can be a problem. The fruits are usually unsuitable for marketing fresh, due to the scarring and the taste is bitter (Akin-Osanaiye, BC personal comm.) compared to the fresh pawpaw fruit from a non-tapped pawpaw tree because of its been force-ripened. This work is therefore aimed at processing the pawpaw fruit waste in which papain has been tapped into alcohol which will have industrial application and maximize profit for the purpose of papain production business.

MATERIALS AND METHODS

Experimental Materials

Carica papaya (pawpaw) fruits in which papain (latex) has been tapped were obtained from National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria papain experimental garden. This was kept in a cupboard for 3 days to ripe and soften. The ripe fruit was peeled and the seeds removed. One kilogram of this prepared sample was pulverized using National blender and then packed in a plastic container. This was then stored in a freezer at 4°C pending analysis.

Chemicals and Reagents

Chemicals and reagents were of analytical grade and products of SIGMA Chemical Company Limited, USA and British Drug Houses (BDH) Limited, Poole, England. Dried bakers yeast (*Sacchromyces cerevisiae*) is the product of GYMA ZI. Le Terradou 84200 CARPENTRAS, TEWEX 431184, France and was purchased from Sabon Gari market, Zaria, Nigeria.

Analytical Methods

Analyses for the proximate composition of the fruit were carried out using AOAC (1980) methods. The pH of the syrup was determined using, EIL 7020 Kent pH meter. The total reducing sugar was determined using dinitrosalicylic (DNS) method described by Miller (1959) with slight modification.

Fermentation

One hundred gram portion of the slurry (pulverized sample) was weighed into 250 cm^3 conical flask and 100 cm^3 distilled water was added. This was pasteurized by boiling in a water bath for 15 min. It was allowed to cool and the inoculums added. The fermentation was then monitored from day 1 (24 h) to day 14. Different concentrations of the yeast were used and the pH of sample was also adjusted with 0.5M NaOH, between 4.2-5.0, at the end of the fermentation, the fermented sample was poured into a cheese cloth to drain out the fermented broth. This procedure was carried out in triplicates.

Analysis of the Percentage Alcohol Produced

The alcohol content was determined as described by AOAC (1974). With the aid of the specific gravity values of the distillate the percentage alcohol was determined using a standard alcohol density table (AOAC, 1974). The overall distillate obtained was then pooled together and rectified. The percentage purity of the rectified (distillate collected at 78-80°C) was determined.

RESULTS AND DISCUSSION

Results in Table 1 and 2 represent the values obtained from the analyses of the pawpaw waste. It is 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 may be a good source for ethanol production.

The pH of the substrate (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 7.6 g/100 g of the sample which shows that not all the carbohydrate in the sample is available as fermentable sugar.

The ethanol yield obtained from the substrate when fermented at its initial pH and room temperature (27±3°C) different duration of fermentation and different yeast concentrations are shown in Table 3. 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 ($p \geq 0.05$) even when the pH was adjusted to 4.5 (Fig. 1-4). Also, the yield was increased when the concentration of the yeast was increased.

Figure 1-4 show 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 is 4.5. The result in this study agrees with what was reported by

Table 1: Proximate composition of pawpaw fruit waste (n = 3, ±SD)

Parameters	g/100 g
Lipid extracts	2.60±0.31
Protein	1.63±0.08
Ash content	4.95±0.07
Carbohydrate	90.82±0.23

Values are means of triplicate experiments

Table 2: Physical and chemical properties of the fruit waste (n = 3, ±SD)

Parameters	g/100 g
Moisture content (wet/weight) (g/100 g)	83.85±0.15
pH	4.30
Reducing sugar (g/100 g)	7.60±0.03
*Alcohol content (% v/v)	3.02±0.05
+Percentage purity of alcohol (%)	88.16±0.12

*: Fermentation of the waste using 1% yeast for 7 days at its initial pH 4.3 and room temperature (27±3°C). +: Percentage purity of the overall alcohol produced after rectification

Table 3: Ethanol yield (%) at different duration of fermentation and different yeast concentrations of pawpaw fruit waste at pH 4.3*

	Ethanol yield (%v\v)			
Yeast concentration	Days			
(%)	1	3	7	14
1	2.64±0.01	3.04±0.08	3.02±0.05	2.82±0.04
2	2.88±0.01	3.30±0.03	3.20±0.07	2.84±0.10
5	3.02±0.03	3.28±0.01	3.08±0.02	2.93±0.04
10	3.06±0.02	3.34±0.03	3.12±0.03	3.02±0.05

Results are means of duplicate experiments. Temp. = Ambient temperature (27±3°C). *:The initial pH of the sample

Table 4: Effect of various nutrient supplements on percentage ethanol yield (v/v) from pawpaw waste

Yeast supplement (0.1 g/100 g)[1]	Ethanol yield
Yeast only	3.31±0.02
$(NH_4)_2SO_4$	3.33±0.03
$CaCl_2$	3.32±0.05
KH_2PO_4	3.36±0.04
Combined nutrient[a]	3.47±0.10
Combined nutrient[b]	6.40±0.10
Combined nutrient[c]	6.60±0.04

Values are means of duplicate experiments [1]: All concentrations were finals in 100 g of medium. a: Combined-made up of 0.1 g each of $(NH_4)_2SO_4$, $CaCl_2$ and KH_2PO_4. b: Comprise-0.1 g KH_2PO_4, 0.1 g NaCl, 0.4 g $(NH_4)_2SO_4$, 0.56 g $CaCl_2$,2.0 g glucose, 1 g peptone (Gilson and Thomas, 1995). C: Comprise: 0.1 g $(NH_4)_2SO_4$, 0.1 g KH_2PO_4, 2 g glucose, 0.6 g peptone (Ameh *et al.*, 1988)

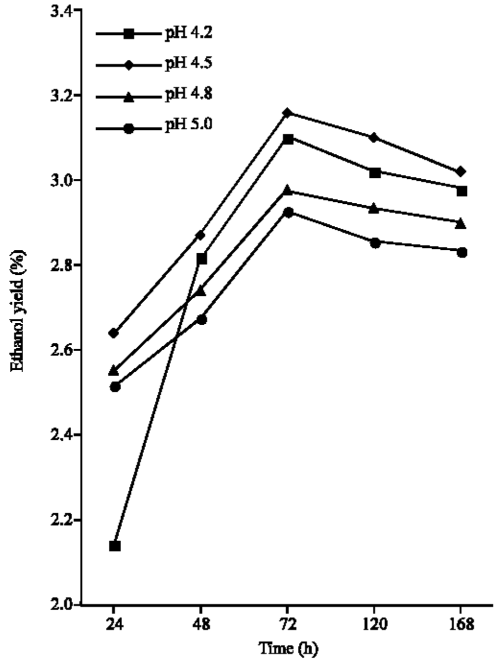

Fig. 1: Effect of pH and duration on % ethanol yield (v/v) from pawpaw fermentation using 1% (w/w) dried active Baker's yeast at 30°C

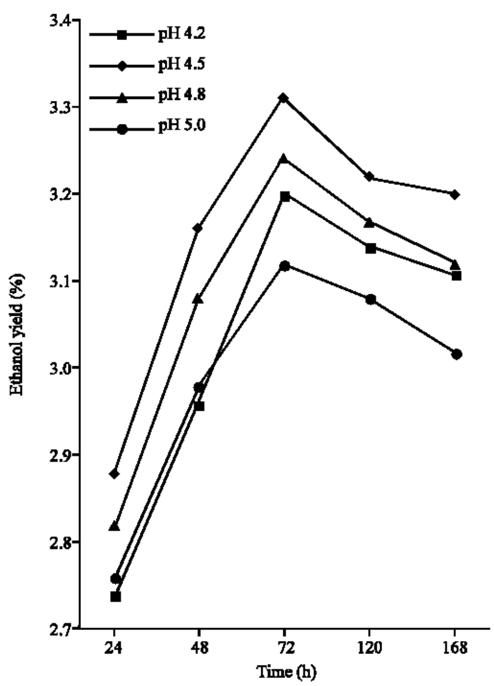

Fig. 2: Effect of pH and duration on % ethanol yield (v/v) from pawpaw fermentation using 2% (w/w) dried active Baker's yeast at 30°C

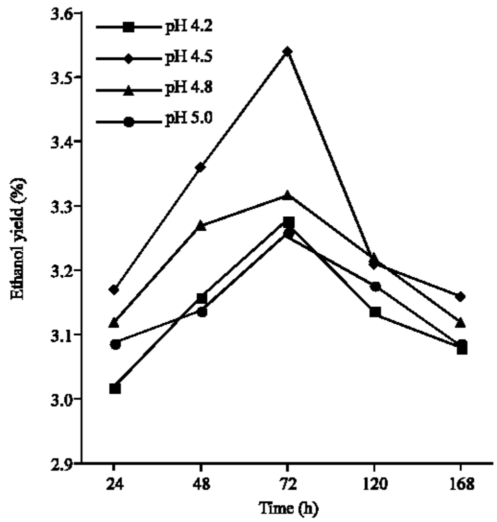

Fig. 3: Effect of pH and duration on % ethanol yield (v/v) from pawpaw fermentation using 5% (w/w) dried active Baker's yeast at 30°C

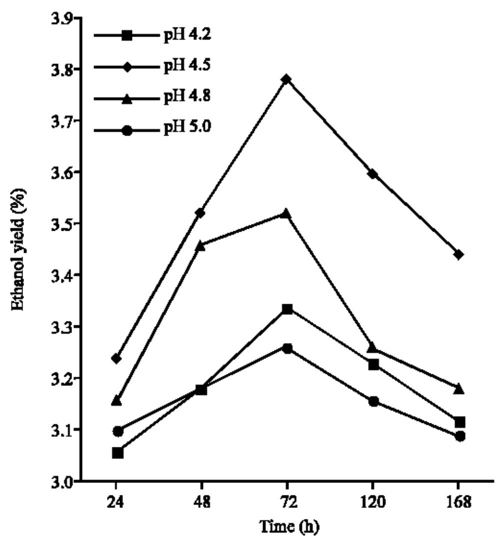

Fig. 4: Effect of pH and duration on% ethanol yield (v/v) from pawpaw fermentation using 10% (w/w) dried active Baker's yeast at 30°C

Ekumankama *et al*. (1997). This pH recorded is within the earlier reported value of 4.5-5.0 (Adams and Flynn, 1982; Morris and Sarad, 1990). In the region outside the optimum pH, the cells of *Sacchromyces cerevisiae* are less tolerant to the environment and hence less active and less efficient in substrate utilization (Ekumankama *et al*., 1997).

Generally, irrespective of the pH, optimum fermentation period was 72 h (Fig. 1-4). It was also observed that there was increase in percentage alcohol yield with increase in yeast concentration up to the 10% (10 g/100 g). 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 (Table 4). It is equally observed that significant increase can only be achieved when these nutrient supplements are combined (combined[a]). Meanwhile, the yields recorded when combined[b] and combined[c] were used were 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 *Sacchromyces cerevisiae* prefers glucose for its utilization to ethanol than the substrate. However, several workers have added glucose or sucrose to their substrates to improve the ethanol yield from such substrates (Gilson and Thomas, 1995; De *et al*., 1999).

CONCLUSION

The results of this study show that the rate of alcohol production through fermentation of pawpaw waste by baker's yeast (*Sacchromyces cerevisiae*) increases with fermentation time and peaked at 72 h. It is also increased with yeast concentration at the temperature of 30°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.

REFERENCES

Adams, M.R. and G. Flynn, 1982. Fermentation ethanol: An industrial profile. Tropical Products Institute, London, pp: 1-19.

Akin-Osanaiye, B.C., H.C. Nzelibe and A.S. Agbaji, 2005. Production of ethanol from *Carica papaya* (pawpaw) agro waste: Effect of saccharification and different treatments on ethanol yield. Afr. J. Biotechnol., 4: 657-659.

Ameh, J.B., R.N. Okagbue, A.A. Ahmadu and C.O. Ikediobi, 1988. Ethanol production from corn-cob wastes and grass-straw. Nigerian J. Biotechnol., 6: 110-112.

AOAC, 1974, 1980. Official Methods of Analysis, Horwits, W. (Ed.). 13th Edn. Association of Official Analytical Chemist, Washington DC.

De, N.B., P.E. Okonofua and A.E. Uwabujo, 1999. Production of Alcohol using the Pulp of *Parkia biglobosa* (Locust Beans). Proc. Biotechnol. Soc. Nig., pp: 48-51.

Ekumankama, E.O., C. Onwuchekwa and O.D. Onukwuli, 1997. Fermentation kinetics of *Carica papaya* using *Sacchromyces cerevisiae*. Conf. Proc. Chem. Eng. Vision 2010, pp: 26-35.

Gilson, C.D. and A. Thomas, 1995. J. Chem. Tech. Biotechnol., 62: 227-232. Cited in combined use of three methods for high concentration ethanol production by *Sacchromyces cerevisiae* by Xu *et al*., 1996. Biotechnol. Lett., 18: 1439-1440.

Greenway, P.J., 1953. The Pawpaw. Its Botany, Cultivation, Diseases and Chemistry. Printed by the Government Printer Dar Es Salaam, pp: 1-10.

Miller, G.C., 1959. Use of the dinitrosalicylic acid reagent for the determination of reducing sugar. Anal. Chem., 31: 420-428.

Morris, W. and R.P. Sarad, 1990. Biotechnology of Biomass Conversion: Fuels and Chemicals from Renewable Resources. Open University Press, Milton Keynes, pp: 235

Nzelibe, H.C., H.A. Salihu and C.M.Z. Whong, 2001. Production and evaluation of wines from English plum and two varieties of grapevine grown in Zaria. J. Trop. Biosci., 1: 124-127.

Ogbonna, C.I.C. and E.O. Asiegbu, 1990. The effects of yeast fermentations on the nutrient contents of *Canica papaya* L. (Pawpaw) and *Olax latifolia* L. (Tsocla). Nigerian J. Biotechnol., 7: 165-167.

Saddler, J.N., 1983. Steam Pretreatment of Lignocellulosic Residue. In: Bioconversion of Forest and Agricultural Plant Residues, Saddler, J.N. (Ed.). A.B. International Wallinford, United Kingdom, pp: 73-92.