



## Fermentation of Pretreated Hydrolyzates of Banana and Mango Fruit Wastes for Ethanol Production

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### ABSTRACT

Laboratory experiments were conducted to evaluate the chemical composition of fruit wastes (pulp and peels) of Banana and Mango in order to explore their potential application in bio-ethanol production. The proximate composition of banana fruit pulp was 76.63% moisture, 5.65% protein, 1.37% lipid, 19.75% ash and 0.632% starch. Similarly for mango, the proximate composition of fruit pulp was 81.26% moisture, 7.96% protein, 1.48% lipid, 13.08% ash and 0.507% starch. The total dietary fibre content ranged from 3.54% to 73.04% in the fruit samples and found at higher level in mango peels. A maximum polyphenol content of 54.45% was observed in mango fruit peels and a minimum of 10.97% was recorded in banana fruit pulp. The dilute acid (H<sub>2</sub>SO<sub>4</sub>) pretreatment (DAP) followed by enzymatic hydrolysis showed maximum reducing sugar yield of 64.27% in the mixed fruit pulps, followed by the banana fruit pulp (57.58%). The banana fruit peels also yielded a maximum reducing sugar content of 36.67% where as the lowest of 31.29% was observed in mango fruit peels. The fermentation of the DAP hydrolysate of mixed fruit pulps showed maximum ethanol production of 35.86% corresponding to a fermentation efficiency of 70.31% at 48 hr of incubation. Similarly, the hydrolysates obtained from the dilute H<sub>2</sub>SO<sub>4</sub> pretreated banana fruit peels yielded a maximum of 13.84% ethanol with a fermentation efficiency of 27.13% at 42 h of incubation. The present study revealed that the fermentation of hydrolysates obtained from the dilute acid pretreatment followed by enzymatic saccharification of mixed fruit pulps (banana and mango) and the banana fruit peels were found to be best for higher ethanol production at optimized conditions.

**KEYWORDS:** Fruit wastes, bioethanol, reducing sugars, saccharification, fermentation

### INTRODUCTION

Fuel ethanol production has been fascinated now, because many countries look for reducing oil imports, boosting rural economies and improving air quality. The world ethanol production has reached about 51,000 million liters [1], being the USA and Brazil the first producers and India stands fourth among the top fuel ethanol producers. Since it is estimated that the fossil fuels will be running out by the next few decades, attention has currently been dedicated to the conversion of biomass into fuel ethanol. Main feedstocks for bioethanol production are sugarcane (in Brazil) and corn grains (in USA), while many other agricultural raw materials are also used worldwide. Among the three major types of raw materials, the production of ethanol from sugary and starchy materials are easier as compared to lignocellulosic materials since it requires additional technical challenges such as pretreatment [2]. Further more, the use of high technology and complicated instrumentation methods with high operating costs may in turn limit their commercialization and industrial application in the developing countries [3]. Research efforts are focused to design and improve a process, which would produce a sustainable transportation fuel using low cost feed stocks. Many agricultural raw materials rich in fermentable carbohydrates were tested worldwide for bioconversion from sugar to ethanol, but the cost of carbohydrate raw materials has become a limiting factor for large scale production by the

industries employing fermentation processes. Since the price of feedstock contributes more than 55% to the production cost, inexpensive feedstocks such as lignocellulosic biomass and agri-food wastes, are being considered to make bioethanol competitive in the open market [4]. The production of ethanol from comparatively cheaper source of raw materials using efficient fermentative microorganism is the only possible way to meet the great demand for ethanol in the present situation of energy crisis [5]. The ripen fruit biomass as raw materials for fermentation, enzymatic hydrolysis using microbial enzymes could be a possible solution to reduce the energy and input costs in ethanol production [6]. Among the fruit crops, banana occupies the fourth world rank and mango is at the fifth rank of the most significant foodstuffs after rice, corn and milk. These pulpy fruits are more prone to spoilage due to their nature and this spoilage occurs at the time of harvesting, storage, marketing and processing resulting in wastes. According to India Agricultural Research Data Book 2004, the estimated fruit and vegetable production in India was 150 million tones and the total waste generated was 50 million tones. The extent of total losses in these commodities is approximately estimated as 20-30% of the total production, amounting to a loss of Rs. 30,000 crore per annum. According to FAO [7], the total waste generated from fruits was estimated as 3.36 million tones (MT) out of the total production of 16.8 MT and particularly for banana it was 6.4 MT. The failure or inability to salvage and reuse such materials economically results in the unnecessary waste and depletion of natural resources [8]. The solid wastes generated by fruit processing industries can serve as potential raw materials for the production of secondary metabolites of industrial significance by microorganisms. Peels are the major by-products obtained during the processing of various fruits and these were shown to be a good source of various bioactive compounds which possess various beneficial effects. But, significant quantities of fruit peels (20-30% for banana and 30-50% for mango) are discarded as waste by the processing industries which cause a real environmental problem [9]. These fruit-processing wastes can be used as potential feedstock for bioethanol production and this could also be an attractive alternate for disposal of the polluting residues [10]. Some few research articles deal with different practical applications of these fruit wastes (banana and mango), e.g., production of microbial enzymes for industrial uses [8], production of alcohol [6], production of wine, vinegar, production of biogas [11] and food for livestock [12]. The amount of reviews covering ethanol production from other types of feedstocks like sucrose-based or starchy materials is more reduced. However, little effort has been made on ethanol production from pretreated enzyme saccharified fruit wastes by simple fermentation techniques. Hence, the present study was framed to determine the effect of acid pre-treatment, enzymatic saccharification by microbial enzymes and further ethanol fermentation of the obtained hydrolyzates from banana and mango fruit biomass by *Saccharomyces cerevisiae* yeast. The proximate composition of fruit pulp and peels of Banana and Mango have also been studied in order to explore their potential application in bio-ethanol production.

## MATERIALS AND METHODS

### Preparation of plant materials

Fully ripened un-marketed fruit samples of banana (B) and mango (M) were collected from the local wholesale fruit market. The banana variety taken for the present study was *Robusta* which is a high yielding cultivar with poor keeping quality. The mango variety used for the present study was *Alphonsa* (King of Mango), commonly cultivated in a large area for commercial production of juices, jams and wines. The fresh plant materials were brought to the laboratory, washed and separated into pulp and peels. The fruit pulps were homogenized by using simple wet milling technique without adding water and the pulverized puree was taken for further experimental studies. A mixed fruit pulp sample was also prepared by mixing both the fruits in equal ratio (B1:M1 w/w). The fresh ripened banana and mango fruit peels were chopped into small pieces (2-3 cm) and dried in a hot air oven at 65°C for 24 h. The dried materials were ground in a Wiley mill to pass through a 1 mm screen and the powdered materials were stored at room temperature for further analysis. A composite sample of both the fruit peels was also prepared in the same manner.

### Chemical analysis

The ash content was estimated according to the methods described by USDOE [13] by heating the residue of moisture determination at 550°C for 24 h and the moisture content was determined by drying the sample to a constant weight at 105°C. The crude protein content was determined by the standard Kjeldahl method [14] and converted using a nitrogen factor of 6.25. The acetone extracts of the fruit samples were analysed for polyphenol content using the method of Swain and Hillis [15] and the starch content was determined by using the iodometric method of Hassid and Neufield [16]. The total lipid content was determined by Soxhlet extraction method. The dietary fiber content was estimated by an enzymatic gravimetric method in which, the homogenized fruit samples (0.5 g in 20 ml Na<sub>2</sub>PO<sub>4</sub> buffer)

were subjected to enzymatic digestion by amylase, protease and amyloglucosidase. The insoluble dietary fiber (IDF) fractions were separated by filtration and the filtrate was subjected to alcohol precipitation to obtain soluble dietary fiber (SDF). The fractions were dried, weighed and expressed as % DM (w/w) [17].

#### Microbial enzyme production and extraction

The thermoresistant culture strains of *Bacillus subtilis* (NCIM 2655) and *Aspergillus niger* (NCIM 616) were procured from the National Chemical Laboratory, Pune, India and used as the microbial enzyme sources for  $\alpha$ -amylase and glucoamylase, respectively. The production medium used for  $\alpha$ -amylase contained (% w/v) starch 0.5, peptone 2, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.1 and K<sub>2</sub>HPO<sub>4</sub> 0.3 in 250ml Erlenmeyer flasks. The medium was inoculated with 1 ml of inoculum collected from 48 h grown culture of *Bacillus subtilis* on TSA slants at 35±2°C [18]. The medium for glucoamylase production comprised (% w/v): starch 1, peptone 0.1, malt extract 0.1, yeast extract 0.2, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.4, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.1, FeSO<sub>4</sub>.7H<sub>2</sub>O 0.001, K<sub>2</sub>HPO<sub>4</sub> 0.2 and KHPO<sub>4</sub> 0.06. The medium was inoculated with 1 ml of inoculum collected from 72 h grown culture of *Aspergillus niger* on PDA slants at 30±2°C [19]. Inoculated production media were incubated under static conditions at 35±2°C for *Bacillus* sp for 3 d and at 30±2°C for *Aspergillus* sp for 5 d and enzymes production were checked at 24 h interval using starch as substrate. Enzymes were extracted using 50 ml sodium acetate buffer (0.1M) on a rotary shaker at 250 rpm for 30 min. The content was filtered through muslin cloth and the filtrate was used as the enzyme source.

#### Pretreatment methods

##### Liquid hot water treatment (LHW)

The fresh pulp puree and milled peels (B, M, B1:M1) were slurried with distilled water using a solid to liquid ratio of 10% (w/w) and autoclaved at 121°C, 15 lbs for 15 min. The thermal pretreated samples were cooled to room temperature and used for further analysis.

##### Dilute acid pretreatment (DAP)

The dilute acid pretreatment (DAP) was performed in a similar manner as described in Section 2.4.1 using dilute H<sub>2</sub>SO<sub>4</sub> (0.05N). The acid pretreated samples were cooled and the pH of the hydrolysate was adjusted to 6 with 10 N NaOH. The reducing sugar content in the hydrolysates was determined by DNS method [20].

##### Enzymatic saccharification

The hydrolysates obtained from the pretreated (banana, mango and mixed fruits) pulps and peels were subjected to a two step enzymatic saccharification. The crude enzyme filtrate of  $\alpha$ -amylase obtained from *Bacillus subtilis* (2% v/v) was added to the acid pretreated hydrolysates and the mixture was heated to 93°C for 1h. The mixture was then cooled to 60°C and the glucoamylase enzyme filtrate obtained from *Aspergillus niger* (2% v/v) was added. The concentration of the microbial enzymes (2% v/v) was taken on the basis of the maximum productivity of enzymes (U ml<sup>-1</sup>) in the crude filtrate tested within the incubation period (3 – 5 d). The temperature of the reaction mixture for saccharification by glucoamylase was maintained at 60°C for 1h in order to facilitate the enzymatic catalysis. The hydrolysis was performed in flasks in thermostated water both with shaking (150 rpm). Finally, insoluble residues were removed by filtration, and the clear hydrolysates were used for further fermentation studies. The reducing sugar content in the enzymatic hydrolysates was determined using DNS method [20] to find out the net yield of fermentable sugars.

##### Fermentation and ethanol production

The fermentation studies were carried out using baker's yeast (*Saccharomyces cerevisiae*) in the hydrolysates obtained from pretreated, enzymatic hydrolyzed fruit pulps and peels. The hydrolysates were autoclaved at 121°C for 15 min and the flasks were then cooled to room temperature. The pH of the fermentation medium was adjusted to 6 and the yeast was added at a rate of 1g yeast/ 1000ml of mixture. In order to study the effect of enzymatic hydrolysis on ethanol yield in fruit pulps, a separate set of fermentation experiment was carried out in a similar manner using the hydrolysates obtained from both the pretreatments (LHW & DAP) (with out enzymatic saccharification). Fermentation was allowed for 3 days (72 h) at 35°C and samples from the medium were withdrawn periodically at 6h interval from the replicated fermentar flasks to determine yeast cell growth, ethanol productivity and residual sugar content. The ethanol concentration was determined based on the density of alcohol distillate at 20°C and expressed in weight % (w/w) [21]. The fermentation efficiency is calculated using the following formula [22]

$$FE (\%) = \frac{\text{Ethanol yield obtained}}{\text{Theoretical maximum ethanol yield from sugar}} \times 100$$

The yeast cell growth was determined gravimetrically. The cells were separated by centrifuging the samples at 14000 rpm for 5 min, repeatedly washed with distilled water. The separated cell mass was weighed after dried at 80°C 120°C [5].

**Statistical analysis**

The experimental design was completely randomized, with three replicates. All data were expressed as mean values ± SE. The comparison between the mean values were tested using Duncan's new multiple range test and the ANOVA was also performed to find out the LSD ( $p < 0.05$ ) using Number Crunch Statistical Software (NCSS, 2000).

**RESULTS AND DISCUSSION**

**Proximate composition**

The proximate composition of the ripened fruit pulp and peels of mango and banana is shown in Table 1. The highest moisture content of 82.3% was observed in mango fruit pulp and the lowest was in mango peels (60.8%) and in banana, the moisture content was 74.8% in pulp and 66.8% in peels. It clearly appears that the mango has higher dry matter than banana. The dry matter (DM) ranged from 10.97% to 26.89% in fruit samples and it was found to be high in banana fruit pulps. The overall DM showed comparatively high values in pulps than peels. This might be due to an increase in water content of the pulp, derived from carbohydrates utilized during breathing and osmotic transfer from the peel to pulp due to rapid increase in the sugar content in the pulp [23]. The starch content ranged from 0.507% to 0.632% in the fruit pulps and from 1.074 to 1.706 % in fruit peels of mango and banana, respectively. Several authors have reported that the degradation of starch to free sugars during the ripening process due to combined action of several enzymes [23, 24]. A considerable decline in starch content from 20-23 to less than 1% and increase in soluble sugar from less than 1% to 20 % was observed by Forster et al. [25] in fruit pulps during ripening and the degradation of starch reserve in fruit pulps appears to be relatively rapid where as in peels the conversion is rather gradual. Lima et al. [26] have reported that even though starch is the main carbohydrate present in the mature green mango fruit, but as the fruit becomes over-ripe, only traces of starch can be detected. The banana fruit pulp has showed highest ash content of 19.75% and the mango pulp has the lowest of 13.08%. The observed results in the present study on ash content are in agreement with the literature findings of Hammond et al. [6] in the dessert banana peels.

The crude protein content ranged from 5.65 to 7.65% in banana and 4.27% to 7.96% in mango fruit biomass (Table 1). Essien et al. [8] have reported 7.8% protein content in banana peels has good agreement with our results. According to Esseien et al. [8], the high protein content and carbohydrate content of these fruits could serve as the main source for fermentative ethanogenic microbial growth. The highest lipid content of 6.5% was observed in banana fruit pulps and the lowest was recorded in banana peels (1.37%) (Table 1).

**Table 1: Proximate Composition (%) of ripened banana and mango fruits**

Sample	Fruit Parts	Moisture	DM	Lipid	Crude Protein	Starch	Ash
Banana	Pulp	76.63abc	26.89abc	1.37c	5.65abc	0.632cdb	3.46abc
	Peel	69.42abd	15.20abd	6.50dba	7.65ad	1.706abd	7.89abd
Mango	Pulp	81.26acd	19.38acd	1.48c	7.96ad	0.507cdb	6.27acd
	Peel	59.98bcd	10.97bcd	3.20b	4.27bcd	1.074bac	1.87bcd
LSD ( $p < 0.05$ )		0.0116*	0.0015*	0.0027*	0.0179*	0.0234*	0.0156*

Values are expressed on dry weight basis. All data are the mean of three replicates. Mean value followed by different letters in the same column differs significantly

The crude lipid of the fruit peels was found to be lower than those of some other fruit peels (2.2% for citrus) [27]. The results observed in the present study on the proximate composition are in agreement with the literature findings of Ajila et al. [28] on different mango varieties and Emaga et al. [23] and Hammond et al. [6] on banana varieties.

The fruit samples were extracted with 80% (v/v) acetone and the phenolic content in the extracts were determined. The

maximum polyphenol content was observed in the peels of mango (54.45%) and the minimum was observed in the pulp of banana (10.97%) (Table 2). The results are comparable with previous reports by Larrauri et al. [29] and Ajila et al. [28] in which they have reported polyphenol content of 70 mg/g and 100 mg/g in ripe peel of different mango varieties. According to Ueda et al. [30], the total polyphenol content was higher in the peel than the pulp at any stage of mango fruit development. The total polyphenol content in grape pomace extracts was reported to range from 68.8 to 98.3 mg [31] which is comparably high than the polyphenol content in mango peel observed in the present study, whereas in apple pomace it was reported as 33.42 mg [32] much lower than mango peels. John et al. [33] have reported polyphenol content ranged from 0.049 to 0.127 mg/ml in the sap of different mango varieties, which might have potential action over microbial growth during fermentation. Banana, tomato and mango peels are reported to be a good source of carotenoids and polyphenols [29].

**Table 2: Polyphenol and dietary fiber content (%) of ripened banana and mango fruits**

Sample	Fruit Parts	Polyphenol	TDF	IDF	SDF	IDF/TDF ratio (%)	SDF/TDF ratio (%)
Banana	Pulp	10.97ba	3.54bca	1.63ca	1.91	46.04	53.95
	Peel	16.60ba	52.11dba	40.52dba	11.59	77.75	22.24
Mango	Pulp	42.02dca	23.07dca	10.61ca	12.46	45.99	54.00
	Peel	54.45dcb	73.04dbc	53.59dbc	19.45	73.37	26.62
LSD (p<0.05)		0.0213*	0.0134*	0.0192*	0.0159*		

Values are expressed on dry weight basis. All data are the mean of three replicates. Mean value followed by different letters in the same column differs significantly

**Dietary fiber content**

The total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) contents of both fruit samples are given in Table 2. Higher values of TDF were observed in mango peels (73.04%) and in banana peel (52.11%). It appears that in all fruit biomass samples, IDF was the dominant fiber fraction, where it accounted 73.37% of TDF in mango peels and 77.75% in banana. The observed results in the present study on dietary fiber content are in agreement with the previous findings of Gorinstein et al. [34] and Emaga et al. [23] in different banana varieties, plantains and other fruits. The characteristics feature of mango peel is that it has high content of soluble dietary fiber, which is reported to have more health beneficial effects. According to Zhang et al. [9], the peel of bananas and plantains could be a rich, low cost source of dietary fiber, mainly hemicelluloses and pectin polysaccharides. Similar to our results, a high IDF of 43.4% and 50% were reported in mango peels by other workers [28,29]. SDF is found to be high in fruit pulps (>54%) compared to the peels. SDF content in apple waste was reported to be 23% of the TDF, while it was 36% in orange byproducts [35].

**Effect of pretreatments on sugar yield**

The reducing sugar yields from the banana and mango fruit pulps and peels by different pretreatment methods are shown in Figure 1. The results showed that the sugar yield was not affected significantly by pretreatment methods in fruit pulps where as in peels the dilute acid pretreatment significantly increased the sugar release by nearly 20% over the LHW. Sirkar et al. [36] have reported that acid pretreatment method was found to be optimal for better yield of fermentable sugars from fruit peels. Acid, alkaline pretreatment of biomass has been extensively studied and in an experiment using agricultural wastes, Patle and Lal [37] has observed reducing sugar yield of 49 84 g L<sup>-1</sup> and ethanol production of 23 32 g L<sup>-1</sup> in the acid pretreated fruits and vegetable residues and the enzymatic hydrolyzate yielded 36 123 g L<sup>-1</sup> of reducing sugars and 11 54 g L<sup>-1</sup> of ethanol. An initial pretreatment stage, in case of fibrous peel residues, is needed to breakdown its structure to make it more susceptible to an enzymatic

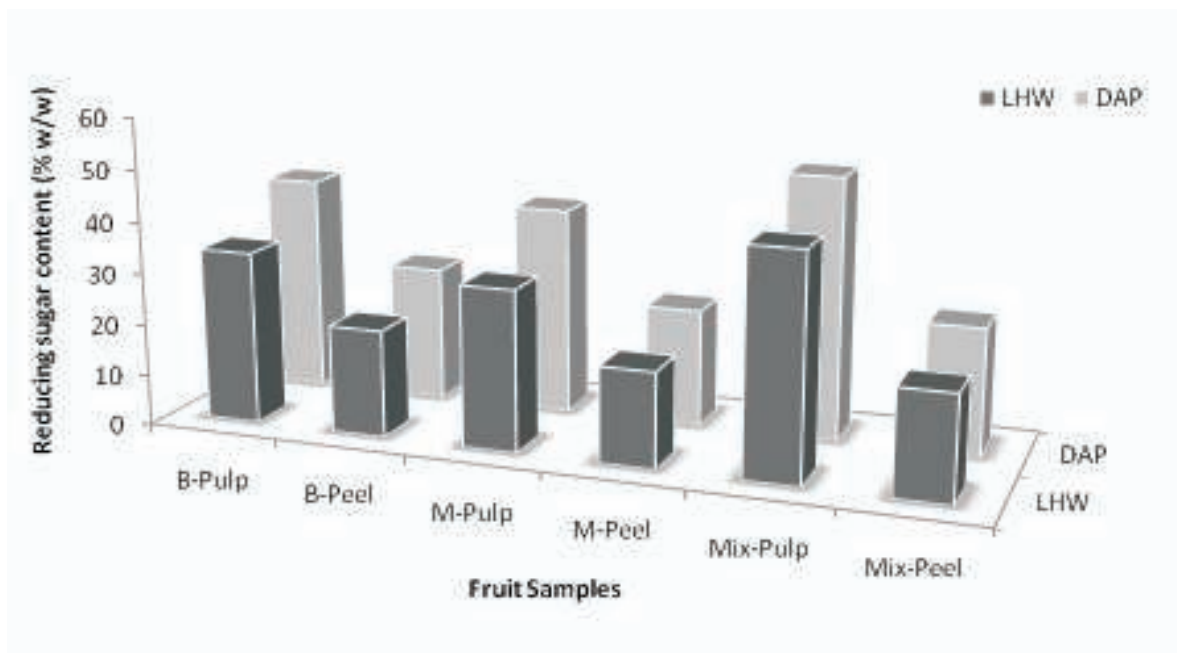


Fig 1. Effect of liquid hot water (LHW) and dilute acid (DAP) pretreatments on reducing sugar yield from fruit samples

attack, whereas for pulp residues, it is required to avoid the need for reducing the size of the biomass and also to limit the degradation of compound that inhibits growth of the fermentative microorganisms. Aden et al. [38] pointed out that the main advantage of dilute acid pretreatment related to other pretreatment methods is the higher recovery of sugars derived from hemicellulose. The dilute acid pretreatment has the advantage of not only solubilizing hemicelluloses but also converting solubilized hemicelluloses to fermentable sugars in wheat straw and it thus, eliminates or reduces the need for use of hemicellulase enzymes mixtures [39]. In a similar fermentation experiment, Nigam [40] has reported more than 60% sugar yield in the hydrolysates obtained from water hyacinth by dilute acid treatment. The National Renewable Energy Laboratory (NREL) of the US Department of Energy, which currently is developing ethanol production technologies from biomass, has preferred the dilute acid pretreatment for the design of its process alternatives [38]. Campo et al. [4] have found that the soft hydrothermal pretreatment (110°C for 5 min) as optimal for a valuable amount of single sugars for tomato residues. According to Ogier et al. [41] and Laser et al. [42], the LHW or thermohydrolysis may be a promising pretreatment that presents elevated recovery rates of pentoses which does not generate inhibitors.

#### Liquefaction and saccharification

The two stage enzymatic hydrolysis treatment was carried out in the pretreated fruit samples with the aim of first liquefying the starch at higher temperature (90°C) by amylase and then the second stage was performed at a low temperature (60°C) by glucoamylase to convert the starch completely to monomeric sugars. The liquefaction of starch by  $\alpha$ -amylase from *Bacillus subtilis* under high temperatures (90–110°C) and saccharification of the liquefied starch by glucoamylase from *Aspergillus niger* or *Rhizopus* sp. at low temperatures (60–70°C) have been extensively studied by many authors [43,44].

The reducing sugar content obtained from enzymatic hydrolysis of the pretreated fruit samples are shown in the Table 3. The results on sugar recovery after dilute acid pretreatment followed by enzymatic hydrolysis in fruit pulps showed a maximum sugar release of 64.27% in mixed fruit pulps and 57.58% in banana fruit pulp. Similarly in fruit peels, the maximum reducing sugar was observed in banana (36.67%) and the minimum was observed in mango (21.68%). It was observed from the present study that a valuable amount of reducing sugars are liberated only after the enzymatic hydrolysis, and this also revealed that both the banana and mango residues require more severe pretreatment conditions to release maximum fermentable sugars. Singh et al. [45] has found that the fermentation of enzymatic hydrolysates showed better fermentation efficiencies in comparison to acid hydrolysates of agricultural residues.

**Table 3: Effect of pretreatment and enzymatic hydrolysis on reducing sugar yield (% w/w) and ethanol production (% w/w) in the fruit samples**

Sample	Fruit Parts	Reducing sugar content (% w/w)		Maximum ethanol content (% w/w)		Fermentation efficiency (%) <sup>b</sup>	Ethanol Productivity (% w/w h <sup>-1</sup> ) <sup>b</sup>
		LHW + ES <sup>a</sup>	DAP + ES <sup>b</sup>	LHW + ES <sup>a</sup>	DAP + ES <sup>b</sup>		
Banana	Pulp	53.93cea	57.58ceab	21.36bac	28.45cae	55.78	0.593
	Peel	22.56dfb	36.67a	8.66edf	13.84dfb	27.13	0.330
Mango	Pulp	51.39cea	55.18ceab	19.62bac	26.50caeb	52.00	0.552
	Peel	20.48dfb	31.29b	7.82edf	9.68dfb	18.96	0.230
Mixed fruit	Pulp	56.38cea	64.27ceadf	28.72adb	35.86caed	70.33	0.747
	Peel	25.57dfb	33.90b	8.32edf	11.94dfb	23.40	0.284
LSD (p<0.05)		0.0208*	0.0376*	0.0224*	0.0137*		

All data are the mean of three replicates. Mean value followed by different letters in the same column differs significantly.

<sup>a</sup>Results obtained by Liquid hot water pretreatment followed by enzymatic saccharification.

<sup>b</sup>Results obtained by Dilute acid pretreatment followed by enzymatic saccharification.

The two microbial enzymes used in the present study exhibited a high efficiency in the conversion of starch from fruit peels, which was comparable with the results obtained by many other researchers [21, 46]. The saccharification of different agro-wastes has been also reported by other workers employing enzymes from different microorganisms [47]. Karakastanis and Liakopoulou-Kyriakides [48] have observed 96% of starch conversion in corn by using amylase and glucoamylase, simultaneously. Dettori-Campus et al. [49] have reported 80% of starch conversion in barley, corn and rice using amylases from *Bacillus* species. Sharma et al. [50] have reported a maximum yield of 63 g L<sup>-1</sup> reducing sugar after enzymatic saccharification and 0.426 gg<sup>-1</sup> ethanol after fermentation in a mixture of banana peels and kinnow waste. Hammond et al. [6] have reported an increased sugar recovery and ethanol production from bananas and banana wastes using commercial  $\alpha$ -amylase and glucoamylase.

### Ethanol Production

The fermentation efficiencies and ethanol production in the hydrolysates of fruit samples are presented in Table 3. Ethanol yield in fruit pulps varied significantly between the fruit samples and the highest yield was 35.86% in the mixed fruit pulps sample, followed by 28.45% in banana pulp and the lowest yield was 26.5% in mango pulp. The fermentation of enzymatic hydrolysate of acid pretreated mixed fruit pulps (banana and mango) by yeast showed an incubation period of 48 h as optimum for maximum ethanol of 35.86% corresponding to a fermentation efficiency of 70.33%. In peels samples, the maximum yield was 13.84% in banana and 9.68% in mango at 42 h of incubation. The results on ethanol yield are in concert with the observations of Sirkar et al. [36] in banana. The fermentation studies on the hydrolysates of fruit pulps obtained from both the pretreatments (LHW & DAP) with out enzymatic hydrolysis have showed poor ethanol yield and the ethanol yield was 25% lower than the normal fermentation process of hydrolysates obtained after saccharification (Fig 2). The results are in good agreement with the previous report of Hammond et al. [6], where he has reported a ethanol yield reduction of 13.4% from the ripen banana pulp without enzymatic hydrolysis. Joshi et al. [51] in a fermentation study with flocculating yeast (*S. uvarum*) observed that waste banana peels are capable of providing enough sugar for the fermentation and hence can be economically utilized for ethanol production. Onwuka and Awam [12] have reported 19–24% of fermentable sugar and alcohol content of 8–81.5 brix (9.96–11.25%) in cooking banana and plantain.

The fermentation studies showed a maximum ethanol productivity of 0.747% h<sup>-1</sup> in mixed fruit pulps and minimum of 0.230% h<sup>-1</sup> in mango fruit peels (Table 3). A steep increase in the ethanol productivity over the increase of yeast cell growth was also observed and the highest ethanol yield was observed when the yeast biomass was recorded as 10.23 g L<sup>-1</sup>, 4.06 g L<sup>-1</sup> during the fermentation of mixed fruit pulps and peels respectively (Fig 3). Similarly for banana and

mango fruit pulps the yeast growth was maximum at 48 h incubation and for fruit peels the maximum yeast growth was observed at 42 h incubation (Fig 4 & 5). Previous reports showed a fermentation period of 36 h as optimum for ethanol production in water hyacinth [52] and 24 h for acid and enzymatic hydrolysate of agricultural wastes by *S. cerevisiae* [22]. A rapid bioconversion of sugars to ethanol during the initial stages could also be observed in all the fruit samples from the increased cell mass of yeast and also from the decreasing trend in the amount of residual sugars in the fermentation medium (Fig 3 - 8). This observation is consistent with the report of Akin-Osanaiye et al. [53], which indicated that the amount of yeast influenced ethanol production in *Carica papaya* agro wastes. The decline in the ethanol production beyond 48 h of incubation in fruit pulps and 42 h of incubation in fruit peels might be probably due to reduced substrate concentration or due to decrease in the number of viable yeast cells or because of the denaturation of enzyme by the ethanol produced during fermentation. The current observations are in good agreement with similar results reported by Pramanik and Rao [5] in grape waste. The reduction in the alcohol yield in mango might be due to the inhibitory effect of high polyphenol content and or less availability of fermentable sugar after even saccharification. A maximum concentration of ethanol from mango pulp (7.85%) was reported by Reddy and Reddy [54] using yeast fermentation. It is comparably low with the current findings on ethanol production from mango.

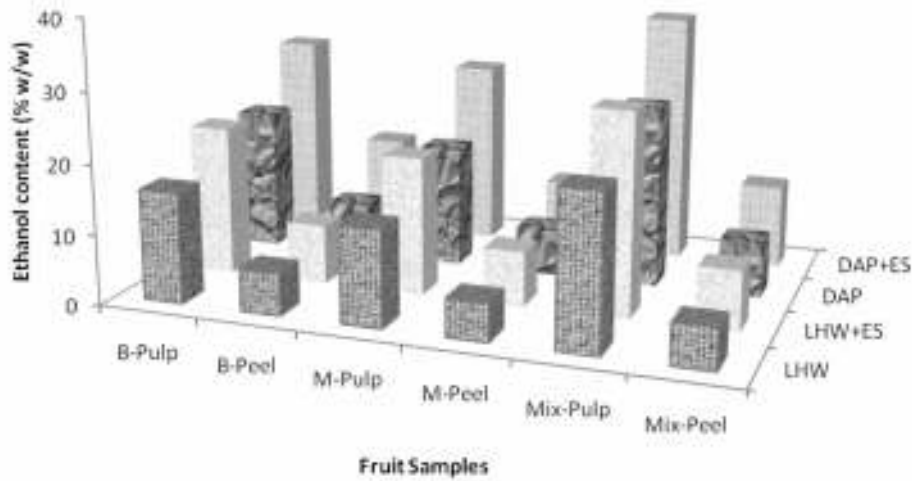
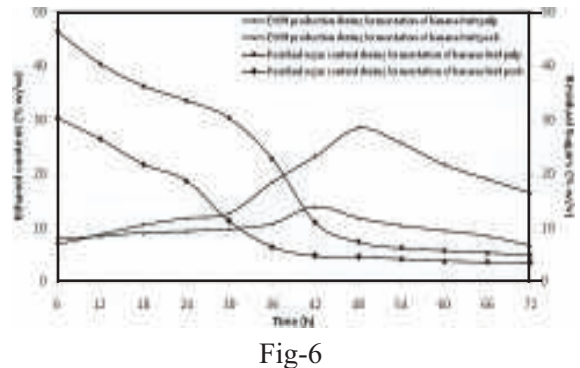
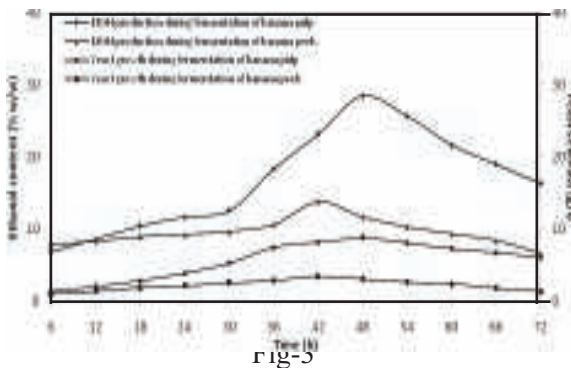


Fig 2. Effect of pretreatments and enzymatic hydrolysis on ethanol production in banana and mango fruit biomass





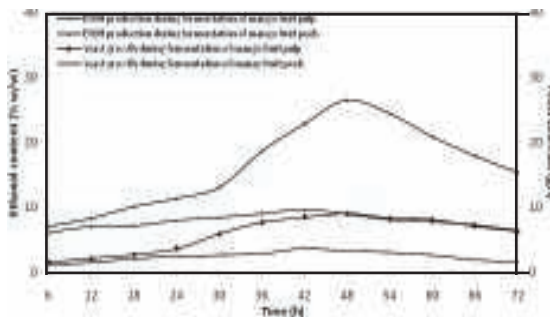


Fig4

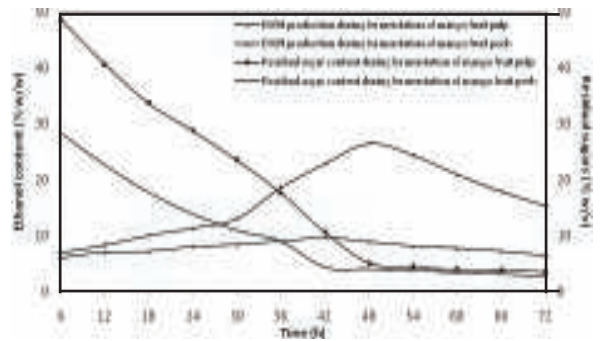


Fig-7

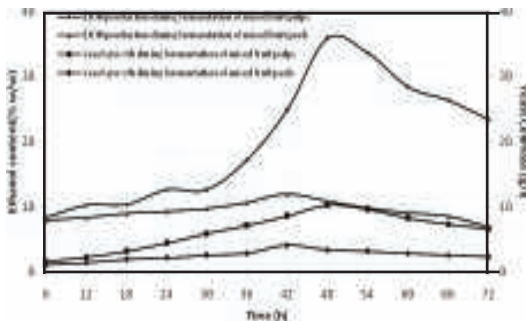


Fig-5

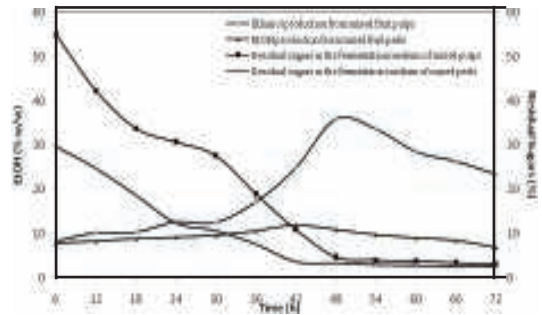


Fig-8

- Fig 3. Ethanol production and Yeast growth during fermentation of fruit pulp and peels of banana
- Fig 4. Ethanol production and Yeast growth during fermentation of fruit pulp and peels of mango
- Fig 5. Ethanol production and Yeast growth during the fermentation of mixed fruit pulps and peels
- Fig 6. Ethanol production and Residual sugar content during the fermentation of fruit pulp and peels of banana
- Fig 7. Ethanol production and Residual sugar content during the fermentation of fruit pulp and peels of mango
- Fig 8. Ethanol production and Residual sugar content during the fermentation of mixed fruit pulps and peels

**CONCLUSION**

The massive utilization of fuel ethanol in the world requires that its production technology be cost-effective and environmentally sustainable. The current research tendencies for improving fuel ethanol production are linked to the nature of employed raw materials, processing steps, and related process engineering issues. Agro-processing techno-economic activities are to be generated for conservation and handling of agricultural produce and make it usable as food, feed, fibre, fuel or industrial raw material. In this present study, efforts were made to identify the fruit wastes as potential raw material for bioethanol production and the results showed that mixed ripened fruit biomass of banana and mango can yield 36% of ethanol and similarly the banana fruit peels treated with dilute acid and microbial enzymes showed a potential production of 14% ethanol. The high non structural carbohydrates, reserve starch content and low fiber contents showed the potentiality of bananas as a good feedstock for ethanol production. Even though the ethanol obtained was comparably lower than other starchy, sugary and lignocellulosic feedstocks but, the production of ethanol from these cheap low cost materials can be improved by using suitable technologies i.e., using genetically engineered strains that are capable of converting multiple sugar in to ethanol. Increasing available sugars of the feedstock at a fixed pretreatment level might result in reduced capital and operating costs for higher ethanol yield. The major costs for a biorefinery are feedstock collection, storage, transportation, and pretreatment, hence, optimizing these unit operations through using fruit peels and un-marketed fruit wastages should prove beneficial.

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