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Bioethanol Production from Different Varieties of Over-ripen Mango (*Mangifera indica* L.) of Bangladesh

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Abstract: Rapid population growth of Bangladesh, exponential growth on industrialization load on fossil fuel resources and this large economic growth has created an increased demand for energy than the current output. Bioethanol is an attractive, renewable, environment friendly fuel which is considered one of the most promising substitute for fossil fuels. As an agricultural country it produces a large amount of mangoes (*Mangifera indica* L.) every year. However, Bangladesh is the 8th largest mango producing country in the world. The aim of this research was to find out the potentiality of bioethanol production from different over-ripen mango varieties of Bangladesh. About 100 g of sample mango pulp was blended with 300 ml of distilled water and then sterilized. For fermentation, 200 ml ($1x10^5$ cell/mL) 24-hours old yeast culture (Sacc*haromyces cerevisiae*) was added to make the total volume 500 ml and incubated at 35°C for 6-days. Five different local mango varieties (Lengra, Khershapat, Amropali, Fazli and Lakhna) were assessed for bioethanol production. Out of these mango varieties a highest yield and purity of bioethanol was found in Khershapat (*Mangifera indica* L.) 77.67 g/L with 32% (v/v) purity. This research proved that Bangladesh has a good opportunity of bioethanol production from over-ripen mangoes.

Keywords: Bioethanol, Over-ripen Mango Pulp, Saccharomyces Cerevisiae, Distillation and Alcoholic Fermentation

1. Introduction

Bioethanol is an organic liquid biofuel made by microbial fermentation, mostly from carbohydrates produced in sugar or starch-bearing plants such as corn, sugarcane, sweet sorghum or lignocellulosic biomass and more recently from microalgae. That is also biodegradable, less toxic, oxygenated (35% oxygen), thus providing the potential to reduce automobile greenhouse gas emissions generated in fossil fuel combustion and lessen dependence on oil imports [1-3]. Bioethanol is simply ethanol, it is the same chemical as the alcohol in beer, wine or any spirit.

The demand for bioethanol is being rising day by day due to its potential of being used as transportation fuel and has a great advantage over conventional fuels in various feasible ways, directly or blend with gasoline called "gasohol" [4]. It has higher octane rating and it is safer to use. Air quality will improve for its clean and proper burning quality. Bioethanol and gasoline blended fuel reduces production energy and total greenhouse gas emission as well as total hydrocarbon and nitrogen oxide emission decreased up to 34.5% to 35.6% with increased oxygenated fuel and one of the best tools to fight vehicular pollution, as it contains 35% oxygen that helps complete combustion of fuel and thus reduces harmful tailpipe emissions [5, 6].

Mango (*Mangifera indica* L.) is one of the most important fruit marketed in the world with a global production exceeding 47.13 million tons in 2017 and estimated production was 1,047,850 tons in 2019. This accounts for about 3.9% of the total production of the world [7]. It is rich in essential nutrients like carbohydrates, sugar, dietary fibers, pectins, irons, vitamins A, B-6 and C. Rajshahi, Chapainawabganj and Dinajpur are the main mango growing regions of Bangladesh. Varieties available in Bangladesh are

Fazli, Gopalbhog, Lengra, among several others. In mango, the edible tissue makes up 33–85% of the fresh fruit, while the peel and kernel amount to 7–24% and 9–40%, respectively [8]. When mango processed at a maximum extent and producing a high quality of solid and liquid wastes. This contributes about 40 to 50% of total fruit waste, in which, 5 to 10% is pulp waste and 15 to 20% is kernel [9-11].

The primary challenge in biomass conversion to bioethanol is achieving yields that make it cost-competitive with the current fossil-based fuels. The costs of ethanol production by fermentation are significantly influenced by the cost of the raw materials, which accounts for more than half of the production costs [12]. To date, numerous biomass resources have been investigated for bioethanol production such as: sugar juice, starchy crops, and lingocellulosic biomass and it can broadly be classified into: i) first generation used sugarcane, sugar beet, wheat, fruits, corn, potato, rice, sweet potato or barley [13, 14]; ii) second generation involves lingo-cellulosic biomass [15, 16]; iii) third generation comes from micro and micro algae biomass and new investigations also done from fecal waste [17, 18]. Its production process involves a fermentation that transforms carbohydrates into ethanol [19]. Zabed et al. [20] studied on the potential of various biomass sources, technological approaches, role of microorganisms and factors affecting ethanol production process.

The over-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 [21]. Production of ethanol from sugary materials is easier than lignocellulosic materials, since it requires additional technical challenges such as pretreatment [22]. Majority of rotten fruit and vegetables are available from processing industries are seasonal and not decompose rapidly. The annual availability of these wastes amounts to 1.05 billion tons [9]. Scientists are doing research to convert food waste or inedible parts of fruits into bioethanol. Ethanol production from orange peel, banana and pineapple peels were also investigated [23-25]. Now it has gained considerable attention in recent years due to escalating price of petro-fuel throughout the world. It has been also stated that bioethanol up to 5-20% can be blended with conventional fuel and the fruits processing industry disposes of around million tons of waste each year, but many researchers have shown that it is possible to convert these wastes into bioethanol [26, 27].

Mango peel is difficult to decompose, as it takes a very long time, because of its complex composition. Suitability of mango peel for bioethanol production was investigated by Madhukara et al. [10]. Currently, there is no economically viable method for obtaining ethanol from cellulose as the extraction procedure requires large amounts of energy input. However, ethanol fermentation from fruit and vegetable wastes appears to give better returns. Over-ripen mango pulp can be a great substance for bioethanol production. If we can alternate use of biofuel from mango pulp then it will be economically beneficial, environment will be pollution free and scarcity of fuel will be reduced. Conventional fermentation is the process that converts the sugars from sugar-rich feedstocks (fruit juices, pomace and grains, such as corn and sweet sorghum) into alcohol in the brewing and beverage alcohol industries. In order to keep distillation costs low, the appropriate microorganism is selected based on the need to achieve high ethanol yield while also withstanding inhibition from accumulating toxic substances and autointoxication from increasing ethanol concentration. Organisms such as yeast (*Saccharomyces cerevisiae*) or bacteria (*Escherichia coli*) are used to convert these simple sugars to ethanol.

Ethanol is produced commercially by yeast (*Saccharomyces cerevisiae*) because its high ethanol tolerance, rapid fermentation rates and insensitivity to temperature and substrate concentration [28]. The main task is to develop easier techniques by using cheaper source for the production of bioethanol without any pretreatment cost. For this purpose, over-ripen mango fruits were taken as a substrate for the ethanol production with use of yeast *Saccharomyces cerevisiae* [29].

Bangladesh has shown good economic growth, rapid urbanization and industrialization in recent years. As a result, the numbers of bus, truck, taxi and auto-rickshaw have been increasing day by day. These high numbers of automobiles are consuming a huge amount of fuel. Bangladesh imports most of the fuel from abroad by spending valuable foreign currencies. It has created tremendous pressure on Bangladesh's annual budget. However, Bangladesh can easily reduce this expenditure with the production of bioethanol from renewable options like sugarcane, corn, potato, sweet potato and over-ripen fruits. At the current level of production and consumption, it seems to be highly difficult target to meet and it is a good prospect for countries like Bangladesh to invest in to renewable energy sector. If we can utilize this over-ripen mangoes for bioethanol production, then we'll be economically beneficial, our environment will be pollution free and scarcity of fuel will be reduced.

The objective of this study were to find out an easier technique of bioethanol production from over-ripen mango pulps of Bangladesh using ethanol tolerant yeast *Saccharomyces cerevisiae*.

2. Materials and Methods

2.1. Raw Materials Collection

Ripen mango fruits (*Mangifera indica* L.) were collected from local market and farms, Rajshahi city, Bangladesh. Fruits were kept until it was fully ripens and softens in cupboard, at room temperature. It was then washed, peeled out and seeded off manually. Then its pulp was blended, sterilized and used as substrate for experimental purpose.

2.2. Yeast Strain and Culture Media

Yeast (*Saccharomyces cerevisiae* CCD) was obtained from the Spirit Section of Carew and Co., Darsana, Bangladesh. For yeast culture, modified YMPD (Yeast-Malt-Peptone-Dextrose) broth culture media was used. The YMPD media was prepared with yeast extract (3.0 g), malt extract (3.0 g), peptone (5.0 g) and dextrose (10.0 g). All of these ingredients were dissolved in 1000 ml of water and adjusted to pH 6.0 [30].

2.3. Optimum Bioethanol Production from Selected Local Varieties of Mango

Among different local mango varieties, five common and popular varieties (Lengra, Khershapat, Amropali, Fazli and Lakhna) were assessed for bioethanol production. Their pulps were extracted and blended with a blender machine. Then it was used as raw materials for bioethanol production. For optimum bioethanol production 200 ml of two days old yeast was added in 300 ml fruit pulp solution (20%) and incubated for 6 days.

2.4. Bioethanol Production from Over-ripen and Fresh Mango Pulps

About 100 g of fully ripen and fresh pulps were separately blended with 300 ml distilled water was sterilized and cooled at room temperature. Then 200 ml of yeast was added to each treatment and incubated at pH-6.0 and temperature 35°C for 6-days.

2.5. Bioethanol Production from Filtering and Non-Filtering Mango Pulps

Two samples of 100 g pulps were blended with 300 ml distilled water. Then one sample was filtered with a muslin cloth and the other was treated as non-filtered. About 200 ml of yeast inoculum were added. After 6-days of fermentation the crude fermented fruit solution was first centrifuged at 12,000 rpm for five minutes to remove the unused starch and yeast cell. Then, the clear solution was taken into rotary evaporator for separation of ethanol at 78.5°C. All the stated experiments were conducted at pH 6.0 and temperature 35°C.

2.6. Estimation of Total Sugar Before and After Fermentation

It is found that sugar measurement in "On Call Plus" and spectrophotometric method is same. But sugar measurement in "On Call Plus" is easy and time-saving. Therefore, sugar content of different mango varieties was determined by sugar measurement machine named "On Call Plus"(Model: SN 103L1254FDC, Germany) during this research [31].

2.7. Distillation Process

Distillation was carried out using a distillation apparatus (Witeg, Germany). Heating of fermented materials was carried out at 78.5°C. The condensation product was stored at 4°C and collected in a flask for estimation of ethanol concentration [31].

2.8. Measurement of Purity of Produced Alcohol

The percent of purity of produced bioethanol was measured by using an alcohol meter (Jiujingnongduji, China). This meter can measure the alcohol purity from 0-100% [30].

2.9. Statistical Data Analysis

Each treatment consists of three replication and all data were expressed as mean values ± SD (Standard Deviation).

3. Results and Discussion

3.1. Estimation of Bioethanol from Some Local Over-ripen Mango Varieties

Bioethanol production of five local mango varieties (Lengra, Khershapat, Amropali, Fazli and Lakhna) was assessed (Figure 1). The sugar content in local variety of Khershapat (17.40 mmol/L) is higher than other four varieties of Mango (Figure 2). In Figure 3 shows that, Khershapat produces a large amount (77.67 g/L) of bioethanol with 32% (v/v) purity and after fermentation the sugar concentration was reduced to 12.60 mmol/L from 17.40 mmol/L. The lowest amount of bioethanol was produced from Lakhna with purity of 26% v/v (Figure 3).

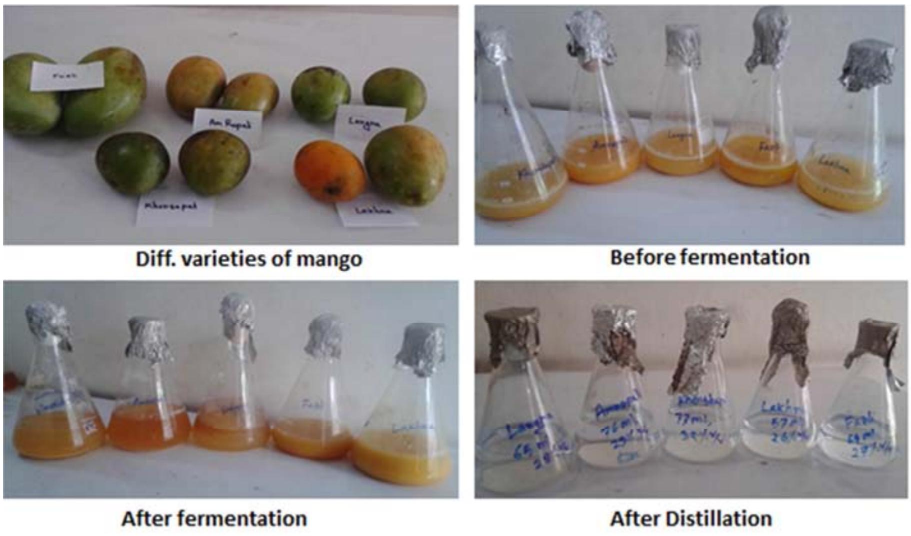

Figure 1. *Different Steps of Bioethanol Production from Mango Varieties.*

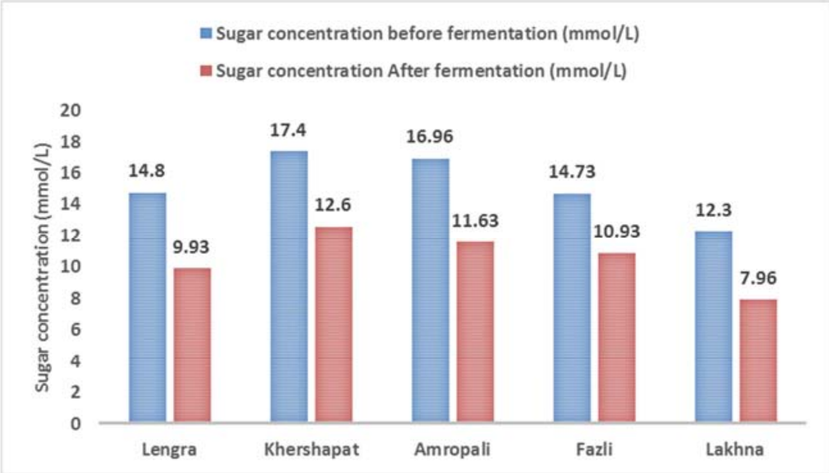

Figure 2. *Sugar Concentrations of Selected Varieties of Mango.*

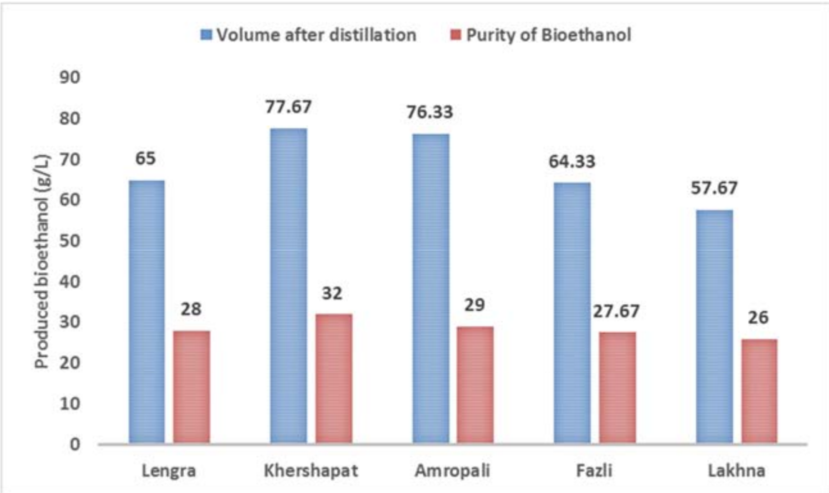

Figure 3. *Produced Bioethanol of Selected Varieties of Mango.*

The result is consistent with Arumugam and Manikandan [32], who found 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. Khandaker et al. [33], investigated on the feasibility of the utilization of mango waste, *Mangifera indica* L. cv Chokanan to produce bioethanol via fermentation by yeast, *Saccharomyces cerevisiae* and found the highest yield from mango pulp in the yeast concentration of 3 g/L at the temperature of 30°C that yielded 15% (v/v) of ethanol followed by mixture at 13% (v/v) and peel at 11% (v/v).

The decline in the ethanol production beyond 6 days of incubation in fruit pulps 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 [34], 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 (78.5% v/v) was reported by Reddy and Reddy [35], using yeast fermentation. It is comparably high with the current findings on ethanol production from mango (32% v/v). Cutzu and Bardi [36], reported that (substrates were apple, kiwifruit, and peaches wastes; and corn threshing residue) vacuum simple batch distillation by rotary evaporation at lab scale at 80°C (heating bath) and 200 mbar or 400 mbar allowed to recover 93.35% (v/v) and 89.59% (v/v) of ethanol, respectively.

3.2. Bioethanol Production from Filtering and Non-filtering Mango Pulp

About 100 g Mango Pulp with 300 ml distilled water was blended and prepared 20% concentration. Then two experiments were done, one was filtered with a moslin cloth and other was kept without filtered. In non-filtered solution the sugar concentration (17.23 mmol/L) and yield (78.33 g/L) of bioethanol is comparatively higher than the filtered solution (15.73 mmol/L and 73.66 ml) as shown in Figure 4.

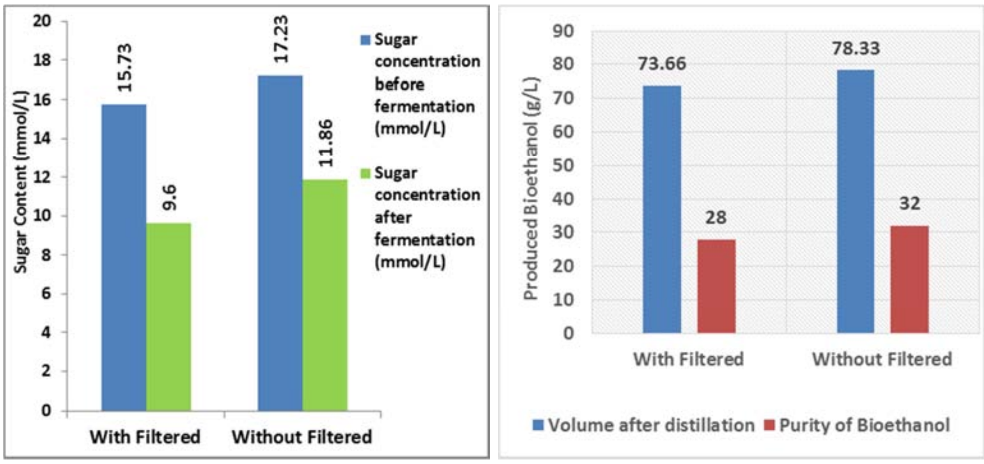

Figure 4. *Sugar Content and Bioethanol Production from Filtered and Non-filtered Mango.*

The ethanol production was increased significantly in non-filtered solution due to release of monosaccharide from mango pulp for fermentation process. Sugar concentration was low in filtered solution because separated top layer of pulp contains some more saccharides. So the produced ethanol is comparatively low in filtered solution than the non-filtered solution. Similar investigations were done by Urbina et al. [37], Navarro et al. [38], Caylak and Vardar [39], they found ethanol concentrations of 52.6, 70 and until 96.71 g/L, respectively.

Jagessar and Fraser [40], used 500 g of ripe fruit mash of banana, pineapple and mango treated with some additives (1%, 5% and 10% Urea, Ammonium Tartrate and Zinc Sulphate under pH 4-5 and 30.5°C temperature) in the presence of uncultured yeast they produced 6.81% (v/v), 6.55% (v/v) and 5.51% (v/v) of ethanol respectively.

Somda et al. [41], found four varieties mangos juice (Amelia, Brook, Wild, Zill) showed that their pulp contained an average 13.12% (w/w) to 25.78% (w/w) of total sugar and pH various to 3.61 to 5.20 and fermented mango juice with *Saccharomyces* and *Shizosaccharomyces* genus showed 12% (v/v) and 5.5% (v/v) of alcohol. These values are lower than those found in this study (32% v/v).

S. cerevisiae is able to get high rates of glycolysis and production of ethanol when optimal conditions are presented, by producing 2.5 g/L more ethanol per hour and per gm of cellular protein. However, this high rate is kept only by brief periods of time during the batch fermentation and decreases gradually while ethanol accumulates in the nutrient medium [42]. Based on previous reports, the ethanol production concentration was 4.2% (w/v) in pineapple peel and 4.02% (w/v) in citrus peel waste [25, 43].

Reddy et al. [44], found that direct fermentation of mango peel extract gave only 5.13% (w/v) of ethanol. The rate of the fermentation was very slow. Nutrients such as yeast extract, peptone and wheat bran extract were tested for the supplementation of mango peel medium and it was observed that the nutrient supplementation increased the ethanol production significantly up to 7.14% (w/v).

3.3. Bioethanol Production from Fresh and Over-ripen Mango Pulp

Fresh and ripen mango pulps were studied for optimum bioethanol production. Among them over-ripen mango pulp shows comparatively high yield of bioethanol (77 g/L with 31.66% v/v purity and fresh mango pulp produces a low yield 74.33 g/L with 28.66% v/v purity (Figure 5).

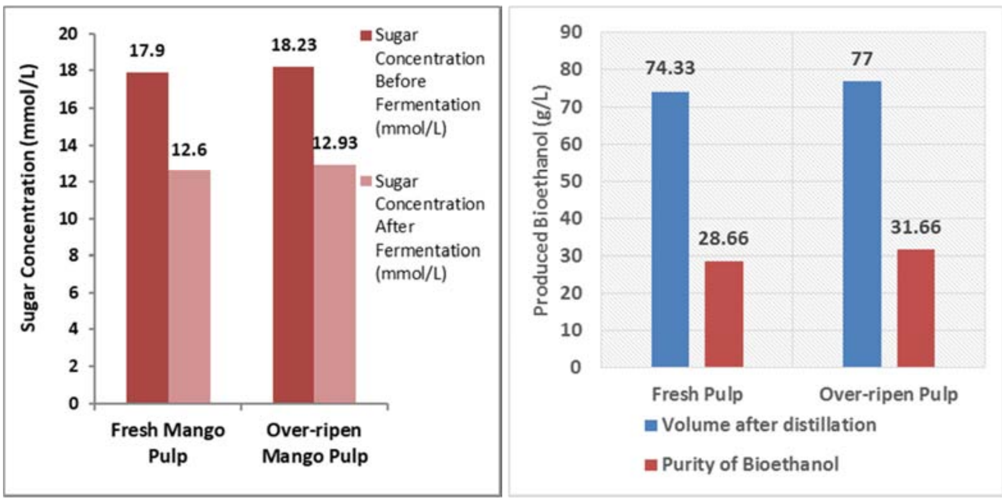

Figure 5. *Sugar Content and Bioethanol from Fresh and Over-ripen Pulps of Mango.*

The present study reveals that maximum production of ethanol is in over-ripen fruit pulp than the fresh pulp of mango. The results of this study is quite similar with Khandaker et al. [33], who showed that the bioethanol production through fermentation of mango waste was the highest in percentage, from mango pulp in yeast concentration of 3 g/L at temperature of 30°C after five days of incubation. This vast variation may due to the larger amounts of fermentable sugars are present in fully ripen pulp.

It is well documented that pulp contained the highest amount of starch, which would then be converted into reducing sugars after fully ripening. So, this huge amount of reducing sugars would generate more bioethanol. According to Reddy and Reddy [45], there are three types of sugar namely glucose, fructose and sucrose, and the major sugar is sucrose in mango fruits.

Lima et al. [46], 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. Because, during ripening process, the starch and sucrose would be degraded into fructose and glucose. So, after undergoing ripening stage, the amount of reducing sugars in mango would increase. This promoted the high production of bioethanol from mango via fermentation process economically. The amount of produced ethanol was mainly dependent on the amount of fermentable sugar present in sample.

4. Conclusion

Over-ripen mango varieties could be used as the potential raw materials for bioethanol production. Out of five mango varieties, Khersapat gave a good yield of bioethanol (77.67 g/L) with purity of 32% (v/v) compare to other varieties. It can be used for small and large scale production of bioethanol because mangoes are available grown everywhere in Bangladesh.

The purity and production of ethanol from the over-ripen mango varieties can be further improved by using genetically engineered yeast strains that are capable of converting multiple sugars into ethanol and high-grade distillation setup is required for the preparation of anhydrous bioethanol.

Conflict of Interests

The authors declare that they have no competing interests regarding the publication of this paper.

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