Korean Chem. Eng. Res., 61(2), 234-239 (2023)
https://doi.org/10.9713/kcer.2023.61.2.234
PISSN 0304-128X, EISSN 2233-9558

Production and Investigation of Parametric Effect on Bio-ethanol by Sapota Using Separation Technique

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(Received 24 August 2022; Received in revised from 14 November 2022; Accepted 1 December 2022)

Abstract – Waste from the food is a challenge to the environment all over the globe, hence there is need to be recycled. There is a great deal of renewable energy potential in the biomass of vegetables and fruits, which can be used to generate power and steam, as well as fuel for human consumption and laboratory solvents. To maintain the nutritional, antioxidative, and functional qualities of sapota fruit, wine was made by fermenting it with wine yeast (Saccharomyces cerevisiae). The wine's approximate composition was as follows: total soluble solids, 2.38°Brix; total sugar, 3.8 g/100 ml tartaric acidity (TA), 1.29 g tartaric acidity total phenolics, 0.21 g/100 mL; pH, 3.02; acid/100 mL; pH, 3.02; total phenolics, 0.21 g/100 mL; 22 g/100 ml -carotene; 1.78 g/100 ml ascorbic acid mg/100 ml; 0.64 mg/100 ml lactic acid; and The ethanol percentage is 8.23% (v/v). The sapota wine was delicious. A DPPH-scavenging 2, 2-diphenyl-1picryl hydroxyl (DPPH) at a dosage of 250 g/ml, the activity was 46%. Infrared alcohols, phenethylamines, and other compounds were discovered via spectroscopy.

Key words: Sapota, Ethanol, Fermentation, Fruit waste, Wine

1. Introduction

As the world's energy supply is rapidly depleting, it is imperative that we find a new source of energy. Global warming and the consequences of greenhouse gases have become worrying as a result of the depletion of oil [1]. However, despite this, the world's energy needs are mostly reliant on finite reserves of fossil-based petroleum.

Global demand for biofuel has grown as a result of concerns about climate change and the depletion of the world's fossil fuel reserves. There is a huge side effect to the use of natural resources for fuel. Global warming is caused by the rapid rise in CO_2 levels in the atmosphere as a result of the use of petroleum-based fuels. Because of concerns about climate change and greenhouse gas emissions, bio-fuels like bio-ethanol are being promoted as a viable alternative or replacement [2,3]. It is also a problem that arises from the disposal of rubbish in an open area, which is harmful to the surrounding natural habitat. Waste can be used to create energy in the form of a solution, which can be produced at a low cost and with high efficiency [4]. Solid biomass, liquid fuels, and biogases are only a few of the rapidly expanding renewable energy technologies in recent years. In contrast to fossil fuels, bio-fuels are generated through biomass rather than through the geological process of oil and fossil fuel creation. Thus, biomass can technically be used as a fuel in its own right. In the context of bio-fuels and biomass, they are used interchangeably [5,6]. Bio-ethanol is made from biomass that contains a complex of free sugars that can be converted later to soluble sugars. Although there are three major categories of feedstock, the starchy crops, which include the products of sugar refineries, and lignocellulosic biomass (LCB), each has a distinct sugar solution. Conventional starch-rich feedstocks (corn, potato) and sugarcane have previously been reported as first-generation bio-ethanol processes [7,8]. In spite of this, they face a number of economic and social challenges. There is a growing interest in the second-generation bio-ethanol process [9]. The second-generation process uses lignocellulosic biomass (corn stover, sugarcane bagasse, straws, stalks, and switch-grass [10-12].

It is one of the most important alternative processes for the generation of bio-ethanol, which may be easily adapted to current engines that have higher octane ratings [13]. Bio-ethanol manufacturing uses any plant material with a large amount of sugar as a raw material. Because sugarcane is a key component of sugarcane, pineapple, and potato, these plants resulted in a high bio-ethanol yield as byproducts.

1-1. Saccharomyces Cerevisiae

Yeast is a type of basidiomycetous or ascomycetous fungi, which means that it reproduces by either budding or fission and forms spores that are not encased in the fruiting body. Cerevisiae is the yeast most commonly used in the production of ethanol because it has a high tolerance for a wide range of pH levels, making it less prone to infection. The ability of yeasts to metabolize compounds containing six carbons is the fundamental factor in the synthesis of bioethanol without allowing the reaction to continue on to its natural conclusion, which is the production of carbon dioxide [14]. Any

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Table 1. Properties of micro-organism used

Component	Characteristic	Volume
Acremonium Cellulase	Powder form and retains the characteristics of water	The same volume as would be occupied by water heaving the same mass as the enzyme
Baker's yeast saccharomyces cerevisiae	Solid form	Volume based on density of the micro organism

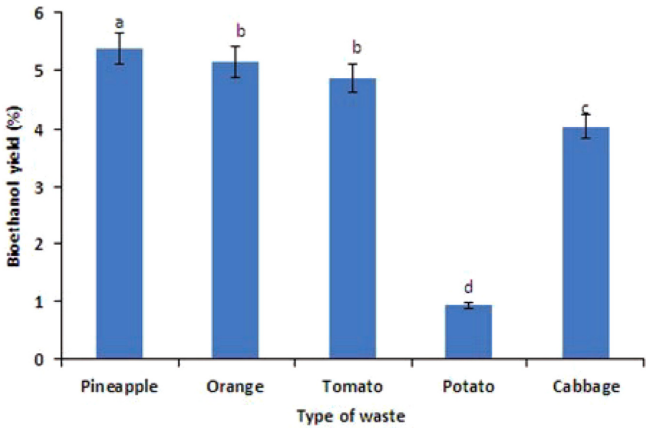

Fig. 1. Wine made from different fruits.

plant material with significant amounts of sugar is utilized as a source of raw materials in bioethanol production.

Table 1 shows the lipid content of different fruits. Any plant material with significant amounts of sugar is utilized as a source of raw materials in bio-ethanol production. Sugarcane, pineapple and potato are one of the major plants that result in a high yield of bio-ethanol as byproducts due to the presence of a large amount of sugarcane in it (Fig. 1).

Diauxic shift and fermentative metabolism are the processes that take place during the generation of alcohol dehydrogenase enzymes that are dependent on bio-ethanol. These enzymes are encoded on the ADH1 locus. ADH1 is responsible for the generation of ethanol and the reduction of acetaldehyde during the fermentation of glucose. ADH1 can also catalyze the reverse reaction, which is the process of converting ethanol to acetaldehyde, but with a reduced catalytic efficiency. Currently, it is grown in all of the tropical countries of the world. Sapota can reach a height of more than 30 metres. 1.5 m diameter is the typical trunk length, encased in a brownish skin. It looks much like a smooth-skinned potato.

Fruits, in particular, are rich in sugars, antioxidants, minerals, and vitamins, and have a range of technological and therapeutic uses. Sapota Among the most significant tropical plants is Achras sapota Linn. Sapotaceous fruits are those that belong to the Sapotaceous family. Currently, it is grown in all of the tropical countries of the world. It looks like a smooth-skinned potato.

The pulp is yellowish, soft, granular, sweet, juicy, and fragrant, and it contains a large amount of latex. Sapota pulp that has matured is a good source of carbohydrates (21.4). g/100 g), dietary fibers (10.9 g/100 g), and tannin (3.16~3.99 g/100 g). 3.45%), ascorbic acid (3.45%), and minerals such as calcium (28%), as well as phosphorus (27 mg/100 g). It is also high in bio-iron, which is necessary for the creation of vitamin A and hemoglobin. Amino acids are the building blocks of life. Glutamic acid, glycine, alanine, methionine, phenylalanine, proline, and hydroxy - are all found in the fruits. Proline, threonine, taurine, tyrosine, serine, and valine are all amino acids. and urea, as well as phosphoethanolamine. Sapota has an important role in Indian culture. It has a unique place among fruit crops; its cultivation takes up a large space. During the 2007~2008 season, 150,000 hectares were planted and 1,238,000 Mt were harvested. 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).

The objective of present research work, primary step fermentation products, was carried out and the product was separated by using the RO-membrane which specifies the concentration of permeate bio-ethanol with other components. This research work also explains the characteristics of production of bio-ethanol using sapota as raw material. As the world's energy supply is rapidly depleting, it is imperative that we find a new source of energy. Global warming and the consequences of greenhouse gases have become worrying as a result of the depletion of oil. However, despite this, the world's energy needs are mostly reliant on finite reserves of fossil-based petroleum.

2. Materials and Methods

Sapota fruits that were fully ripened and healthy were harvested from the Regional Centre's garden. The Central Tuber Crops Research Institute is a non-profit organization dedicated to the study of tubers (RC-CTCRI), May 2012 in Bhubaneswar, Odisha (day temperature 35 ℃, night temperature 32 ℃).

2-1. Yeast for wine

Saccharomyces cerevisiae (stock culture) is wine yeast, was used in the fermentation trials. This yeast culture has previously been used in the production of wine [10-12] from various fruits and other substrates.

2-2. Method

Fruits weighing about 1 kilogram of sapota were harvested. A knife was used to cut the fruit into two halves and the pulp was removed. The thumb rule was used for scooping out the contents. The seeds, as well as starter culture preparation 100 g of healthy grapes [Vitis vinifera (var. Bangalore blue)], were chosen and rinsed under running water from the tap In a mixer-cum grinder; the grapes were mashed (TTK Prestige Limited, Bangalore, India) and a juice

Table 2. Reaction and condition involved

Fermentation	$2C_6H_{12}O_6 \rightarrow 4C_2H_5OH + 4CO_2$	Temperature: 370 ℃ Pressure: Atmospheric Pressure Retention time: 7 Days Enzyme: Zymase Microorganism: Saccharomyces Cerevisiae

squeezer was used to remove the juice. The juice volume (filtered via cotton) cheese cloth was weighed, and an equal volume of water was added. A glass of water was added. The mixture was boiled for ten minutes on a hotplate, then chilled to room temperature (282 ℃). The grape juice was allowed to cool before being used.

S. cerevisiae culture from the stock inoculated (Klenzoides, Mumbai) cultivation in a laminar airflow.

This is the fermentation reaction in which two molecules of glucose are broken down into four molecules of ethanol and four molecules of carbon dioxide in the presence of yeast at optimum temperature 37 ℃ and at atmospheric pressure.

2-3. To Prepare the starter culture

It was incubated at 30 ℃ for 24 hours. Bottling and racking (decanting the top fluid after fermentation), a part of the residue that had settled to the bottom. The experiment was carried out at room temperature [15]. Stacking the shelves:

When TSS was reached, sapota wine was made 2–3 degrees Brix. This procedure was carried out three times. With time intervals of 20 days to discard the deposited at the bottom, there were remnants. Bentonite (0.04%) was added to the mix. Before the final racking to get rid of the remainder of the items residues to be clarified SMS, was sent after the final racking. Before bottling, a preservative (100 g/ml) was added. The wine bottles were filled, corked, and labeled. Beeswax was used to seal the package.

2-4. Analytical biochemistry

Total soluble solids, total soluble phenol, total sugar, titratable acidity (TA), pH ethanol, ascorbic acid, lactic acid, -carotene). The characteristics of the sapota must and wine were determined by Ref. suggested methods. The must and wine were tested for DPPH-scavenging action.

Table 3. Biochemical composition of sapota must and wine

Parameters	Must (%)	Wine (%)
TSS	20.01	2.38
Titratable acidity	0.82	1.29
Total sugar (g/100 ml)	28.1	3.28
pH	4.8	3.02
Phenol (g/100 ml)	0.21	0.21
Ascorbic acid (mg/100 ml)	2.86	1.78
Lactic acid (mg/100 ml)	0.05	0.68
Ethanol	nd	8.23
DPPH	59.4	46.02
B-carotene (ug/100 ml)	35	22.01

3. Results and Discussion

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. 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 the degradation of starch to free sugars during the ripening process due to combined action of several enzymes.

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

Table 4. Proximate composition % of banana and mango fruits

Sample	Fruit parts	Moisture	DM	Lipid	Protein	Starch	Ash
Banana	pulp	76.63	26.89	1.37	5.65	0.632	3.46
	Peels	69.42	15.20	6.50	7.65	1.706	7.89
Mango	pulp	81.26	19.38	1.48	7.96	0.507	6.27
	Peels	59.98	10.97	3.02	4.27	1.07	1.87
LSDP ($p<0.05$)		0.01	0.01	0.002	0.01	0.02	0.015

Table 5. Polyphenol and dietary fiber content (%) of ripened banana and mango fruits [16]

Sample	Fruit parts	polyphenol	TDF	IDF	SDF	IDF/TDF Ratio%	SDF/TDF Ratio%
Sapota	Pulp	71.00	20.6	1.04	4.92	0.423	0.47
Banana	pulp	10.97	3.54	1.63	1.91	46.04	53.95
	Peel	16.60	52.11	40.52	11.59	77.75	22.24
Mango	Pulp	42.02	23.07	10.61	12.46	45.99	54.00
	Peel	54.45	73.04	53.59	19.45	73.37	26.62
LSD ($p<0.05$)		0.0213	0.0134	0.0192	0.0159		

observed by Forster et al. in fruit pulps during ripening, and the degradation of starch reserve in fruit pulps appears to be relatively rapid, whereas in peels the conversion is rather gradual. Lima et al. reported that even though starch is the main carbohydrate present in the ethanol yield in fruit, for mature green mango fruit, 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. 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. reported 7.8% protein content in banana peels, which has good agreement with our results. According to Essien et al., the high protein content and carbohydrate content of these fruits could serve as the main source for fermentative methanogenic microbial growth.

3-1. 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. and Emaga et al. in different banana varieties, plantains and other fruits. The characteristic 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., 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% was reported in mango peels by other workers. 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 byproduct.

3-2. Fermentation parameter

The two microbial enzymes used in the present study exhibited a high efficiency in the conversion of starch from fruit peels, which was comparable to the results obtained by many other researchers. The saccharification of different agro-wastes has been also reported by other workers employing enzymes from different microorganisms. Karakastsanis and Liakopoulu-Kyriakides observed 96% of starch conversion in corn by using amylase and glucoamylase, simultaneously. Dettori-Campus et al. reported 80% of starch conversion in barley, corn and rice using amylases from Bacillus species. Sharma et al. reported a maximum yield of 63 g L^{-1} reduced sugar after enzymatic saccharification and 0.426 g^{-1} ethanol after fermentation in a mixture of banana peels and kinnow waste. Hammond et al. reported an increased sugar recovery and ethanol production from bananas and the fermentation efficiencies and ethanol production in the hydrolysates of fruit.

Table 6 shows the sugar content in different fruits. 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. in banana. The fermentation studies on the hydrolysates of fruit pulps obtained from both the pretreatments (LHW & DAP) with out enzymatic hydrolysis showed poor ethanol yield, and the ethanol yield was 25% lower than the normal fermentation process of hydrolysates obtained after saccharification. The results are in good agreement with the previous report of Hammond et al., who reported an ethanol yield reduction of 13.4% from the ripe banana pulp without enzymatic hydrolysis. Joshi et al., in a fermentation study with flocculating yeast, observed that waste banana peels are capable of providing enough sugar for fermentation and hence can be economically utilized for ethanol production. Onwuka and Awam reported 19 24% of fermentable sugar and alcohol content of 8 81.5 brix (9.96 11.25%) in cooking banana and plantain.

3-3. Treatment

For a suitable comparison, two important fermentation parameters (sugar utilization efficiency, ethanol yield) were considered. The ethanol yield per consumption sugar was 0.435 g/g in coconut milk, 0.47 g/g in pineapple juice and 0.29 g/g in tuna juice (see Table 2). The

Table 6. Effect of pre-treatment and enzymatic hydrolysis on reducing sugar yield % w/w and ethanol production % w/w in fruit sample

Sample	Fruit parts	Reducing sugar content % w/w		Max. ethanol content % w/w		Fermentation efficiency %	Ethanol productivity %
		LHW+ES	DAP+ES	LHW+ES	DAP+ES		
Banana	Pulp	53.93	57.58	21.36	28.45	55.78	0.593
	Peel	22.56	36.67	8.66	13.84	27.13	0.330
Mango	Pulp	51.39	55.18	19.62	26.50	52.00	0.552
	Peel	20.48	64.27	28.72	35.86	70.73	0.747
Mixed fruit	Pulp	56.38	64.27	28.72	35.86	70.33	0.747
	Peel	25.57	33.90	8.32	11.94	23.40	0.284
LSC ($p<0.05$)		0.028	0.037	0.022	0.013	---	---

Table 7. Maximum yield of different substrates

Substrate	Max yield (%)
Pineapple	31.53
Orange	37
Banana	32.21
Pea	31.53
Sapota	16

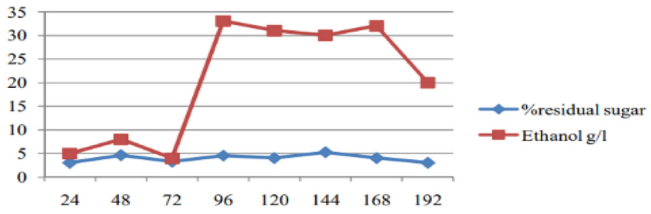

Fig. 2. Progress of ethanol fermentation with sapota as substrate.

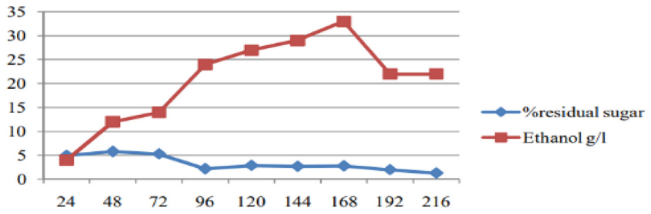

Fig. 3. Progress of ethanol fermentation with coconut milk substrate.

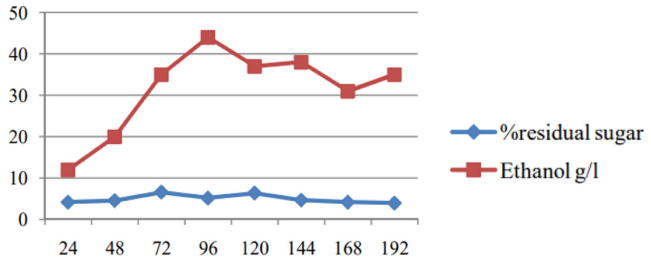

Fig. 4. Progress of ethanol fermentation with pineapple juice substrate.

reduction in tuna juice could be due to several reasons, including the production of compounds other than ethanol like glycerol, acetic acid and CO_2 for tuna juice, which is related with the fact that at 48 h of time course sugar consumption was higher than 96% in all the cases. Results presented here confirmed that biocatalyst process of yeast cells on coconut milk or Pineapple juice for ethanol fermentation was at least as efficient as in other widely used materials like molasses or thick juice. Moreover, ethanol fermentation on coconut milk or pineapple juice has been found to be economically favorable in terms of all presented process parameters as shown in fig 2, 3 and 4.

3-4. Alcohol production from fruit and vegetable waste

The highest ethanol production could be obtained with pineapple peel as substrate (49.34 g/L) at 28 ℃. Also considering that no additional sugar was required. It is necessary to attain high ethanol concentration in order to decrease the costs of ethanol distillation.

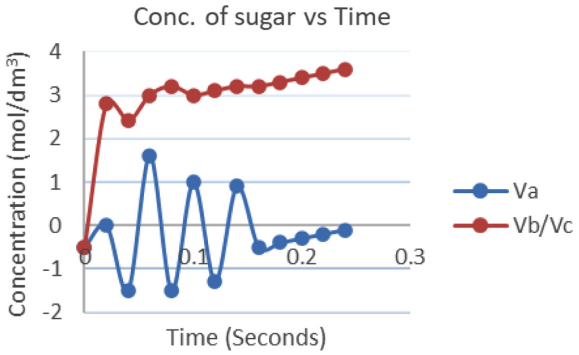

Fig. 5. System without surge arresters.

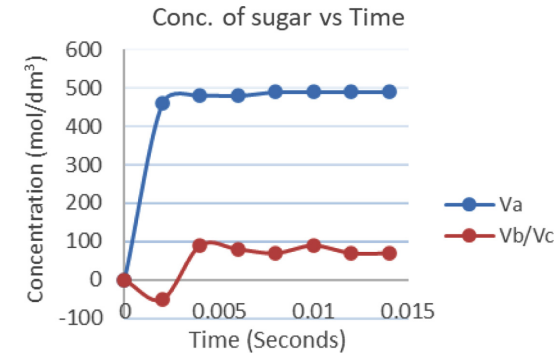

Fig. 6. System with surge arresters.

3-5. Effect of Bio-Ethanol concentration

Figure 5 and 6 show the concentration behavior of the fermenting material with sugar with respect to time. It was observed when time increased gradually, then concentration of ethanol first increased, then decreased due to its primary conversion into desired products and similarly in secondary conversion the percentage of ethanol decreased with sudden increase of the other components. These oscillation curves define the rate of reaction when substrate reacts in the presence of yeast to produce the desired product, which is ethanol at the initial stage when the fermentation reaction takes place then large amount of substrate is required to proceed the reaction. After taking some time, the amount of substrate remained constant with time because at the initial state large amount of enzyme molecules are available with vacant active site. Therefore, at the initial state amount to substrate (sugar) participates in the reaction, after some time the concentration becomes constant.

4. Conclusion

It was observed the formation of bioethanol from sapota has more fuel relevant properties than other fruit wastes. Sapota had more percentage of sugar content in its pre-mature stage. A functional wine made from sapota fruit has been produced and assessed for its biochemical and sensory characteristics in this study. Antioxidant-rich new wine was made from sapota fruit pulp. Per volume, it has an alcohol content of 8.23%. For seasonal availability of sapota fruits in South Asian countries, a sufficient number of countries such as

India, the months of May through June the fruit has a short shelf life of 8~10 days. This research suggestion for making alcohol from these fruits is to protect their worth by using added-value products like wine nutrients, minerals, flavoring agents, and additives. Consumers can purchase them at any time of the year. Recycled agricultural waste and management processes can be used to produce bio-ethanol from vegetables and fruits waste, according to the final conclusions of the study. Bio-ethanol optimum yield was achieved at pH 4, 32 ℃, and 3 g/L yeast use, according to the talks. Because it contains no harmful ingredients and has a low composition, the bio-ethanol derived from rotting pineapple waste was used well in engine automobiles. Food waste can be used to make bio-ethanol as a means of reducing the environmental impact of agriculture. Bioresearch energy was created as a result of the procedure, which helps overcome the issues of fossil fuel depletion. Producing bio-ethanol from agricultural waste, such as that from vegetables and fruits, is an effective way to reduce emissions from an engine. Because of its low cost and wide availability, vegetable and fruit waste makes an excellent bio-ethanol feedstock.

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