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Research Article
Bioethanol Production from Fruit Biomass as Bio-antiseptic and Bio-antifermenter: Its Chemical and Biochemical Properties

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Abstract

Background and Objectives: The exploration of fruit biomass derived biomaterials (bio-antiseptic, biosolvent, biofilm, biofuel) encourages the uses of biomass extensively. Thus, fruits wastes can be reused to generate bioethanol as antiseptic and bio-solvent. The study was carried out to investigate the optimization of bioethanol production and evaluate the bioethanol as anti-fermenter and anti-septic. **Materials and Methods:** Rotten banana, grape and dates biomasses were used through fermentation bioprocess using yeast. Samples were thoroughly washed with distilled water, cut using a sterile knife and were blended by using a sterilized automatic juice blender. **Results:** Bioethanol yield was higher in dates biomass than in grape and banana biomass at 3 g L^{-1} yeast concentration at 30°C. The lowest pH was found in the bioethanol produced from dates biomass. The lower TSS and glucose content was exhibited in the bioethanol produced from banana biomass. The lowest viscosity and acid value was found at 3 mg L^{-1} of yeast concentration in dates biomass. Chemical elements like Ca, P, Fe, Pb, Cu and Si fulfilled the requirement of the standard specification as well. Grape juice mixing with bioethanol showed antifermenter for 2 days while in the 1st day juice started to rot the faster in the control. The lowest bacterial colony formation was observed in the dates biomass derived bioethanol. **Conclusions:** Results explored that produced bioethanol was of good quality and can be used as antiseptic and bio-solvent from fruit biomass.

Key words: Bioethanol, fruit waste, anti-fermenter, anti-septic, *E. coli* bacteria

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Biomass is the biodegradable fraction of bio-products, waste and residues from agriculture like vegetables and animal origin, forestry and related industries as well as industrial and municipal waste[1,2]. Different forms of bio-products like bioethanol[3,4], nano-cellulose[5], biofilm, biofibre etc., can be produced from a wide range of biomass sources for example, agricultural (fruit, vegetable, crops) residues. Bioethanol can be used as antiseptic (disinfectant), biosolvent (anti-fermenter) and biofuel as bioenergy[5]. Pineapple waste have potential for recycling in order to get valuable raw material, convert into useful and higher value products, food or feed after biological treatment and even as raw material for other industries. Pineapple waste was converted to the bioethanol production by fermentation bioprocess[6]. Ethanol is the type of alcohol present in alcoholic beverages and is effective disinfectant for many reasons. Isopropyl alcohol is also known as Isopropanol, 2-propanol or rubbing alcohol. When used as disinfectants, both are typically at a concentration of 70% in water[7,8].

Disinfectants are anti-microbial agents that are applied to the surface of non-living objects to destroy micro-organisms. Disinfectants (anti-septics) destroy micro-organisms on living tissue[9]. Disinfectants work by destroying the cell wall of microbes or interfering with the metabolism sanitizers are substances that simultaneously clean and disinfect. Disinfectants are frequently used in hospitals, dental surgeries, kitchens and bathrooms to kill infectious organisms[10]. Alcohol and alcohol based compounds comprise a class of proven surface sanitizers and disinfectants approved by the Centers for disease control for the use as a hospital grade disinfectant[11]. A mixture of 70% ethanol or isopropanol diluted in water was effective against a wide spectrum of bacteria, though higher concentrations to disinfect wet surfaces[11]. The effect of 29.4% ethanol with dodecanoic acid was effective against a broad spectrum of bacteria, fungi and viruses[12,13].

Many disinfectants are used alone or in combinations (e.g., hydrogen peroxide, acetic acid and alcohol) in the health-care setting efficiently. Ethyl alcohols have been used effectively to disinfect oral and rectal thermometers, hospital pagers, scissors and stethoscopes. Alcohols have been used to disinfect fiberoptic endoscopes. Ethyl alcohol towels have been used for years to disinfect small surfaces such as rubber stoppers of multiple-dose medication vials or vaccine bottles[14]. This is an innovative research of bioethanol which can be used as anti-septic and bio-antifermenter in medical, biomedical and food industries.

The objectives of this study were:

- To investigate the influence of different concentration of yeast and temperatures on bioethanol production by using rotten banana, grapes and dates
- To evaluate the physical, biochemical and chemical (chemical elements) properties of bioethanol for the use of antiseptic and bioantifermenter

MATERIALS AND METHODS

Experiment 1 (Banana waste): The banana wastes (rotten) were bought from the experimental garden, University of Malaya, Kuala Lumpur. The yeast used in this experiment was *Saccharomyces cerevisiae* type II collected from BioChemika with Fluka No. 22180. Only 10% would autolyze in aqueous buffer at 37°C and fast dried to yield 90% active, viable yeast in a convenient solid. The experiment was done in the University of Malaya, Plant Physiology and Biotechnology Laboratory and Biology Laboratory, Hail University, KSA. It took 1.5 years to complete the experiment in both laboratory.

Sample preparation: About 2 kg of rotten banana were thoroughly washed with distilled water, cut using a sterile knife and were blended by using a sterilized automatic juice blender. The banana mash was then dispensed into the total of 9 cylinder with three replicates for each sample for different temperature and days parameter. The 250 mL of water were added into the cylinder (1500 mL) containing banana mash (1000 g). The pH of the banana mash was measured. After that, total soluble solids and glucose of banana mash were determined.

Fermentation using bioreactor: The 1, 3 and 5 g L^{-1} of yeast, *Saccharomyces cerevisiae* was added into each set and all of the bottles were closed to ensure they were made air-tight to provide an anaerobic condition and placed in incubator at 28, 30 and 32°C. The dry active yeasts were rehydrated in water bath at 40°C by using clean water and allowed taking to room temperature before added into the banana mash. Fermentation was carried out for 3 days in the dynamic modeling pH, temperature control and dissolved oxygen concentrations of a continuous yeast fermentation based benchtop bioreactor. After fermentation, the clean sterile cotton cloth was used to sieve the product from the residue. Extracts were collected in sterile plastic containers.

Water and bioethanol separation by rotary evaporator: The raw bioethanol was seperated by vacuum evaporator at 70°C

of water bath temperature. The obtained bioethanol was then taken in room temperature to measure pH (by pH meter), Total Soluble Solid (TSS) [by refractometer] and glucose (by GC). The bioethanol yield was measured by GC-FID.

Glucose determination by GC-FID: The ground samples were filtered and extracts were evaporated to dryness using a rotary evaporator. The residues were taken up into 10 mL of 80% ethanol and stored in the freezer until analysis. Al aliquot of 20 µL sample was taken into the vial and dried them by dryer. Then, 40 µL pyridine including TPB (1, 3, 5 tri-phenyl benzene) 1 mg mL^{-1} as an internal standard, 40 µL HMDS (hexamethyl disilazane) and 40 µL TMCS (chlorotrimethylsilane) were added to the dried samples. The vials were incubated at 60°C for 30 min. About 1 µL of the trimethylsilated sample was injected into a gas chromatograph (GC-FID). The GC condition was as follows: Column temperature: 150-265°C at the increment rate of 10°C min^{-1}. The GC was equipped with a glass column (2.6 mm×2 m) peaked with 1.5% Se-30 coated on Chromosorb WAW DMCS (80-100 mesh) nitrogen was used as carrier gas at the flow rate of 30 mL min^{-1}.

Bioethanol determination by GC-FID: Bioethanol was assessed using GC-FID. The GC conditions were of SRI GC model 8610C, equipped with a 60 m column (Restec MXT-1, Id 0.53 mm, 5 µM), on-column injector and FID conditions: 250°C; H_2, 25 PSI, equivalent to 25 mL min^{-1}; air, 2 PSI, equivalent to 100 mL min^{-1}; gain set to medium. The GC was equipped with an internal air compressor and hydrogen generator. Th N_2 was used as carrier gas with pressure control (24 PSI constant; equivalent to 25 mL min^{-1}). Oven temperature (hence column and injector temperature) was initially set at 50°C and then elevated at the rate of 7°C min^{-1} to 100°C, thus giving a total run time of 7 min. Furthermore, 2 µL was injected manually at time 0, using a 5 µL syringe and temperature cycle was begun. Syringe was thoroughly washed with ethyl acetate between injections to avoid cross-contamination. Bioethanol peak has been appeared at retention time equivalent to 65°C.

Experiment 2 (Grape waste): The grape wastes (rotten) were collected from the experimental garden, University Putra Malaysia, Selangor. The yeast used in this experiment was same as Expt. 1.

Sample preparation was same as Expt 1 except raw materials. In this experiment, sample was used as rotten gapes waste. Other procedures were same as mentioned in the Expt. 1. The same methods were followed for fermentation, water and bioethanol seperation by rotary evaporator, sugar (glucose) determination by GC-FID and bioethanol determination by GC-FID as mentioned in Expt. 1.

Experiment 3 (Dates wastes): The date wastes (rotten) were collected from the experimental garden, King Abdulaziz University, Jedah, KSA. The yeast used in this experiment for fermentation was same as Expt. 1. Sample preparation was same as Expt. 1 except raw materials. In this experiment, sample was used as rotten dates waste. Other procedures were same as the mentioned in the Expt. 1. The same methods were followed for fermentation, water and bioethanol separation by rotary evaporator, glucose determination by GC-FID and bioethanol determination by GC-FID as mentioned in Expt. 1.

Disinfectant experiment as anti-septic using bacteria: *Escherichia coli* bacteria was used in this experiment. The experiment was performed in 1.5 mL tubes, 3 different contact times: 5, 10 and 15 min were also tested. For each tube, 0.1 mL of culture solution was added into 0.9 mL of disinfectant. After certain contact time, a 5000 rpm centrifuge was performed for 5 min to separate the culture from the solution. Supernatant was discarded and then the tube was refilled by deionized water followed by spread plating on each tube. After the experiment, all the result tubes were stored in refrigerator at 4°C. The next day, plate counting was performed on each spread plate after 24 h culturing at 37°C in the incubator.

Bioethanol as biosolvent or (anti-fermenter): Grape juice was used to test the date produced bioethanol as biosolvent. Juice was stored at room temperature for 4 days mixing with bioethanol and without bioethanol (control). Five drops of bioethanol were added into the grape juice vial and observed it's rotten condition at room temperature. Glucose content and bioethanol percent were measured from 1-4 days following the methods mentioned in the Expt.1.

Viscosity, acid value and chemical elemental analysis: Viscosity was measured at the Faculty of Engineering, University of Malaya. For viscosity test, the samples were put in the beaker and heated up at 40°C and then measured by using viscometer. The viscometer was set with the rpm of 30. Then the spindle with the size of 63 was used according to the American Society of Testing Materials (ASTM D 6751) and European Norm for Biodiesel (EN 14214). Total acid value was measured using titration method. An atomic emission (AE)

specification multi-element oil analyzer (MOA) was used to determine the chemical elements like Ca, P, Fe, Pb, Cu and Si content.

Statistical analysis: Data were analyzed statistically. Standard error (SE) and Least significance Difference Test (LSD-Test) were employed.

RESULTS

Bioethanol yield, TSS, pH and glucose determination: Bioethanol yield was higher in dates biomass than in grapes and banana biomass (Table 1). In the case of all biomasses, bioethanol production was lower at 1 and 5 g L^{-1} yeast concentration and higher at 3 g L^{-1} yeast concentration. It has also been shown that pH before fermentation was fixed (5.8) and after fermentation pH was lower for all parameters at fruit different biomass. The lowest pH was found in the bioethanol produced from dates biomass (Table 1). In addition to that TSS (total soluble solids) was higher before fermentation and lower after fermentation for all concentration parameters. After fermentation lower TSS was found in the bioethanol produced from banana biomass compared to the grapes and dates biomass. Glucose content was higher before fermentation and lower after fermentation in the case of all concentrations of yeast. Glucose content was found after fermentation lowest in the bioethanol produced from banana biomass and was highest in the bioethanol produced from dates biomass (Table 1).

Maximum bioethanol yield was found in dates biomass than in grapes and banana biomass (Table 2). For all biomasses, bioethanol production was lower in the fermentation occurred at 28 and 32°C temperature and higher in the fermentation occurred at 30°C temperature for all fruit biomass. The higher bioethanol production was found in the dates biomass at 30°C compared to the banana and grapes biomass (Table 2). It has been observed that pH at the beginning of fermentation was fixed (5.8) and after fermentation pH was lower for temperature parameters at fruit different biomass. The lower pH was found in the bioethanol produced from grapes biomass compared to the dates and banana biomass (Table 2). Moreover, TSS (Total soluble solids) was higher before fermentation and lower after fermentation for all temperature parameters. After fermentation, lower TSS was found in the bioethanol produced from banana and grape biomass compared to the dates biomass at different temperatures. The lowest TSS was found at 30°C in the bioethanol produced from banana biomass (Table 2). Glucose content was higher before fermentation and lower for all temperatures after

Table 1: pH, total soluble solid (TSS) at different concentration of yeast

			pH		TSS		Glucose (%)	
Samples	Parameters (g L^{-1})	Bioethanol yield (%)	Before	After	Before	After	Before	After
Banana biomass	1	7.8[a]	5.8[a]	4.7[a]	12.0[a]	3.93[a]	13.0[a]	3.9[a]
Grapes biomass	3	8.1[a]	5.8[a]	4.6[a]	12.0[a]	4.0[a]	13.0[a]	4.1[a]
Dates biomass	5	8.0[a]	5.8[a]	4.9[a]	12.8[a]	4.0[a]	13.0[a]	4.13[a]
	1	11.5[a]	5.8[a]	4.7[a]	11.0[a]	5.1[a]	14.5[a]	6.0[a]
	3	13.5[a]	5.8[a]	4.4[a]	11.0[a]	4.6[a]	14.5[a]	4.8[a]
	5	12.0[a]	5.8[a]	4.2[a]	11.0[a]	4.1[a]	14.5[a]	5.5[a]
	1	12.0[a]	5.8[a]	3.3[a]	22.0[a]	14.5[a]	17.0[a]	9.0[a]
	3	18.1[b]	5.8[a]	2.8[a]	22.0[a]	13.5[a]	17.0[a]	8.0[a]
	5	17.0[b]	5.8[a]	2.1[a]	22.0[a]	11.4[a]	17.0[a]	7.5[a]

Same letters (a, a) showed no difference at 5% level of significant by least significant difference (LSD) test

Table 2: Bioethanol yield, pH, total soluble solid (TSS) and glucose content in different temperatures

			pH		TSS (%)		Glucose (%)	
Samples	Parameters	Bioethanol yield (%)	Before	After	Before	After	Before	After
Banana biomass	28°C	7.2[a]	5.8[a]	4.3[a]	11.1[a]	3.8[a]	9.0[a]	3.6[a]
Grapes biomass	30°C	8.7[b]	5.8[a]	4.3[a]	11.1[a]	4.0[a]	9.0[a]	4.4[a]
Dates biomass	32°C	7.4[a]	5.8[a]	4.4[a]	11.1[a]	4.2[a]	9.0[a]	3.4[a]
	28°C	12.0[a]	5.8[a]	3.4[a]	11.0[a]	5.8[a]	14.5[a]	6.8[ab]
	30°C	13.0[a]	5.8[a]	2.8[a]	11.0[a]	4.6[a]	14.5[a]	5.0[b]
	32°C	11.3[a]	5.8[a]	3.9[a]	11.0[a]	6.0[a]	14.5[a]	8.0[a]
	28°C	18.5[a]	5.8[a]	4.7[a]	12.0[a]	5.4[a]	13.0[a]	7.7[a]
	30°C	19.0[a]	5.8[a]	4.4[a]	12.0[a]	5.3[a]	13.0[a]	7.6[a]
	32°C	16.6[b]	5.8[a]	4.8[a]	12.0[a]	5.1[a]	13.0[a]	7.1[a]

Same letters (a, a) showed no difference at 5% level of significant by least significant difference (LSD) test

fermentation. After fermentation, glucose content was found lower in the bioethanol produced from banana biomass compared to the grapes and dates biomass and was the highest in the bioethanol produced from dates biomass (Table 2).

Viscosity and acid value determination: As shown in Table 3, the bioethanol produced from dates biomass (it was tested due to the highest yield) was used for the viscosity and acid value analysis. The viscosity was within 1-5 cst which was under the ASTM standard. The lowest viscosity was found at 3 mg L^{-1} (1.09 cst) followed by 1.21 and 1.85 cst at 1 mg L^{-1} and at 5 g L^{-1} yeast concentration. It has been shown from the result, there was a little difference among the acid values for all fermentation in 1, 3 and 5 g L^{-1} of yeast concentration. However, the lowest acid value was found at 3 mg L^{-1} yeast concentration (0.4 mg KOH/g).

Chemical element analysis: It has been exhibited from Table 4 that most of the chemical elements (Ca, P, Fe, Pb, Cu and Si) fulfilled the requirement of the standard specification as well (ASTM D 6751 and EN 14214 methods). The values were 0-4.7 PPM which were under the standard having maximum 5 ppm for P and Ca. In addition, for Pb, Cu, Si, Fe less than 1 PPM.

Glucose correlation: Figure 1 shows the correlation of glucose and bioethanol percent from dates biomass treated with different fermentation period. It has been observed that there was very good correlation found between glucose and bioethanol. When bioethanol yield increased then the glucose yield decreased. R-squared value [for bioethanol (0.86) and glucose (0.77)] showed the good relation between them.

Bioethanol as solvent and anti-septic

As anti-fermenter (biosolvent): From the Fig. 2, it has been seen that glucose content was started to reduce in the first (after 12 h) and bioethanol was started to produce and made rotten the juice faster in the grape juice without produced bioethanol (from dates biomass) at room temperature. Juice mixing with bioethanol showed glucose content was stable for 2 days and from 3 days it was started to rot slowly and bioethanol production (juice rotten percent) was lower than control.

As anti-septic (disinfectant): As shown in Table 5, bacterial, *E. coli* colony/culture was found decreasing trend by

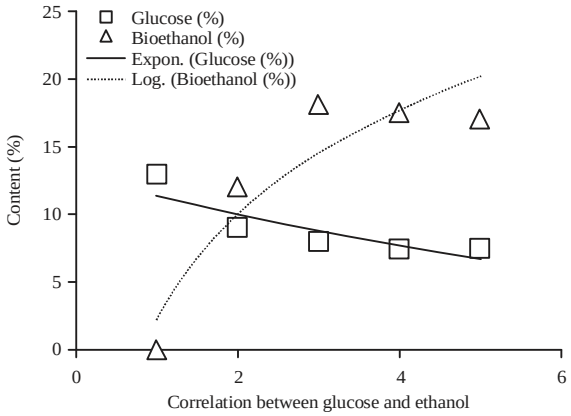

Fig. 1: Correlation of glucose and bioethanol percent from dates biomass treated with different fermentation period
1: Starting, 2: 1st day, 3: 3rd day, 4: 4th day, 5: 5th day

Table 3: Determination of the viscosity and acid value test in dates waste based bioethanol

Amount of yeast (g L^{-1})	Viscosity value (cst)	Acid value (mg KOH/g)	ASTM standard of viscosity and acid value
1	1.21±0.2	0.45±0.03	0-6.0
3	1.09±0.15	0.40±0.02	0-0.5
5	1.85±0.1	0.50±0.02	

Mean±SE

Table 4: Determination of chemical element in date waste based bioethanol

	Chemical element (PPM)						
Amount of yeast (g L^{-1})	Cu	Pb	Fe	Si	P	Ca	ASTM standard value
1	0	0	0.1	0	4.0±0.1	5±0.2	0-5 PPM
3	0	0	0.05	0	3.9±0.2	4.1±0.1	
5	0	0	0.1	0	4.0±0.2	4.7±0.1	

Mean±SE

increasing the time after applying the banana, dates and grape waste based bioethanol. Bacterial colony was lower in the grapes and dates biomass than in banana biomass based produced bioethanol. The lowest colony was observed in the dates biomass derived bioethanol. Figure 3 shows the fruit biomass samples used in the experiment and produced bioethanol.

DISCUSSION

Bioethanol yield was higher in dates biomass than in grapes and banana biomass. It might be due to the high glucose content found in the dates biomass. For all biomasses, bioethanol yield were lower at 1 and 5 g L^{-1} yeast concentration and 28 and 32°C temperature and higher at 3 g L^{-1} yeast concentration and 30°C. This might be due to the optimized fermentation at 3 g L^{-1} yeast concentration

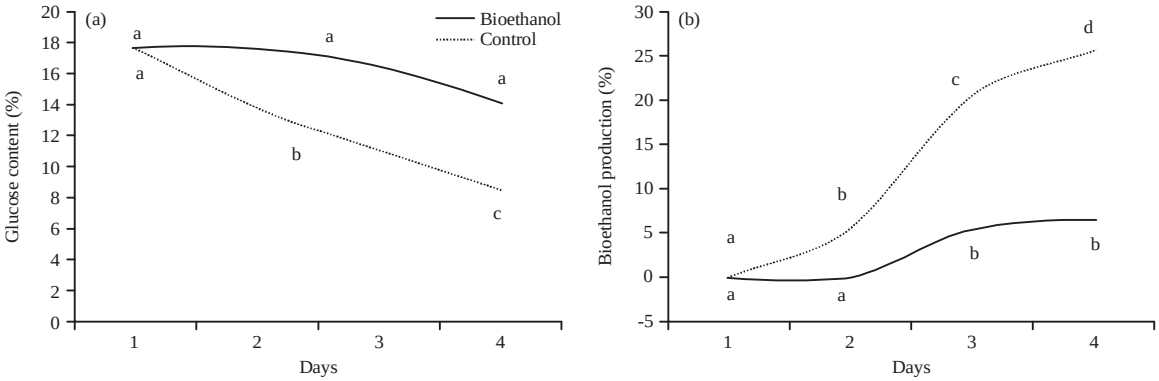

Fig. 2(a-b): Determination of (a) Glucose content and (b) Bioethanol production at different days using dates based bioethanol as solvent (anti-fermenter)

Same letters (a, a) showed no difference at 5% level of significant by least significant difference (LSD) test

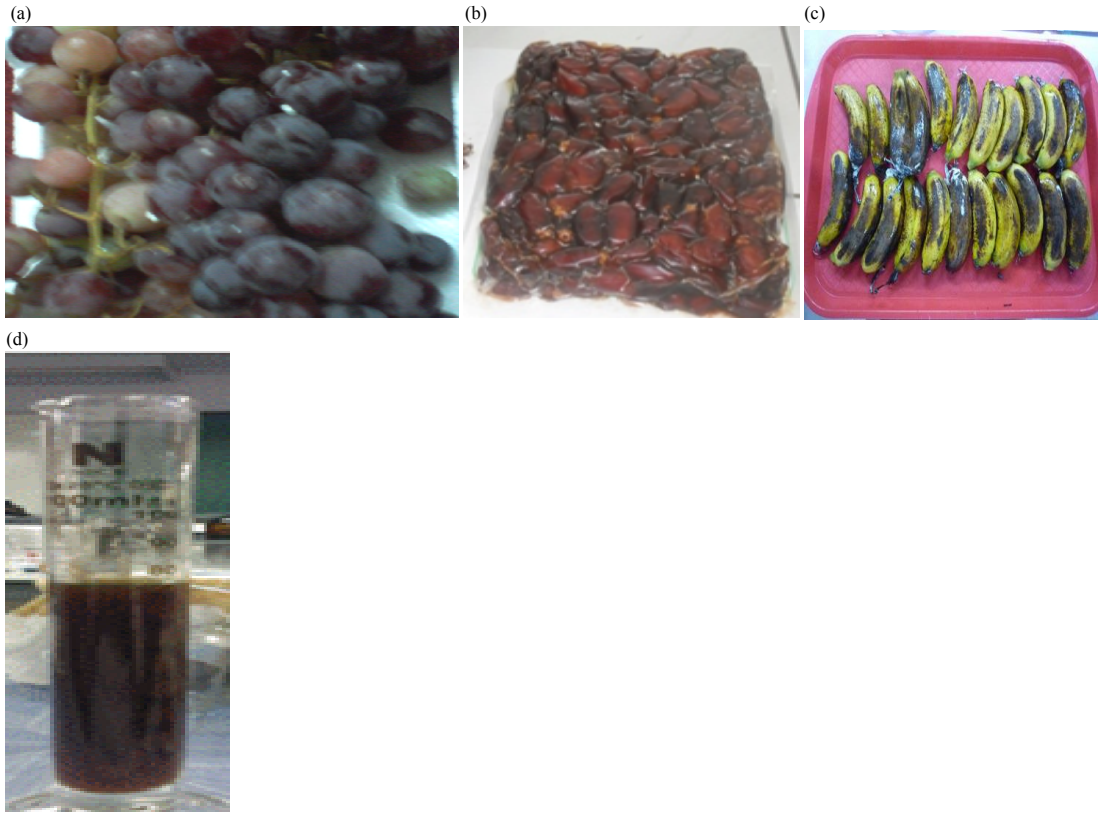

Fig. 3(a-d): Fruit biomass sample and produced bioethanol, (a) Rotten grape (Waste), (b) Dates biomass, (c) Rotten banana and (d) Produced bioethanol

Table 5: Bacterial, (*E. coli)* colony/culture in fruit waste based bioethanol as antiseptic

Exposure time (min)	Disinfectant (log CFU mL^{-1})	Bioethanol banana (log CFU mL^{-1})	Bioethanol grapes (log CFU mL^{-1})	Bioethanol dates (log mean CFU mL^{-1})	Control (log mean CFU mL^{-1})
5	38	2506	1066	1039	10^4
10	14	2032	980	922	10^4
15	NG	2200	768	720	10^4
20	NG	2018	718	690	10^4

and 30°C. It has been shown that after fermentation TSS, glucose and pH was lower for all parameters at fruit different biomass, it might be in order to converting the sugar to the bioethanol in the fermentation. It had been reported that fermentation at 32°C for 48 h yielded the highest bioethanol from sweet Sorghum[15]. At low temperature, (28°C) cells were inactive and longer lag phase was obtained. Thus less ethanol produced by fermentation of glucose to give CO_2 as by-products. At 32°C, cells were at their most active form. Sugar consumption and alcohol production were greater. They were active and have short lag phase and normal log, stationary and death phase. Secondary metabolites to alcoholic fermentation increased as the temperature increased thus bioethanol yield was greater[4] at 32°C. It had been stated that the best parameters for bioethanol obtained were 2 days fermentation using 2 g L^{-1} *S. cerevisiae* at 32°C using rotten apple biomass[4].

As shown in the results, low viscosity value was good for bioethanol used and reduced problem of corrosion. The viscosity of the bioethanol produced was important when considering the production of industrial products, pharmaceutical and cosmetic products. However, the viscosity obtained was maintained under ASTM standard, which indicated best result for this bioethanol produced. Acid value test from samples fermented at different amount of yeast. The lowest acid value was found at 3 mg L^{-1} yeast concentration. The results obtained were in the good range and under ASTM standard specification. It might be due to the fermentation occurred well and produced good quality bioethanol. When bioethanol yield was highest, the glucose content was also lowest at 30°C compared with 28 and 32°C. This indicated good fermentation process where most sugar had been utilized efficiently by *S. cerevisiae* to yield bioethanol. However, in this experiment, bioethanol yield was less compared to the theoretical yield. This might be due to the rate of fermentation of the sugar where small part of sugar was used by yeast to produce new cells and grow[16].

It can be observed that most of the elements (Fe, Pb, Cu, Ca, Si and P) fulfilled the requirement of the standard specification (ASTM) as well. The presence of metals in the bioethanol is undesirable, as this may cause various problems, including promoting bioethanol degradation environmental pollution and subsequent negative effects on human health[17]. The elements whose quantities in bioethanol need to be controlled are calcium (Ca) and phosphorus (P), which originated from the raw materials. The maximum permissible concentrations[18] of while Ca and P is 10 mg kg^{-1}.

It has been shown that bioethanol mixing with juice made delay fermentation while fresh juice (control) rotted 2 days earlier. It might be due to the bioethanol produced from dates biomass mixed with grape juice and acted as antifermenter. Hossain[4] suggested that bioethanol produced from rotten apple biomass might be produced commercially as biosolvent in the laboratory, pharmaceutical, cosmetic, medical and biomedical industries for the substitute of ethanol.

As shown in the result, the lower bacterial colony was observed in the dates biomass derived bioethanol compared to the banana and grapes biomass based bioethanol. It might be used as disinfectant (anti-septic). It has been reported that disinfectants (anti-septics) which destroy microorganisms on living tissue[9]. Disinfectants work by destroying the cell wall of microbes or interfering with the metabolism. Ethyl alcohol and alcohol based compounds had been used as surface sanitizers and disinfectants approved by the centers for disease control for the use as a hospital grade disinfectant[11]. A mixture of 70% ethanol or isopropanol was effectively used against a wide spectrum of bacteria[11]. It has been reported that 29.4% ethanol with dodecanoic acid was effective against a broad spectrum of bacteria, fungi and viruses[12].

CONCLUSION

It can be concluded that production of bioethanol derived from dates and grapes biomass was higher than banana biomass at 30°C using 3 g L^{-1} yeast concentration. Bioethanol derived from dates biomass was the best bio-antiseptic (bio-disinfectant) and bio-solvent (anti-biofermenter). In addition to the it is suggested that bioethanol can be used widely as bio-antiseptic (bio-disinfectant) and bio-solvent (anti-biofermenter).

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