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ORIGINAL ARTICLE

Bioethanol Production from Fruit Biomass as Bio-antiseptic (antibacterial) and Bio-antifermenter: Its Physical, Chemical and Biochemical Properties as an Innovation

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ABSTRACT
The study was carried out to investigate the optimization of bioethanol production and evaluate the bioethanol as antifermenter and antiseptic using banana, grape and dates biomasses through fermentation bioprocess using yeast. Bioethanol yield was higher in dates biomass than in grape and banana biomass at 3g/l yeast concentration at 30°C and lower at 1 and 5 g/l yeast concentration at 28°C and 32°C. Moreover, the lowest pH was found in the bioethanol produced from dates biomass. The lower TSS was found in the bioethanol produced from banana biomass. In addition, the glucose content was lowest in the bioethanol produced from the banana biomass and highest in the bioethanol produced from the date biomass. The lowest viscosity and acid value was found at 3mg/l 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 first day juice started to rot the faster in the control. The lowest bacterial colony formation was observed in the dates biomass derived bioethanol. Results explored that produced bioethanol was of good quality and can be used as antiseptic and bio-solvent from fruit biomass.
Keywords: bioethanol, fruit waste, antifermenter, antiseptic, E.coli bacteria

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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 (antifermenter) 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 percent in water [7, 8].

Disinfectants are antimicrobial agents that are applied to the surface of non-living objects to destroy microorganisms. Disinfectants (antiseptics) destroy microorganisms 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].

The objectives of this study were

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

MATERIAL 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

Sample preparation: Two 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 six cylinder with three replicates for each sample for different temperature and days parameter. The 250 ml of water were added into the cylinder (1500ml) containing banana mash (1000g). 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 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

Raw bioethanol was seperated by vaccum evaporator at 70 ^{0}C of water bath temperature. The obtained bioethanol was then taken in room temperature to measure pH (by pHmeter), 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 in 10 ml of 80% ethanol and stored in the freezer until analysis. Al aliquot of 20µl sample was taken into the vial and dried them by dryer. Then, 40µlpyridine including TPB (1,3,5tri-phenyl benzene) 1 mg/ml 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^{0}C for 30 min. One µl of the trimethylsilated sample was injected into a gas chromatograph (GC-FID). The GC condition was as follows: colunm temperature: 150-265 ^{0}C at the increment rate of 10 ^{0}C/min. The GC was equipped with a glass column (2.6mmx2m) peaked with 1.5% Se-30 coated on ChromosorbWAW DMCS (80-100 mesh). nitrogen was used as carrier gas at the flow rate of 30 ml/min.

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; air, 2 PSI, equivalent to 100 ml/min; gain set to medium. GC was equipped with an internal air compressor and hydrogen generator. N_2 was used as carrier gas with pressure control (24 PSI constant; equivalent to 25 ml/min). Oven temperature (and hence column and

injector temperature) was initially set at 50°C and then elevated at the rate of 7°C/min to 100°C, thus giving a total run time of 7 min. Furthermore, 2µL 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 Purta 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 separation 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 antiseptic using bacteria

E.coli (Escherichia coli) bacteria was used in this experiment. The experiment was performed in 1.5ml tubes, three different contact times: 5min, 10min, 15minwere also tested. For each tube, 0.1ml of culture solution was added into 0.9ml of disinfectant. After certain contact time, a 5000rpm centrifuge was performed for 5min 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^{o}C. The next day, plate counting was performed on each spread plate after 24h culturing at 37 ^{o} C in the incubator.

Bioethanol as biosolvent or (antifermenter)

Grape juice was used to test the date produced bioetahnol 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 one to four 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 AND DISCUSSION

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 yeast concentration and higher at 3g/l 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°C 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 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 to 5 cst which was under the ASTM standard. The lowest viscosity was found at 3mg/l (1.09cst) followed by 1.21 and 1.85 cst at at 1mg/l and at 5g/l 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 of yeast concentration. However, the lowest acid value was found at 3mg/l 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 & 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 antiseptic

As antifermenter (biosolvent)

From the Figure 2, it has been seen that glucose content was started to reduce in the first (after 12 hours) 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 antiseptic (disinfectant)

As shown in Table 5, bacterial, *E.coli (Escherichia coli)* colony/culture was found decreasing trend by 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.

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 yeast concentration, and 28°C and 32°C temperature, and higher at 3g/l yeast concentration and 30°C. This might be due to the optimized fermentation at 3g/l yeast concentration 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 hours 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 CO2 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 at 32°C [4]. It had been stated that the best parameters for bioethanol obtained were two days fermentation using 2g/l *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 3mg/l 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°C 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 of while Ca and P is 10mg/kg [18].

Table 1. Showing the pH, total soluble solid (TSS) at different concentration of yeast. Same letters (a, a) showed no difference at 5% level of significant by Least significant difference (LSD) test.

Sample	Parameter (g/l)	Bioethanol yield, (%)	pH		TSS		Glucose (%)	
			Before	After	Before	After	Before	After
Banana Biomass	1	7.8a	5.8a	4.7a	12.0a	3.93a	13.0a	3.9a
	3	8.1a	5.8a	4.6a	12.0a	4.0a	13.0a	4.1a
	5	8.0a	5.8a	4.9a	12.8a	4.0a	13.0a	4.13a
Grapes Biomass	1	11.5a	5.8a	4.7a	11.0a	5.1a	14.5a	6.0a
	3	13.5a	5.8a	4.4a	11.0a	4.6a	14.5a	4.8a
	5	12.0a	5.8a	4.2a	11.0a	4.1a	14.5a	5.5a
Dates Biomass	1	12.0a	5.8a	3.3a	22.0a	14.5a	17.0a	9.0a
	3	18.1b	5.8a	2.8a	22.0a	13.5a	17.0a	8.0a
	5	17.0b	5.8a	2.1a	22.0a	11.4a	17.0a	7.5a

Table 2. Showing the bioethanol yield, pH, total soluble solid (TSS) and glucose content in different Temperatures. Same letters (a, a) showed no difference at 5% level of significant by Least significant difference (LSD) test.

Sample	Parameter	Bioethanol yield, (%)	pH		TSS (%)		Glucose (%)	
			Before	After	Before	After	BF	AF
Banana Biomass	28°C	7.2a	5.8a	4.3a	11.1a	3.8a	9.0a	3.6a
	30°C	8.7b	5.8a	4.3a	11.1a	4.0a	9.0a	4.4a
	32°C	7.4a	5.8a	4.4a	11.1a	4.2a	9.0a	3.4a
Grapes Biomass	28°C	12.0a	5.8a	3.4a	11a	5.8a	14.5a	6.8ab
	30°C	13.0a	5.8a	2.8a	11a	4.6a	14.5a	5.0b
	32°C	11.3a	5.8a	3.9a	11a	6.0a	14.5a	8.0a
Dates Biomass	28°C	18.5a	5.8a	4.7a	12a	5.4a	13.0a	7.7a
	30°C	19.0a	5.8a	4.4a	12a	5.3a	13.0a	7.6a
	32°C	16.6b	5.8a	4.8a	12a	5.1a	13.0a	7.1a

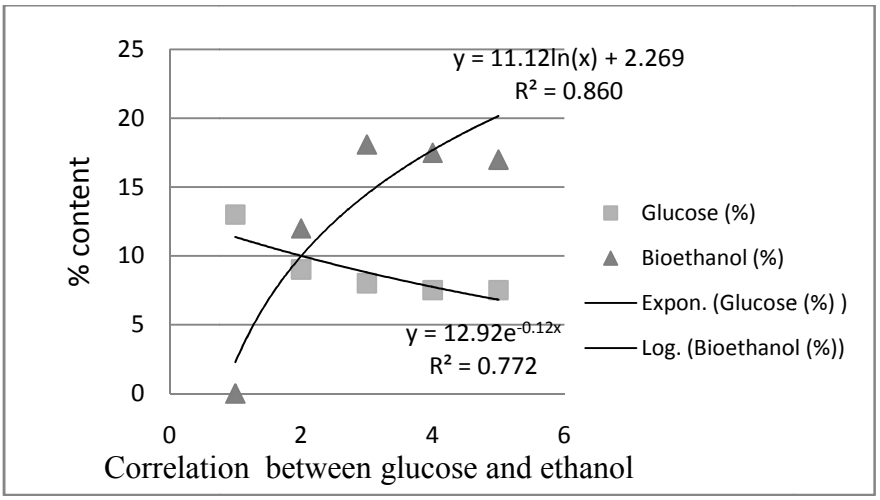

Figure 1. Correlation of Glucose and bioethanol percent from dates biomass treated with different fermentation period. (1= starting, 2= 1st day, 3= 3rd day, 4= 4th day, 5 = 5th day).

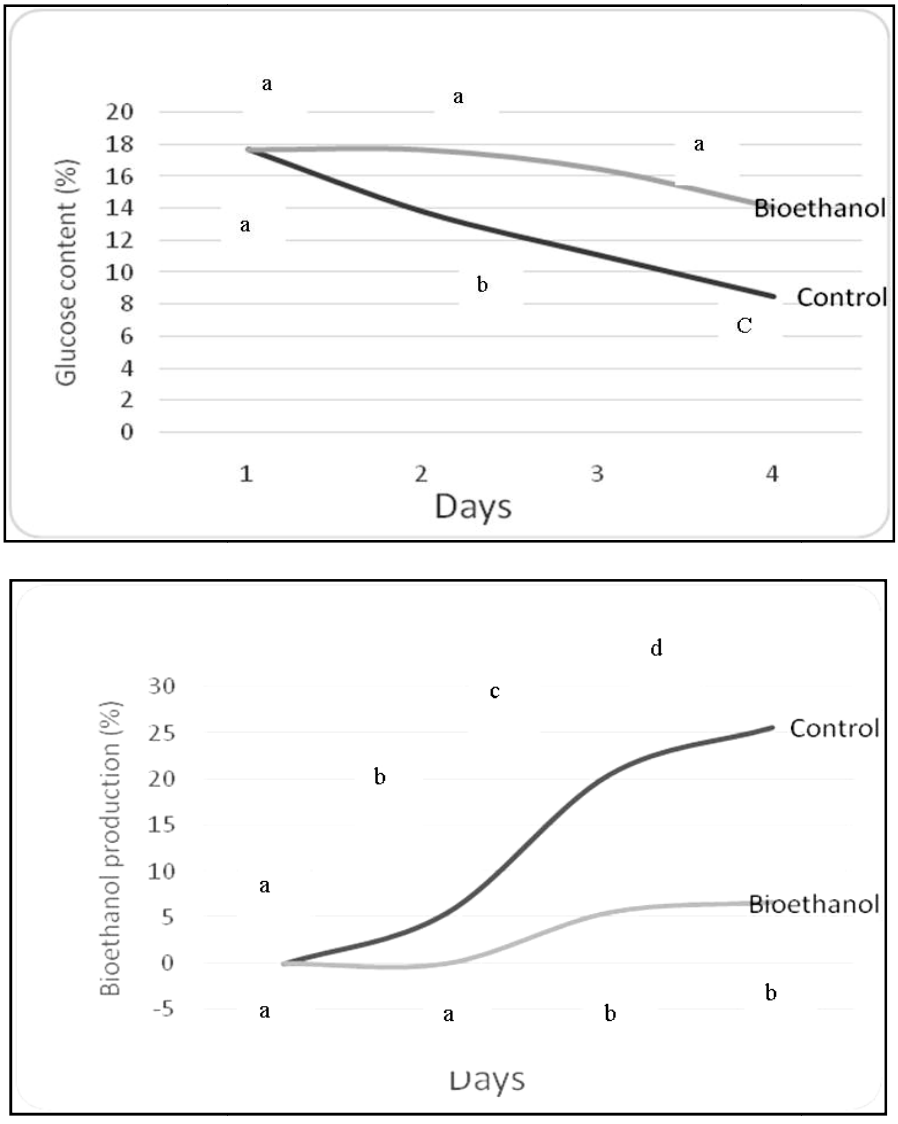

Figure 2: Determination of glucose content and bioethanol production at different days using dates based bioethanol as solvent (antifermenter). Same letters (a, a) showed no difference at 5% level of significant by Least significant difference (LSD) test.

Table 3. Determination of the viscosity and acid value test in dates waste based bioethanol. Mean ± SE.

Amount of yeast (g/l)	Viscosity value (cst)	Acid Value (mgKOH/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	and 0 - 0.5
5	1.85±0.1	0.50±0.02	

Table 4. Determination of chemical element in date waste based bioethanol. Mean ± SE.

Amount of yeast (g/l)	Chemical element (PPM)						ASTM standard value
	Cu	Pb	Fe	Si	P	Ca	
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	

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

Exposure time Min	Disinfectant log cfu/ml	Bioethanol banana log cfu/ml	Bioethanol grapes log cfu/ml	Bioethanol dates log Mean cfu/ml	Control log Mean cfu/ml
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

Rotten grape (Waste) Dates biomass Rotten banana

Produced bioethanol

Fig. 3 Photographs show the fruit biomass sample and produced bioethanol.

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 disinfactant (antiseptic). It has been reported that disinfectants (antiseptics) 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 30^0C using 3 g/l yeast concentration. Bioethanol derived from dates biomass was the best bioantiseptic (biodisinfactant) and biosolvent (antibiofermenter). In addition to the it is suggested that bioethanol can be used widely as bioantiseptic (biodisinfactant) and biosolvent (antibiofermenter).

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