RESEARCH ARTICLE

Production and quality evaluation of bioethanol from corn (*Zea mays* L.) cobs: simultaneous fermentation and process optimization

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ABSTRACT

Bioethanol is a renewable fuel and has been lauded as a viable alternative to petroleum-based fuels, offering less pollution to the environment; hence, considered as a sustainable source of energy. Present study was designed to produce bioethanol from corn grains and to find out the most suitable corn mash concentration for maximum ethanol production and to analyze the quality of ethanol. Bioethanol was made from different concentration (10, 20, 30 and 40%) of corn mash. To obtain corn ethanol, ground corn was subjected to hydrolysis using enzyme amylase, fermented with yeast and distilled. Physicochemical properties such as ethanol concentration, pH, electrical conductivity, total soluble solids, titratable acidity, reducing and total sugar of distilled bioethanol were analysed. Determination of ethanol content by both specific gravity and spectrophotometer technique revealed that peak ethanol productivity was at 30% of corn mash content. Other quality parameters changed according to the ethanol produced in the sample. It can be concluded that maximum amount of bioethanol (13.6%) can be produced at 30% of corn mash concentration.

Keywords: *Bioethanol, distillation, fermentation, hydrolysis, physic-chemical properties*

INTRODUCTION

Energy crises around the world is a very big challenge in recent times and this is given mainly due to the incapability of government and energy sectors to balance supply of energy with the upsurge in human population and emergent demand for energy by the industries (Lambert and Middleton, 2010). Therefore, it calls for the urgent development of alternative energy sources which are clean burning, cost effective and environmentally friendly and competitive well with the fossil fuel. Alcohol has been proposed as a potential alternative source of energy given the fact that it could be easily produced from different sources that are simply available in unindustrialized countries (Uthman and Jimoh, 2012).

Bioethanol is an alcohol usually obtained from the conversion of carbon-based feedstock by microbial fermentation, mostly from carbohydrates produced in sugar or starch bearing plants. Ethyl alcohol can be produced efficiently from a variety of feed stocks such as sugar cane, corn, sorghum, barley, sweet potatoes, cassava, wheat, several fruits, molasses and other biomasses and wastes (Onuki *et al*., 2008). Corn (*Zea mays*) based ethanol has been used as a liquid transportation fuel for more than a century and is known as a popular feedstock for ethanol production in the United States and other developed countries due to its abundance and comparatively easiness of conversion to ethanol. Conversion includes grinding, cooking with enzymes, fermentation with yeasts, distillation to remove water and solid particles. For fuel ethanol two more steps are included; molecular sieving to remove the last of the water and denaturing to make the ethanol undrinkable.

Commercial production of bioethanol is undertaken by either wet mill or dry grind process (Sapna *et al.*, 2012). In wet milling the grain kernel will be separated into its constituents (starch, fibre, protein, and corn germ) prior to fermentation by heating in sulfurous acid solution for 2 d (Butzen and Haefele, 2008) whereas in dry mill process, the entire grain kernel is ground into flour.

The starch in the flour is converted to ethanol during the fermentation process, creating carbon dioxide and dried distiller grain with solubles (DDGS) (Bothast and Schlicher, 2005). During the bioethanol production process, starch will be mixed/slurried with hot water (60 ºC) to form a mash or slurry. Once the corn mash is made it goes through cooking and liquefaction. When the mash is subjected to cooking stage gelatinization of starch will occur. When the temperature of corn mash is more than 60 °C water interacts with the starch granules and forms a viscous suspension. In order to accomplish liquefaction, the pH of the mash is maintained in the range of 5.9 – 6.2, and ammonia and sulfuric acid are added to the tank to maintain the pH. The cooked corn mash is then allowed to cool to 80 – 90 °C (175 – 195 °F), additional enzyme (α-amylase) is added to enhance starch hydrolysis (Mosier and Ileleji, 2015).

The next step in the process of making ethanol is saccharification. This is the process of further hydrolysis to glucose monomers. Gluco-amylase enzyme is used during saccharification. The final step in producing ethanol from the starch is fermentation. The biochemical reaction of fermentation yields two moles of ethanol and two moles of carbon dioxide from one mole of glucose. To source the fermentation, yeast (*Saccharomyces cerevisiae*) is added (Sapna *et al.*, 2012). The last phase of ethanol production process is distillation. It involves separation of ethanol from the solids and water in the mash. The distillation unit utilises the differences in the boiling points of ethanol (78 ºC) and water (100 ºC) (Bothast and Schlicher, 2005). Bioethanol can be used as fuel by blending with gasoline at the rate of 5, 10 and/or 85% which are known as E5, E10 and/or E85 blends.

Ethanol concentration in the samples can be determined by densitometry (pycnometer and hydrometer), spectrophotometry and gas chromatography techniques (Furtado *et al.*, 2015). Acidity and/or pH and solids present in the samples will control the ethanol production rate during fermentation (Shyam *et al.*, 2015). There are number of importance of producing corn bioethanol such as resulting from a renewable resource and reduction of greenhouse gas emissions (USDA, 2016). Further, blending bioethanol with gasoline will help extend the life of diminishing oil supplies and ensure greater fuel security, avoiding heavy reliance on oil producing nations (Bekunda *et al.*, 2009). Hence, bioethanol production is promoted in many countries worldwide due to these benefits. Many of the above benefits are particularly attractive to developing nations in Asia as they have a large agriculture base and many are also increasingly net importers of energy fuel for their rapidly growing economies. Thus, many countries in the region have already implemented ambitious targets and/or mandates to promote biofuels (Shi and Goto, 2013). In an effort to face the energy crisis, it is timely that Sri Lanka also takes initiatives to look at these feasible alternative fuels for transportation of which bioethanol is the most common (Gunawardena, 2009).

Ethanol production in Sri Lanka is approximately 12 million litres per year and is produced using sugarcane molasses. Fuel grade ethanol should be produced locally at least for partial replacement of petrol in Sri Lanka. Among the crops grown in Sri Lanka, rice, cassava and maize, with a high starch content and sugar cane, with a high sugar content, and wasted fruits and vegetables also can be fermented into bioethanol. Nonetheless, bioethanol production in Sri Lanka is still in its infancy (Nisskanka and Konaris, 2010).

Therefore, there is a need to study the development of bioethanol production processing system. In this present study, efforts have been made to incorporate corn into ethanol from various processes. Therefore, this research study was carried out to produce renewable, alternative and sustainable energy source as a bioethanol and to find out most suitable corn mash concentration for maximum ethanol production and to carry out the physicochemical property analysis.

MATERIALS AND METHODS

Location

This study was conducted at the Department of Agricultural Chemistry, Faculty of Agriculture, Eastern University, Sri Lanka for a period of six months.

Materials and methods

Dried corn kernels and commercial baker's yeast were purchased from the Departmental store and active amylase enzyme was bought from S.K. Laboratory, Colombo. Dried corn kernels were manually de-stoned, ground and sieved through a one mm sieve to get uniform size flour. Dissolved solutions of amylase and yeast were prepared at the concentration of 3 and 5%, respectively. Corn bioethanol was prepared by using different concentration of corn mash such as 10, 20, 30 and 40% using 10, 20, 30 and 40 g of corn flour in the treatments of T_1, T_2, T_3 and T_4 respectively, with constant amounts of amylase enzyme (2 mL) and yeast (5 mL) to formulate different formulations of ethanol sample

Different concentrations of corn mash (10, 20, 30 and 40%) were prepared and they were cooked for 2 h at 100 °C. Then, hydrolysis of starch was allowed to take place with the help of amylase enzyme (2 mL/100 mL of corn mash). Hydrolysis was undertaken for about 15 – 24 h at 60 °C. After the hydrolysis, 5 mL of dissolved yeast was added for each treatment to conduct the fermentation of hydrolysed starch. Fermentation was allowed to occur for around 2 – 3 d at 30 – 32 °C. Entire process was conducted in a sealed container and occasionally samples were shaken to facilitate the even reactions. Finally distillation was carried out to separate ethanol from samples. This experiment was carried out in triplicate.

Determination of physicochemical qualities of distillated bioethanol

All the physicochemical parameters such as specific gravity, pH, electrical conductivity (EC), total soluble solids, titratable acidity, reducing sugar and total sugar were analysed using the recommended AOAC (2002) methods.

Ethanol concentration of the samples was analysed by both the specific gravity (Bakalinsky and Penner, 2003) and spectrophotometric (Caputi *et al.*, 1968) methods. During the fermentation and after the distillation pH of each sample was analysed. Amount of total soluble solids in the samples was measured before fermentation and after the distillation. Other parameters were measure after the distillation of ethanol.

Statistical analysis

Treatments were assigned in Complete Randomized Design (CRD). Data of the chemical analysis was subjected to Analysis of Variance (ANOVA) (α = 0.05) and mean separation was undertaken with Duncan's Multiple Range Test (DMRT). Statistical analysis was carried out using Statistical Analysis System (SAS) software.

RESULTS AND DISCUSSION

Physicochemical properties

Ethanol Concentration by Specific Gravity Method

According to the results shown in Figure 1, there was no significant ($P>0.05$) difference between the values of T_2 and T_3 distilled ethanol samples. As shown in the Figure 1, ethanol concentration increased from 8.3

to 13.6% gradually with the increase in corn mash concentration and it suddenly dropped to 11.5% with further increase in mash concentration.

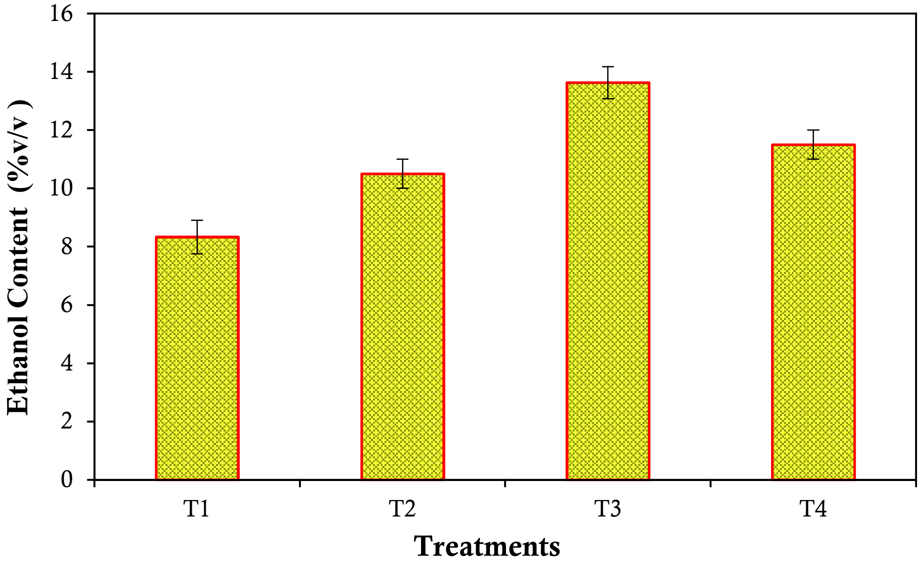

Figure 1: Ethanol concentration of distilled sample.

The values are means of triplicates and the vertical bars indicate the standard errors. T_1–10% mash concentration; T_2–20% mash concentration; T_3–30% mash concentration; T_4– 40% mash concentration.

The highest amount of ethanol was obtained from T_3 (13.6%) and T_1 had the lowest value of 8.3%. These ethanol concentration values are compared well with the values (8.5 – 16.0%) reported for bioethanol produced from corn by other authors (Gumienna *et al.*, 2016; Sapna *et al.*, 2012). Due to the increase in mash concentration, ethanol production during fermentation is increased until 30% of mash content but above 30% sudden drop of ethanol production was clearly visible. The reason for the drop of ethanol production in higher mash concentration (at 40%) is due to the limited enzyme and yeast activity and eventually hydrolysis and fermentation were also inhibited. The reaction rate involving enzymes increases as the substrate concentration increases. However, the number of enzyme active sites available is limited. At specific enzyme concentrations or high substrate concentrations, all of the accessible enzyme active sites could be occupied with substrates. Consequently, increasing the substrate concentration further will not change the rate of reactions and also initial increase in intercellular ethanol concentration deactivates the enzyme and its effects (Hosny *et al.*, 2016).

Ethanol content by spectrophotometer

This experiment was conducted to confirm the ethanol concentration which was obtained from the specific gravity method. After the experimentation, results obtained were compared each other for the reliability of outcomes. Out of four mash concentrations used for ethanol production, T_3 produced the highest ethanol content which is shown in the Table 1. This result also further confirms the results obtained from specific gravity method. From the results obtained from both methods spectrophotometric determination is most trustworthy due to the small amount of standard deviation. This statement is in agreement with Sayyad *et al*. (2015) who reported that dichromate oxidation is a consistent and reliable method for quantitative determination of ethanol in fermented beverages. Further, it was observed that ethanol determination by specific gravity method is also somewhat accurate and it is preferred by many manufacturers because of its easy handling. Therefore, from the above methods determination of ethanol concentration by spectrophotometry method is more accurate.

Table 1: Comparison of ethanol content of the distilled sample by spectrophotometer and specific gravity methods.

Treatments	Ethanol content (% v/v)	
	By Spectrophotometer	By Specific Gravity
T_1	8.45^a	8.33^a
T_2	10.72^b	10.50^b
T_3	13.83^c	13.63^c
T_4	11.65^b	11.50^b

The values are means of triplicates.
The means with the same letters are not significantly different at 5% level.
T_1–10% mash concentration; T_2–20% mash concentration; T_3–30% mash concentration; T_4–40% mash concentration.

Titratable acidity of distillate

There was a significant increase (P<0.05) in titratable acidity content from 0.51 to 0.74% when the mash concentration was increased from 10 to 30% (Figure 3). This may be due to the increase in the level of ethanol produced from 10 to 30%. The highest acidity (0.74%) was evident in T_3. T_1 had the least acidity value of 0.51%. The results showed that acidity changed with the ethanol content. This may be due to the conversion of ethanol to acetic acid via further fermentation. Similar observations have been reported during ethanol production from corn starch using *Saccharomyces cerevisiae* by Graves *et al*. (2006).

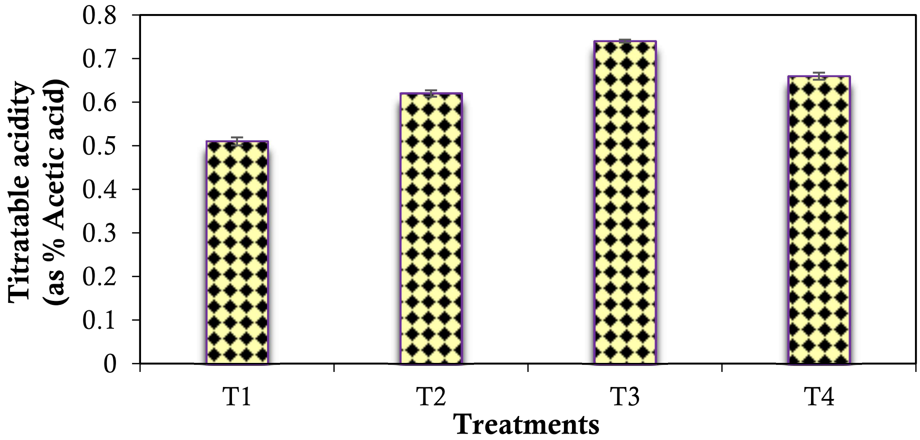

Figure 2: Titratable acidity of the distilled ethanol from different treatments. The values are means of triplicates and the vertical bars indicate the standard errors.
T_1–10% mash concentration; T_2–20% mash concentration; T_3 – 30% mash concentration; T_4 – 40% mash concentration.

pH

The pH values of samples during fermentation and after the distillation are presented in Table 2. The highest titratable acidity and the lowest pH in the distilled samples were recorded in T_3. Because titratable acidity and pH were inversely correlated.

Table 2: pH and electrical conductivity of bioethanol samples

Treatment	pH During Fermentation	pH After Distillation	Electrical Conductivity (µs/cm)
T_1	6.07a	4.39^a	57.67^d
T_2	5.68^b	4.19^b	48.33^c
T_3	5.07^d	3.76^d	36.67^b
T_4	5.45^c	4.13^c	43.67^a

The values are means of triplicates.
The means with the same letters are not significantly different at 5% level.
T_1–10% mash concentration; T_2–20% mash concentration; T_3–30% mash concentration; T_4 – 40% mash concentration.

There was a significant reduction in pH ($P<0.05$) when increasing the concentration of mash because production of ethanol was increased in higher mash concentration and in turn the acidity also increased. For the distilled samples the highest pH value 4.39 was observed in the T_1 and T_3 had the least pH value. During fermentation, the lowest pH value (5.07) observed in T_3 and the highest value was witnessed in T_1. Maximum ethanol yield perceived at around pH of 5. The yield was lower at a slightly acidic pH like 6 – 5.5. This is similar with the investigations of Ogbonda and Kiin-Kabari (2013). Yeast is an acid-tolerant microorganism and their growth is best at lower pH conditions. As a result, fermentation by yeast will be faster at lower pH values. The best pH range for yeast fermentation is 4.5 – 5.5 (Balcerek *et al.*, 2016).

Electrical conductivity

Table 2 shows the EC measurements of bioethanol samples after distillation. The EC of the treatments significantly decreased ($P<0.05$) from 57.67 to 36.67 µs/cm with the gradual increase in the mash concentration in the samples. According to Personna *et al.* (2013), the decrease in EC in the ethanol-water mixture as ethanol concentration increases. Since pure ethanol is highly resistive to conduct electric current, its addition to other solutions reduces the mixture's EC. With the increase in mash concentration EC of distilled ethanol decreased due to increase in ethanol content in those samples and further increase in mash concentration to 40% increased the EC of distilled ethanol to 43.67µs/cm.

Total soluble solids of mash and distilled ethanol

Figure 3 shows the amount of TSS measured before the fermentation and after the distillation of mash. The amount of TSS before fermentation and after distillation had increased gradually according to the increase in mash content from 10 to 40.

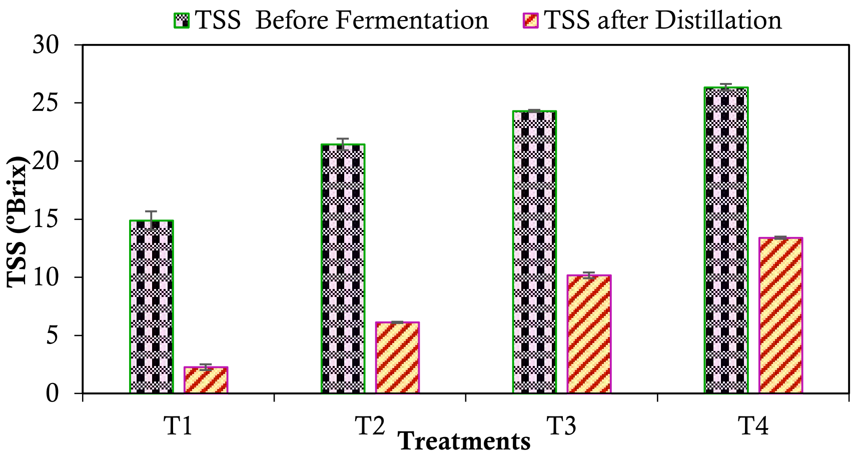

Figure 3: Total soluble solids before fermentation and following distillation

The values are means of triplicates and the vertical bars indicate the standard errors. T_1–10% mash concentration; T_2–20% mash concentration; T_3–30% mash concentration; T_4–40% mash concentration

According to the results shown in Figure 3, TSS values increased significantly (*P*<0.05). Values of TSS measured before fermentation increased gradually from 14.87 °Brix to 26.33 °Brix and after the distillation also TSS values increased from 2.2 ° Brix to 13.4 °Brix. Similar increasing trend in total soluble solids have been reported by Khandaker *et al.* (2018) in bioethanol production from fruit and vegetable wastes. Amount of conversion of total soluble solids present in the samples during fermentation decreased with increase in the mash content. T_1 showed the highest amount of conversion and T_4 showed the lowest. At the end of fermentation, TSS content of corn mash decreased because sugars present in the hydrolysed mash converted into ethanol after fermentation (Zabed *et al.*, 2014). According to the results, increase in ethanol production was observed with increase in total soluble solid up to 24 °Brix (T_3) and thereafter it decreased. Higher sugar concentration can inhibit the fermentation process due to osmotic stress (Jones *et al.*, 1981).

Reducing sugar

Reducing sugar content of distilled ethanol samples increased significantly (*P*<0.05) from 0.39 to 0.63% with the increase in the concentration of mash content from 10 to 40% (Figure 4). Ethanol produced from 40% mash content (T_4) had the highest amount of reducing sugar (0.63%) and T_1 (Ethanol from 10% mash content) had the least amount (0.39%). Reducing sugar content present in the distilled ethanol sample is increased with increase in substrate concentration (Fakruddin *et al.*, 2012).

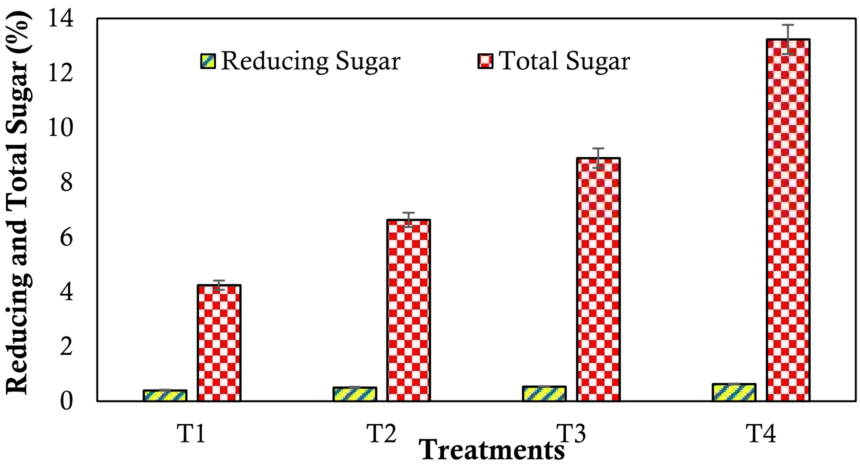

Figure 4: Reducing sugar and total sugar in bioethanol after distillation

The values are means of triplicates and the vertical bars indicate the standard errors.

T_1–10% mash concentration; T_2–20% mash concentration; T_3–30% mash concentration; T_4–40% mash concentration.

Total sugars

Increasing trend in total sugar with the mash concentration was observed (Figure 4). Total sugar significantly (P<0.05) differed between each treatments. T_4 (40% mash content) had the highest value of total sugar (13.23%) and T_1 (10% mash content) had the least amount of total sugar (4.24%). Bioethanol production increased with the treatment until 30% of mash. After that ethanol production limited because high initial substrate concentration may inhibit substrate utilization and/or decrease end product yields (Jessen and Orlygsson, 2012).

CONCLUSIONS

In this study, attempts have been made to produce bioethanol from corn. Maximum amount of ethanol could be produced with 30% of mash content of corn. These studies have shown the potential of producing bioethanol from corn. The amount produced can be further processed and blended with gasoline at required amounts and used as an alternative petroleum based fuel.

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