JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2024, 25(1), 187–194
https://doi.org/10.12911/22998993/175038
ISSN 2299–8993, License CC-BY 4.0

Received: 2023.10.31
Accepted: 2023.11.15
Published: 2023.12.04

Linear Correlation Analysis of Production Parameters of Biofuel from Cacao (*Theobroma Cacao* L.) Mucilage

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ABSTRACT

The present study aimed to analyze the linear correlation between the production variables of biofuel based on cocoa (*Theobroma cacao L.*) mucilage in the city of Calceta - Manabí. The issue addressed was the generation of waste from cocoa farming, leading to contamination of aquifers and the land surface. The CCN-51 cocoa variety was used for the research, following the guidelines of the Completely Randomized Design (CRD), with the proportion of yeast (*Saccharomyces cerevisiae*) as the studied factor, in three quantities: 0.5 kg, 0.1 kg, and 0.025 kg, and two methods of sample dehydration: saline distillation and molecular sieves the interaction between factors generated six treatments, each of which was repeated three times. The study found significant differences in the variables of alcohol content and yield, while there were no differences in pH and ratio/biomass. Treatment T6 was identified as the most feasible for biofuel production, with a pH of 5.86, 83% alcohol content, 76.67 mL ratio/biomass, and 58.10% yield. Regarding the relationship between the production variables, the analysis of linear correlation revealed a strong, directly proportional correlation for all variables, with values ranging from 0.94 to 0.98.

Keywords: association, biofuel, ethanol, CCN-51, mucilage.

INTRODUCTION

The extended use of fossil fuels has become one of the most concerning issues for environmental protection organizations in recent years. The continuous burning of fossil fuels is a primary driver of climate change (Intergovernmental Panel on Climate Change [IPCC], 2014, 2019). Therefore, limiting the average global warming to 1.5–2°C above pre-industrial levels is a goal of the Paris Agreement (No. 2.1) on climate change (United Nations, 2018), calling for a significant phasing out of fossil fuels (McGlade and Ekins, 2015; Welsby et al., 2021).

From 2019 onwards, approximately 80% of the global primary energy demand was covered by fossil fuels (Johnson et al., 2019). The rate of fossil fuel production increased to the point that by 2020, the 'budget' for burning fossil fuel to limit the temperature increase to 1.5°C by 2030 was exceeded by over 120% (Stockholm Environment Institute [SEI] et al., 2020). In this context, multilateral organizations are attempting to develop alternatives that allow for these activities to be conducted more cleanly, thanks to first and second-generation biofuels obtained from agricultural products, microalgae, animal fats, and vegetable oils. Vegetables and biomass also play a key role (Hackenberg, 2008; Morelos, 2016).

The global generation of waste continues to increase due to the effects of population growth, urbanization, and the improvement of economic and industrial activity. Despite various efforts to curb this trend, waste management has been one of the perennial problems facing humanity in recent decades (Awogbemi and Kallon, 2022). For example, the total amount of waste generated worldwide in 2016 was 2.02 billion tons. This figure is expected to rise to 2.59 billion tons in 2030 and 3.4 billion tons in 2050 (Statista, 2018). The global waste management market was valued at $1.61 trillion in 2020 and is expected to reach $2.5 trillion in 2030, with the highest volume of waste in East Asia and the Pacific.

Agricultural waste is an integral part of these wastes. Available statistics showed that approximately 998 million tons of agricultural waste are generated annually, and most of these wastes are either dumped in landfills or incinerated, leading to adverse environmental consequences (Obi et al., 2016). Agricultural waste is classified into crop residues, farm waste, industrial waste, animal waste, and food waste (Guo et al., 2021; Pattanaik et al., 2019). Transforming these agricultural wastes into useful forms seems to be the ecological, economical, and sustainable way of dealing with the waste. The value addition of agricultural wastes as a type of waste transformation and recycling strategy not only contributes to a clean environment, social and economic development, resource conservation, and recycling but also helps achieve energy security and the economic cycle (Chilakamarry et al., 2022).

The use of renewable energy for various applications is one feasible option for reducing the unpleasant environmental impact of the extraction, refining, and use of fossil fuels (Statista, 2022). In line with this approach, this study is based on the production of biofuels from agricultural waste such as cocoa pulp, a new possibility in the national supply that has so far shown limited production of this type of alternative (Navas, 2014). Among other things, we aim to harness the characteristics of Ecuador's significant cocoa operations, which generate large quantities of waste that, if not properly managed, can lead to cross-contamination of water and soil (Arteaga, 2013; Peso, 2015). In this context, the purpose of the research was to utilize cocoa mucilage (*Theobroma cacao L.*) as an alternative for the production of biofuel using yeast and dehydration methods.

MATERIALS AND METHODS

For the development of the study, an experimental research approach was employed, utilizing a bioreactor and 18 experimental units, each composed of 700 mL of CCN-51variety cocoa mucilage in combination with yeast and the dehydration method. Within the experimental design, two study factors were taken into consideration: Factor L - Yeast Extract and Factor D - Dehydration Method. Regarding Factor L - Yeast Extract, three levels of concentration were used: L1 - 0.5 kg, L2 - 0.1 kg, and L3 - 0.025 kg. Meanwhile, for Factor D - Dehydration Method, two methods were employed: D1 - Saline Distillation and D2 - Molecular Sieves. After combining factors L and D, six treatments were obtained, as shown in Table 1.

To identify the potential of cocoa mucilage in ethanol production, the guidelines of a 3×2 two-factor completely randomized design (CRD) (with 3 replications) were followed, through which ethanol concentrations were determined, aiming to delve into the kinetic parameters of fermentation using models to predict and enhance yields (Delgado et al., 2018). For the analysis of laboratory results, inferential statistics were employed to determine the significance among treatments (ANOVA and Tukey's multiple mean comparison test at a 5% error probability).

Subsequently, the Pearson linear correlation coefficient was used at a 5% significance level, which measures the strength of the linear relationship between the parameters of biofuel production. If there are n pairs of data in the form (x_i, y_i), the interval measuring the correlation is between $-1 \leq r \leq 1$. If r is close to -1, then there is a strong negative linear relationship (inversely proportional), and if r is close to 1, then there is a strong positive linear relationship (directly proportional) (Gutiérrez and De la Vara, 2012). For this purpose, a comparison matrix was created using the InfoStat 2020 software – free version.

The characterization of the sugary material in cocoa mucilage involved taking a sample and evaluating the collected mucilage sample by determining the sugars (Brix degrees), pH, Acidity, and moisture. In determining the dehydration

Table 1. Treatments stated in the research

Treatments	Nomenclature	Mucilage dosage
$L_1 \times D_1$	T_1	700 mL
$L_1 \times D_2$	T_2	700 mL
$L_2 \times D_1$	T_3	700 mL
$L_2 \times D_2$	T_4	700 mL
$L_3 \times D_1$	T_5	700 mL
$L_3 \times D_2$	T_6	700 mL

method and the amount of yeast that enhances bioethanol production, a record of the variables was kept, determining the scope of the characteristics for bioethanol production. The following variables were considered: pH, alcohol concentration, alcohol yield about biomass, and finally, consumption or yield.Principio del formulario

RESULTS AND DISCUSSION

Characterization of Cocoa Mucilage

Table 2 details the physicochemical parameters of the different analyzed samples, considering factors such as pH, moisture, Brix, and acidity, along with the average achieved for each of these factors. In line with the results in Table 2, it is important to note that the optimal characteristics for bioethanol production are achieved following the parameters of the Ecuadorian Institute of Standardization (INEN) 2 478:2009 standard.

The pH reached an average of 3.39, a value that indicates an optimal characterization of the samples, as according to Álvarez et al. (2010) and Araujo et al. (2010), the optimum pH of cocoa mucilage should fall between 3.2 and 3.5. Regarding the Brix degrees, according to Coronado (2001) and Gutiérrez (2002), cocoa mucilage should reach between 10 and 15 Brix degrees to fall within the safety range, thereby avoiding fermentation and fungal development. The sample achieved an average of 11.67° Brix, falling within the established ranges. The acidity level, decreased as the sugar percentages increased, ultimately resulting in an average acidity of 0.65%, which falls within the appropriate range. According to Sandoval (2002) and Jahurul et al., (2013), it should not exceed 0.8%. Finally, for the moisture parameter, sample values between 53.12% and 82.92% were recorded. It should be noted that the highest sugar level of 45% was used for the samples with the highest moisture content. The average moisture content of 63.98% aligns with Arteaga (2013), Vallejo et al. (2015), and Villa (2015), who explains that cocoa mucilage can reach a moisture content of up to 84.5%.

Determination of the treatment that improves bioethanol production

Once the experimental stage has been completed, Table 3 is compiled, grouping the results of each of the variables analyzed about the treatments addressed in the study, which have been obtained through category analysis using the Tukey test with a 5% margin of error.

pH

Based on the ANOVA conducted for the pH variable, the p-value for the independent factors of yeast and dehydration methods is 0.001, which is < 0.05. However, in the interaction of these factors, a p-value of 0.1709 is achieved, which is > 0.05 as the margin of error. This leads to accepting

Table 2. Parameters evaluated in the cocoa mucilage samples

Parameter	Unit	Sample 1	Sample 2	Sample 3	Method	Results
pH	-	3.41	3.54	3.22	Potenciometría	3.39
Brix degrees	-	10	13	12	Refractometría	11.67
Acidity	%	0.83	0.58	0.55	Bureta	0.65
Moisture	%	55.89	82.92	53.12	Tensiómetro	63.98

Table 3. Results of the interaction between treatments

Treatments	pH	Categories	Alcohol grades (%)	Categories	Ration/biomass (mL)	Categories	Yield (%)	Categories
T1	5.04	-	51.33	D	46.33	-	53.93	D
T2	5.19	-	65.67	C	54.67	-	45.97	C
T3	5.39	-	73.33	B	65.67	-	51.33	B
T4	5.62	-	77.67	A B	71.00	-	54.37	A B
T5	5.75	-	80.33	A B	72.67	-	56.23	A B
T6	5.86	-	83	A	76.67	-	58.10	A

the null hypothesis that there is no significant difference in the means of pH in the study treatments. Figure 1 represents the data concerning the mean pH values achieved in the experiment, with T6 reaching the highest pH level at 5.86. Overall, the recorded pH values ranged from 5.04 to 5.86, with greater variability observed between T1 and T6. The achieved results fall within the acceptable pH ranges in the bioethanol production process, as detailed by Suárez et al. (2016), where yeast responds better to a slightly acidic environment with pH levels between 5.1 to 6.

However, there are records from other authors who have attained different pH levels depending on the temperature employed and the fermentation times, such as Delgado et al. (2018), who reached a pH of 4 in their study to obtain bioethanol from cocoa mucilage. In this regard, it should be argued that attaining high citric acid contents is favorable, which, combined with low oxygen levels, facilitates yeast growth and consequently yields ethanol with desirable characteristics.

Alcohol concentration

According to the ANOVA, the p-value for the independent factors of yeast and dehydration methods is 0.0001, while the p-value for the interaction of these factors was 0.0052, both of which are < 0.05 within the margin of error. Thus, the alternative hypothesis is accepted, determining that all sources of interest show statistical differences in the alcohol concentration of the analyzed treatments. Values between 51.33% and 83% were recorded, indicating significant variations in the combination of factors L and D (Figure 2). In

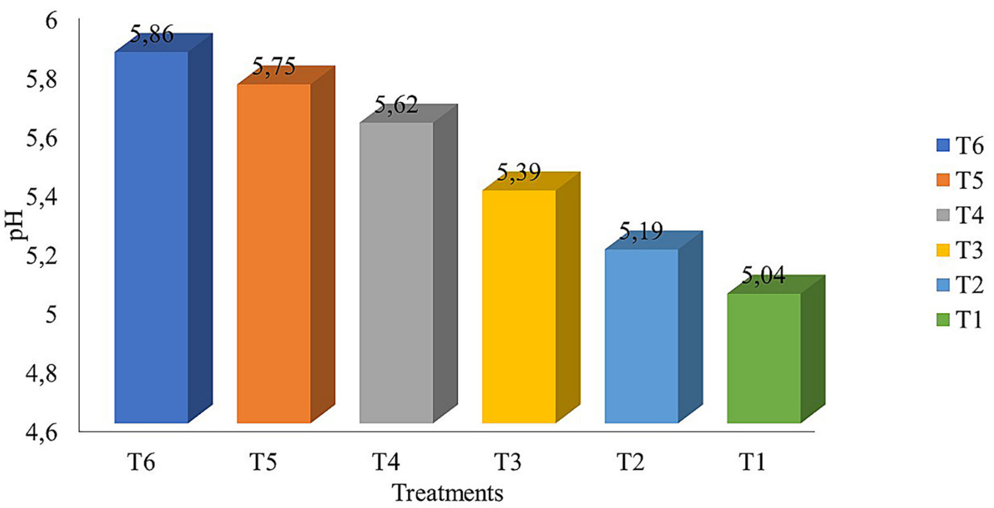

Figure 1. Graphical representation of the mean pH values

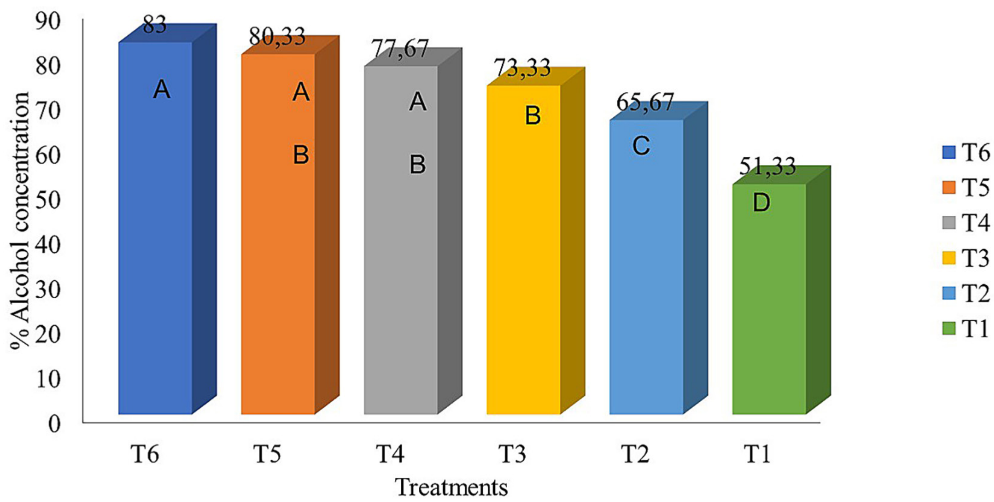

Figure 2. Graphical representation of the mean concentration values

this case, it can be noted that T6 is where higher alcohol levels are achieved in the bioethanol production from cocoa mucilage, while T1 had the lowest rates with a 51.33% alcohol concentration. These results fall within the appropriate ranges, as according to Castillo et al. (2012), ethanol is the most widely used biofuel in the world, typically reaching a concentration of up to 85%.

Additionally, Pérez and Garrido (2011) have explained that to enable the use of ethanol as fuel, it is necessary to remove the water present in it, thereby reaching alcohol levels of up to 92%. The ranges achieved in the study align with the applicability framework in Ecuador, as in the country, the bioethanol fuel is E85, which is 85% ethanol and 15% gasoline, making it a viable alternative.

Alcohol yield of biomass

According to the ANOVA, the p-value for the yeast factor is 0.0001, while that for the dehydration methods is 0.0013, both of which are < 0.05 within the margin of error. Therefore, it is established that these factors show significant differences independently. On the other hand, in the interaction of the factors, a p-value of 0.4612 was reached, which is > 0.05, leading to the acceptance of the null hypothesis that there is no statistical difference in the combination of these factors.

According to Figure 3, despite no significant difference existing between the samples concerning the biomass ratio, a numerical difference is recorded, with T6 being the treatment that obtained the highest amount of alcohol. It is also detailed that the greatest difference was found between T1 and T6. Based on this data, Cárdenas (2017) was able to determine that within the evaluation of the biomass ratio, cocoa mucilage can generate up to 13 mL of alcohol per 100 mL of sample, a value that is related to the data obtained within the present study. Pacheco and Trujillo (2019), on the other hand, carried out a bioethanol production process from cocoa mucilage, determining that for every 500 mL of biomass, between 60 and 80 mL of ethanol can be reached, depending on the strain used, the amount of yeast, and the dehydration method.

Yield

For the yield variable, the ANOVA indicates a p-value of 0.0001 for both the yeast factor and the dehydration method. Furthermore, the p-value corresponding to the interaction of the factors was 0.0052, which is < 0.05, leading to the acceptance of the alternative hypothesis that there is a significant difference in yield in the analyzed treatments, according to the interaction of the yeast extract and dehydration method factors.

In this case, values between 35.93% and 58.10% are recorded, indicating a significant difference in yield among each of the treatments (Figure 4). Based on this, it can be concluded that T6 shows the highest yield, followed by T5 and T4, while the lowest yield corresponds to T1. This shows that a lower amount of yeast extract results in a higher ethanol yield.

About results from other authors, significant variations in yield are noted. For example, Angulo (2017) achieved yields of up to 42%, Vera and Zambrano (2018) achieved a yield of 19.49%, and Cárdenas (2017) achieved yields of up to 13%. According to López (2013), these variations are

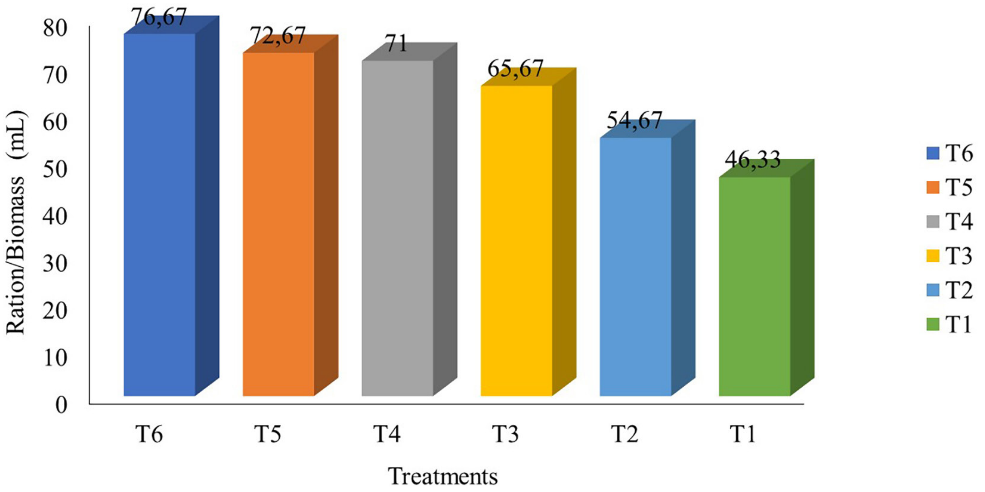

Figure 3. Graphical representation of the mean values of biomass ratio

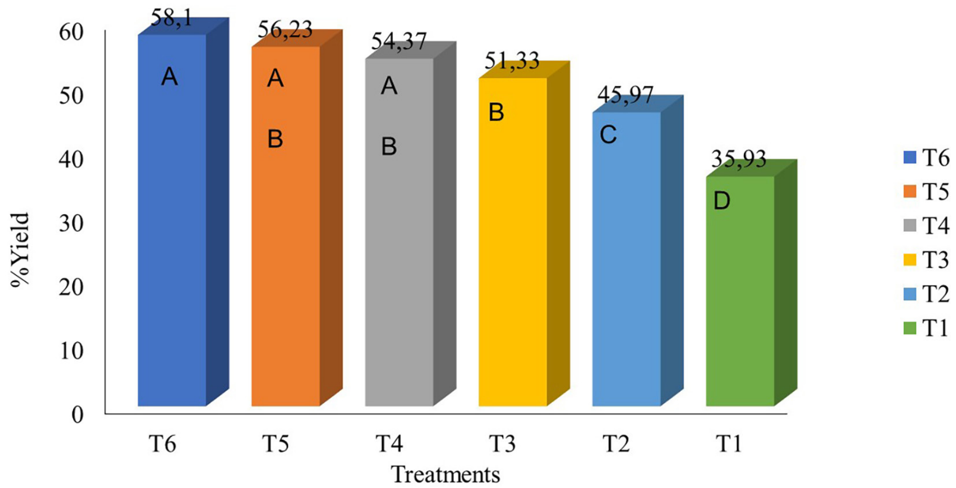

Figure 4. Graphical representation of the mean yield values

Table 4. Results of the interaction between treatments

Parameter	pH	Alcohol grades (%)	Ration/biomass (mL)	Yield (mL)
pH	1	0.94	0.96	0.94
Alcohol grades (%)		1	0.98	1
Ration/biomass (mL)			1	0.98
Yield (mL)				1

due to the percentage of yeast used and the fermentation times, which influence the yield of the finished product (ethanol) from cocoa mucilage.

Correlation of production parameters

As a result of the experiment's execution and the analysis of the obtained data, the linear relationship between the production variables involved in biofuel production was determined with a 5% margin of error, as indicated in Table 4. Once the statistical test was applied, it was determined that all resulting values were equal to or greater than 0.94, indicating a strong positive correlation, suggesting that the relationship is directly proportional, which was influenced by the type of biomass used and the fermentation process applied in the research.

According to Cortés et al., (2019), there is a close relationship in the production parameters of bioethanol such as the incubation periodicity, as prolonged times would generate a high concentration of bioethanol, ultimately becoming toxic. Similarly, fermentation is sensitive to changes in pH, resulting in reduced nutrient permeability in yeast, with an optimal pH of 6.5. Meanwhile, Llenque et al., (2020) state that the parameters evaluated for bioethanol production depend on the type of plant residue used. In acidic treatments, the pH ranges around 2, alcohol levels vary between 7 and 9, and Brix degrees range from 14 to 22, at an ambient temperature of 25°C, after 7 days of fermentation.

CONCLUSIONS

Regarding the characterization of the sugary material, it was found that cocoa mucilage meets the optimal parameters of pH, Brix degrees, acidity, and moisture according to the INEN 2 478:2009 standard, allowing its conversion to alcohol through anaerobic fermentation.

Within the study, bioethanol was obtained from cocoa mucilage through an experiment in which six treatments were designed with yeast concentrations of 0.5 kg, 0.1 kg, and 0.025 kg, subjecting the samples to saline dehydration and molecular sieves. It was determined through ANOVA and Tukey's test that with T6 (0.025 kg yeast extract and dehydration through molecular sieves), the best indicators were obtained in variables such as pH, concentration, biomass ratio, and yield. The analysis of linear correlation shows values between 0.94 and 0.98, explaining the existence of a strong-directly proportional correlation for all biofuel production variables, results that will favor the construction of the regression model and the application of a specific plant residue.

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