Chapter

Evaluation of Ethanol Production Process by the Fermentation of Blue Cabuya Juice (*Agave americana*), using Yeast Strains (*Saccharomyces cerevisiae*) as Potential Application for Blue Agave Liquor

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Abstract

The blue cabuya (*Agave americana L.*) grows wild on large tracts of land in various regions of Peru. The research is aimed at taking advantage of this renewable plant biomass as a potential substrate in one of its possible applications such as cabuya azul liquor, for which, firstly, the yields of the anaerobic fermentation process of this biomass at temperatures of 26, 28 and 30 C and a pH of 3.5 and 4.0, in a batch bioreactor of 120 liters and nutrient concentrations of diammonium phosphate (DAP) of 1 and 2 g/L and a substrate with reducing sugars (AR) of 60 g/L from the district of La Merced, province of Churcampa, Huancavelica Region. The experimental tests were carried out in the laboratories of the Faculties of Biological Sciences and Chemistry and Chemical Engineering of the Universidad Nacional Mayor de San Marcos. Using *Saccharomyces cerevisiae yeast strains* (D 47, LALVIN-Canada) at 28 C, pH 3.5 and DAP nutrient concentration of 2 g/L during 26 h of fermentation, was obtained total biomass yields $Y_{(x/s)}$ =. 0.1606 and ethanol $Y_{(p/s)}$ = 0.4739. It is concluded that the production of ethanol for use in its industrialization as blue cabuya liquor is viable and for the transfer of technology environmental aspects and economic studies must be integrated.

Keywords: blue cabuya, substrate, *Saccharomyces cerevisiae*, diammonium phosphate

1. Introduction

Given the particularity of different microclimates in Peru, the blue cabuya (*Agave americana L.*) grows wild mainly in the Regions of Huancavelica, Huánuco, Ancash, and Cajamarca where their inter-Andean valleys have mild weather between 18 C to 26 C. In addition to this, the favorable conditions of the composition of organic

nutrients and compounds of mineral origin of the soils are ideal for the development of *Agave americana L*. Thus, the maturation time of this plant fluctuates between five to ten years less than the seven to nine years required for the maturation of the same variety in Mexico.

In studies carried out in Mexico, the authenticity of parameters was determined in agricultural soils belonging to the region that grants the Denomination of Origin Tequila (DOT), characterizing 26 samples belonging to Jalisco, Michoacán, Nayarit, and Sinaloa. The characterization consisted of determining the texture, apparent density, color, pH, humidity, organic matter, total carbon, and trace metals. The trace presence of the minerals Cr, Ba, and Zr are useful as auxiliary tracers to determine the authenticity of soils and agaves belonging to the DOT region [1].

The preferable soils for growing tequilana agave from Blue Weber variety must have good drainage. The sucker roots of the agave explore the surface soil when water is available and are practically eliminated in dry seasons. For proper functioning, gas exchange is required in the soil. Therefore, flood areas must be avoided. It is essential that the pH of the soil is not very acidic, since at a pH below 5.5 the plants present nutritional imbalances that if not managed properly can cause damage such as the red ring, a disease that causes the appearance of red areas in the leaves, which form a belt around them that constricts them, many times a hardening and delay in the growth of the plant occurs [2].

Recently, new auxiliary analytical techniques have been analyzed in the current verification process for the detection of counterfeits and/or adulterations of tequila. According to the Official Mexican Standard NOM-006-SCFI-2012, tequila is a regional alcoholic beverage obtained by distillation of musts and prepared directly from the extracted material in the factory facilities of an authorized producer. Tequila is made from tequilana Weber Agave from the blue variety, cultivated in specific protected regions of Mexico that make up the geographical territory of Denomination of Origin Tequila (DOT). According to Mexican regulations, Tequila has a classification based on its category (100% agave Tequila and Tequila) and its classes (silver, gold, extra-aged, and ultra-aged). Based on 2021 data from the Tequila Regulatory Council (CRT), the tequila industry produced 352 million liters and exported to 120 countries [3].

The authenticity and traceability of Tequila are established through a permanent inspection carried out by the CRT at the facilities of the tequila producers, verifying the production process in all its stages and according to the Official Mexican Standard NOM-006-SCFI- 2012. However, the consumption of tequila has increased in volume, being marketed in more countries around the world. To achieve this, certain competitive criteria must be met to demonstrate the quality, safety, and authenticity of the Tequila.

The diversity and dynamics of yeasts and bacteria during small-scale spontaneous fermentations of agave juice have been identified and described. Observing a high heterogeneity in the microbial populations and fermentation parameters that shows the bacteria have a greater diversity than the yeast. The central microorganisms identified were *Saccharomyces cerevisiae* and *Lactobacillus fermentum*. Bacterial growth and the concomitant production of lactic acid were associated with low ethanol production. Therefore, bacteria could be defined as contaminants in tequila fermentation and efforts to control them should be implemented [4].

The most important stage in tequila processing is the fermentation of sugar, mainly fructose, which is transformed into ethanol and volatile compounds that give tequila its unique characteristics [5]. Tequila companies often prefer to commercially inoculate *Saccharomyces cerevisiae* strains that provide batch-to-batch homogeneity; however, low yields are still generated as the strains are not well adapted to agave juice, a totally different environment from the origin of the strain [6].

The *Agave tequilana* Weber of blue var. is a semelparous plant, that is, once it blooms, it dies. Its reproduction can be sexual by seeds; or asexual by shoots of rhizomes or bulbils of the inflorescence. For Tequila production, instead of allowing the plant to develop the flower to attract pollinating birds, bats, and moths that feed on the nectar, the flower stem is cut as soon as it begins to bud. Thus, interrupting the process of flowering. The reason is that during the flowering process, the plant consumes its carbohydrate reserve and then dies [7].

The biological cycle of agave cultivation oscillates between six and eight years. During that time of its growth, the pineapple or head can weigh between 35 kg and 120 kg, although some reach up to 150 kg depending on the conditions of the cultivation process. The maturity of the plant is manifested in the appearance of the so-called "quiote", but this must be cut quickly since its permanence in the plant consumes the sugars accumulated for years [8].

Lavado, Robles, and Yenque (2015), in Peru, studied the physicochemical properties of blue cabuya juice obtained in the Huanca Huanca district, Angaraes province, Huancavelica Region [9]. **Table 1** shows the physicochemical properties of blue cabuya juice.

The water-soluble carbohydrates (WSC) contained in the agave heads were: 28,3 ± 0,1% g/100 g (fresh weight) and 86,7 ± 1,3% g/100 g (dry weight). [10]. These high values are consistent with previous reports by other researchers [11, 12].

It has been studied that the variables that influence the alcoholic fermentation process are mainly temperature, degrees Brix (°Brix), consumption of AF fermentable sugars (fructose and glucose), biomass concentration, and nitrogen concentration [13]. On the other hand, It has been studied the effect of pH and airflow on the production of biomass, ethanol, the synthesis of aromatic compounds, the consumption of reducing sugars, the yields, and speeds of production of biomass and ethyl alcohol of two strains of *Saccharomyces cerevisiae* cultivated continuously [14]. Thus, the effect of the dilution speed and the addition of nutrients on the fermentation and synthesis of volatile compounds of two native genera of *Saccharomyces cerevisiae*, S1 and S2, in continuous cultures fed with *Agave tequilana* juice have also been studied [15].

Other researchers studied the effect of temperature and pH variables on the yield of alcoholic fermentation of Agave cocui must. First, they studied the temperature: 27, 31, 33, 35, and 37 C at constant pH. Then, they evaluated the pH at values of 3.0, 4.0, and 5.0 at a constant temperature. The results obtained indicated that the maximum yield of alcoholic fermentation is at a temperature of 33 C and a pH of 4.0 with an ethanol productivity of 1.14 g/(L.h) and a yield of 81.5% [16].

For modern literature standardization purposes, fermentation is defined in microbiology as the type of metabolism of a carbon source in which energy is generated by phosphorylation at the substrate level where organic molecules function as the final acceptor of electrons (or as acceptors of reducing equivalents), generated during the decomposition of carbon-containing compounds or catabolism. As is well known,

Inulin	°Brix	Density	Viscosity
(g/100 g juice)	(ss/100 mL sol.)	(g/mL)	(cp)
56,60	14,00	1,27	1,56

Source [9].

Table 1.
Physicochemical properties of blue cabuya juice.

the process is called respiration when the final acceptor is an inorganic compound. Respiration is called aerobic if the final acceptor is oxygen and anaerobic when it is some other inorganic compound apart from oxygen, for example, sulfate or nitrate [17].

The musts for alcoholic fermentation are inoculated within the range of 5 x 10^6 cells/ml, equivalent to 1.5 g/L to 6.0 g/L in wet weight and 0.3–1.2 g/L in dry weight. The inoculum is related to the gravity of the wort and should produce as fast fermentation as possible without compromising the quality of the beer or the size of the yeast crop. The pitching rate influences both the fermentation rate and the degree of yeast growth [18].

In this context, the research was oriented to evaluate the production process of ethanol by fermentation of the juice of the blue cabuya (*Agave americana L.*) using yeast strains (*saccharomyces cerevisiae*). First, the temperature and pH of the alcoholic fermentation process must be determined to achieve the best production yield. Then, the concentration of diammonium phosphate, which is a nutrient, will be determined. Finally, the ethanol production yield will be determined by its potential application as a blue cabuya liquor.

2. Methodology, results, and discussion

2.1 Methodology

The development of the research was carried out at the laboratory level and was planned with the purpose of evaluating the yield of ethanol production by fermentation using blue cabuya juice as a substrate and a yeast *saccharomyces cerevisiae*, for which experimentation is used by manipulating and controlling the independent variables and analyzing their effects on the yield of ethanol production (dependent variable).

2.1.1 Type of research

According to the classification proposed by Hernández Sampieri, Fernández, and Baptista [19], this research is descriptive and explanatory with independent variables such as temperature, pH, substrate concentration, and nutrient concentration (diammonium phosphate, DAP).

2.1.2 Research design

The research design is experimental with a 3 x 2 x 2 factorial arrangement with experimental tests carried out with two repetitions. The temperature was considered with three levels: 24 C, 28 C, and 32 C, the pH with two levels: 3.5 and 4.5; and the nutrient concentration with two levels: 1 g/L and 2 g/L. The response variable was the ethanol concentration.

Table 2 shows the experimental design of the first stage of the alcoholic fermentation process of blue cabuya juice at different conditions: temperature, pH, and DAP nutrient concentration, for a substrate concentration of 60 g/L [20].

2.1.3 Obtaining cabuya juice

The development of organoleptic properties such as sweetness, acidity, aromas, and flavorstakes place in the state of complete maturity of the blue cabuya; therefore, it is important to extract the juice when the plant is fully mature. The blue cabuya completes its life cycle after flowering, so the juice is extracted before the floral scape comes out.

Test	Temperature °C	pH	Nutrient (DAP), (g/L)
1	28	4,5	1
2	28	4,5	2
3	32	4,5	1
4	32	4,5	2
5	28	3,5	1
6	32	3,5	2
7	32	3,5	1
8	28	3,5	2
9	24	3,5	1
10	24	3,5	2
11	24	4,5	1
12	24	4,5	2

Table 2.
Experimental design of the first stage of alcoholic fermentation of blue cabuya juice with a substrate concentration of 60 g/L.

Some physical characteristics that indicate that the plant is mature according to the ancestral knowledge of the inhabitants of the La Merced district, Junín region, Peru are described below:

- The leaves of the plant become thicker.
- The outer leaves of the plant droop downward.
- The trunk of the plant is more voluminous.
- The leaves around the upper part of the trunk are thinner and more erect.

Once the mature plant has been selected, the external leaves are cut in such a way that there is free access to the trunk, leaving the rest of it with its respective leaves. Then, a hole is made approximately 30 centimeters (cm) deep and 20 to 25 cm in diameter in the upper part of the pineapple or trunk to accumulate the juice [21].

Depending on the size of the mature plants, the juice is collected between two to three months, with the collection time being longer in larger plants. The collection is carried out two to three times a day, depending on the size of the plant: the first extraction is carried out between 4 and 5 in the morning; the second, at noon; and the third extraction, between 3 and 5 in the afternoon. In each extraction, about three liters of juice obtained. After each extraction, the walls of the hole are scraped so that they do not seal and prevent the juice from accumulating in the hole, while it is cleaned to avoid fermentation of the juice.

2.1.4 Determination of the physicochemical properties of blue cabuya juice

The blue cabuya juice was characterized to determine the following physico-chemical parameters: °Brix, density, pH, and viscosity. The following laboratory

instruments were used in the measurements: portable refractometer, series X0015RURCN; pycnometer; Milwaukee brand potentiometer, MW 102, pH/Meter, USA, and QUIMIS viscometer, Brazil. The results are shown in **Table 3**.

2.1.5 Alcoholic fermentation process

To carry out the alcoholic fermentation process, the yeast *Saccharomyces cerevisiae* variety ellipsoideus, D 47 - LALVIN - Canada was used. This yeast is lyophilized and comes in 5 g sachets, so it was necessary to reproduce it up to the concentration necessary to inoculate 20 L of cabuya juice.

To obtain the inoculum to 3 L of blue cabuya juice, 6 g of DAP was added as a nutrient. Subsequently, the solution was sterilized at 110 C and transferred to a 5 L flask where the aeration system was installed. The solution was cooled to the incubation temperature of 24 C, the yeast strains were added, and sterile air was supplied at a flow of 1 vvm for 24 h until reaching a concentration of 1,15 g of biomass/L. This biomass concentration is equivalent to 2875 x 10^7 cells/mL, which is within the range required for must fermentation that considers 1,0 g of cells ≅ 1,4 x 10^7 cells [22].

The experimental tests of alcoholic fermentation were carried out in the environmental microbiology and biotechnology laboratories of the Faculty of Biological Sciences and Unitary Operations of the UNMSM. A 120 L capacity bioreactor equipped with automatic temperature control and a 0.5 HP motor was used, with a stirring speed of 90 rpm and a supply of sterile air with a variable volumetric flow rate of 20 L/min. at 60 L/min. For each experimental test, 20 L of cabuya juice (minimum amount of bioreactor operation) was used. The cabuya juice was sterilized at 110 C in the bioreactor before carrying out the experimental tests of the alcoholic fermentation process at different temperatures, pH, and DAP nutrient concentrations.

The tests were carried out under the following conditions: constant substrate concentration, AR of 60 g/L, pH of 3.5 and 4.5 [23], at temperatures of 24 C, 28 C, and 32 C, and DAP nutrient concentration of 1 and 2 g/L. To 20 L of cabuya juice, previously sterilized, the yeast inoculum was supplied at a concentration of 1.15 g/L (dry basis), sterile air at a flow of 1vvm during the first 8 h of the process and the agitation speed was 90 rpm. The process was monitored by taking samples every 2 h for 26 h for the respective analysis of biomass growth, sugar consumption and ethanol production.

2.1.6 Measurement of reducing sugars, ethanol, and microbial biomass

Reducing sugars were determined by Miller's method using 3,5-Dinitrosalicylic acid (DNS), and ethyl alcohol concentration was determined by spectrophotometry using potassium dichromate, at a maximum wavelength of 580 nm.

To measure the concentration of microbial biomass, a 10 ml sample is taken from the broth during fermentation and centrifuged at 4000 rpm for 15 minutes. The sediment is deposited in a crucible and 10 mL of 0,85% w/v alkaline NaCl solution is

Fructans	°Brix	Density	Viscosity
(mg/mL)	ss/100 mL solution	(g/mL)	(cp)
37,34	14,40	1,29	1,58

Table 3.
Physicochemical properties of blue cabuya juice.

added. Then, this solution is dehydrated in the oven at a temperature of 80 C for 6 h until it reaches a constant weight. The amount of biomass is determined by the difference in weight between the crucible with dry biomass and the crucible without sample.

2.2 Results

Table 4 indicates the tests carried out in the investigation at different temperatures, pH, and DAP concentrations for a substrate concentration of 60 g/L of the blue cabuya juice to determine the conditions in which the highest ethanol yield is obtained.

Figure 1 shows the biomass yields of the experimental tests from the blue cabuya juice alcoholic fermentation process. As shown below, test 8 has the highest biomass yield.

Figure 2 shows the graphs of Ln (biomass) Vs time in hours of the alcoholic fermentation process experimental tests. It is observed that the kinetics of microbial growth is greater at a temperature of 28 C, pH 3.5, and DAP concentration of 2 g/L (test 8).

In **Figure 3**, the AR substrate consumption of the experimental tests of the alcoholic fermentation of cabuya azul juice is shown. It is observed that the speed of consumption of AR is greater in the conditions of test 8.

In **Figure 4**, the ethanol production of the experimental tests from the alcoholic fermentation process is observed. The highest yield of ethanol is obtained in test 8.

From the experimental results, it can be appreciated that test 8 at a temperature of 28 C, pH 3.5, Diammonium phosphate (DAP) nutrient concentration of 2 g/L, and substrate concentration (AR) of 60 g/L represents the best conditions in ethanol and biomass yield with Yp|s = 0.4739 and Yx|s = 0.166, respectively, as shown in **Figure 5**.

2.2.1 Analysis of the results of the consumption of reducing sugars

Table 5 shows the statistical treatment of the experimental tests performed on reducing sugar consumption.

Test	Temperature °C	pH	Nutrient DAP, g/L
1	28	4,5	1
2	28	4,5	2
3	32	4,5	1
4	32	4,5	2
5	28	3,5	1
6	32	3,5	2
7	32	3,5	1
8	28	3,5	2
9	24	3,5	1
10	24	3,5	2
11	24	4,5	1
12	24	4,5	2

Table 4.
Experimental tests performed at different temperatures, pH, and DAP concentrations for a substrate concentration of 60 g/L.

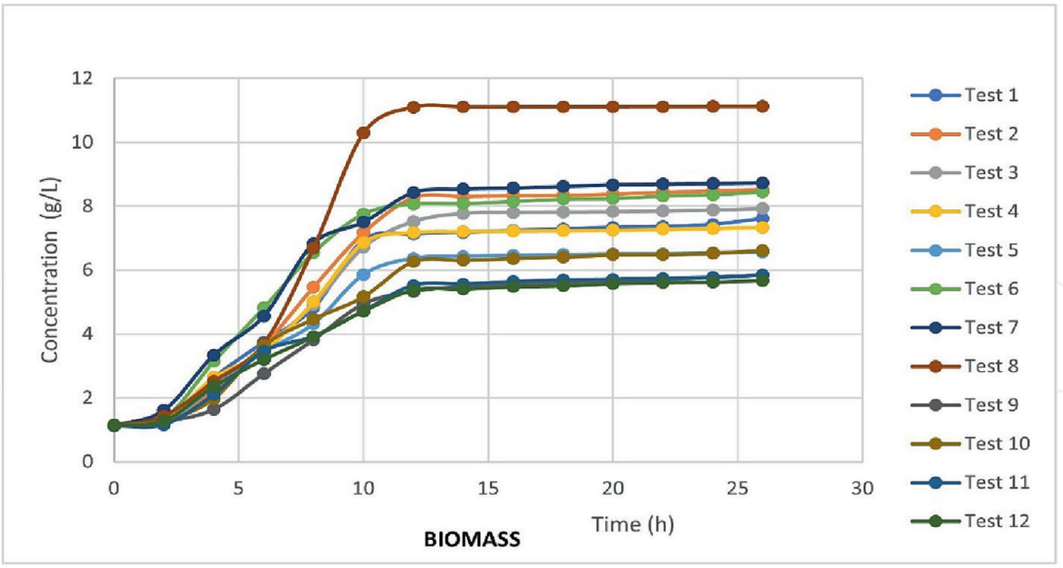

Figure 1.
Biomass production of alcoholic fermentation process in the first stage of experimental tests.

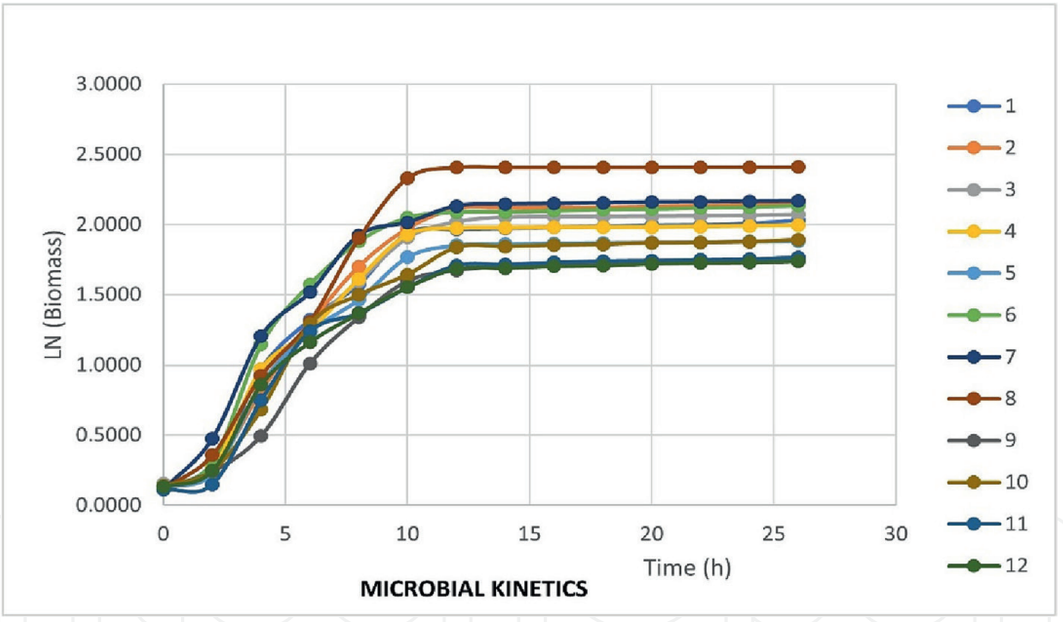

Figure 2.
Microbial kinetics from the 12 experimental tests of the alcoholic fermentation process.

In **Table 5** of inter-subject effects, the significant value of 0.000 ˂ 0.05 is observed. Therefore, Ha is accepted. This value indicates that there is a significant difference between the groups (Test and time).

Figure 6 of estimated marginal means shows that test 8 has the highest consumption of reducing sugars.

2.2.2 Analysis of biomass yield results

Table 6 shows the statistical treatment of the biomass production in the experimental tests performed.

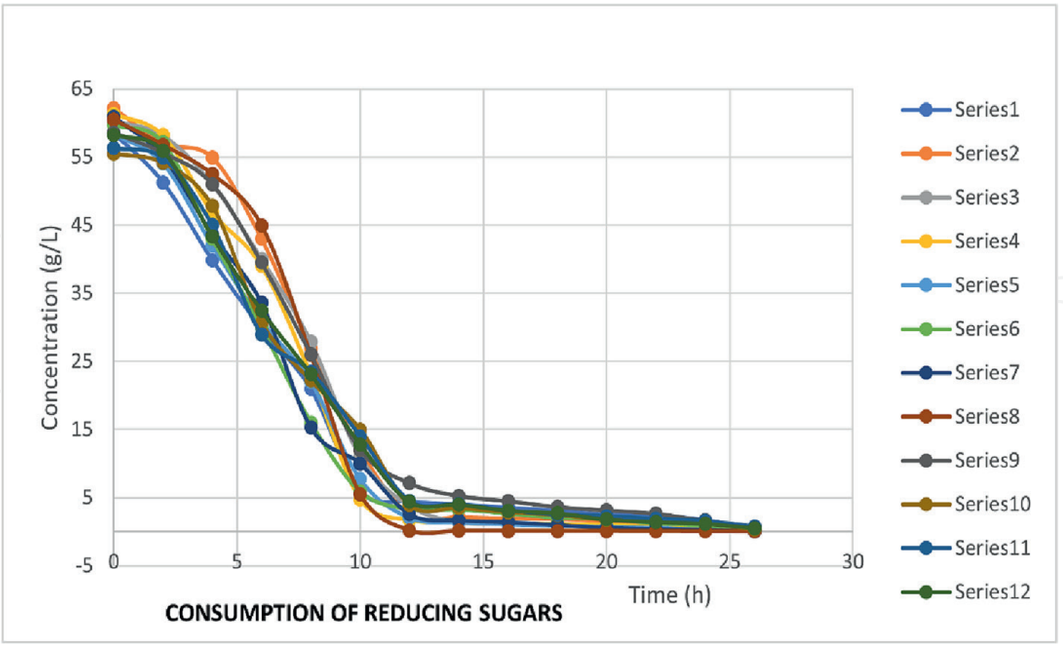

Figure 3.
Consumption of reducing sugars from the 12 experimental tests of the alcoholic fermentation process.

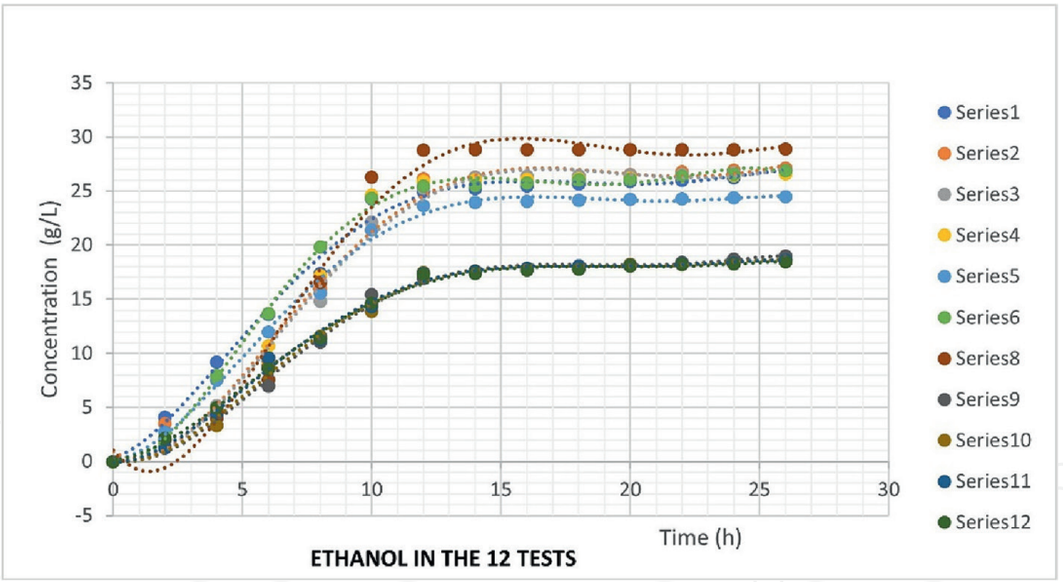

Figure 4.
Ethanol production in the 12 experimental tests of the alcoholic fermentation process.

In **Table 6**, showing the inter-subject effects, a significant value of 0.000 ˂ 0.05 is observed. Therefore, Ha is accepted. This value indicates that there is a significant difference between the groups (Test and time).

In **Figure 7** of estimated marginal means, it is shown that test 8 has the highest biomass yield.

2.2.3 Ethanol yield results analysis

Table 7 shows the statistical treatment of the ethanol yield from the experimental tests performed.

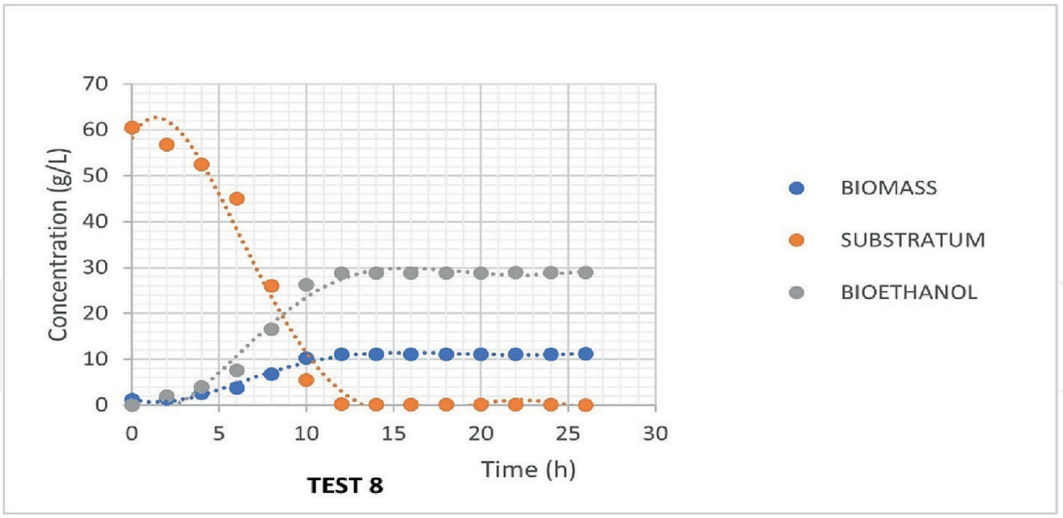

Figure 5.
AR consumption, biomass, and ethanol concentrations during the alcoholic fermentation process of test 8.

between-subjects effects tests					
dependent variable: Reducing sugars					
Origen	**Type III sum of squares**	**gl**	**Root mean square**	**F**	**Sig.**
corrected model	77548,985[a]	24	3231,208	509,808	,000
Intersection	50686,980	1	50686,980	7997,202	,000
Test	201,498	11	18,318	2,890	,002
Time	77347,487	13	5949,807	938,738	,000
Mistake	906,347	143	6,338		
Total	129142,312	168			
Total corrected	78455,332	167			

aR squared =,988 (R adjusted squared =,987).

Table 5.
Statistical treatment of AR consumption of the 12 experimental tests carried out at different temperatures, pH, and DAP concentrations.

In **Table 7** of inter-subject effects, a significant value of 0.000 ˂ 0.05 is observed. Therefore, Ha is accepted. This value indicates that there is a significant difference between the groups (Test and time).

In **Figure 8** of estimated marginal means, it is shown that trial 8 has the highest yield of ethanol.

2.3 Discussion

About 20 or more species of maguey are found in all the states of the Republic of Mexico, except in Tabasco and the Yucatan peninsula. A large part of the harvested

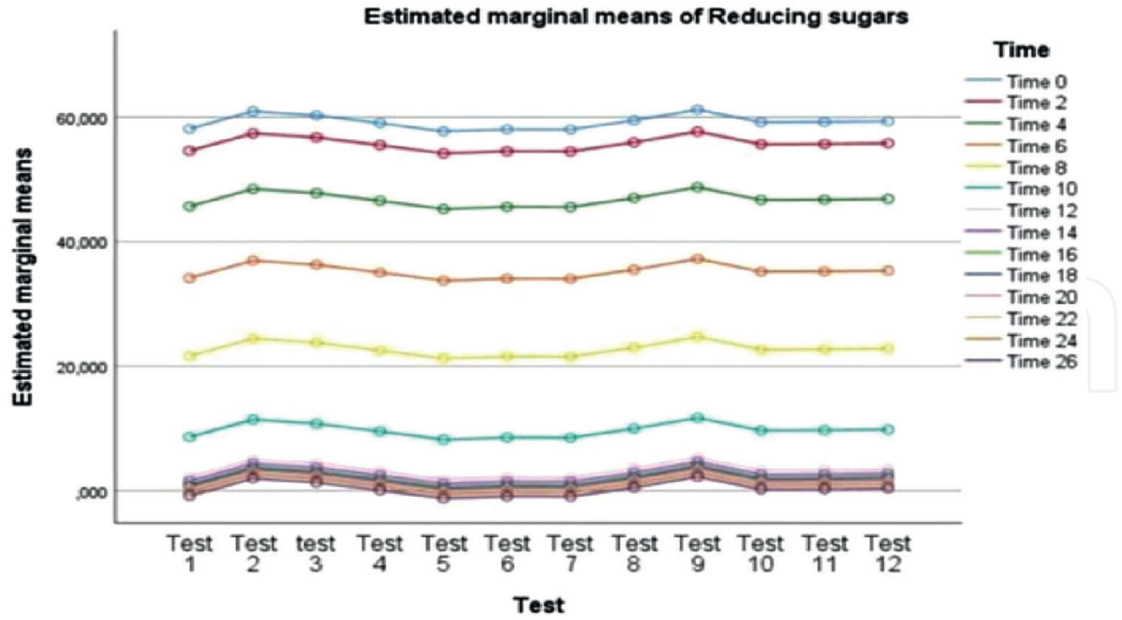

Figure 6.
Estimated marginal means from the 12 experimental tests performed on the consumption of reducing sugars.

between-subjects effects tests					
Dependent variable: Biomass					
Origen	**Type III sum of squares**	**gl**	**root mean square**	**F**	**Sig.**
corrected model	1142,363[a]	24	47,598	95,298	,000
Intersection	5369,762	1	5369,762	10750,927	,000
Test	202,802	11	18,437	36,912	,000
Time	939,561	13	72,274	144,701	,000
Mistake	71,424	143	,499		
Total	6583,549	168			
Total corrected	1213,787	167			

[a]*R squared =,941 (R adjusted squared =,931).*

Table 6.
Statistical treatment of the biomass yield of the 12 experimental tests carried out at different conditions of temperature, pH, and DAP concentration.

area corresponds to the species of the Americana group, mostly used to produce alcoholic beverages, mainly tequila, which has a denomination of origin [24].

Among the few varieties of cabuya existing in Peru, only two grow in large areas of rural land: the blue cabuya (*Agave americana L.*), which is distinguished by the bluish-green color of its leaves, and the other variety is the lineño (green cabuya), which belongs to the *A. angustifolia* Haw complex that is generally used in rural areas for the manufacturing of ropes, while in Mexico it is used to produce a distilled beverage that belongs to the mezcal family [25].

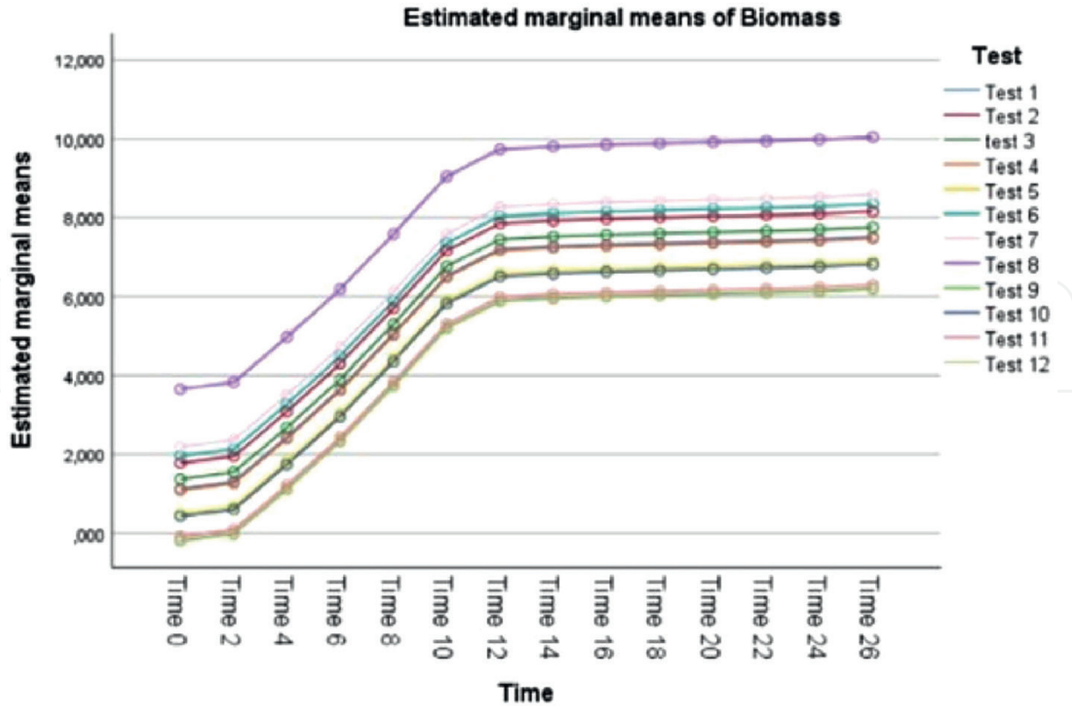

Figure 7.
Estimated marginal means from the 12 experimental tests performed on biomass yield.

between-subjects effects tests					
dependent variable: Ethanol					
Origen	**Type III sum of squares**	**gl**	**Root mean square**	**F**	**Sig.**
corrected model	13992,299[a]	24	583,012	160,230	,000
Intersection	50642,558	1	50642,558	13918,149	,000
Test	1556,292	11	141,481	38,883	,000
Time	12436,007	13	956,616	262,908	,000
mistake	520,320	143	3,639		
Total	65155,177	168			
Total corrected	14512,619	167			

[a]*R squared =,964 (R adjusted squared =,958).*

Table 7.
Statistical treatment of the ethanol yield from the 12 experimental tests performed at different temperatures, pH, and DAP concentration.

Even though blue cabuya grows wild in large tracts of land in the inter-Andean valleys of Peru with temperate climates (Cajamarca and Huánuco Regions), this resource is not used. In the southern regions of the country such as Huancavelica, Ayacucho, and Apurímac, blue cabuya is used for the artisanal production of chancaca (a solid sugar concentrate) and the sale of the juice as a drink. While in Mexico, some 120,000 hectares of blue agave [26] are intensively cultivated with an average of 2500 to 2800 plants per hectare. In 2018, according to the Tequila Regulatory Council (CRT), 1,138,800 tons of tequilana cabuya were used to produce 309.1 million liters of tequila at 40% alcohol volume.

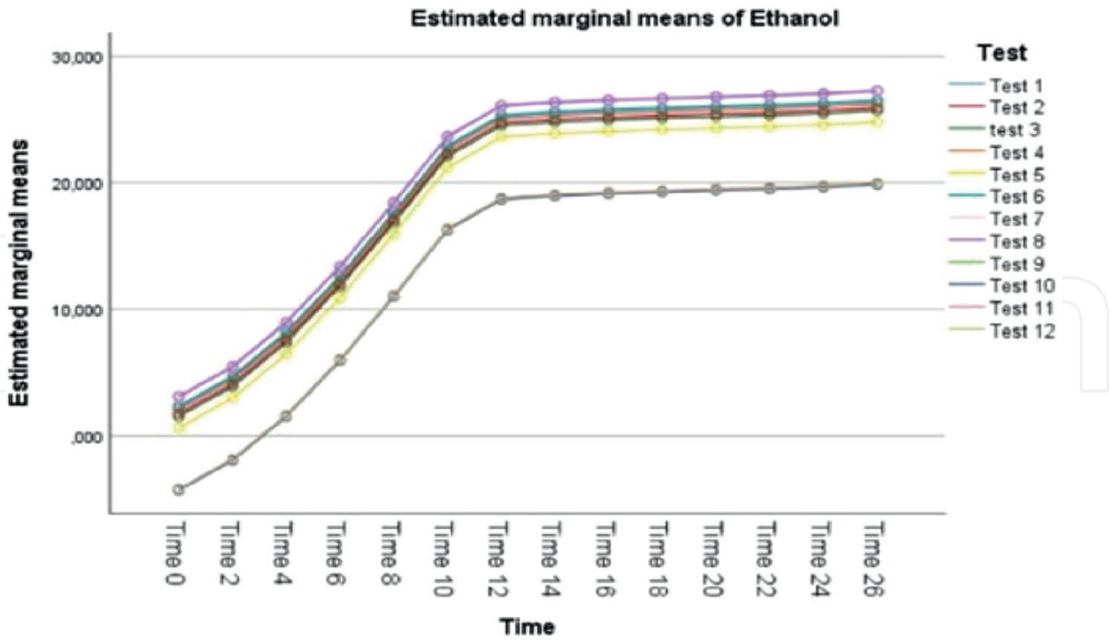

Figure 8.
Estimated marginal means from the 12 experimental tests performed on ethanol yield.

Due to climatic differences, land composition, and geographical location in which the blue cabuya grows in Peru; its physicochemical, physiological, metabolic, and morphological properties differ from the Mexican plants. One of the main differences is the maturation time of the plant. In our country, it takes 5 to 10 years but matures in less time in the inter-Andean valleys with temperate and rainy climates, which are between 1500 to 2200 meters above sea level. In Mexico, the plant matures in an average of 8 years [27]. Therefore, this shorter maturation time is an advantage for agro-industrial exploitation in Peru.

In Mexico, the cabuya tequilana is exploited on a large scale. Therefore, the pineapple plant is generally treated in ovens for the hydrolysis of the polysaccharides present in the juice [28]. Then, add water for the extraction of the juice in the milling at the ratio of 1 to 2.5 L per kg of pineapple pulp to obtain about 120 L of juice per pineapple. However, obtaining blue cabuya juice in Peru is done by hand. The juice is extracted about three times a day in an average of 3 L for each collection for two to three months depending on the plant size. Thus, an average of 270 L of juice is collected per month per plant. This constitutes an advantage for its exploitation on a small scale to produce the liquor-type tequila.

The industries that produce alcoholic beverages are based on the fact that, under proper fermentation conditions, sugar is transformed into alcohol. The organoleptic characteristic of the finished product depends on the raw material that provides the sugar and the conditions for transforming the sugar into alcohol. In the investigation, the factors that affect the alcoholic fermentation process of the blue cabuya juice were examined to obtain the highest ethanol yield, which was: substrate concentration of 60 g/L; temperature 24, 28, and 32 C; pH 3.5 and 4.5; and DAP nutrient concentration 1 and 2 g/L, during 26 h of the process, with an air supply with a flow of 1 vvm, during the first 8 h of the process, these results being very satisfactory. and they agree with the results obtained by other researchers who have worked in similar conditions but with different substrates.

The must was inoculated with *saccharomyces cerevisiae* yeast strains, genus D 47–Lalvin, at a concentration of 1,15 g/L (4,3 x 10^7 cells/mL). This genus of yeast was used for its property of fermenting white and red musts, in addition to having a good performance at low and high fermentation temperatures, which has been demonstrated with the statistical analysis, see **Figure 8** of estimated marginal means that shows that trial 8 has the highest yield of ethanol.

From the experimental results obtained, 12 tests, it is observed that test 8 is the one that gave the highest yield of biomass Y(x/s) = 0.1606 and ethanol Y(p/s) = 0.4739 at the conditions of substrate concentration, AR of 60 g/L; temperature 28 C; pH 3.5; and nutrient concentration DAP 2 g/L. The ethanol yield obtained represents approximately 93% of the theoretical maximum yield of alcoholic fermentation, which is 51.0% [29].

3. Conclusion

The ethanol yield in the alcoholic fermentation process of cabuya azul juice was 47.4%, which represents approximately 93% of the theoretical yield of ethanol production by fermentation, observing that it meets the minimum yield, 45%, that requires a fermentation industry for the production of liquors, therefore, the yield obtained in the investigation is very good.

The experimental results showed that the yield of ethanol in the fermentation process of cabuya azul meets the minimum requirements for its industrialization, it is a product of biology from renewable biomass, which will allow this natural resource to be used in liquor production as part of a sustainable development program.

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