

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Elaboration Of An Alcoholic Drink From The Aerobic Fermentation Of Mucilage Of Cocoa (*Theobroma cacao* L.)

Marx Iván García¹, Angélica Tigre¹, Vicente Dominguez², Ulices Barragán Vinueza³, Jhoan Guamán^{1,4}, Rivelino Ramón¹, Jagger Segura¹, Favian Bayas-Morejón^{1*}.

¹Universidad Estatal de Bolívar, Centro de Investigación y Desarrollo Biotecnológico, Departamento de Investigación, Facultad de Ciencias Agropecuarias Recursos naturales y del Ambiente, 020150, Guaranda (Ecuador)

²Universidad Estatal Amazónica, Departamento de Ciencias de la Tierra, 160150, Puyo (Ecuador)

³Universidad Estatal de Bolívar, Centro de Investigación y Desarrollo Biotecnológico, Departamento de Investigación, Facultad de Jurisprudencia, 120150 Guaranda (Ecuador)

⁴Universidad Estatal de Bolívar, Centro de Investigación y Desarrollo Biotecnológico, Departamento de Investigación, Facultad de Ciencias de la Salud y del Ser Humano, 120150 Guaranda (Ecuador)

ABSTRACT

The present investigation was carried out in the city of Guaranda (Ecuador). The objectives set out in this investigation were: to establish the best time of fermentation of the mucilage, to determine the best percentage of yeast and time of fermentation. The experimental material used was mucilage of cocoa from two cocoa varieties (Nacional and CCN51). A completely randomized block design with a 2x3x3 factorial arrangement with two repetitions was applied. The functional analysis was based on a 5% Tukey test to compare averages of the treatments. When performing the sensory evaluation of the organoleptic characteristics of the attributes; taste, smell, aroma, texture and acceptability when comparing treatments, panelists have selected T6 (A1B2C3) and T15 (A2 B2 C3) as better treatments. The two treatments were characterized by physicochemical analysis, finding values within the parameters required in the control regulations (INEN 362).

Keywords: Alcoholic drink, mucilage, cocoa

**Corresponding author*

INTRODUCTION

Cocoa is a long-standing crop in Ecuador, since it was cultivated from before the arrival of the Spanish, taking great importance, which in some places was used as currency for its high value. Of all the varieties of cocoa, the three most important are: *the creole*, *the foreigner* and *the trinitario* (UNIDA, 2009). World cocoa production is concentrated essentially at 10 degrees in the North and 10 degrees in the South of Ecuador. Finding its origins in South America, cocoa appears first in Spain thanks to Hernán Cortés in 1528. In order to satisfy the demand of the Spanish classes, the first attempts of plantations are undertaken in Los Caribes, without success, then particularly in Ecuador around 1635 by the Capuchin brothers (ZONADIET, 2011).

Consequently, in view of the industrialization of cocoa, various by-products or derivatives are obtained, among which stand out: chocolate cocoa powder and cocoa liquor; in the latter, worldwide in relation to the general meaning of the production of derivatives of the product under study (cacao) reach levels by addition, it is, that the derivative itself is little expended in its specific presentation, but rather it is worth one of the essential ingredients for the production of chocolate (MAGAP, 2011).

In Ecuador, cocoa is the third most important non-oil traditional product of Ecuador's exports, having about 70% of the world production of fine aroma cocoa (ANECACAO, 2011).

The mucilage, or pulp, is broken down into liquid substances when the cocoa fermentation process is incurred. During fermentation, the mucilage, or pulp, decomposes into liquid substances. The sugar in the pulp is transformed first into alcohol, and then into acetic acid. Much of the pulp escapes in the form of exudate. The alcohol concentration in the exudate is approximately 2-3% and that of the acetic acid is 2.5%. The total dry matter content of the exudate is around 8%, with a crude protein content of about 20%. The total volume of exudate is considerable, but no practical use has been found (Loureiro et al., 2017).

Considering the previously described, the objective of this work was to take advantage of the cocoa mucilage by making a fermented alcoholic drink.

MATERIALS AND METHODS

The present research work was carried out in the processing plant of the agribusiness of the State University of Bolívar (Guaranda-Ecuador), for which, mucilage of cocoa obtained from cocoa pods cultivated in Las Naves (Ecuador) was used as raw material.

Analysis in the raw material

In the mucilage, analyzes were performed such as: Brix degrees according to the INEN-273 standards and pH analysis according to the INEN 389 standard.

Alcoholic drink elaboration process

Plastic containers with a capacity of 1L were used to transport the mucilage, which was previously frozen to avoid possible fermentation. To obtain the alcoholic drink, formulations were made according to the following combination of factors, for an experimental design (table 1).

Table 1: Combination of factors for the preparation of the alcoholic drink

Methods	Factors	Levels
Varietyof cocoa	A	A1: National A2: CCN51
Yeast percentage	B	B1: 0% B2: 5% B3: 10%
Fermentation time	C	C1: 5 days C2: 10 days C3: 15 days

The applied experimental design was: Design completely random blocks (DCRB) in factorial arrangement 2x3x3 with 2 repetitions.

The experimentation was according to the following diagram:

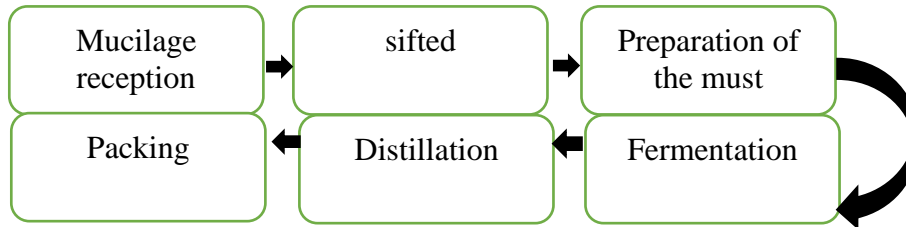


Figure 1: Process diagram for obtaining the alcoholic drink

Analysis in the alcoholic drink

In the product obtained the pH (INEN-389), alcoholic degree (INEN-340) was analyzed, a sensory analysis was also carried out to determine the acceptance of the product, for which, a group of 10 tasters was semi trained, the parameters analyzed were: odor, flavor, color, acceptability of the drink.

Statistical analysis

For the determination of the best treatment, the medias test was applied, and for this specific case the Tukey test was used at 5% (confidence level).

Physical-chemical analysis the best treatment

In the best drink, analyzes were performed such as: determination of acidity (INEN-341) and determination of methanol (INEN 347).

RESULTS AND DISCUSSION

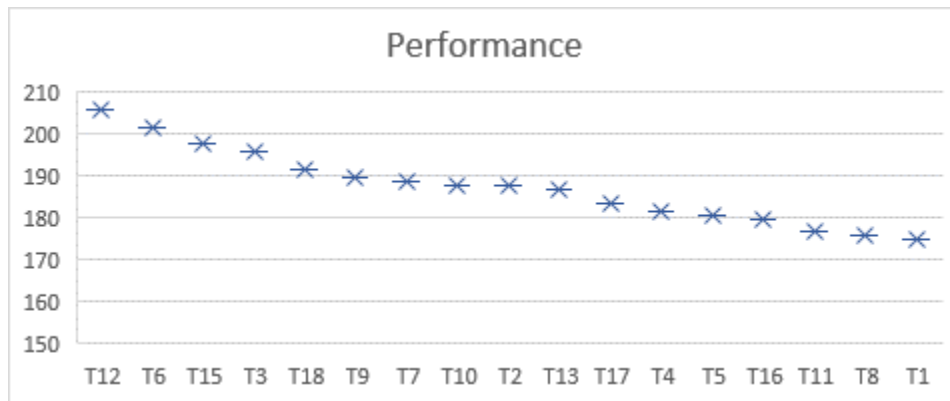
In the raw material

The results of °Brix and pH, it is appreciated that in the variety National cocoa obtained 15.3 °Brix, and a pH of 4.1 while in the results of cocoa CCN-51 was observed 14.2 °Brix and a pH of 4.2; in both cases, the pH values turned out to be acidic, this especially due to the presence of citric acid (Loureiro et al., 2017). Lower pH results were obtained in other works carried out in Ecuador by Villagómez and Arguello (2013) and Balladares et al. (2016) with 3.79 and 3.58 respectively. pH values lower than 4.5 and high content of total soluble solids, with values of around 14-18 °Brix. These two factors promote the growth of microorganisms that act in the fermentation (Penha and Matta 1998; Puerari et al. 2012).

Analysis in the finished product

In the finished product, analyzes were carried out such as: yield, pH, alcoholic degree and sensory evaluation.

Performance



DSM = 8.94

Figure 2: Average values for the performance of the alcoholic drink

In what corresponds to the performance tests, for the comparison of means by Tukey's method at a level of 5% of significance, it can be seen that there are highly significant differences, the best treatment being T12 corresponding to levels (A2B1C3) at mucilage of cocoa variety CCN-51, 0% yeast at 15 days of fermentation, followed very behind by treatments T6 and T15.

pH

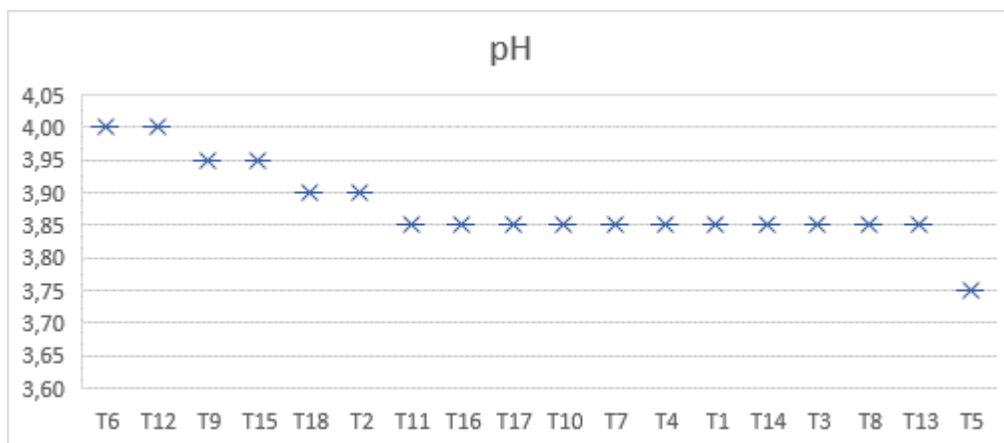


Figure 3: pH of the alcoholic drink obtained

In the pH tests, for the comparison of means (Tukey 5%), it is observed that there are significant differences being the T6 that corresponds to the levels (A1B2C3) to the mucilage of the National variety, to 5% of yeast to 15 days of fermentation.

Alcohol content (GL)

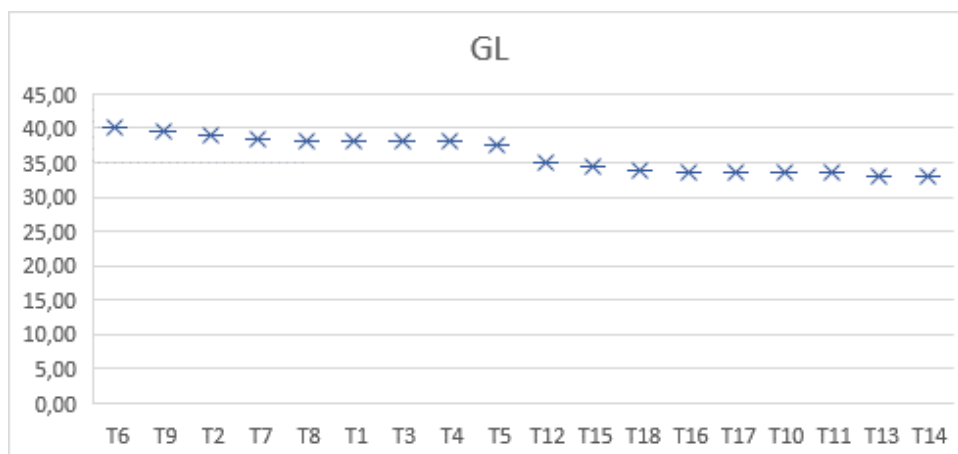


Figure 4: GL of the alcoholic drink obtained

In what correspond to the GL tests, for the comparison of means (Tukey 5%), it is observed that there are significant differences between the treatments, being the T6 (A1B2C3: mucilage of the National variety, to 5% of yeast to 15 days of fermentation) which presented a higher GL value than the other treatments.

Sensory evaluation

In what corresponds to the averages obtained by the tasters, but according to the standards indicated above, mention that: -All alcoholic beverages must have the color, aroma and flavor characteristic of the fruit from which it comes, considering the parameters of analysis was determined that the treatments with greater acceptability by the tasters were T6 (A1B2C3: mucilage of the National variety, 5% of yeast to 15 days of fermentation) and T15 (A2B2C3: mucilage of the CCN51 variety, 5% of yeast 15 days of fermentation).

Analysis in the best treatments

Table 2: Physical-chemical analysis in the best treatments

Analysis	T6 (A1B1C3)	T15 (A2B2C3)
pH	3.7	4.1
Temperature	17.5	20.5
Alcohol content	40	36
Titratable acidity	0.74	0.43
Methanol	negative	negative

In the Physical Chemical analyzes of the best liquor (T6 and T15), it can be seen that the pH of the CCN-51 variety is 4.1, whereas the pH of the national variety is 3.7, these results indicate that are acid, in a work carried out by Thanh Tinh et al. (2016) in a wine type liquor of mucilage of cocoa obtained a pH value of between 4 -4.1.

The alcoholic strength of the mucilage of cocoa of the CCN-51 variety was 20.5, in terms of the alcoholic strength of the cocoa mucilage of the national variety was 40, these results indicate that they are acceptable for consumption; the titratable acidity of the mucilage of cocoa of the CCN-51 variety was 0.43, as for the titratable acidity of the mucilage of cocoa of the national variety was 0.74, these results indicate that they are within the ranges according to the INEN 362 standard, ranges allowed in alcoholic degrees are between 15 to 85 ° GL; titratable acidity of 40 * mg / 100 cm3 and methanol of 10 * mg / 100 cm3 of alcohol.

It is the first time that has been worked with parameters of comparison of regulated factors to obtain a fermented drink from mucilage of cocoa in Ecuador.

GRATITUDE

We thank the State University of Bolivar, Department of Research, especially the Ecuador-Spain debt swap project for funding this research.

REFERENCES

- [1] ANECACAO. Cacao ecuatoriano fue galardonado en dos categorías en el Salón de París (en línea). Quito, Ecuador. 2011. Consultado 20 dic 2011. Disponible en <http://www.anecacao.com/>
- [2] Balladares C, Chóez-Guaranda I, García J, Sosa D, Pérez S, González J, Viteri R, Barragán A, Quijano-Avilés M, Manzano P. Physicochemical characterization of *Theobroma cacao* L. sweatings in Ecuadorian coast. *Emirates Journal of Food and Agriculture*. 2016; 28(10): 741-745. DOI: 10.9755/ejfa.2016-02-187.
- [3] Loureiro G, Reis de Araujo Q, Valle R, Andrade G, Moreira S. Influence of agro-environmental factors on the quality of cacao (*Theobroma cacao* L.) clone PH-16 in the cacao region of Bahia, Brazil. *Ecosist. Recur. Agropec*. 2017; 4(12):579-587. DOI: 10.19136/era.a4n12.1274.
- [4] MAGAP (Ministerio de Agricultura, Acuicultura y Pesca, Ecuador). Agrocadena de cacao y elaborados - Panorama Internacional. 2011; Consultado 20 dic 2011. Disponible en http://www.magap.gob.ec/sinagap/charts/cacao_panoramaint.htm
- [5] NTE INEN 0273. (1990) Melazas. Determinación de la densidad en grados Brix. Pages 8. Identifier-arkark:/13960/t8jd65t25
- [6] NTE INEN 0340. (1994) (Spanish): Bebidas alcohólicas. Determinación del grado alcohólico, pages 17. <http://studylib.es/doc/5147131/nte-inen-0340--bebidas-alcohol%C3%B3licas.-determinaci%C3%B3n-del-grado>.
- [7] NTE INEN 0341 (1978): Bebidas alcohólicas. Determinación de la acidez, Identifier ec.nte.0341.1978.
- [8] NTE INEN 0347 (1978): Bebidas alcohólicas. Determinación del metanol, Identifier ec.nte.0347.1978
- [9] NTE INEN 0389 (1986): Conservas vegetales. Determinación de la concentración del ión hidrógeno (pH), pages 7. Identifier-arkark:/13960/t6vx1p202
- [10] Penha EM, Matta VM. Características físico-químicas e microbiológicas da polpa de cacau. *Pesquisa Agropecuária Brasileira*. 1998; 33: 1945-1949.
- [11] Puerari C, Karina TM, Schwan RF. New cocoa pulp-based kefir beverages: Microbiological, chemical composition and sensory analysis. *Food Research International*. 2012; 48: 634-640.
- [12] ThanhTinha T, Nguyen TA, Ho TT, Nguyen TT. A study of wine fermentation from mucilage of cocoa beans (*Theobroma cacao* L.) *Dalat University Journal of Science*. 2016; 6; 3: 387-397.
- [13] UNIDAD DE INVESTIGACIÓN Y DESARROLLO EN ALIMENTOS (UNIDA). 2009. Calzada Miguel Ángel de Quevedo #2779 Col. Formando Hogar Veracruz, Ver C. P. 91860 <http://www.buenastareas.com/ensayos/Evaluaci%C3%B3n-De-La-Producci%C3%B3n-De->
- [14] Villagómez S and Argüello F. Optimización y aprovechamiento del residuo (exudado del mucílago) de la almendra fresca del cacao (*Theobroma cacao* L.) CCN51 en la elaboración de vinagre. *Tsafiqui - Revista de Investigación Científica UTE*. 2013; 4: 1-12.
- [15] ZONADIET. 2011. Bebidas alcohólicas destiladas (en línea). Consultado 20 dic 2011. Disponible en <http://www.zonadiet.com/bebidas/destilacion.htm>