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Evaluation of the Cocoa Bean (*Theobroma Cacao L.*), using Two Fermentators, Orellana and Sucumbios Provinces, Ecuador

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Abstract— The study was conducted in the provinces of Orellana and Sucumbíos, where cocoa is one of the most important agricultural items and seeks to position itself as a product with differentiation because they are in the area of influence of the Yasuni Biosphere Reserve. The purpose of this work was to define the quality of the National and Trinitarian cocoa (CCN51) obtained with the process of fermentation in sacks, a method used at the local level by small producers, and with the use of Rohan boxes as an alternative for the handling of small amounts of cocoa. The physical characteristics analyzed were percentage of fermented beans, seed index, number of almonds in 100 g, percentage of seed coat, cotyledon pH and seed pH. The results that showed significant differences with respect to the influence of the type of fermentation were the percentage of fermented beans (Rohan boxes: 79.6%, in bags: 60.4%), pH of testa (Rohan boxes: 7.02, in bags: 6.05) and pH of cotyledon (Rohan boxes: 5.61, in bags: 4.96), while the type of cocoa produced significant differences in the seed index (National: 1.34, CCN51: 1.61) and number of almonds in 100g (National: 65.5, CCN51: 80). The percentage of fermented beans with the use of the Rohan box reached the rank of the ASSS category while the fermentation in sacks that of the ASN category. The pH of the cotyledon suggests a good fermentation in the Rohan boxes while with the sacks, the fermentation was incomplete..

Keywords— Cocoa, fermentation, varieties, Amazon, Ecuador.

I. INTRODUCTION

Ecuadorian cocoa is a product that is strategically positioned on an international scale because it is fine and scented (Schwan & Wheals, 2004). Currently, the cultivation of cocoa is one of the most important items for Ecuador, occupying the third place in non-oil exports (Rivera et al., 2012). According to data from the National Association of Cocoa Exporters [ANECACAO] (2015), the country exported around 260 thousand metric tons of cocoa beans and derivatives, 10% higher than in 2014, generating an item of \$ 812 millions. The classification of cocoa is made according to

the demands of international markets. The INEN 176: 2018-2 standard establishes three categories for fine cocoa, the first is Arriba Superior Summer Selecto (A.S.S.S), Arriba Superior Selecto (A.S.S) y Arriba Superior Época (A.S.E); for the first category the 100 almonds should weigh over 130 g and have a percentage of fermentation greater than 75%.

At the national level, cocoa crops are distributed in 16 provinces (García, Sarabia, & Almeida, 2015) being the main cocoa producing area in the coastal region, headed by the province of Guayas with 49,233 metric tons per year, covering the 28% of national production, followed by the Sierra and Amazonía (Corporación Financiera Nacional [CFN], 2018).

In the Ecuadorian Amazon there are around 41,815 hectares planted with cocoa, with a production of 11,229 tons per hectare, the provinces with the largest area planted with cocoa in the region are Sucumbíos, Napo and Orellana (National Institute of Statistics and Census [INEN]). 2016). However, in the region the quality of cocoa is affected by poor post-harvest handling, since there is no record in the sector that indicates the correct way of fermenting the almond, however, on the coast the fermentation processes are carried out in jute bags, wooden boxes and piling up the almond (Rivera et al., 2012; E Portillo, Graziani, & Betancourt, 2005).

The application of a suitable post-harvest, provides a good fermentation process of the bean, since a series of biochemical transformations are produced that give origin to the aroma and flavor precursors typical of a fine cocoa and aroma (Rivera et al., 2012). These changes in the almond allow us to characterize the different types of cocoa existing in the region and in the country. (Espín, Samaniego, Wakao, & Jiménez, 2007).

During fermentation, the pigment cells containing the polyphenols of the anthocyanin type are released by the

exudate, changing the color of the almond, from purple to brown (Wollgast & Anklam, 2000); Epicatechin and catechin are oxidized to quinones and the condensation of proteins and polyphenols occurs, decreasing astringency (Zapata, Tamayo, & Rojano, 2013).

While in the alkaloids constituted by theobromine and caffeine no transformation has been seen, but they lose 20% of the theobromine, which causes the decrease of the bitter. The synergy between these alkaloids and diketopiperazines are responsible for the bitter taste of cocoa (Espin et al., 2007). All these compounds affected during fermentation in addition to the volatile acidity (acetic acid) (Rivera et al., 2012) and the volatile fraction are indicators of the organoleptic quality of cocoa, resulting in floral and fruit notes (Elvis Portillo et al., 2009). However, the lack or insufficient fermentation of the almond negatively impacts the content and concentration of the volatile fraction, the sensory profile and physical characteristics (Pineda et al., 2012). For this reason, the objective of this study was to evaluate the effect of two fermenters on the physical characteristics of the cocoa bean (*Theobroma cacao*) in the Ecuadorian Amazon.

II. MATERIALS and METHODS

Materials – Study Area.

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The research was conducted in the northern region of the Ecuadorian Amazon, in the provinces of Sucumbíos and Orellana. The average temperature was 23 ° C, rainfall 3,500 mm per year, with a relative humidity of 89% (Vargas Tierras et al., 2018)

In the locality, 20 farms were selected, with cocoa plots (*Theobroma cacao L.*), belonging to the project "Contribution to the construction of sustainable agroforestry development in the Ecuadorian Amazon (AFAM)", developed by the Tropical Agronomic Center for Research and Teaching (Catie) and the National Institute of Agricultural Research (Iniap).

The selected farms had at least one type of cocoa, Nacional (own materials of the producer) or Trinitario (CCN51), between 5 to 10 years of age and at least two hectares of the crop in production, in addition the interest of the producers in participating in the study. In Figure 1 the location of the farms of the study is indicated.



Figure 1. Location of the farms of the study, with distinction of the fermenter used.

Experiment management

In the months of October and November, the research team had two main plots, each consisting of 10 farms, the first with production of Trinitarian cocoa (CCN51) and the second with production of National cocoa. The main plots were divided into two subplots of 5 farms. From each unit of the sub-plot a collection of three samples of 45 kg of slime cocoa was carried out, which were fermented and dried to obtain independently 1 kg of dry cocoa at 7% humidity, which was analyzed in duplicate in the Food laboratory of the Iniap - Central Experimental Station of the Amazon

Sack fermentation

The fermentation in bags was carried out according to the procedure used by the producers of the farms under study, which consisted of placing a 45 kg cocoa mass in polypropylene bags with a banana leaf cover, for 3 days, with removal at 24 and 48 hours.



Figure 2. Cocoa fermentation in sacks

Fermentation in Rohan boxes

Fermentation in Rohan boxes was carried out for 5 days, with a cocoa mass of 45 kg, with removals at 24 and 48 hours. The Rohan boxes that were used were Laurel wood with a dimension of 100x100x15 cm (length x width x height) with square compartments of 15x15x15 cm (length x width x height) and a flat base with slits of 0.5 cm to allow the runoff of the mucilage.



Figure 3. Rohan box for cocoa fermentation

Variables in study

The experimental design that was applied was divided plots, the main plots were formed by 2 varieties of cocoa and the subplots constituted the 2 fermenters, 5 replications were made (farms). The variables were: percentage of fermented beans (%), seed index, number of almonds in 100 g (units), percentage of seed coat (%), cotyledon pH and seed pH. These were analyzed in the three baba cocoa samples that were obtained from each experimental unit.

Physical parameters

The percentage of fermented beans, seed index, number of almonds in 100g and percentage of husk or testa was analyzed according to the methodology of (Jiménez Barragán et al., 2011), while the pH of testa and cotyledons was determined according to the method used by (Afoakwa, Budu, Mensah-Brown, Takrama, & Akomanyi, 2014).

The equipment used to analyze the percentage of moisture in dry cocoa was the moisture measuring equipment for KPM cocoa beans, Model Aqua-Boy KM, for the percentage of fermented beans, a Magra 12 guillotine, Teserba model, B-Matthaei, for the seed index, weighed weight, cotyledon weight, a Citizen analytical balance, model CX 220 and for the determination of the pH of the seed coat and of the cotyledon, a Boeco potentiometer, model PT-380.

Statistic analysis

The results were obtained by descriptive statistics, normality tests, and analysis of variance. The differences between treatments were estimated with the Tukey test, with a level of significance of 0.05 in the statistical software InfoStat version 2018 (Di Rienzo et al., 2015)

II. RESULTS AND DISCUSSION

Table 1 shows the results of the analysis of variance that were obtained in the physical characterization of two varieties of cocoa, registered at the end of the fermentation process in sacks and Rohan boxes. The normality tests justify the use of parametric statistics.

Variety and fermenters	% of fermented beans	Seed index (g)	N ° of almonds in 100g	% of testa	pH testa	pH cotyledon
Variedades						
Nacional	70.8a	1.34b	80.95a	12a	6.53a	5.10a
CCN51	69.2a	1.61a	65.50b	11.9a	6.53a	5.46a
E.E	2.6	0.04	3.1	0,3	0.11	0.16
Fermentadores						
Sacos	60.40b	1.48a	70.35a	10.8b	6.05b	4.96b
Caja Rohan	79.6a	1.47a	76.1a	13.10a	7.02a	5.61a
E.E	4.77	0.08	3.56	0.46	0.16	0.18
CV (%)	21.54	16.14	15.36	12.05	7.61	10.96

Motility Post freezing

Percentage of fermented beans

The percentage of fermented beans according to the analysis of variance did not show significant differences between the National and Trinitario cocoa. The percentages reached exceeded 53% and 55%, the minimum requested by the NTE INEN 176 standard (Ecuadorian Normalization Service

[INEN], 2018) for the National and Trinitarian type cocoa respectively.

In Table 1 it can be seen that the results of the percentage of fermented beans, show significant differences between the fermentation methods that were used, being the fermentation in boxes Rohan a better alternative with respect to the fermentation in sacks because this fermenter is suitable for small volumes of fresh cocoa mass (Jiménez Barragán et al., 2011) and in turn the 5 days of fermentation used coincide with the time suggested by (Moreno & Sánchez, 1989) for the alien cocoas with Criollo legacy.

Bag fermentation is one of the methods used by small producers to benefit cocoa in the provinces of Orellana and Sucumbios, as well as in the province of Esmeraldas (Torres, 2016) and cocoa areas in other countries such as Venezuela (Contreras, Ortiz de Bertorelli, Graziani de Fariñas, & Parra, 2004), however the fermentation carried out by the producers on the farm is usually not very efficient because the different influencing factors are not controlled (Torres, 2016) as type the volume of the dough and turning of the beans (Graziani de Fariñas, Bertorelli, Álvarez, & Trujillo de Leal, 2003). In addition, according to what was reported by (Contreras et al., 2004) with the fermentation in sacks, a lower percentage of fermentation was found with respect to the drawers or drawers and this was attributed to the fact that the sack does not meet the adequate conditions for aeration and drainage.

Seed index

Physical characteristics such as seed index, weight of 100g of cocoa and percentage of testa are decisive since they allow to infer yields in the chocolate industry and also the quality in the processes of transformation of the raw material (Aguilar, 2016).

The seed index did not show significant differences according to the fermentations that were made, but if among the variety of cocoa. The seed indexes obtained in the analyzed cocoa from the provinces of Orellana and Sucumbios exceeded the reference values of the NTE INEN 176 standard (Ecuadorian Normalization Service [INEN], 2018) for both Fine cocoa and CCN51. The cocoa seed index CCN51, 1.61, as the Cocoa National index, 1.34, coincided with the results obtained by (Vera Chang & Vallejo Torres, 2014), who indicated for the CCN-51, 1.4 and for the clones of cocoa type National EET-575 and EET-576 averages between 1.2 and 1.3 and with that reported by (Quintana Fuentes, Gómez Castelblanco, García Jerez, & Martínez Guerrero, 2015) who indicates an average bean of CCN51 of 1.6g.

The seed index of the cocoa under study is higher than what according to (Moreno & Sánchez, 1989) guarantees a good ear index. The coefficient of variance of this variable is 16.14, which suggests uniformity in the size of the bean, an important attribute for the cocoa processing industry since the greater the size and uniformity of the bean, the higher the yield, as well as the quality of the toasting (Aguilar, 2016)

Number of almonds in 100g

The number of almonds in 100 g is inversely proportional to the seed index, so it also presented significant differences

between the types of cocoa, with the Cocoa National having the highest number of almonds in 100g, a result analogous to that of (Zambrano et al., 2010) that indicate that in 100g of sample, the number of almonds was 78 for the cocoa Forastero, while for the Cocoa Trinitario reports 66 almonds per 100g, considering that the Cocoa National has ancestry of Forastero while the CCN51 of Cocoa Trinitario (Escuela Politécnica del Litoral [ESPOL], 2016).

The number of almonds for the Cocoa National was 80.95, while for the CCN51 it was 65.50, data that differ with that reported by Vera Chang & Vallejo Torres (2014), who carried out the harvest in the months of February-July and they indicate results for the Trinitario cocoa of 46.35 and for the Cocoa National of 40.62. The average weight of the almond decreases in summer, since it depends on the adaptation of genetics and the environment, ranging from 20 to 60 almonds per ear (Sánchez, 2007)

Proportion of testa

The percentage of testa did not present significant differences with respect to the type of cocoa, like the study of (Sánchez, García, Dugarte, Mendoza, & Rivas-Echeverría, 2017) where it is indicated that genetic diversity is not an influential factor in the amount of husk. In addition, the values obtained for Cocoa Nacional and CCN51 coincide with the reference given by (Chocolate, Biscuits & Confectionery of Europe / Ergocean Cocoa Association / FCC [CAOBISCO / ECA / FCC], 2015) which is 11-12% of the total bean weight, which in turn indicate that this percentage varies according to the type of cocoa and post-harvest practices, which agrees with the significant difference between the types of fermentors in this study.

The amount of testa or husk is inversely related to bean size (Aguilar, 2016), a premise that is confirmed in this study. The percentage of testa to be an important index for the calculation of yield in the processing plant is relevant to compare it with the characteristics of cocoa in the region, so it is shown that the value obtained for CCN51 11.9% is similar to that of the neighboring country since (Quintana Fuentes et al., 2015) obtained values between 11-12% for the same variety in the Santandereana mountain, Colombia.

Testa pH

The pH of the testa obtained for Cocoa Nacional and Trinitario was 6.53, a value similar to what it obtained (Vera Chang & Vallejo Torres, 2014), for National cacao (EET-103) 6.1 and CCN51 6.87, however this author refers to a great variability of data obtained by different authors but coincides with (Steinau Dueñas, González Rosales, & Castañeda de Abrego, 2017) in stating that the pH of testa is subject to genetic variability and fermentation methods, which explains the significant difference that existed between the fermentations (sacks 6.05 and Rohan box 7.02), in addition to monitor parameters such as pH and temperature allows greater control in fermentation (Gutierrez Seijas, 2012)

Cotyledon pH

The chocolate industry considers pH a critical parameter in the quality of cocoa (Álvarez, et al., 2010) because a high level

of acidity is usually associated with a pH of 5 or less and in cocoas with a good fermentation the perception of the acid taste is very low, however, it is necessary to indicate that the pH of cocoa greater than 5 does not guarantee the good taste of chocolate (Chocolate, Biscuits & Confectionery of Europe / Ergocean Cocoa Association / FCC [CAOBISCO / ECA / FCC], 2015).

As indicated in Table 1, the pH of the cotyledon showed no differences between the varieties of cocoa, as reported by (Sanchez et al., 2017), which conducted an analysis between Creole, Trinitarian and foreign cocoa whose values fluctuated between 4.6 and 5.1. However, the results indicate that the type of fermentor significantly influences the pH of the cotyledon (Sacks 4.96, Rohan box 5.61), results similar to those obtained (Quevedo & Romero, 2016), who show the same tendency in this parameter, that is, a lower pH value with fermentation in sacks than with fermentation in wooden boxes. In addition, the pH values obtained in the Rohan boxes are similar to those reported by (Álvarez, et al., 2010), who fermented in plastic and wood boxes and obtained values between 5.37 and 5.59 respectively.

III. CONCLUSION

The percentage of fermented beans according to the cut test for the National and Trinitario cocoa in climatic conditions of the province of Orellana and Sucumbíos was better when using the Rohan box for a time of 5 days than the one made in sacks for 3 days.

According to the percentage of fermented beans and according to the norm NTE INEN176: 2018-2 the cocoa fermented in boxes Rohan complies with the requirement of the ASSS category while the cocoa fermented in bags comply with the requirement of the category ASN.

The pH of the cotyledon obtained with the use of Rohan boxes suggests a good fermentation since it exceeds the value of 5, while the fermentation in bags had a pH lower than 5, indicative of an incomplete fermentation.

The physical characteristics of the cocoa according to the statistical analysis of variance showed no significant differences in the type of fermentor interaction and type of cocoa.

According to the weight parameter of 100 beans of the standard NTE INEN176: 2018-2, the Cocoa Trinitario (CCN51) meets the requirement of the CSS category while the Cocoa National, is categorized as ASSS.

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