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Research Article

Evaluation of the Fermentation Time on the Physical Characteristics of Cocoa (*Theobroma cacao* L), Clones Fedecacao Tame 2, Fedecacao Lebrija 3 and Fedecacao Saravena 12 in the Town of San Vicente de Chucuri

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Abstract: The aim of this project was to determine the influence of the fermentation time and the final physical-chemical characteristics of the regional cocoa clones (FLE3, FTA2 and FSA12), due to a big variety of clones, agroforest systems, selection systems of crops and fermentation, it was necessary the establishment of the fermentation times according to the grain size. To verify the final quality of the cocoa it was evaluated the influence of the fermentation time in the physical- chemical characteristics, taking into account the grain index as a reference factor for the cocoa clones FLE3, FTA2 and FSA12, it was searched the ideal fermentation time for the three clones at 72, 120 and 168 h to guarantee a final physical quality for the cocoa grains, this process was made on the experimental farm Villa Monica property of Fedecacao, located on San Vicente del Chucurí, in the determination of the physical-chemical characteristics it was taken samples of the first harvest of 2017 in triplicated. The fermentation index for the first harvest of 2017, increase with time, obtaining values higher than 60% after the 120 h, the clone with higher values was FTA2. These fermentation percentages are adequate and within the expectative of a 60%-80% for the 120 h, assuring that there is no over fermentation getting an adequate final quality.

Keywords: Clone, grain, physical, postharvest, variety

INTRODUCTION

The cocoa is known as the seed of the tree denominated botanically *Theobroma cacao L*, this tree was cultivated on its beginning in South America, it´s a small tree that grows up preferably between the 0 and 1200 *m.a.s.l* and it´s principally developed inside the 17° of the Equator latitude, nowadays it is produced all over the world on these latitudes (Beckett, 1998).

In 2000 decade, Fedecacao began the "Selection, conservation and evaluation of the materials with high performance on production and quality", a project focused on the rescue and conservation of the genetic diversity of the cocoa in Colombia. (Fedecacao, 2012).

In 2014, Fedecacao presented to the cultivator community eight clones: FSV 41, FEC 2, FLE 2, FLE 3, FTA 2, FEAR 5, FSA 12 and FSA 13, that was evaluated and characterized for 10 years, this evaluation and tracing was made in 27 plots of Colombia, for the execution of this study it was selected three of it that are FLE 3, FTA 2 and FSA 12.

One of the critical points for the guarantee of the quality is the post-harvest management, starting off with the recollection, fermentation, dried and storage. The step of most interest in this study is the fermentation.

The benefit, cure or fermentation of the cocoa is the biochemical process by which the seeds after being extracted of the fruit and placed on special deposits, on appropriate conditions, suffer the physical and chemical transformations that are generated by the products of this process for the improvement of its characteristics, easing the dry and the conservation and achieving a better presentation of the final product. The cotyledon

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components of the almonds suffer deep changes during fermentation. These changes are accompanied by losses on the astringency for the diminution of some of its components, transformation on new compounds and the diffusion of the purple pigment from the exterior of the cells and the cotyledons to the uncolored adjacent tissues, including the radicle and plumule. At the same time, the purple coloration becomes partially brown, particularly in the almonds drying (Amores, 2010; Fedecacao, 2012).

The fermentation of the seeds is a necessary process because all the factories know that:

> *"the real aroma it´s not developed only on the toasted unless the almonds have been fermented and dried appropriately*" (Amores, 2010)

In general, there are many ways to ferment cocoa, the most common methods in bulk are in heap, in bags with fresh cocoa to let it hanging with the finality to ease the wring and in drawers that are built on unitary type wood or stairs type fabricated with cedar or walnut, but also can be fermented on other manners: in baskets, gutters, rotating barrels that allow the airing of the cocoa mass without the reactor being opened, rounded special designs.

The factors that influence an adequate fermentation are the presence or absence of oxygen and the temperature. The absence of oxygen stimulates the anaerobic phase and when the twirls are made it is favored the aerobic phase. It can be affirmed that the twirls serve to homogenize the development of the biochemical processes that are manifested in the course of the fermentation, which immediate consequence is the uniformity on the temperature of the cocoa mass in fermentation (Cruz *et al*., 2013).

The temperature goes up rapidly during the first 48 h of cocoa fermentation (34-40°C). Once the 48 h have passed there are little changes on the temperature (42-45°C) until the fermentation is complete (4 or 7 days); then the temperature falls rapidly to acquire the room temperature (Cruz *et al*., 2013). It is estimated that temperatures higher than 43-44°C eliminates the majority of the organisms with a critical point that seems to stay around the 45°C.

It has been determined that due to the transformations generated by the growing up of different types of microbial flora that consume sugars and the rest compounds, it is generated the production of acids increasing its concentration with the time and being modified during the time (Cruz *et al*., 2013).

In a good fermentation, the majority, but all the almonds die at the end of the first or second day due to the penetration of the acetic acid inside the external layers of the almond. Then the oxygen starts to penetrate the husk making the cotyledons surface get brown or purple. When the almonds are transferred to the drying platform this process goes on until the almond is dried; it will be then of a uniform brown color.

The duration and the fermentation method are crucial for the formation of flavor compounds and precursors of the flavor, bitter notes are generated by the theobromine and caffeine, together with diketopiperazines form starting with the toast between the thermal decompositions of proteins (Afoakwa *et al*., 2008, 2013; Kongor *et al*., 2016).

Based on the biochemical reactions and the color changes that occur during a good fermentation, the grain must be put to dry when it just appears the brown ring of the cut cotyledon. Due to all the grains are not exactly the same and the deviations of the ideal conditions, all within a drawer are not going to be in the same state at any moment so that the fermentation times influence on the final quality of the grain (Amores, 2010).

The study of physical variables is very important to establish the effects of fermentation in the final grain quality to recommend conditions and times of fermentation in a particular way for the three clones studied like it has been carried out in multiple investigations all over the world.

The biochemical changes are accompanied by the color changes of the cotyledons, the following description is referred to a grain with purple cotyledons, in one part of the fermentation drawer where the changes in temperature and pH are of the whole of all the grains and it is representative of the changes that are found in the practice of a normal plantation (Amores, 2010).

Sandhya *et al*. (2016) compared a fermentation with a known inoculum and a spontaneous fermentation. The fermented cocoa grains were collected at a fixed time (0, 24, 48, 72, 96, 120, 144 and 168 h), with this procedure and the inoculation of a starter culture it can be improved the fermentation period decreasing it to three days according to the results of the presented transformations on the cocoa grains.

In three Venezuelan crops, the cocoa grains were fermented for 4 days on wood square drawers with a volume of 60 cm^3 and with five fermentation times (0, 24, 48, 72 and 96 h). The cocoa mass on fermentation was covered with banana leaves and yute bags. The result was that the aromatic intensity, acidity and fruity flavor were lower on the grains that were not fermented and the bitterness and astringency notes were the higher (Portillo *et al*., 2006).

In Ecuador, in 2014, to evaluate the national cocoa, three parcels were selected from the localities: Río Chico, Ricaurte and Calceta of the Manabi province. The parcels contain cocoa type national, clonal. All the analyzed materials presented similar sensorial characteristics and the cocoa type national the best profile for the fruity flavor.

In order that the product can be stored with the security that it is not going to be affected by a fungus attack, it is necessary to condition its humidity content near to 7%. Moreover, it must be taken into account that during the drying process of the grain, it continues the development of the physical and chemical transformation processes, that can't be completed while the grain is on fermentation, it is during this step that oxidation and transformation of the polyphenols end, disappearing completely the purple color of the almonds turning it into brown, generating the wished organoleptic characteristics.

For the sun-dried, it is used structures like trays, house type Elba, wood stretchers or slides cars type Elba. It must not be used either cement courtyards nor paved areas because it can produce contamination by harmful elements. In the drying process, it must be removed the cocoa mass frequently for the uniform distribution of the heat and achieve a uniform drying (Fedecacao, 2012).

The grain index or grain size play an important role on this process due to it is going to ease the penetration of the generated compounds on the fermentation to the interior of the grain, each one of the selected clones has a specific index corresponding to the low, middle and high index.

The objective of this project was to evaluate the influence of the fermentation time and the grain index under the final physical-chemical characteristics of the regionals cocoa clones (FLE3, FTA2 and FSA12) produced in San Vicente del Chucurí-Santander.

MATERIALS AND METHODS

Of the eight regional clones it has been already studied in similar way to the proposed for the clone FSV 41 FEC 2, FLE 2, FEAR 5 and FSA 13, the three that are going to be studied have not had an evaluation of this kind and it corresponds to the clones FLE3, FTA2 and FSA 12.

For the three selected clones, its origin is presented in Santander and Arauca, all are considered hybrids of Trinitarian origin, with performance by hectare superior to the one thousand kilograms, it is required 15 to 18 cobs of mature cocoa to obtain 1 kilogram of dried cocoa, it possesses a grain index between the 1,3 g to 1,8 g, the color of its mature cobs goes of yellow to red, it has optimal husk percentages for the performance of production processes according to with Beckett (1998), the relation, theobromine caffeine is an indicator of its smooth flavor, due to its Trinitarian origin Ramos *et al*. (2013) and Perea *et al*. (2013). The acronyms used to identify the clones are FLE 3: Fedecacao Lebrija 3, FTA 2: Fedecacao Tame 2 and FSA 12: Fedecacao Saravena 12 (Fedecacao, 2012).

The samples were collected in the town of San Vicente del Chucurí, which are related to Table 1, it was taken in account for its selection by grain index the characterization made by Perea *et al*. (2013), where it was established the grain index per clone, which gives a starting point for the design of the clones and the fermentation time to evaluate.

Table 1: Clones classification by the grain index

Genotype	Grain index	Classification
FTA 2	1.8	Large
FLE 3	1.6	Medium
FSA 12	1.3	Small

Perea *et al*. (2013)

The fermentation process was made in the experimental farm of Villa Monica, owned by Fedecacao, located in the town of San Vicente del Chucurí and contemplating the next aspects:

Harvest: It was collected the cobs with an adequate mature degree, for the FLE 3 the cob must have an orange-yellow color, the FTA 2 a light red and the FSA 12 an orange-yellow.

Cutting and grain extraction: It was made on the site destined to the fermentation in an adequate manner to avoid the contamination by strange materials.

Fermentation: It was made on a wood drawer, retiring the samples for each clone in the 3, 5 and 7 days to evaluate the characteristics. The drawer's method has been the most used in South America, Central America, Ceilan and Indonesia and its capacity can vary between the 100 to 1500 kg, having variables dimensions to obtain these volumes according to Hardy (1961).

Drying: The retired samples of the fermenter drawer were dried naturally in Elba house during the necessary time to achieve a humidity level of maximum 7%.

The type of drawer that is going to be used in the fermentation process is a linear drawer of three compartments that allows the execution of the twirls in the established time periods of 48, 72, 96 and 120 h.

The weight of fermented and dried grain: Before beginning each test, it was verified the humidity percentage with the measurement equipment FARMEX to guarantee a percentage not superior to 7%. The fermented and dried cocoa grain was weighted according to with the procedure specified on the NTC 1252 of 2003, using a precision scale Sartorius trademark. Grain index (average weight of the grains in grams, taking a sample of 100 g of dried cocoa). It was taken three samples of 100 g chosen by random with shell and without toast, it is weighted each grain of each sub-sample to make an average of the three, this average is divided between 100 and its value will be the grain index of each one of the samples (Stevenson *et al*., 1993; DeZaan, 2009).

Almond percentage and grain's husk: Of each sample was taken and weighted 50 grains with husk and without toast which it is retired the husk and put on separated recipients husk and cotyledon, then it is weighted the husk and the cotyledon, with this data, it can be calculated the husk percentage, having in account the starting weight of the 50 grains (Stevenson *et al*., 1993; DeZaan, 2009).

Husk and cotyledon pH determination: It was taken 10 grains with husk, added 100 mL of deionized boiling water, mixed and cooled, once it is cool it was determined the pH introducing the electrode on the solution, the same procedure was made to determine the cotyledon's pH (Stevenson *et al*., 1993; DeZaan, 2009).

Fermentation index: This test allows to determine the level of fermentation and guide about determined defects that cause negatives flavors in the cocoa almond. The fermentation level is classified in the next categories: Brown color almonds, brown and purple color almonds, totally purple almonds, gray-green almonds, moldy almonds, infested almonds, germinated almonds and flat almonds (Stevenson *et al*., 1993). For each clone was taken 300 cocoa grains at random, which were broken longitudinally with a guillotine Magra 12, these grains were visually analyzed, making the count of the well-fermented, medium-fermented, purple and gray-green grains. The fermentation index is obtained by summing the totality of well-fermented grains and divide it between 300 that is the total number of almonds (Jiménez *et al*., 2011; Stevenson *et al*., 1993; DeZaan, 2009).

It is expected to establish the physical and chemical characterization and a sensorial profile for each one of the three clones to determine the final quality of the cocoa liquor, with this results it is going to be contributed to the confirmation and recommendation of the adequate fermentation time, an input to the flavor library of cocoa liquors and to the national characterization of the regional cocoa in Colombia.

An ANOVA analysis was made to establish if there are significates differences between the grain index, the fermentation index, husk's and cotyledon's percentage, husk's and cotyledon's pH of each clone on each fermentation time, for the differences it was applied a Tukey test with a reliability of 95%, to determine which samples are the different ones. It was used the software STAT GRAPHIS Centurion for the data processing.

RESULTS AND DISCUSSION

Figure 1 it is observed an increase in the pH of the grain coverage with the time until it is evened at the end with all pH of the grain, the husk has a stable pH during the 7 fermentation days and the cotyledon has a decrease after the third day, stabilizing around a pH of 4 to 5.

The grain index for the three clones on the three fermentation times in the first harvest of 2017 do not vary in a significant manner with a reliability level of 95%, maintaining it for the three clones on similar values. Figure 2 presents the behavior of the averages at 72, 120 and 168 h, observing principally variation on the clone FTA 2 at 120 h, for the clones FSA 12 and

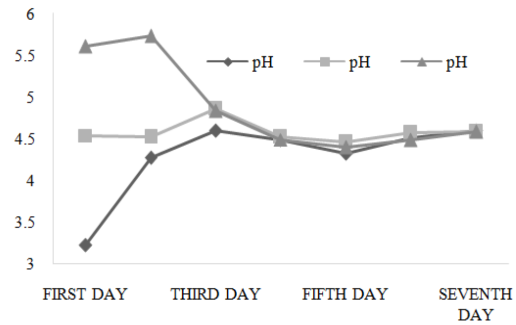

Fig. 1: pH-behavior

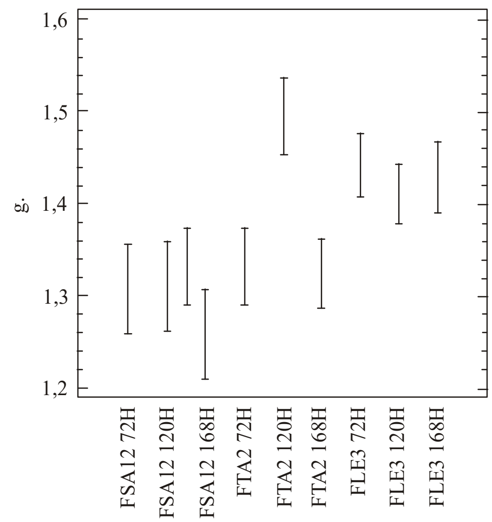

Fig. 2: Graphics of the grain index averages

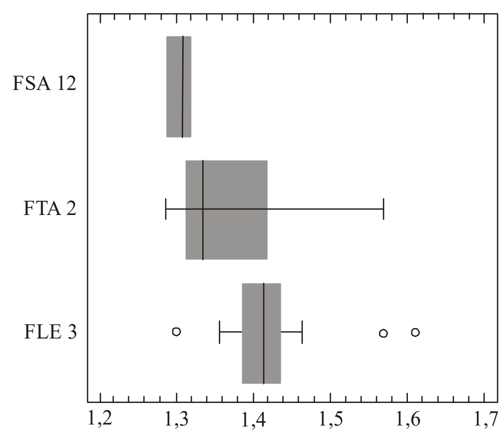

Fig. 3: Box and whiskers diagram for the clones FSA12, FTA2 and FLE3

FLE 3 the averages are maintained at the three moments.

As it is observed in Fig. 3, it is obtained the values for the grain index for the first harvest of 2017, for the FSA 12 in an average near to 1,3 g, FTA 2 between 1,3-1,4 g and FLE 3 between 1,4 to 1,45 g.

The grain index is an important factor in the fermentation process, it conditions the entry of the

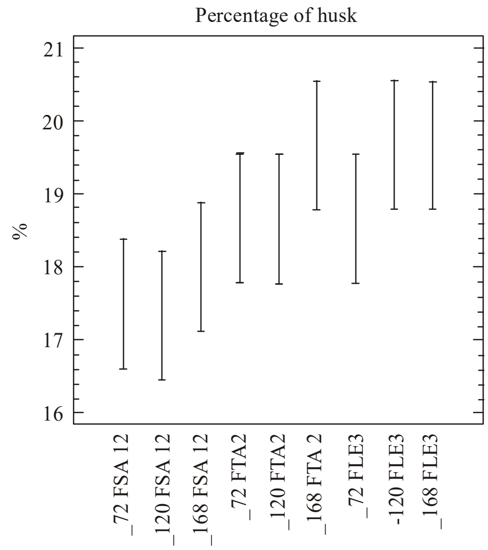

Fig. 4: Husk percentage graphic

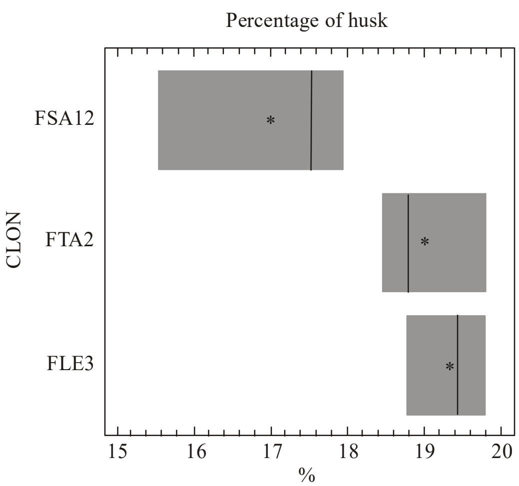

Fig. 5: Box and whiskers diagram for the husk percentage of each clone

fermentation sub products to achieve the cotyledon's transformation and the generation of flavor's precursors and aroma of cocoa (Afoakwa *et al*., 2013, 2008; Kongor *et al*., 2016; Mayorga-Gross *et al*., 2016). In this case the grain index did not suffer any variation on the three evaluated time periods with a reliability of 95% and the grain index of the three clones are different, although two of it are found in the medium grain range, like the FTA 2, contrary to the reported by Perea *et al*. (2013) like a clone with a high grain index.

Husk percentage in the grain: The husk's percentage of the first harvest of 2017 were similar for the three clones without any significant difference with a reliability level of 95%, in Fig. 4 it is observed that it was obtained values between 17% and 20% for all the

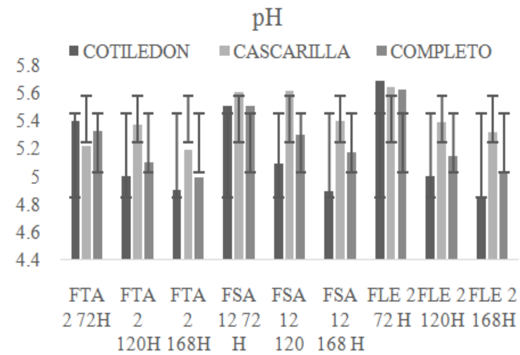

Fig. 6: pH-behavior in the fermentation days

treatments and clones, without any significant difference with a reliability level of 95%.

Figure 5 shows that the clone with the lowest percentage was the FSA1, the clone with the higher percentage was the FLE3 and the next with values between 18,5 and 19,8 was the FTA 2.

The husk percentage found corresponds with the reported by Perea *et al*. (2013) for the clones FTA 2 and FLE 3 with a percentage superior than 18% for both, in the case of the clone FSA 12 that is reported like a clone with a medium husk percentage between 11% and 12%, it was obtained values between 17% and 18% being superiors for what it was expected (Stevenson *et al*., 1993).

Determination of the husk and cotyledon pH: The pH behavior for the three studied clones described on Fig. 6 was similar for all and it is observed a decrease in its values at 72, 120 and 168 h, the higher values were always obtained by the husk.

The pH-values are very important because it allows evaluating the development of the fermentation process, it was obtained a decrease on the value for the cotyledon, husk and complete grain for the first harvest of 2017, verifying, in this way, an adequate fermentation process (Kadow *et al*., 2015; Hue *et al*., 2016; León-Roque *et al*., 2016; Evina *et al*., 2016). In studies made by Reyes *et al*. (2015), Soto *et al*. (2016) and Reyes *et al*. (2016) it was achieved similar values for this variable of the process, beginning with pH-values around 6-7 and ending with values between 4 and 5.

Fermentation index: The fermentation index for the first harvest of 2017, as can be observed on Fig. 7, increase with the time, getting superiors values to 60% after the 120 h, the clone with higher values in this index was the FTA2. These fermentation percentages are adequate and are within the expectations of 60 to 80% for the 120 h, securing that there is not over-fermentation (Stevenson *et al*., 1993; DeZaan, 2009; Quintana and Gómez, 2013; Lucas *et al*., 2015).

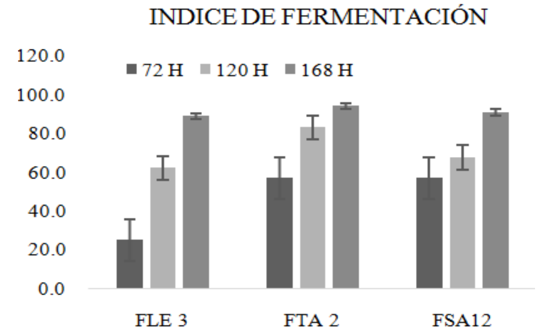

Fig. 7: Fermentation percentage of the three evaluated times

CONCLUSION

The physical and chemical characterization for each one of the three clones allows to determine the final quality of the cocoa for the first harvest, with these results it is contributed to the confirmation and recommendation of the adequate fermentation times, in the case of the physical indexes its variation is not significate for the first evaluated harvest of 2017.

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