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Sensory analysis of cacao liquor (*Theobroma cacao* L.) in cultivars with different origins grown in the Colombian tropics

Análisis sensorial del licor de cacao (*Theobroma cacao* L.) en cultivares de diferentes orígenes cultivados en el trópico colombiano

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Regional cacao genotype.

Photo: N.C. Martínez-Guerrero

ABSTRACT

The sensory profile of 16 cacao cultivars with different origins from the producing areas of the departments of Arauca, Huila and Santander in Colombia was evaluated. This study used the Laboratorio de Calidad Integral de Cacao of the Instituto Nacional de Investigaciones Agropecuarias (INIAP) at the Estación Experimental Tropical Pichilingue (Quevedo-Ecuador), with a panel of four evaluators who analyzed the typical flavor attributes of cacao liquor, such as: cocoa, acid, bitter, astringent, fruity, floral, nutty, sweet and green/raw. The mean values of the scores assigned by the evaluators showed that the sensory profile varied considerably between genotypes and locations. Some genotypes had more complex flavor profiles than the control, CCN 51. The introduced genotypes EET 8, ICS 1, ICS 39 and ICS 60 had a low intensity in the evaluated attributes, while most of the selected regional materials had greater intensity in the fruity, cocoa and nutty notes, especially FLE 3, FSV 41, FEC 2 and FEAR 5. The sensory profiles demonstrated the potential of Colombian cacao with its fine aroma.

Additional key words: quality; sensory profile; flavors; aromas.

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RESUMEN

Se evaluó el perfil sensorial de 16 cultivares de cacao de diferentes orígenes de las zonas productoras de los departamentos de Arauca, Huila y Santander en Colombia. Este estudio utilizó el Laboratorio de Calidad Integral de Cacao del Instituto Nacional de Investigaciones Agropecuarias (INIAP) en la Estación Experimental Tropical Pichilingue (Quevedo-Ecuador), con un panel de cuatro evaluadores que analizaron los atributos de sabor típicos del licor de cacao como: cacao, ácido, amargo, astringente, frutal, floral, nuez, dulce y verde/crudo. Los valores medios de las calificaciones asignadas por los evaluadores mostraron que el perfil sensorial varió considerablemente entre genotipos y localidades en estudio. Algunos genotipos presentaron perfiles de sabor más complejos que el control, CCN 51. Los genotipos introducidos EET 8, ICS 1, ICS 39 e ICS 60, presentaron baja intensidad en los atributos evaluados, mientras que la mayoría de los materiales regionales seleccionados presentaron mayor intensidad de notas frutales, cacao y nuez, especialmente FLE 3, FSV 41, FEC 2 y FEAR 5. Los perfiles sensoriales demostraron el potencial del cacao colombiano con su fino aroma.

Palabras claves adicionales: calidad; perfil sensorial; sabores; aromas.

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INTRODUCTION

Chocolate is a key ingredient in many types of food and is listed as one of the most preferred flavors by people around the world. The particular flavor of chocolate is due to the mixture of hundreds of compounds that originate from precursors in cocoa beans during postharvest treatments (Di Carro *et al*., 2015). The global market for cocoa beans has two main categories: “fine” and “bulk” or “ordinary”. According to Afoakwa *et al*. (2008), Latin America and the Caribbean provide 80% of fine cacao, which is widely recognized by processors and covers a small amount of production, equivalent to 5-8% of global supply of cacao, which, according to the FAO, reached 5,756,953 t in 2020, with Colombia contributing 63,416 t (FAO, 2022). The International Cacao and Chocolate Organization (ICCO) classified Colombian cacao as fine aroma, which offers more economic and social benefits to cacao producers than to regular cacao producers (Ríos *et al*., 2017).

Quality classification is primarily based on the aromatic composition of cacao, where genotype is the most important factor; however, the agroclimatic conditions of a crop and harvesting, fermentation, drying and processing processes are factors that have significant effects on the formation of volatile and non-volatile components and define the final quality of the product (Sim *et al*., 2016; Palacios *et al*., 2021). Different studies carried out in countries such as Ghana, Trinidad and Tobago, Mexico, Venezuela, Peru and Ecuador have shown that the sensory profile of cacao varies with genotype and conditions in the origin region (Frauendorfer and Schieberle, 2008; Pino *et al*., 2011; González *et al*., 2012; Ordoñez *et al*., 2020).

More than 500 volatile compounds have been detected in cacao, whose mixture includes alcohols, aldehydes, ketones, acids, esters and pyrazines, among others, which are developed during fermentation, drying and roasting, generate the characteristic attributes of chocolate, and have differences in the volatile compounds in cacao with different geographical origins (Argout *et al*., 2008; Cambrai *et al*., 2010; Batista *et al*., 2016).

According to Argout *et al*. (2008), flavor is one of the more important quality criteria for chocolate manufacturers, which is why important advances have been made in its assessment in recent years; however, because of the difficulty in taking these measurements, technical techniques have been used, including sensory and chemical evaluations with chromatography. Sensory evaluation is an important technique for determining quality based on sensory properties, which has been used by trained panels of evaluators who use precise measurement techniques for human responses to food. This technique has been widely used for food, including product development, classification studies, and quality determination for products with high commercial value (Vázquez-Ovando *et al*., 2012; Cevallos-Cevallos *et al*., 2018).

Global standards and protocols have been developed for the evaluation of cacao and chocolate flavor quality by the Cacao of Excellence Program (CoEx, 2015), which recognizes the quality and diversity of cacao in producing countries (International Standards for the Assessment of Cocoa Quality and Flavour – ISCQF, 2022). These standards established the flavor profile by identifying and measuring the intensity of basic and complementary attributes that determine the attractiveness of the final product and therefore the desirability of cacao and chocolate products (Streule *et al*., 2022). In recent years, consumer interest in high-quality foods with a clear geographic identity has grown rapidly (Luykx and Van Ruth, 2008; Cevallos-Cevallos *et al*., 2018).

Given the advances in determining cacao quality in the global environment, the fact that Colombia has been recognized as a country that produces fine aroma cacao (ICCO, 2022), and this crop's increased relevance in agricultural development, comparative evaluations of the expression of quality characteristics are needed, taking into account clone diversity from different cacao producing regions. This study aimed to elucidate the sensory profiles of cacao cultivated in Colombia.

MATERIALS AND METHODS

Environment assessments

This research prioritized three of the main cacao-producing departments in Colombia: Arauca, Huila and Santander. Samples were taken on Santa Elena experimental farms in the municipality of Arauquita, Department of Arauca, at 162 m a.s.l. (7º01'06.03" N and 71º23'06.17'' W), with 1,969 mm of annual precipitation, an average temperature of 27ºC, and79% relative humidity; Alto Magdalena in the municipality of Gigante in Huila at 960 m a.s.l. (2º23'20" N and 75º32'17" W), with 1,108 mm of precipitation, an average temperature of 20ºC, and 79% relative humidity; and Villa Mónica located in the municipality of San Vicente de Chucurí, in the Department of Santander, at 879 m a.s.l. (6º51'48.27" N and 73º24'7.97" W), with 2,022 mm of annual precipitation, an average temperature of 23ºC, and 80% relative humidity, as representative areas of cacao production in Colombia.

Cacao genotypes

Table 1 lists the 16 cacao genotypes used in this study, of which eight were introduced to Colombia and are in current commercial use, and eight clonal selections were obtained in different regions of Colombia by Fedecacao and Agrosavia with participatory varietal selection on farms with good yield. The controls were the commercial materials CCN 51, with high productivity, resistance to diseases, large fruits and seeds with a high butter content, and ICS 39, with good quality characteristics (Perea *et al*., 2017; Boza *et al*., 2014; Palacios *et al*., 2021). In each location, the cacao clones were established in rows of 15 plants, taking samples from 10 trees and leaving the remaining five plants as borders.

Sensorial analysis

To carry out the sensory quality analysis, the cacao fruits were harvested in a state of maturity in the same season in the three locations. To confirm the state of maturity of the cacao, the change in fruit color was used as an indicator, where green, immature fruits turn yellow, and purple ones change to orange. In each location, the microfermentation of samples was carried out in a fermentation dough matrix as suggested by Jiménez *et al.* (2011), the process was carried out by placing the grains (2.5 kg) of each of the 16 genotypes in plastic mesh inside drawers for the 6-d fermentation process, with two removals at 24 and 72 h and local temperature. An Elba dryer was used until reaching close to 7% moisture (Jiménez *et al*., 2011).

The processing was carried out according to the protocols of the Laboratorio de Calidad Integral de Cacao of the Instituto Nacional de Investigaciones Agropecuarias - INIAP, at the Estación Experimental Tropical Pichilingue (Quevedo-Ecuador), where the beans were roasted at 112ºC for 12 min, cooled, shelled and ground until obtaining cacao paste or liquor that was refrigerated for a week to carry out the sensory evaluation.

The liquors were evaluated with quantitative descriptive analysis or sensory profile evaluation of NTC 3929 (ICONTEC, 2021), using a four-person sensory evaluation panel that was trained according to ISO 8586 (ISO, 2012). Liquor samples of each material were evaluated, performing three repetitions for each sample. The panel of judges assigned scores according to the intensity of each attribute on a scale

Table 1. Identification and origin of the cacao genotypes.

Nomenclature	Identification	Origin
Introduced genotypes		
ICS 1, ICS 39 (control), ICS 60, ICS 95	Imperial College Selection	Trinidad, Nicaragua and Venezuela (Bekele *et al*., 2006)
EET 8 = UF 650	United Fruit Company	Costa Rica (Bekele *et al*., 2006)
CCN 51 (control)	Colección Castro Naranjal	Ecuador (Boza *et al*., 2014)
IMC 67	Iquitos Mixed Calabacillo	Peru
TSH 565	Trinidad Selection Hybrid	Trinidad (Johnson *et al*., 2009)
Regional genotypes, Trinidadian hybrids (Perea *et al*., 2017)		
FLE 2, FLE 3	Fedecacao	Lebrija, Santander, Colombia
FTA 2	Fedecacao	Tame, Arauca, Colombia
FEAR 5	Fedecacao	Arauquita, Arauca, Colombia
FEC 2	Fedecacao	El Carmen, Santander, Colombia
FSV 41	Fedecacao	San Vicente, Santander, Colombia
SCC 61	Selección Colombia Corpoica,	Santander, Colombia
FSA 13	Fedecacao	Saravena, Arauca, Colombia

of 0 to 10, with 0 being the absence of the attribute, 1 to 2 low, 3 to 5 medium, 6 to 8 high and 9 to 10 very high, according to protocols of ISCQF and Cacao of Excellence Program (CoEx, 2015). Nine attributes were evaluated for cacao liquor: cocoa, acid, bitter, astringent, fruity, floral, nutty, sweet and green/raw. The panelists also determined the presence of other characteristics such as off-flavors or contamination, e.g. mold, dirt, and over-fermented.

Statistical analysis

A Principal Components Analysis (PCA) was carried out to discriminate the 16 cacao genotypes based on the effect of the environment on the expression of sensory attributes evaluated in the three locations, classifying food quality for different geographical regions (Luykx and Van Ruth, 2008).

RESULTS AND DISCUSSION

The correlation matrix (Tab. 2) shows the relationship between each pair of sensory attributes: cocoa, acid, astringent, bitter, fruity, floral, nutty, sweet and green/raw, with high positive correlations between astringent and bitter (r=0.85), astringent and green/raw (r=0.78), and bitter and green/raw (r=0.77), demonstrating a direct relationship between the astringent, bitter, and green/raw notes in the cacao liquor. Another high and positive correlation was established between fruity and sweet, which are perceived in well-fermented fine cacao (Counet *et al*., 2004). Another high and positive correlation was found between cacao and fruity (CoEx, 2015).

Several authors have reported that the presence of these attributes is associated with high polyphenol contents that are important components of cocoa flavor, responsible for bitterness and astringency that differs between genotypes, environmental and that are present in low-fermented cacao beans, which significantly affects the sensory qualities of chocolate (Afoakwa *et al*., 2012; Owusu *et al*., 2012; Ramos *et al*., 2014; Menezes *et al*., 2016; Sim *et al*., 2016; Ordoñez *et al*., 2020). According to Menezes *et al*. (2016), there is a positive correlation between the level of fermentation and the expression of floral, fruity and sweet aromas. High and negative correlations were found between bitter and fruity, bitter and nutty, fruity and green/raw, and nutty and astringent, demonstrating an inverse relationship between these attributes. These inverse relationships indicate that, when fermentation is adequate, aromas such as nutty, fruity, and floral can be expressed and are not masked by attributes related to bottom fermentation, allowing chocolate to develop all its characteristic aromas (Rodríguez-Campos *et al*., 2011; Ramos *et al*., 2013).

Table 2. Correlations (*r*) between sensory attributes of 16 cacao genotypes evaluated in three locations in Colombia.

Attribute	Cocoa	Acid	Astringent	Bitter	Fruity	Floral	Nutty	Sweet	Green
Cacoa	1	0.380[+]	-0.374	-0.587	0.609	0.107	0.355	0.436	-0.495
Acid	0.380	1	0.288	0.119	0.267	0.218	-0.278	0.189	0.163
Astringent	-0.374	0.288	1	0.854	-0.458	0.001	-0.673	-0.305	0.780
Bitter	-0.587	0.119	0.854	1	-0.636	-0.171	-0.630	-0.474	0.772
Fruity	0.609	0.267	-0.458	-0.636	1	0.218	0.350	0.737	-0.631
Floral	0.107	0.218	0.001	-0.171	0.218	1	0.080	0.316	-0.005
Nutty	0.355	-0.278	-0.673	-0.630	0.350	0.080	1	0.303	-0.559
Sweet	0.436	0.189	-0.305	-0.474	0.737	0.316	0.303	1	-0.363
Green	-0.495	0.163	0.780	0.772	-0.631	-0.005	-0.559	-0.363	1

[+] Pearson’s correlation coefficient of 48 observations = 16 genotypes × 3 environments.

According to the Principal Component Analysis (PCA), two components were obtained that had a vector or eigenvalue greater than 1. CP1 had an eigenvalue of 4.34, and CP2 had 1.83, which explained the behavior of the genotypes in the three locations, accumulating 68.52% of the total variance and indicating that they are the more important in terms of absolute variance. In the first component, the greatest contribution was from the green/crude, bitter, astringent, fruity, nutty, and cocoa attributes, and the second component was from the acid, floral, and sweet attributes (Fig. 1).

Figure 1 shows that the first component accumulated 48.18% and was represented on the positive axis by the astringent, green/crude, bitter and acid attributes. On the negative axis of component 1, this component was represented by the floral, sweet, fruity,

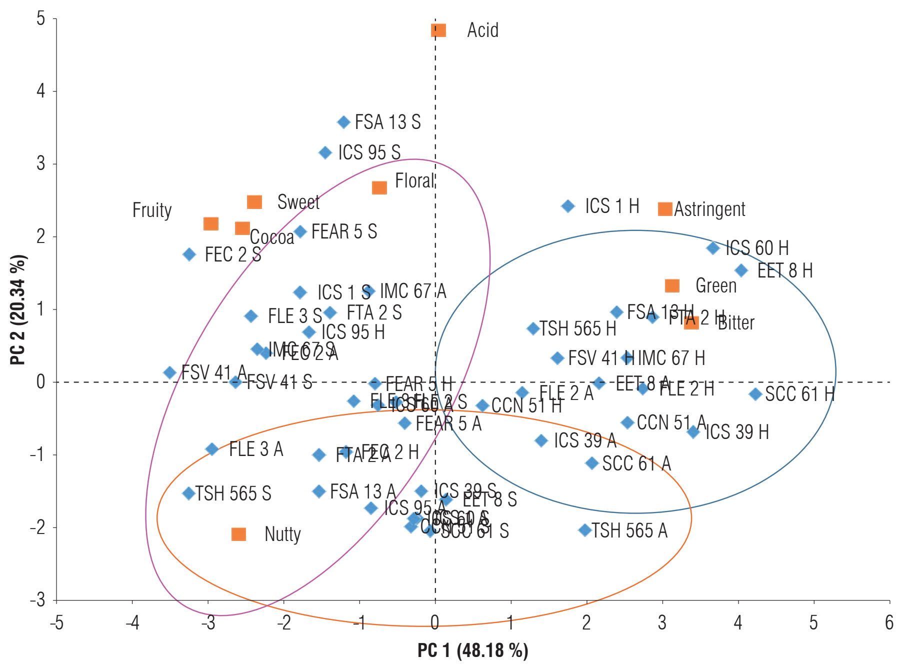

Figure 1. Representation of the first two principal components for the sensory attributes of 16 cacao genotypes in three regions of Colombia. The final letters of the genotype names correspond to the departments: S = Santander, A = Arauca and H = Huila.

cocoa and nut attributes that are perceived in cacao when fermentation has allowed the components that define cacao quality to form. These results demonstrate the inverse relationship between the bitter, astringent, acid and green/crude attributes with cocoa, fruity, floral, nutty and sweet notes, which have also been seen in other studies. These results led to the suggestion that the expression of attributes that describe the pleasant sensory impression of cacao can be masked by the presence of unpleasant attributes that affect the final quality of chocolate (Rodríguez-Campos *et al*., 2011; Batista *et al*., 2016).

Similarly, figure 1 shows that the genotypes formed three groups that coincided with the regions where they were evaluated, where most of the genotypes with the final letter S that were evaluated in Santander were in the negative quadrant of the first component and the positive quadrant of the second one. The genotypes evaluated in Arauca with the final letter A were in the negative axis of the second component, and those from Huila (H) were in the positive quadrants of the first and second components. The genotypes in the Santander group presented attributes such as fruity, cocoa, sweet and floral. The Arauca group had nutty notes, and the Huila group had astringent, green and bitter notes.

The samples from Huila presented profiles where the attributes were related to low fermentation as a result of various causes that may be related to the temperature reached in the aerobic phase, the populations of microorganisms, the water content, and the amount of mucilage around the grains (Streule *et al*., 2022). According to Sukha *et al*. (2008), these variations raise questions related to the importance of the genotype-environment relationship and how their interaction during fermentation and drying affect cacao flavor attributes.

The groupings by regions were consistent with those reported by other authors who evaluated the expression of chemical and sensory compounds of cacao in different regions, demonstrating that there are differences in sensory profiles influenced by environmental and management conditions (Ramos *et al*., 2013; Menezes *et al*., 2016). Carrillo *et al*. (2014) observed considerable variations that resulted from altitude in the concentrations of cacao volatile compounds from different geographic regions of Colombia. These compounds are correlated with the aromatic profile of cacao after being fermented and dried, as has been described by several authors (Frauendorfer and Schieberle, 2008; Rodríguez-Campos *et al*., 2011; Menezes *et al*., 2016; Sim *et al*., 2016).

The differences in the regions may also have been generated by microorganisms present during cacao fermentation that are responsible for the production of metabolites and flavor precursors that affect quality (Batista *et al*., 2016; Streule *et al*., 2022). According to Moreira *et al*. (2013) and Ramos *et al*. (2014), the bacterial communities present in the fermentation process vary according to the conditions of the pulp, such as sugar and moisture content, that can change with the genotype and the environmental conditions where the fermentation and drying process takes place.

Additionally, there is high genetic variability in Colombian cacao, represented in the genotypes evaluated in this study, which may play an important role in the variability of sensory profiles and grouping by region. According to Ramos *et al*. (2013), different varieties can offer characteristics that favor the growth of diverse communities of microorganisms during the fermentation process, which must be taken into account in management for the production of high-quality cacao.

According to the results obtained in several recent studies, since cacao varieties influence the quality of chocolate, different varieties should be fermented separately using yeast starter cultures to improve standardization of the cacao fermentation process (Ramos *et al*., 2014; Batista *et al*., 2016; Menezes *et al*., 2016; Bastos *et al*., 2019).

The results found in the present work demonstrated what has been established by several authors, where genotype and environmental and management conditions where the crop is developed influence the sensory profile and the quality of the chocolate (Luna *et al*., 2002; Cambrai *et al*., 2010; Porras *et al*., 2019). These results will aid future classifications according to the geographical area of cacao-producing regions or "terroir", such as that established for wine, coffee, and other foods, in addition to providing a scientific basis for quality certification programs, as suggested by Bertoldi *et al*. (2016).

Table 3 show the results of the sensory evaluation panel for each genotype in the three locations. In the three localities, typical fruity notes of Trinitario-type cacao stood out. According to Johnson *et al*. (2009), the ICS and TSH 565 materials have intense fruity, floral, nutty and chocolate notes with pleasant secondary aromas such as molasses, caramel and raisins. According to the organoleptic evaluation carried out by Boza *et al*. (2014) and Menezes *et al*. (2016), CCN 51 exhibits a moderate amount of cacao flavor and high levels of bitterness and astringent, consistent with the profile found in the three locations. This clone is not appreciated in the fine cacao market.

The EET 8, ICS 1, ICS 39 and ICS 60 genotypes presented low intensity in the evaluated attributes, when compared with those of the other cultivars, possibly because the fermentation process was affected by the large size of the grains. On the other hand, most of the clonal selections presented attributes with greater intensity in the fruity, cocoa and nutty notes, especially FLE 3, FSV 41, FEC 2 and FEAR 5. This result is consistent with the report by Sukha *et al*. (2008), who observed that the sensory profile varied between the cacao genotypes from Ghana and Trinidad. The latter had moderate cocoa flavor values and intense acid, floral, and fruity flavors. According to the results, the FEC 2 genotype had the highest intensity for the cocoa note and the lowest astringency. ICS 1 and FEC 2 presented floral notes, the FLE 3 and FSV 41 genotypes had nutty notes, and FTA 2 and FSA 13 presented fruity and sweet notes.

In general, each genotype had a different sensory profile, which, according to Afoakwa *et al*. (2008),

Table 3. Description of the sensory profile of 16 cacao genotypes according to the panel of judges in three locations.

Genotypes	Sensory profile concept
Introduced commercial genotypes	
ICS 1	Balanced, highlighting fruity notes in Santander, Huila had floral notes, astringency and bitterness, and Arauca presented low values in all attributes
ICS 39	Astringent sensation and a bitter taste in Huila and Arauca, while Santander had nutty tones
ICS 60	Huila reached very high values in acid, astringent and bitter, qualified as unpleasant; Arauca and Santander presented low values in all attributes
ICS 95	Santander presented an excellent profile with intense citrus notes and a good cacao background, typical of Trinitario cacao; Arauca presented nutty notes, and Huila had fruity and caramel notes
EET 8	The three localities presented high intensity in the acid, bitter and astringent attributes, with very low values in pleasant aromas typical of cacao
CCN 51	Notes of chocolate and low tones of ripe fruits in Santander; in Huila and Arauca, astringency stood out
IMC 67	Arauca and Santander stood out for presenting notes of dried fruit, while Huila presented greater intensity in the bitter and astringent attributes, with a fruity undertone
TSH 565	Santander presented an intense fruit flavor and notes of nuts and chocolate, while in Arauca, the intensity of cocoa notes was lower, and, in Huila, the acidity, astringency and bitter taste stood out
Regional genotypes	
FLE 2	An intense fruit flavor with very pleasant notes of chocolate and bitterness was in Santander and Arauca; Huila presented a similar trend with lower intensity in cocoa and walnut
FLE 3	Balanced with nuances of nuts, fruit and caramel, however, Huila presented greater intensity in astringency and bitterness, and Santander had floral notes that stood out
FTA 2	Excellent profile in Santander, with notes of sweet fruits, caramel, spices and nuts, similar to that of Arauca. Huila presented greater intensity in the bitter, astringent and green notes, with a consequent lower intensity of pleasant attributes
FEAR 5	Fruity citrus and floral notes of aromatic herbs and excellent cacao flavor; samples from Arauca and Huila also presented fruity and cocoa notes
FEC 2	Santander presented very pronounced chocolate and balanced floral, fruity and nutty notes, similar to Arauca and Huila
FSV 41	Santander presented notes of wine, with intense ripe fruit, nutty tones and a bit sweet and soft, while Arauca presented floral notes, and Huila had astringent, bitter and green attributes that predominated
SCC 61	Low intensity in most attributes in the three regions, highlighting bitter and astringent notes in Huila
FSA 13	High notes of cocoa, fruity and sweet in Santander; Arauca had nutty tones, and Huila had low fruity, astringent and bitter notes

was mainly due to quantitative differences caused by aroma precursors, which are inversely proportional to the polyphenol content, as well as the sugar content and the enzymatic breakdown of polysaccharides, which are a significant source of precursors.

CONCLUSIONS

Some regional genotypes surpassed the CCN 51 genotype in the sensory profile, demonstrating the potential of regional clones for crop development and as a genetic resource in Colombia. Genetic and environmental conditions influenced the development of cacao flavor characteristics, and Colombian cacao has fine attributes. Sensory evaluations are a fundamental tool for evaluating the organoleptic quality of cacao and for geographical classifications as a basis for the development of denominations of origin for specialty markets.

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