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Scientific Note

Development of color guides to evaluate the maturity of cacao clones by digital image processing[1]

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ABSTRACT

Raw material homogeneity is one of the most requested characteristics in any production industry, and the cacao industry is no exception. However, there are many factors that affect the final quality of fruits, among them the variety and maturity stage. The present study aimed to create color tables for evaluating the maturity index of the ICS06, CCN51 and EET8 cacao clones, using digital image processing, in order to contribute for the quality and final homogeneity of the fruits and their by-products.

KEYWORDS: *Theobroma cacao* L., maturation index, cacao quality.

RESUMO

Desenvolvimento de guias de cores para avaliar a maturação de clones de cacau por meio de processamento digital de imagens

A homogeneidade da matéria-prima é uma das características mais solicitadas em qualquer indústria produtiva, e a indústria do cacau não é exceção. No entanto, há muitos fatores que afetam a qualidade final dos frutos, dentre eles a variedade e o estágio de maturação. Objetivou-se criar guias de cores para avaliar o índice de maturação dos clones de cacau ICS06, CCN51 e EET8, utilizando-se processamento digital de imagens, a fim de contribuir para a qualidade e homogeneidade final dos frutos e seus derivados.

PALAVRAS-CHAVE: *Theobroma cacao* L., índice de maturação, qualidade do cacau.

Cacao is a fruit cultivated all around the world, with Africa being the largest producer (71.1 %), followed by the Americas (16.1 %), Asia (11.9 %) and Oceania (1 %) (FAO 2020). The cacao market distinguishes between two categories of beans: fine and flavor cacao and ordinary or bulk cacao. Such distinctions are due to the organoleptic properties that the bean offers, with fine cacao being highly appreciated (ICCO 2019). Colombia is among the top ten fine cacao producing countries worldwide (Cubillos Bojacá et al. 2019, Rojas et al. 2020).

Variety, soil characteristics, maturity stage and processing conditions are key factors for obtaining high organoleptic cacao quality (Kongor et al. 2016). In cacao farming, the selection of the maturity stage of the cacao pod is crucial for obtaining high quality cacao beans, because, during the ripening of the fruit, compounds such as sugars, organic acids, methylxanthines, polyphenols and proteins, all of which play an important role in the generation of aroma precursors, are formed or modified (Dang & Nguyen 2019). Similarly, physicochemical parameters such as the pH and titratable acidity of the pulp will determine the fate of those compounds and, thus, the performance of the fermentation process (Cubillos Bojacá et al. 2019). Therefore, the appropriate maturity stage of the cacao pods and their homogeneity will contribute to a good fermentation process and, ultimately, to the quality of the cacao beans.

In cacao farming, the selection of pods during harvest is a highly empirical task, based mainly on the appreciation of the residual color of the husk, which has been shown to change according to the maturity stage (Cubillos-Bojacá et al. 2019). Then,

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it is important to have a practical and reliable tool to establish the maturity stage of the cacao pod and harvest the appropriate cacao pods to get good quality and homogeneous cacao beans.

Color scales is one of the most widely used non-destructive methods for characterizing the fruits maturity stage, as it enables the association of physicochemical parameters such as sugar content, pH, acidity, maturity index and fruit peel color. This method is widely used when determining the maturity stage of apples (Cárdenas-Pérez et al. 2017) and olives (Avila et al. 2015). However, this tool does not exist for cacao, as there are many different varieties that develop different color patterns, what means that each clone needs its own proper color scale. The development of a color scale for every cacao clone would be a key tool in reducing the heterogeneity of the raw material in the production of cacao for the chocolate industry.

To accurately detect color, systems such as the CIELAB have been established. This system is based on three coordinates: luminosity (L*), difference between green (-a*) and red (+a*) and between blue (-b*) and yellow (+b*) (Sahin & Summu 2006), which allow the characterization of colors in the visible spectrum.

In the present study, color scales for three cacao clones (EET8, CCN51 and ICS60) were created, using digital image processing, in order to contribute for the quality and final homogeneity of the fruits and their by-products.

A total of 54 cacao pods, being 18 pods per variety, were collected in El Carmen de Chucurí, in Santander, Colombia (6º42’0’’N, 73º31’1’’W and altitude of 768 m), in the second harvest of 2017.

Three pods were harvested per maturity stage from each clone, according to days after anthesis (DAA). All samples were taken between 120 and 200 DAA, with differences of 15 days for each maturity stage (López-Hernández et al. 2021). However, it was not possible to get CCN51 clones in the maturity stage 6, as, at 195 DAA, they were over ripen and not adequate for fermentation.

The cacao fruits were classified according to DAA and transported at 18 ºC to the a photographic studio, considering four stages: adaptation to the conditions of the photographic study and image capturing, processing and analysis.

In the first stage, two flashlights with power of 500 W s^{-1} (Elinchrom, BRX 500), balanced at 125 W s^{-1} and synchronized through radio frequency transceiver units, were used to adapt the photographic studio. The modeling accessories used in the flashes were two Rotalux Softboxes that had the purpose of blurring light to avoid glare and strong shadows. These flash units were matched at the same light unit by two lamps of warm light, which were used to achieve an image without dominant shadows and a flat contrast, that is, a light:shadow ratio of 1:1. To verify that the same amount of light was available for each flashlight, it was measured with an incident light meter with retracted lumisphere (Sekonic l-308x Flashmate), to achieve a punctual measurement in each one and in an independent way. This step is important, so that the light sources do not suffer the same wear during their work and, therefore, vary in their power. After balancing the two flashlights in power and position, the camera was placed.

In the second stage, a photographic capture was made with the camera in a zenithal position and lights in a lateral position near the cacao fruit at a distance of 1.50 m from the camera to the object. The camera (Canon EOS 60D) was adjusted to the manual mode, ISO 100, and the shots were taken at a shutter speed of 1/125 and aperture of 11, with a 0.060 m focal length prime lens. The camera was supported by an extensor arm, thus achieving a zenithal frame, conditioned to a three-section tripod. The temperature setting on the camera was defaulted for flash.

In the third stage, the image processing was carried out by dividing each cacao fruit into three sections, due to the diversity of colors in the same fruit (upper, equatorial and lower section). Likewise, these sections were divided into three subsections each one, giving a total of nine areas (Figure 1).

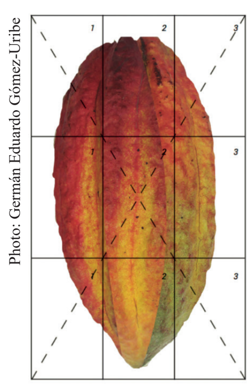

Figure 1. Grid used to homogenize the information collection methods for the color analysis of each cacao fruit.

Then, with the dropper tool of the Adobe Photoshop CC 19.0.0 software (Adobe, California, United States), the predominant hexadecimal value for each subsection was determined, and the L* (luminosity), a* (green to red) and b* (blue to yellow) parameters were determined with the Adobe Color online tool (Adobe, California, United States).

In the fourth stage, the obtained colors were categorized according to Table 1, which allowed the determination of predominant tones for each maturity stage. Due to the chromatic diversity in the cacao pods, the main five prevalent colors present in these three clones were categorized according to a* and b* values.

The soluble solids (SS)/titratable acidity (TA) ratio, known as maturity index, is widely used in fruits, and will be proposed as a harvest maturity indicator in the cacao crop. The fruit pods were opened and the pulp separated, to measure the soluble solids content, which was determined with a digital refractometer (Atago PAL-1, Tokyo, Japan). The titratable acidity content was determined following the protocol established by AOAC 942.15 (AOAC 2005), using a Hanna brand potentiometer (HI2020-01; Woonsocket, Rhode Island, USA). An acid-base titration of 2 mL of the previously filtered juice obtained from cacao beans, with a NaOH 0.1 M solution, was carried out until a pH of 8.2 was obtained. The titratable acidity content was expressed as percentage of citric acid (Cubillos-Bojacá et al. 2019).

To determine the maturity stage effect of each cacao variety in the parameters L*, a* and b* obtained during the digital image processing, Kruskal-Wallis ($p \leq 0.05$) and Mann-Whitney U tests ($p \leq 0.005$ for five maturity stages and $p \leq 0.0033$ for six maturity stages) were carried out, the latter in case of finding significant differences. Additionally, to identify the effect of the maturity stage on the maturity index, an analysis of variance ($p \leq 0.05$) with a subsequent Tukey test ($p \leq 0.05$) was performed, complying with assumptions of normality (Shapiro-Wilk test; $p \geq 0.05$) and homoscedasticity (Leven's test; $p \geq 0.05$). All the aforementioned tests were carried out by the Infostat software (Info Stat, Cordoba, Argentina, 2010).

Table 1. Criteria for determination of the predominant color from the coordinates a* and b*.

Tone	Criteria	
	a* value	b* value
Green	$-31 \leq a^* \leq -14$	-
	$-14 < a^* \leq -9$	$-b < 50$
Yellow	$-14 < a^* \leq -9$	$-b \geq 50$
	$9 < a^* \leq 22$	-
Orange	$22 < a^* \leq 31$	$b \geq 30$
	$31 < a^* \leq 48$	$b \geq 38$
Red	$48 < a^* \leq 66$	-
Purple	$22 < a^* \leq 31$	$b < 30$
	$31 < a^* \leq 48$	$b < 38$

The three cacao clones showed color changes according to the maturity stage. However, such changes were not uniform throughout the fruit, since mixtures of tones such as green, yellow, orange, red and purple were evidenced throughout all the maturity stage. Table 1 shows the intervals of a* and b* corresponding to the five more common color tones shown by the three clones throughout the ripening process. Despite this variability, the green tone gradually disappeared in the advanced maturity stages of the three clones. Such behavior has been documented in fruits such as green apple *Malus domestica* cv. Golden Delicious (from green to yellow, as a* and b* increased) (Cárdenas-Pérez et al. 2017), coffee berries (green to red) (Herrera et al. 2011), bilberry (green to black) (Arteaga et al. 2014) and purple passion fruit (green to purple) (Jiménez et al. 2011). These studies showed that the color change was due to the increase of a* and decrease in L* and b*. According to the authors, such changes are due to the degradation of pigments such as chlorophylls, as well as the appearance of other compounds such as carotenoids or anthocyanins, which are possibly masked in the first ripening stages. In this case, despite the disappearance of the green color, each clone presented a different distribution in the other tones. This contrast may be caused by the presence of different anthocyanin and carotenoid profiles, which can generate colors such as yellow and orange (predominant in ICS60; Figure 2A), red and purple (predominant in CCN51; Figure 2B) (Sigurdson et al. 2017). Additionally, although there is a high variability of tones in the pods, the average color coordinates for each ripening stage gave a tone within the range of colors of the pod, showing a predominant palette of colors for each variety.

The ICS60 variety presented green tones, mainly in the maturity stages 1, 2 and 3, which were replaced by yellow tones in the maturity stages 4

Figure 2. Color table according to the maturity stage for the ICS60 (A), CCN51 (B) and EET8 (C) cacao clones. It was not possible to get CCN51 clones in the maturity stage 6, as, at 195 days after anthesis, they were over ripen and not adequate for fermentation.

and 5, with orange tones appearing in the maturity stage 6 (Figure 2A). The changes are supported by the coordinates a* and b*, which increased significantly ($p < 0.05$) in 37.12 and 29.81 units, respectively, between the maturity stages 1 and 5 (Table 2). Moreover, there was a significant increase in the maturity index between the maturity stages 1 and 5, which would indicate that the color change in the husk reflects changes in the physicochemical characteristics of the pulp, and, therefore, manifests the maturation of the ICS60 cacao. Additionally, the L* coordinate remained unchanged from the maturity stage 2, what leads to discarding luminosity as a ripening criterion. Therefore, it cannot be associated with the change in the color distribution according to the fruit ripening.

For the CCN51 cacao (Figure 2B), the green tones disappeared at the maturity stage 5, giving way to yellow and orange tones. Likewise, the purple color decreased throughout the maturity stage, while the red tone was predominant in the advanced stages. Such changes in color were only evidenced in the b* coordinate, which increased significantly by 61.94 and 97.85 %, respectively for the maturity stages 4 and 5, if compared to the maturity stage 1 (Table 2). Regarding the physicochemical characteristics, the maturity index increased for the maturity stages 4 and 5, if compared to that determined for the stage 2.

Table 2. Color parameters and maturity index (SS/TA) for the cacao clones.

Clone	Maturity stage	L*	a*	b*	Main tone	SS/TA
CCN51	1	49.70 ± 13.69[a]	27.15 ± 29.87[a]	22.78 ± 12.04[c]		13.51 ± 1.61[yz]
	2	44.93 ± 16.53[a]	38.96 ± 22.26[a]	31.00 ± 17.06[bc]		11.70 ± 1.41[z]
	3	46.15 ± 15.60[a]	38.56 ± 25.61[a]	30.56 ± 18.77[bc]		13.63 ± 1.10[yz]
	4	48.04 ± 17.19[a]	33.37 ± 28.98[a]	36.89 ± 18.84[ab]		15.28 ± 1.93[y]
	5	50.63 ± 16.20[a]	37.44 ± 22.67[a]	45.07 ± 19.12[a]		14.90 ± 1.56[y]
	6	-	-	-	-	-
ICS60	1	38.59 ± 11.02[b]	-18.93 ± 3.84[bc]	29.78 ± 9.28[c]		11.32 ± 1.67[z]
	2	54.63 ± 16.07[a]	-22.00 ± 4.23[c]	45.96 ± 15.95[b]		13.52 ± 0.82[yz]
	3	58.41 ± 14.42[a]	-18.44 ± 6.14[bc]	49.85 ± 14.39[ab]		12.69 ± 1.27[yz]
	4	58.11 ± 16.34[a]	-13.41 ± 14.74[bc]	50.15 ± 21.31[ab]		14.73 ± 2.75[yz]
	5	65.19 ± 12.87[a]	-4.81 ± 16.76[b]	60.67 ± 13.90[a]		16.80 ± 3.33[y]
	6	62.63 ± 12.38[a]	18.19 ± 15.21[a]	59.59 ± 10.01[a]		14.08 ± 3.44[yz]
EET8	1	43.44 ± 17.27[bc]	23.56 ± 24.86[b]	11.89 ± 7.45[d]		8.98 ± 1.36[z]
	2	42.70 ± 15.73[bc]	25.67 ± 21.67[b]	18.44 ± 11.19[cd]		10.62 ± 2.30[yz]
	3	41.26 ± 10.10[c]	41.11 ± 13.53[a]	26.33 ± 14.22[c]		11.32 ± 2.26[yz]
	4	52.37 ± 13.25[ab]	31.22 ± 23.18[ab]	46.41 ± 14.25[ab]		13.77 ± 3.25[y]
	5	48.00 ± 10.32[b]	42.74 ± 15.67[a]	44.52 ± 10.74[b]		14.18 ± 3.10[y]
	6	57.59 ± 8.94[a]	30.33 ± 10.80[b]	56.78 ± 9.32[a]		10.84 ± 1.47[yz]

It was not possible to get CCN51 clones in the maturity stage 6, as, at 195 days after anthesis, they were over ripen and not adequate for fermentation. The CIE L*, a* and b* coordinates and the maturity index are represented by the mean and its standard deviation. Equal letters in the same row mean that there is no significant difference with respect to the maturity stage. [(a-c)] CIELAB coordinates according to the Kruskal-Wallis ($p \leq 0.05$) and Mann-Whitney U ($p \leq 0.005$) tests. [(y-z)] Maturity index according to the Anova ($p \leq 0.05$) and Tukey ($p \leq 0.05$) tests.

Therefore, the color table presented in this study could identify these maturity stages from the b* coordinate of the CIELAB space and their respective maturity index.

As for the other varieties, the EET8 cacao (Figure 2C) presented green tones only in the maturity stages 1 and 2, while, for the advanced maturity stage 5 and 6, the main tones were yellow, orange and red, showing the disappearance of purple tones. The main tones of this variety showed similarities in distribution, regarding CCN51 (Figure 2B) in the early maturity stage, while resembling ICS60 in the maturity stage 6 (Figure 2A). The EET8 variety presents color distribution characteristics like the other varieties studied. Thus, it is necessary to use other morphological characteristics during the differentiation between clones. However, unlike the other varieties, the EET8 clone showed significant differences between the maturity stages 3, 4 and 5 for luminosity (L*), increasing according to a greater presence of orange tones.

The color parameters (L*, a* and b*; main tone) and maturity index (SS/TA) for each one of the studied clones and maturity stages are depicted in table 2. The main tone corresponds to the tone that covers most the surface of the cacao pod, while L*, a* and b* represent the color parameters of that main tone.

Such color changes were also reflected by increasing the a* and b* coordinates. Likewise, an increase in the maturity index was evidenced between the maturity stages 1 and 4 and 5, at 4.79 and 5.2 units (Table 2). Therefore, the color table for EET8 differentiate between an early maturity stage 1 and a late maturity stage 4 or 5, from the ripening index and the coordinates L*, a* and b*.

The color tables of this study give an approach toward the application of digital image processing in the cacao sector and are an alternative to extrapolate it to other types of products, to which the determination of the optimal harvest time is critical, but do not have the right tools to determine the maturity stage.

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