UNIVERSIDAD NACIONAL DE SAN MARTÍN

RAA
https://revistas.unsm.edu.pe/index.php/raa
e-ISSN: 2710-0510

Original Article
Artículo Original
Jul-Dec, 2025

# Physicochemical and Sensory Characterization of 20 Wild Cacao Genotypes from the Germplasm Bank of the Instituto de Cultivos Tropicales - ICT, Peru

## Caracterización Fisicoquímica y Sensorial de 20 Genotipos de Cacao Silvestre del Banco de germoplasma del Instituto de Cultivos Tropicales-ICT, Perú

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**Received:** 23 Feb. 2024 | **Accepted:** 15 Jun. 2024 | **Published:** 20 Jul. 2025
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**How to cite this article:** Medina-Ramírez, M. E., Arévalo-Gardini., E., Arévalo-Hernández, C. O., Mendieta-Taboada, O. W., Baligar, V. & Zhang, D. (2025). Physicochemical and Sensory Characterization of 20 Wild Cacao Genotypes from the Germplasm Bank of the Instituto de Cultivos Tropicales - ICT, Peru. *Revista Agrotecnológica Amazónica, 5*(2), e924. https://doi.org/10.51252/raa.v5i2.924

## RESUMEN

Cocoa (*Theobroma cacao* L.*)* is a key crop for the Peruvian economy and society, with a wide genetic diversity, especially in the Amazon. However, many wild genotypes have not yet been characterized, limiting their use. This study evaluated 20 wild cacao genotypes from seven river basins in Loreto, Peru, through physical, chemical, and sensory analyses. The samples, from the germplasm bank of the Institute of Tropical Crops (ICT), were analyzed in terms of grain size, moisture, fat, acidity, pH and cadmium. A strong correlation was found between genotypes, with some commercial varieties outperforming commercial varieties in the bean index, suggesting their potential for the chocolate industry. Fat content ranged from 41.6% to 56.9%, within the ideal range for high-quality chocolate, while cadmium levels remained below European limits. The sensory analysis identified well-defined flavor profiles, highlighting the PAS-100, MACH-26 and AYP-1 genotypes for their aromatic complexity. These results underscore the importance of conserving the biodiversity of wild cacao and its potential in the production of premium chocolate, encouraging further studies on its genetic and industrial value.

**Palabras clave:** physicochemical composition; wild genotypes; aromatic profile; sensory profile; genetic variability

## ABSTRACT

El cacao (*Theobroma cacao* L.*)* es un cultivo clave para la economía y sociedad del Perú, con una amplia diversidad genética, especialmente en la Amazonía. Sin embargo, muchos genotipos silvestres aún no han sido caracterizados, limitando su aprovechamiento. Este estudio evaluó 20 genotipos de cacao silvestre de siete cuencas fluviales en Loreto, Perú, a través de análisis físicos, químicos y sensoriales. Las muestras, provenientes del banco de germoplasma del Instituto de Cultivos Tropicales (ICT), fueron analizadas en términos de tamaño de grano, humedad, grasa, acidez, pH y cadmio. Se encontró una fuerte correlación entre genotipos, con algunos superando a variedades comerciales en el índice de frijol, lo que sugiere su potencial para la industria chocolatera. El contenido de grasa osciló entre 41.6% y 56.9%, dentro del rango ideal para chocolate de alta calidad, mientras que los niveles de cadmio se mantuvieron por debajo de los límites europeos. El análisis sensorial identificó perfiles de sabor bien definidos, con los genotipos PAS-100, MACH-26 y AYP-1 destacándose por su complejidad aromática. Estos resultados subrayan la importancia de conservar la biodiversidad del cacao silvestre y su potencial en la producción de chocolate premium, incentivando más estudios sobre su valor genético e industrial.

**Keywords:** composición fisicoquímica; genotipos silvestres; perfil aromático; perfil sensorial; variabilidad genética

## 1. INTRODUCTION

Cacao (*Theobroma cacao* L.) is native to the Americas, with the most genetic diversity in the Amazon (Zhang & Motilal, 2016). In Peru, 198,169 hectares of cacao are cultivated, yielding 167 thousand tons (FAOSTAT, 2024). Cacao is one of Peru's flagship products and, as of December 2024, is considered the most dynamic export commodity, valued at 740 million dollars with a growth variation of 241% (CIEN, 2024).

According to the International Cocoa Organization (ICCO), based on production volume, Peru, Ecuador, and the Dominican Republic are considered among the main exporters of fine and aromatic cacao (ICCO, 2024). This sector involves more than 83,505 families and 16 cacao-producing regions, with the department of San Martín leading production, accounting for over 70,000 hectares and 65,000 tons (MIDAGRI, 2024).

Since 2008, various expeditions have been conducted in the Peruvian Amazon by the Tropical Crops Institute (ICT), in collaboration with the Sustainable Perennial Crops Laboratory (USDA-ARS, SPCL) and INCAGRO-MINAGRI, aiming to collect wild cacao genotypes to expand the genetic base for resistance to pests and diseases, productivity, and quality in flavor and aroma. The diverse cacao accessions collected and documented are preserved in the ICT cacao germplasm bank in Tarapoto, Peru (Meinhardt et al., 2011; Zhang & Motilal, 2016; Arevalo-Gardini et al., 2019; Arévalo-Gardini et al., 2023).

This study highlights the relevance of wild cacao genotypes as a powerful tool for the obtention of accessions with unique physical, chemical, and sensory traits. According to Motamayor et al. (2008), these genotypes represent important genetic diversity for cacao; however, systematic research has been scarce. Zhang et al. (2013) emphasized that as the data collected on this knowledge is limited, it is important to have sufficient collections of the previously mentioned genotypes (except for per samples), e.g., varieties such as Cacao Blanco de Piura and Cacao Nacional from Ecuador, and wild genotypes from the Santiago and Morona River basins in Peru. The close range between these areas indicates potential common origins by local ecological conditions.

However, wild genotypes' physical, chemical, and sensory characteristics are understudied. This gap was revealed by Quelal-Vásconez et al. (2020), who are currently highlighting the need to evaluate the quality of cacao beans to inform market prospects.

In Peru, these types of evaluations correspond to specific technical guidelines: GIP 103: NTP-ISO 2451:2018 is for physical and chemical analysis, and GIP 116: NTP 107.311-2021 is for sensory evaluation, both defined by the National Institute of Quality, INACAL.

This work aims to characterize the physical, chemical, and sensory characteristics of 20 wild cacao genotypes from seven river basins in Loreto, Peru. Using the state-of-the-art in genomics, we hope to expand our knowledge of cacao genome diversity and begin to demonstrate the promise of future research and development in the chocolate-industrial sector. This study is relevant as it provides information on genetic diversity and potential uses of cacao's by-products, which have a history of low economic use.

## 2. MATERIALS AND METHODS

### 2.1. Experiment Location

The study was developed between 2021 and 2022 in the "Instituto de Cultivos Tropicales" (ICT) agri-laboratory located at 6.51°S, 76.48°W, at 350 m.a.s.l. The genotypes studied were sourced from the ICT cacao germplasm bank at the "El Choclino" Experimental Station (6.47°S, 76.33°W, 540 m.a.s.l.).

Annual precipitation in the region is 1,200 mm, mean temperature (26ºC), and relative humidity (87%) (Arévalo-Gardini et al., 2015).

The soils where collected genotypes are preserved are characterized as clay loam, with pH 5.6, conductive electrical 4.3 mS/m, phosphorus 6.5 ppm, potassium 107.5 ppm, and organic matter of 3.6% (Arévalo-Gardini et al., 2015).

## 2.2. Plant material

A total of 20 cocoa genotypes were employed, specifically selected based on the presence of fruit at the time of assessment, as detailed in Table 1:

**Table 1.** List of genotypes evaluated for physical-chemical and sensorial characterization

| N° | Genotype | River | N° | Genotype | River |
|---|---|---|---|---|---|
| 1 | AYP-1 | Aypena | 11 | UGU-127 | Ungurahui |
| 2 | AYP-12 | Aypena | 12 | UNG-53 | Ungumayo |
| 3 | MACH-26 | Marañón-Charupa | 13 | UNG-60 | Ungumayo |
| 4 | NUC-134 | Nucuray | 14 | UNG-61 | Ungumayo |
| 5 | PAS-100 | Pastaza | 15 | UNG-74 | Ungumayo |
| 6 | PAS-105 | Pastaza | 16 | UNG-79 | Ungumayo |
| 7 | PAS-83 | Pastaza | 17 | UNG-80 | Ungumayo |
| 8 | PAS-88 | Pastaza | 18 | URI-161 | Urituyacu |
| 9 | UGU-113 | Ungurahui | 19 | URI-164 | Urituyacu |
| 10 | UGU-117 | Ungurahui | 20 | URI-184 | Urituyacu |

## 2.3. Sample Processing

The collected fruits for each genotype were harvested. After washing them with water, the fresh beans were extracted to undergo the micro fermentation process following the methodology of Bittenbender et al. (2017). This process involves placing fresh beans with mucilage into one-liter polyethylene containers, which serve as fermenters. Fermentation was conducted in an air-circulating incubator at 48°C, MEMMERT brand. The containers were sealed for 48 hours to create anaerobic fermentation conditions, after which small holes were made in the lids to allow mucilage drainage.

After the first turn, the beans were stirred every 24 hours, and the fermentation lasted six days (144 hours). After fermentation, the beans were sun-dried for six days until reaching a moisture content of 7.5%, measured using a Draminski Twist Bean Moisture Meter (SN: 29607).

## 2.4. Physical Analysis

By evaluating the physical characteristics of cacao, it is possible to determine whether post-harvest processing was carried out correctly. The physical analysis of the beans considered the following attributes: color, assessed visually; odor, evaluated directly through olfaction; and bean size and weight, determined by the average weight of 100 cacao beans. Bean size classification was conducted according to NTP-ISO 2451–201 standards, which define size based on bean count per 100 g as follows:

a) Standard-sized beans: ≤100 beans per 100 g

b) Medium-sized beans: 101–110 beans per 100 g

c) Small-sized beans: 111–120 beans per 100 g

d) Very small-sized beans: >120 beans per 100 g

Moisture content was determined using the NTP-ISO 2451–2018 methodology with a Draminski Twist Bean Moisture Meter (SN: 29607).

### 2.5. Chemical Analysis

The following parameters were considered: pH, measured using a potentiometer in a 1:5 suspension (sample: water), with prior calibration in a buffer solution, following the NTP 203-070 standard, with an optimal range between 5.0 and 5.5.

According to De Melo et al. (2013), oils and fats were quantified as a percentage using the Soxhlet extraction method (n-Hexane), with an optimal range between 48% and 60%.

Acidity, expressed as a percentage, was determined following the methodology of De Melo et al. (2013), with an optimal range of <1%.

The cadmium concentration was quantified using an Atomic Absorption Spectrophotometer, establishing a <0.8 µg/g threshold (European Union, 2014).

### 2.6. Sensory Analysis

#### a. Sample Preparation

Bean samples of each genotype were prepared after systematic roasting, dehusking, grinding, and coding. Relevant procedures were implemented to obtain cocoa liquor from each genotype and to facilitate sensory evaluation.

For each genotype, a 300 g sample was meticulously selected, ensuring that the moisture content was maintained within the range of 7.0 to 7.5%. The roasting procedure was carried out in an oven preheated to 120 °C for 20 minutes. The sample was then cooled to a tolerable temperature before dehusking manually. The cleaned beans were placed in sterile containers.

Each sample was then ground in a bean mill to obtain cacao liquor, ensuring the mill was cleaned of residual liquor from the previous sample before processing a new one. Once prepared, the liquor was poured into molds, cooled, and stored at 4°C until sensory evaluation. Before tasting, the liquor was reheated to maintain a temperature range of 45–50°C.

Each genotype was assigned to a unique code for identification. The samples were served in glass or transparent plastic containers with lids to prevent cross-contamination. Individual spoons were used for sampling. The sensory evaluation followed the Quality Laboratory of the ICT sensory evaluation form, which detailed the profile of each sample.

#### b. Sensory Evaluation Procedure

The sensory evaluation was conducted according to the methodology outlined in NTP 107.303 – 2018, which establishes the sensory analysis process used to assess the sensory characteristics of cacao liquor obtained from each genotype. This process involved descriptive qualitative analysis by consensus.

The panelists quantified their perception of basic flavors, aroma, acidity, bitterness, astringency, defects, flavor, aftertaste, cacao, sweetness, nutty notes, dried fruit, fresh fruit, floral notes, panelist score, and other attributes using a 0 to 10 scale (Cacao of Excellence, 2021).

#### c. Tasting Panel

A trained tasting panel evaluated the samples following the established protocol. Once the cacao liquors were served, the panel was convened for assessment.

### 2.7. Statistical Analysis

The data obtained were analyzed using descriptive statistics and analysis of variance (ANOVA), the media test was Scott & Knotte ($P<0.05$), utilizing the Infostat software (Di Rienzo et al., 2020).

## 3. RESULTS AND DISCUSSION

### 3.1. Physical quality of the bean

The results of the physical analysis of the beans of 20 cocoa genotypes collected in seven riparian basins reveal significant diversity in key attributes for the chocolate industry, and they are presented in Table 2.

**Table 2.** Average physical attributes ± Standard error of the grains of 20 wild genotypes

| River | Genotype | Color | Smell | Weight of 100 beans) | Bean length (mm) | Bean Width (mm) | Bean thickness (mm) | Bean Index (g) | Qualification | Bean size (N° beans/100g) | Qualification | Moisture (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aypena | AYP-1 | Light brown | Characteristic- | 153.4±0.71 | 23.9±0.38 | 12.5±0.17 | 9.6±0.20 | 1.53±0.05 | Middle | 65.3±3.06 b | Standard | 7.3±0.09 |
| Aypena | AYP-12 | Dark brown | Characteristic | 145.5±1.62 d | 23.6±0.03 | 13.2±0.18 | 8.9±0.10 | 1.46±0.05 | Middle | 68.8±5.51 b | Standard | 7.3±0.09 |
| Marañon | MAR-26 | Dark brown | Characteristic | 100.6±0.15 g | 23.6±0.79 | 12.6±0.17 | 9.7±0.15 | 1.01±0.01 | Small | 85.0±13.53 a | Standard | 6.6±0.20 |
| Nucuray | NUC-134 | Reddish | Characteristic | 117.5±0.65 f | 23.7±0.49 | 12.5±0.09 | 8.8±0.35 | 1.18±0.01 | Regular | 85.0±3.00 a | Standard | 7.3±0.29 |
| Pastaza | PAS-100 | Light brown | Characteristic- | 161.5±1.19 b | 25.1±1.53 | 12.7±0.12 | 9.7±0.12 | 1.62±0.01 | Middle | 62.3±5.77 b | Standard | 7.1±0.17 |
| Pastaza | PAS-105 | Light brown | Characteristic | 161.0±0.94 b | 24.2±0.30 | 12.5±0.23 | 9.6±0.06 | 1.61±0.01 | Middle | 62.7±2.08 b | Standard | 7.1±0.12 |
| Pastaza | PAS-83 | Dark brown | Characteristic | 146.4±1.51 d | 23.5±0.71 | 12.5±0.13 | 8.4±0.18 | 1.46±0.01 | Middle | 68.7±6.66 b | Standard | 7.3±0.15 |
| Pastaza | PAS-88 | Dark brown | Characteristic | 147.6±2.03 d | 23.0±1.41 | 12.2±0.23 | 8.4±0.09 | 1.48±0.01 | Middle | 68.3±4.93 b | Standard | 7.1±0.20 |
| Ungumayo | UGU-113 | Light brown | Characteristic | 152.5±0.83 c | 24.9±0.43 | 13.0±0.38 | 8.7±0.20 | 1.53±0.00 | Middle | 66.0±4.00 b | Standard | 7.1±0.12 |
| Ungumayo | UGU-117 | Dark brown | Characteristic | 151.9±1.22 c | 24.4±0.64 | 13.3±0.26 | 8.8±0.67 | 1.52±0.01 | Middle | 66.7±6.03 b | Standard | 7.3±0.15 |
| Ungumayo | UGU-127 | Dark brown | Characteristic | 153.3±2.89 c | 24.5±0.20 | 12.9±0.23 | 9.2±0.23 | 1.53±0.03 | Middle | 65.3±1.53 b | Standard | 7.2±0.03 |
| Ungurahui | UNG-53 | Dark brown | Characteristic | 162.3±1.24 b | 24.5±0.42 | 12.9±0.26 | 9.4±0.26 | 1.62±0.01 | Middle | 61.7±1.15 b | Standard | 7.2±0.15 |
| Ungurahui | UNG-60 | Dark brown | Characteristic | 161.2±0.84 b | 23.3±0.46 | 11.8±0.21 | 9.4±0.19 | 1.61±0.01 | Middle | 62.3±2.08 b | Standard | 7.0±0.06 |
| Ungurahui | UNG-61 | Dark brown | Characteristic | 166.1±1.62 a | 24.1±0.41 | 12.0±0.35 | 9.1±0.15 | 1.66±0.02 | Big | 60.3±0.58 b | Standard | 7.1±0.06 |
| Ungurahui | UNG-74 | Dark brown | Characteristic | 151.0±0.99 c | 22.9±0.84 | 12.7±0.12 | 8.2±0.12 | 1.51±0.01 | Middle | 66.7±0.58 b | Standard | 7.2±0.15 |
| Ungurahui | UNG-79 | Dark brown | Characteristic | 151.3±0.75 c | 22.7±1.44 | 12.3±0.20 | 8.4±0.13 | 1.51±0.01 | Middle | 66.3±0.58 b | Standard | 7.2±0.18 |
| Ungurahui | UNG-80 | Dark brown | Characteristic | 154.3±0.87 c | 23.2±0.32 | 12.5±0.31 | 8.7±0.32 | 1.54±0.01 | Middle | 65.0±3.46 b | Standard | 7.3±0.15 |
| Urituyacu | URI-161 | Reddish | Characteristic | 137.4±1.57 e | 23.8±0.35 | 13.2±0.15 | 8.7±0.20 | 1.37±0.01 | Small | 73.0±2.00 b | Standard | 7.1±0.20 |
| Urituyacu | URI-164 | Light brown | Characteristic | 139.0±0.78 e | 25.4±0.32 | 12.7±0.23 | 9.1±0.32 | 1.39±0.01 | Small | 72.0±2.00 b | Standard | 7.2±0.20 |
| Urituyacu | URI-184 | Dark brown | Characteristic | 135.0±0.32 e | 22.7±0.63 | 12.5±0.23 | 9.8±0.09 | 1.35±0.00 | Small | 74.3±1.53 b | Standard | 7.2±0.06 |
| *p valor* | | | | *<0.0001* | *0.332* | *0.0017* | *0.0001* | *<0.0001* | | *<0.0001* | | *0.3634* |
| *CV* | | | | *1.51* | *5.29* | *3.09* | *4.69* | *2.27* | | *6.72* | | *3.71* |

*Averages joined by the same lowercase letter in a column do not differ significantly depending on the Scott & Knott *(p<0.05)*

25% of the genotypes evaluated have light brown beans (AYP-1, PAS-100, PAS-105, UGU-113, and URI-164); 10% reddish brown (NUC-13 and URI-161), and the rest of the characteristic dark brown. The smell of the beans mostly had the characteristic smell of cocoa, while 10% of the beans emitted a characteristic acidic smell. The quantitative characteristics evaluated presented statistical significance ($p \leq 0.5$) except for bean width and moisture percentage.

The seed caliber fluctuated between 100.6±0.15 g (MAR-26) and 166.1±1.62 g (UNG-61). The length of the beans ranged from 22.7±1.44 mm (UNG-74 and URI-184) to 25.4±0.32 mm (URI-164). The bean width fluctuated between 11.8±0.21 mm (UNG-60) and 13.3±0.26 mm (UGU-117). The bean thickness is 8.2±0.12 mm (UNG-74) and 9.8±0.09 mm (URI-184).

The seed index (SI), a key indicator in the selection of genotypes for processing, presented values higher than 1 g in all cases. The lowest SI was recorded for MAR-26, 1.01 g, with a small rating, while the highest SI was presented by UNG-61, 1.66±0.02 g, with a large rating; the rest of the genotypes studied medium SI.

According to Afoakwa et al. (2008), a high SI is associated with higher yields in the transformation of cocoa into chocolate paste and liquor, suggesting that higher SI genotypes could be preferred for the industry.

Compared to commercial varieties, the CCN-51 clone has an SI of 1.4 g, the IMC-67 1.2 g, and the ICS-95 has an SI of 1.3 g (García, 2014). This indicates that some wild genotypes have a competitive SI, suggesting their industrial potential.

Regarding bean size, all genotypes presented a standard size because it was below 100 beans per 100 g; The size ranged from 60.3±0.58 beans per 100 g (UNG-61) to 85.0±13.53 beans per 100 g (MAR-26 and NUC-134).

Finally, the moisture content of the bean in all genotypes was below the maximum allowable of 7.5% according to NTP-CODEX/CAC/RCP 72:2018 (INACAL, 2021); this characteristic may vary according to the fermentation and drying processes. Fermented beans typically have a lower moisture content (5.8%) than unfermented beans (6.5%). The ideal moisture content for dried cocoa beans is between 7% and 10% on a wet weight basis. This range is crucial to prevent mold growth and maintain bean quality during storage (Raju et al., 2025).

### 3.2. Chemical characteristics of beans

The results of the chemical characteristics evaluated for the 20 cocoa genotypes can be seen in Table 3.mThe samples showed a pH of 4.7±0.23 (UGU-117) to 5.7±0.15 (PAS-100); 75% showed pH values between 5 and 5.5, considered optimal for cocoa fermentation and flavor. A pH of around 5.0 contributes to optimal conditions for microbial growth (Pokharel, 2023) and is responsible for the breakdown of polyphenols and proteins, thereby decreasing the bitterness and astringency of cocoa (Kim-Ngoc et al., 2022).

The average acidity in cacao bean liquor was under 1%, representing an optimum phase in the whole genotypes under consideration. Because acidity is one of the most important factors in determining the flavor of chocolate, this discovery signals an important quality trait for cocoa. The results vary between 0.5±0.03 (UNG-79 and UNG-80) and 0.9±0.03 (PAS-100), likely attributable to genetic diversity. Such natural variation can be harnessed for breeding programs focused on cacao with the best level of acidity (Adeigbe et al., 2021).

The fat content, another important quality factor of cocoa beans, ranged from 41.6±0.52% (UNG-74) to 56.9±0.55% (PAS-100).

Interestingly, 65% of the analyzed samples were grouped into the appropriate range to produce higher-grade chocolate (48%–60%) (Aprotosoaie et al., 2016).

Variations in fat composition can be attributed to genetic polymorphisms and biochemical pathways controlling lipid biosynthesis throughout cacao bean development (Li et al., 2019). for instance, of these two, CCN-51, with a fat content of 41.4%, is higher in fat compared to other commercial varieties, while BMI-67 has a lower fat content, at 32.1% (Vera Chang et al., 2015).

The fat content strongly influences the viscosity and melting profile of chocolate, an important characteristic in terms of both manufacturing and consumer preferences (Barbosa de Melo et al., 2020).

For cadmium, all the concentrations measured in the current study were ≤ 0.8 µg/g, which is less than the value defined by Regulation 488/2014 (European Union, 2014) (SAFETY LIMIT). These ranged from 0.07±0.01 µg/g (AYP-12 and URI-164) to 0.23±0.01 µg/g (NUC-134).

Different genotypes of cacao differ in their ability to take up, translocate, and accumulate cadmium. Some genotypes confine cadmium accumulation into roots, while others facilitate cadmium to translocate into aerial tissues and reproductive structures (Meléndez-Mori et al., 2023). For instance, INDES-38 is a leaf-restricted cadmium hyperaccumulator, which makes it a candidate for this purpose, as the cadmium is fixed in the roots at high levels. A similar pattern has been observed in EET-61 and PA-46, which were categorized into differentiated cadmium translocation groups, with EET-61 being more mobile (Galvis et al., 2023).

Using two hybrid populations (based on 100 cacao accessions), Lewis et al. (2018) demonstrated that genotypic background significantly affected cadmium concentrations between leaf and cacao beans.

**Table 3.** Chemical characteristics of the beans of 20 wild cocoa genotypes

| River | Genotype | pH (5:1) | Acidity (%) | Fat (%) | Cadmium (µg/g) |
|---|---|---|---|---|---|
| Aypena | AYP-1 | 5.5±0.20 a | 0.8±0.07 | 54.3±2.18 a | 0.05±0.01 c |
| Aypena | AYP-12 | 4.8±0.26 b | 0.7±0.07 | 50.0±3.37 a | 0.07±0.01 c |
| Marañón | MAR-26 | 5.2±0.09 a | 0.8±0.06 | 50.7±0.98 a | 0.16±0.03 b |
| Nucuray | NUC-134 | 5.0±0.12 a | 0.7±0.06 | 49.5±2.28 a | 0.23±0.01 a |
| Pastaza | PAS-100 | 5.7±0.15 a | 0.9±0.03 | 56.9±0.55 a | 0.08±0.01 c |
| Pastaza | PAS-105 | 5.1±0.06 a | 0.7±0.06 | 50.5±2.88 a | 0.14±0.01 b |
| Pastaza | PAS-83 | 4.8±0.06 b | 0.6±0.12 | 51.2±3.59 a | 0.15±0.02 b |
| Pastaza | PAS-88 | 5.2±0.03 a | 0.7±0.03 | 50.1±1.62 a | 0.16±0.01 b |
| Ungumayo | SS-113 | 5.0±0.15 a | 0.7±0.09 | 47.5±1.67 b | 0.21±0.02 a |
| Ungumayo | U-117 | 4.7±0.23 b | 0.7±0.15 | 47.0±4.83 b | 0.09±0.02 c |
| Ungumayo | SS-127 | 5.2±0.15 a | 0.5±0.13 | 51.1±3.00 a | 0.16±0.01 b |
| Ungurahui | UNG-53 | 5.1±0.35 a | 0.6±0.15 | 48.6±0.37 a | 0.18±0.02 b |
| Ungurahui | UNG-60 | 5.5±0.18 a | 0.6±0.07 | 45.4±2.23 b | 0.18±0.03 b |
| Ungurahui | UNG-61 | 5.3±0.23 a | 0.6±0.09 | 50.4±3.66 a | 0.21±0.01 a |
| Ungurahui | UNG-74 | 5.5±0.30 a | 0.6±0.12 | 41.6±0.52 b | 0.12±0.02 c |
| Ungurahui | UNG-79 | 4.9±0.06 a | 0.5±0.03 | 42.3±0.67 b | 0.15±0.01 b |
| Ungurahui | UNG-80 | 5.2±0.24 a | 0.5±0.03 | 43.6±0.79 b | 0.17±0.01 b |
| Urituyacu | URI-161 | 5.0±0.12 a | 0.7±0.07 | 50.7±3.82 a | 0.15±0.01 b |
| Urituyacu | URI-164 | 5.2±0.20 a | 0.7±0.07 | 43.9±1.11 b | 0.07±0.01 c |
| Urituyacu | URI-184 | 5.4±0.26 a | 0.6±0.09 | 44.8±3.55 b | 0.14±0.02 b |
| P value | 0.0328 | 0.1078 | 0.0093 | <0.0001 | |
| CV | 6.46 | 22.76 | 9.09 | 19.72 | |

*Averages joined by the same lowercase letter in column do not differ significantly according to

### 3.3. Sensory characteristics

The sensory analysis of the 20 wild cacao genotypes showcased a rich diversity of aromas and flavors, highlighting the genetic wealth of cacao in this region. Each genotype presented unique sensory attributes, reflecting the complexity and potential of these native varieties for high-quality chocolate production.

Figure 1 shows the sensory characteristics of the 20 wild cocoa genotypes evaluated in this study. From the flavor and aroma profiles, general trends and key differences between genotypes can be identified as reflect the genetic richness of cocoa in the region.

Aroma and Olfactory Complexity: Genotypes such as AYP-1, MACH-26, and PAS-100 present the most intense aromas (scores of 7 or 8), standing out for deep notes of cocoa and fresh fruits. In contrast, genotypes such as AYP-12, UGU-117, and URI-164 have more moderate aromatic intensities (scores of 5), with a less pronounced profile in terms of complexity.

Genetic and fermentation factors mainly determine the complexity of the aroma of cocoa beans; for example, the Nacional variety of Ecuador is known for its fine or aroma cocoa, and genetic studies have identified specific genes associated with floral and fruity aromas (Colonges et al., 2022), it is also essential to consider the overall market and consumer preferences.

The increase in demand for differentiated flavored cocoa is being propelled by consumer proclivity for distinctive, premium chocolate commodities (Fouet et al., 2022). This prevailing trend underscores the critical necessity of conserving genetic diversity and refining post-harvest processing techniques to sustain and amplify the aromatic characteristics of cocoa beans (Fouet et al., 2022).

Unique Flavors — Exploring varieties of cacao and their applications as fine-flavored chocolate could provide a springboard for commercializing many other unique beans. Indeed, by selecting specific genotypes, producers can reach a saturated market for low-end cocoa and allow consumers a sensorial experience between both worlds (low and high) in terms of flavor.

Acidity and Bitterness: The acidity was well-balanced, with a score between 5 and 6, making the taste pleasant without acidic sharpness. Genotypes such as AYP-1, MACH-26, and PAS-100 had higher bitterness (score of 6), while AYP-12 and UGU-117 were less bitter (score of 4) and milder on the palate. The acidity in cacao beans is primarily associated with organic acids produced during the fermentation process, which are essential for developing the final flavor profile (Pokharel, 2023).

Cacao bitterness is chiefly derived from alkaloids, including theobromine, caffeine, and polyphenolic compounds, notably flavan-3-ol and diketopiperazines. The levels of these compounds depend on cacao's origin and processing methods (Kauz et al., 2021; Stark et al., 2006).

Astringency and Flavor: The astringency of genotypes ranged from moderate (AYP-1 and PAS-108) to high intensity (MACH-26, PAS-100, scores 6–7). MACH-26 and PAS-100 exhibited the highest (8) scores in overall flavor complexity, suggesting they possessed more aromatic compounds and a more pronounced overall sensory profile. Theobromine concentration also significantly influences the flavor expression of cacao (Goya et al., 2022).

Genetic diversity directly impacts cacao flavor, making the flavor profiles of cacao unique to each region. For example, Peru's famous "Chuncho" variety presents many flavor notes, highlighting genetics' high impact on sensory attributes (Eskes et al., 2018). With this great genetic diversity comes the potential for fine-flavored chocolate, offering farmers and chocolate makers a chance to differentiate their products in a highly specialized sector.

Dominant Sensory Notes: In our evaluations, AYP-1, PAS-100, and PAS-88 have dominant fresh fruit notes, representing a matured fruit profile. AYP-1, PAS-100, and MACH-26 had floral notes, indicating more sensory complexity, and the most intense genotypes in walnuts and nuts were AYP-1, MACH-26, and PAS-83, displaying enhanced perception of unctuousness and mouth persistence.

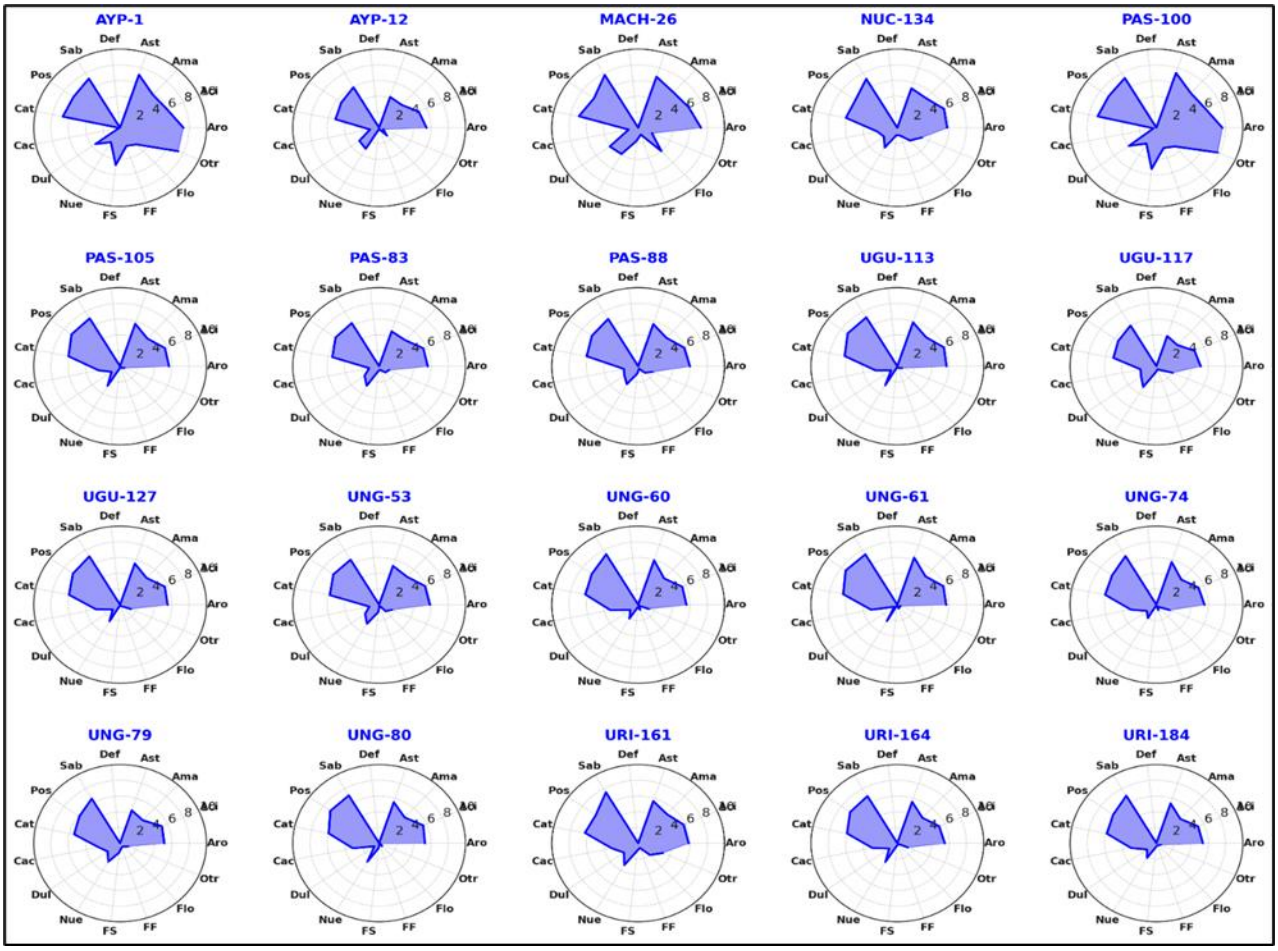


**Figure 1.** Flavors and aromas associated with each of the 20 cocoa genotypes evaluated in this study. Ast: astringency, Ama: bitterness, Aci: acidity, Aro: aroma, Otr: other, Flo: Floral, FF: Fresh fruits, FS: dry fruits, Nue: nut, Dul: sweet, Cac: cacao, Cat: taster, Pos: Aftertaste, Sab: taste, Def: defects.

The sensory attributes of cacao beans are mainly attributed to their genetic background. However, chocolate's final aroma and taste are also influenced by its fermentation, roasting, and environmental conditions (Colonges et al., 2022). The interaction of these factors can cause variations even within the same variety, highlighting the complexity and richness of cacao as a raw material for chocolate production. For example, Peruvian varieties such as "Señorita" and "Achoccha", both Chuncho cacao variants, exhibit fine cacao profiles with balanced bitterness, astringency, and fresh fruit notes (Ordoñez et al., 2020).

Persistence and Post-Taste: The most persistent genotypes were AYP-1, PAS-100, and MACH-26, scoring 7, and retained flavor longer [duration] than the others. Meanwhile, AYP-12 and UGU-117 were rated lower in post-taste (5), which reflects their lower flavor persistence.

Genetic features, chemical composition, and processing approaches significantly determine cacao bean's primary flavor and post-taste (Clapperton et al., 1994). Differences between cacao varieties produce different sensory profiles—bitterness, astringency, and acidity—that are essential to the overall tasting experience.

Sensory analysis indicated that PAS-100, MACH-26, and AYP-1 were the most moldable and well-balanced genotypes. They deliver a fine structure of acidity and bitterness, with fruity and floral notes. Their strong aromatic profile and depth of flavor make them especially promising as potential candidates for gourmet chocolate making.

Conversely, genotypes AYP-12, UGU-117, and URI-164 show a more neutral profile regarding reducing aroma and flavor intensity. These features make them better suited to blends or chocolates that are softer and more delicate in flavor.

By studying these sensory attributes, cacao producers and chocolatiers can better decide on the best possible uses for any given genotype, whether high-quality single-origin chocolates or balanced blends that appeal to wider audiences.

## CONCLUSIONS

This work demonstrates the extraordinary genetic variation in 20 wild cacao genotypes from the Instituto de Cultivos Tropicales (ICT) germplasm collection, which is relevant to the chocolate industry.

Some of the genotypes had greater seed size than the commercial genotypes, indicating a potential productivity advantage. The fat content analyzed from the chemical compositions (41.6% to 56.9%) corresponded with the ideal range required for the premium level in the chocolate industry.

The cadmium levels were also well below European safety limits, ensuring food safety standards without compromising the quality of cacao beans.

Genotypes like PAS-100, MACH-26, and AYP-1 displayed distinctive flavor profiles identified through sensory analysis with aromatic complexity and lingering taste, properties most sought after by producers of fine chocolate.

They underscore the significance of preserving and leveraging wild cacao genetic diversity to improve growing quality and sustainability, creating novel commercial prospects while underscoring the need for more research to enable their industry applications.

## FINANCING AND ACKNOWLEDGMENT

We thank the USDA-ARS-ICT for supporting the field trial activities under Project Agreement Number/FAIN 58-8042-2-042-F. We are also grateful to the "Programa Nacional de Investigación Científica y Estudios Avanzados" (National Program for Scientific Research and Advanced Studies) PROCIENCIA) for financial support (Contract No. 188-2020) that was possible to execute the Thesis of MSc. "Caracterización física, química y organoléptica de genotipos silvestres cacao (*Theobroma cacao* L.) del Banco de Germoplasma del Instituto de Cultivos Tropicales. We extend our gratitude to all technicians who participated in the collection and establishment of the germplasm bank at the Instituto de Cultivos Tropicales (ICT) in Tarapoto, as well as to ICT for providing the field and laboratory facilities necessary for this study.

## CONFLICT OF INTEREST

No existe ningún tipo de conflicto de interés relacionado con la materia del trabajo.

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