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Optimization of fermentation and roasting parameters of fine-flavor cocoa using a Taguchi experimental design to improve the physicochemical and sensory quality of chocolate

Optimización de los parámetros de fermentación y tostado del cacao fino de aroma mediante un diseño experimental Taguchi para mejorar la calidad fisicoquímica y sensorial del chocolate

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Abstract

The purpose of this study was to evaluate and optimize the physicochemical and sensory quality of chocolate produced from fine-flavor cocoa (*Theobroma cacao* L.) originating from different cocoa production zones in Chone, Manabí province, Ecuador, by applying different fermentation and roasting protocols. A Taguchi L9 (3^4) experimental design was employed using four factors at three levels: cocoa origin (low, medium, and high altitude), fermentation time (4, 5, and 6 days), turning intervals during fermentation (24 h/48 h, 48 h/24 h, 48 h/48 h), and roasting temperature (130, 135, and 140 °C). Physicochemical parameters (pH, titratable acidity, moisture, and fat content) were evaluated, and descriptive sensory analysis was performed using a trained panel. The results showed an average pH of 5.59, whereas the optimal combination reached a value of 6.02 under high-altitude cocoa and prolonged fermentation conditions. The highest fat content (42.50%) was obtained under moderate fermentation conditions, while the lowest acidity (0.11%) and moisture (1.20%) values were associated with specific fermentation and roasting combinations. Sensory evaluation indicated that treatments T1, T3, and T9 achieved the highest overall sensory quality. Taguchi contribution analysis showed that cocoa origin was the dominant factor affecting pH, fat content, and moisture variability, whereas fermentation turning intervals exerted the greatest influence on titratable acidity.

Keywords: Altitudinal zone, fermentation, roasting, physicochemical quality, sensory quality.

Resumen

El propósito de este estudio fue evaluar y optimizar la calidad fisicoquímica y sensorial de chocolate elaborado a partir de cacao fino de aroma (*Theobroma cacao L.*) proveniente de diferentes zonas de producción de cacao del cantón Chone, provincia de Manabí, Ecuador, mediante la aplicación de diferentes protocolos de fermentación y tostado. Se empleó un diseño experimental Taguchi L9 (3^4) utilizando cuatro factores a tres niveles: origen del cacao (baja, media y alta altitud), tiempo de fermentación (4, 5 y 6 días), intervalos de volteo durante la fermentación (24 h/48 h, 48 h/24 h y 48 h/48 h) y temperatura de tostado (130, 135 y 140 °C). Se evaluaron parámetros fisicoquímicos (pH, acidez titulable, humedad y contenido de grasa) y se realizó un análisis sensorial descriptivo mediante un panel entrenado. Los resultados mostraron un pH promedio de 5.59, mientras que la combinación óptima alcanzó un valor de 6.02 bajo condiciones de cacao de alta altitud y fermentación prolongada. El mayor contenido de grasa (42.50%) se obtuvo bajo condiciones moderadas de fermentación, mientras que los menores valores de acidez (0.11%) y humedad (1.20%) estuvieron asociados con combinaciones específicas de fermentación y tostado. La evaluación sensorial indicó que los tratamientos T1, T3 y T9 alcanzaron la mayor calidad sensorial global. El análisis de contribución Taguchi mostró que el origen del cacao fue el factor dominante que afectó la variabilidad del pH, contenido de grasa y humedad, mientras que los intervalos de volteo durante la fermentación ejercieron la mayor influencia sobre la acidez titulable.

Palabras clave: zona altitudinal, fermentación, tueste, calidad físico-química, calidad sensorial.

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1 Introduction

Cocoa (*Theobroma cacao L.*) is a crop with a high historical, economic and cultural significance, whose history of use has over 5,500 years and is one of the oldest agricultural products in international trade. Worldwide, cocoa is generally classified into conventional cocoa and fine-flavor cocoa. Although fine chocolate accounts for only 5% of global production, its complex sensory characteristics and distinct aromatic profiles make it a strategic raw material for producing high-quality chocolate (Vargas *et al*. 2021; Sornoza *et al*. 2022). Ecuador plays an important role in this segment, contributing about 70% of the world's fine-flavor cocoa production and about 5% of the world's cocoa production. The main production regions are coastal areas, especially in the provinces of Guayas, Los Ríos, Cañar, and Manabí. From a production perspective, around 35% of the cultivated area is fine-flavor cocoa, while 65% is CCN51 (Moreno et al., 2021). This combination of diverse varieties and specific environmental and altitudinal conditions contributes greatly to Ecuador's chocolate quality and differentiation (FAO, 2021; Sornoza *et al*., 2022).

Chone Canton, in the province of Manabí, shows marked agricultural and ecological heterogeneity, characterized by variations in altitude, soil type and climatic conditions. These factors directly affect the development of cocoa beans and the development of sensory-related characteristics, which are crucial for the final quality of chocolate. However, this potential depends not only on the agricultural and ecological origins of cocoa, but also on the conditions of processing and post-harvest processing, particularly fermentation and roasting. These phases are considered critical operations because they strongly determine the physical and sensory characteristics of the final product (Barberán and Moreira, 2023; Garay *et al*., 2024; Mancera-Castro *et al*., 2024). Despite the importance of cocoa production in the region, concerns remain regarding the progressive loss of genetic and productive diversity, the variability of post-harvest practices, and the lack of standardized fermentation and roasting protocols. Climate change and agricultural expansion threaten product quality and long-term sustainability (Campues, R. and Valencia N., 2022; Mancera-Castro *et al*., 2024). Consequently, integrated information must be generated by linking cocoa agroecological origin to processing conditions, using experimental approaches capable of identifying and optimizing the factors with the greatest impact on chocolate quality.

Therefore, this study aimed to evaluate and optimize the physicochemical and sensory quality of chocolate produced from cocoa grown in different cocoa production zones in Chone canton, Manabí, Ecuador, using various fermentation and roasting protocols. This work seeks to establish clear relationships between cocoa origin, fermentation and roasting conditions, and final chocolate quality, providing technical criteria to improve processing practices and contribute to the sustainability of the Ecuadorian cocoa sector (Yalçın & Ertürkmen, 2024).

2 Materials and methods

2.1 Study location

The study was carried out in two stages. Chocolate production was conducted at the facilities of KAACAO S.A., while physicochemical characterization was performed in the Bromatology laboratories. Both locations are situated at the Escuela Superior Politécnica Agropecuaria de Manabí Manuel Félix López (ESPAM MFL), El Limón campus, Calceta, Bolívar canton, Manabí province, Ecuador.

2.2 Experimental design

A Taguchi L9 (3^4) orthogonal design was applied, corresponding to a fractional factor arrangement in which four factors (A, B, C and D) were evaluated at three levels with nine experimental runs. This design was selected because it was effective in reducing the number of experimental tests and maintaining the ability to identify factors that have the greatest influence on the response variables. The combination of the factors generated nine treatments, each replicated three times, creating a total of 27 experimental units. For each experimental unit, 40 fine flavor cocoa pods of similar size were used. All treatments followed the same chocolate formulation consisting of 68% fine-flavoured cocoa, 3% cocoa butter, 0.5% soybean lecithin and 28.5% white sugar. The combinations of the factors evaluated in the study are shown in Table 1.

2.2.1 Altitudinal zones

Low zone (0–100 m a.s.l.): El Mate site, Santa Rita Parish, Chone Canton

Medium zone (101–200 m a.s.l.): Garrapata site, Ricaurte Parish, Chone Canton

High zone (201–300 m a.s.l.): Río Santo site, Ricaurte Parish, Chone Canton

2.3 Experimental production

The processing facilities of KAACAO S.A. received fine-flavor cocoa beans from three altitudinal production zones in the canton of Chone (Factor A: cocoa origin).

Table 1. Treatments evaluated using the Taguchi L9 orthogonal array.

Treatments	Factor A (Altitudinal zone)	Factor B (Fermentation time)	Factor C (Turning intervals during fermentation)	Factor D (Roasting temperature)
T1 (A1B1C1D1)	Low zone	4 days	1st 24 h + 2nd 48 h	Low (130 °C)
T2 (A1B2C2D3)	Low zone	5 days	1st 48 h + 2nd 24 h	High (140 °C)
T3 (A1B3C3D2)	Low zone	6 days	1st 48 h + 2nd 48 h	Intermediate (135 °C)
T4 (A2B1C2D3)	Medium zone	4 days	1st 48 h + 2nd 24 h	High (140 °C)
T5 (A2B2C3D1)	Medium zone	5 days	1st 48 h + 2nd 48 h	Low (130 °C)
T6 (A2B3C1D2)	Medium zone	6 days	1st 24 h + 2nd 48 h	Intermediate (135 °C)
T7 (A3B1C3D2)	High zone	4 days	1st 48 h + 2nd 48 h	Intermediate (135 °C)
T8 (A3B2C1D3)	High zone	5 days	1st 24 h + 2nd 48 h	High (140 °C)
T9 (A3B3C2D1)	High zone	6 days	1st 48 h + 2nd 24 h	Low (130 °C)

Note: All roasting temperatures (Factor D) were applied for 45 minutes.

The plant material, obtained from local cocoa varieties with recognized productive value and a long cultivation history, was carefully selected to ensure the absence of physical damage from harvesting or pest damage. Post-harvest processing, including fermentation (Factor B) and fermentation turning intervals (Factor C), was carried out using an experimental research-scale microfermentation system with cedarwood boxes (10 × 20 × 10 cm). Each treatment was processed independently using approximately 2.8 kg of fresh cocoa beans in pulp, corresponding to approximately 1 kg of dry-fermented cocoa beans after post-harvest processing. Drying was performed under standardized conditions for 8 days using the Rohan method until a final moisture content of 7% was achieved. All procedures were conducted according to the experimental design described in Table 1 and in compliance with NTE INEN 176:2021 requirements to ensure appropriate cocoa bean quality and classification.

2.3.1 Roasting

Roasting was carried out in an electric oven (Teka, model HLB840) according to the temperature levels established for Factor D (Table 1). Each treatment was roasted for 45 min under controlled thermal conditions. This stage was performed to evaluate the influence of roasting temperature on the physicochemical and sensory attributes of the cocoa and the resulting chocolate.

2.3.2 Crushing and winnowing

Cocoa beans were first processed in a low-speed Oster blender for 30 s. Subsequently, a COCOATOWN huller was used to separate cocoa shells. In order to further reduce the particle size to approximately 0.16 mm, a second grinding step was performed in the high-speed blender for 3 min until the desired particle size was achieved. Particle size reduction was monitored based on the operational characteristics of the equipment and visual homogenization of the cocoa particles; however, no standardized sieve system was used during this stage.

2.3.3 Grinding

The resulting cocoa mass was transferred to an electric grinder (COCOATOWN, model ECCGC–12SL-11), where it was processed for 2 h until a homogeneous consistency was obtained, with an average particle size of approximately 0.04 mm.

2.3.4 Conching

The conching was done with a Spectra Melanger and consisted of refining and homogenizing the cocoa mass over a long period of 12 h at an approximate operating temperature of 50–55 °C, achieving a final fineness of about 30 μm. During this process, the equipment's granite stones performed a continuous mechanical action to reduce the size of cocoa, sugar and other ingredients while promoting the release of undesirable volatile compounds and the development of more homogeneous and balanced flavor characteristics. This step was essential to obtain a creamy texture and superior sensory quality in the final chocolate.

2.3.5 Tempering

During this phase, texture, gloss, consistency, and snap (the characteristic sound produced when high-quality chocolate is broken) were promoted. After conching, the chocolate mass was allowed to cool gradually under ambient laboratory conditions until reaching approximately 45 °C. The melted chocolate was then transferred into a glass container (11.5 cm high, 24.0 cm diameter), and the temperature was maintained at 45 °C using intermittent microwave heating when necessary. Subsequently, the chocolate mass was divided into four equal portions and spread over a marble surface. Using stainless steel spatulas, chocolate crystallization was induced through continuous manual agitation and controlled cooling for approximately 30 min until a final temperature of 31 °C was achieved, as measured using a Milwaukee digital thermometer.

2.3.6 *Molding and packaging*

The tempering chocolate mass was poured into polypropylene molds with predetermined weights to obtain chocolate bars. The air bubbles formed during the molding were gently removed by pressing the moulds, and then the samples were refrigerated at temperatures between 10 °C and 15 °C until the product reached 20 °C.

2.3.7 *Packaging and storage*

The chocolate bars were wrapped in BOPP (biaxially oriented polypropylene) packaging suitable for chocolate and stored at 21 °C in ziplock bags at the laboratories of KAACAO S.A. until physicochemical and sensory characterization.

3 Quality control and analytical validation procedures

3.1 *Physicochemical analyses*

Cocoa beans subjected to the different experimental treatments were characterized through physicochemical analyses (Table 2) to determine their quality and compositional profiles according to NTE INEN 176:2021 requirements. Moisture determinations were performed using a Conterm LED drying oven (Conterm, Ecuador), whereas pH measurements were conducted using a Milwaukee digital pH meter (Milwaukee Instruments, USA). Fat content was determined using a Soxhlet extraction system with petroleum ether as extraction solvent. Titratable acidity was quantified by neutralization titration using analytical-grade sodium hydroxide (NaOH) and phenolphthalein indicator (Merck, Germany). All reagents employed during the analyses were of analytical grade. These analyses were used as baseline reference parameters to establish traceability between the raw material and the final chocolate products and to verify compliance with quality standards. The physicochemical results obtained after the experimental phase are presented in Table 3.

3.2 *Sensory analysis*

Sensory evaluation was conducted to characterize the sensory profiles of the nine chocolate formulations using a quantitative descriptive analysis (QDA) based on the official Cocoa of Excellence sensory evaluation form. The analysis was conducted at the Integral Quality Laboratory of the KAACAO company in collaboration with the specialized sensory panel of ESPAM MFL. Seven trained and certified cocoa tasters with previous experience in fine-flavor cocoa evaluation participated in the assessment. Although larger sensory panels are commonly recommended for descriptive sensory studies, the present evaluation was conducted using the trained assessors available within the specialized sensory panel at the time of the study.

Table 2. Physical analyses performed on cocoa beans.

Analysis	Description	Method
Moisture (%)	A sample of fermented and dried cocoa beans was weighed and dried in a Conterm LED oven at 130 ± 5 °C for 1 hour. Moisture content was determined gravimetrically.	(Bioversity International, 2020)
Testa (%) and Cotyledon (%)	Both analyses were performed gravimetrically using a sample of 30 fermented and dried cocoa beans. For testa, the percentage of shell covering the cocoa bean was estimated by calculating the ratio between the weight of the testa and the total weight of the 30 beans, expressed as a percentage. The same approach was applied to quantify cotyledon percentage.	(Lucero, 2021)
Fermentation (%)	This parameter was determined using the cut test, which consists of performing a longitudinal cut on 100 randomly selected cocoa beans. The beans were placed on a white surface and classified according to the criteria established in NTE INEN 176:2021 for cocoa beans.	(López and Morales, 2022)
Seed index	Three hundred fermented and dried cocoa beans were weighed for each treatment using a high-precision analytical balance (Milwaukee). The total weight was divided by 300 to obtain the average weight per bean, referred to as the seed index.	(Lucero, 2021; López and Morales, 2022)

Table 3. Physicochemical analyses of the treatments.

Analysis	Description	Method
pH	pH was measured using a Milwaukee pH meter equipped with a hydrogen ion–sensitive electrode.	AOAC 970.21
Fat (%)	Ether extract of chocolate, mainly composed of lipids, fatty acids, and unsaponifiable matter, was determined using a Soxhlet extraction system. A solid–liquid extraction method was applied, using petroleum ether as solvent.	AOAC 963.15
Titratable acidity (%)	Organic acids present in the chocolate were neutralized by titration using a standardized NaOH solution with phenolphthalein as indicator.	AOAC 970.21
Moisture (%)	A chocolate sample was weighed and dried in a Conterm LED oven at 130 ± 5 °C for 1 hour. Moisture content was determined gravimetrically.	(Bioversity International, 2020)

Encoded chocolate samples were evaluated under blind testing conditions using a sensory intensity scale from 0 to 10, where 0 represented a non-perceptible attribute and 10 an extremely intense perception. The evaluated sensory parameters included basic taste attributes (cocoa flavor, acidity, bitterness, and astringency), positive aromatic descriptors (fresh fruit, dried fruit, floral notes, woody notes, spices/nuts, and caramelized sugar), defect attributes associated with undesirable sensory perceptions such as moldy, smoky, earthy, over-fermented, or excessively acidic notes (off-flavors), and roasting intensity. Sensory evaluations were performed under controlled lighting and temperature conditions (22–24 °C), and standardized instructions were provided before each evaluation session to ensure consistency among panelists.

3.3 *Statistical analysis*

Data organization and preprocessing were performed using Microsoft Excel 2019. Statistical analyses were conducted in RStudio (version 4.5.1) within the R statistical environment. The Taguchi L9 (3^4) orthogonal array was generated and experimental runs were randomized using the DoE.base package. Signal-to-noise (S/N) ratios were calculated according to the Taguchi methodology to evaluate the influence of the experimental factors on the response variables and to identify treatment combinations associated with reduced variability and improved process robustness. Depending on the response variable, "larger-the-better" or "smaller-the-better" S/N criteria were applied. In addition, main effects plots and a Taguchi-based analysis of variance contribution analysis were performed to estimate the relative influence of the evaluated factors on the physicochemical and sensory responses. Because the L9 (3^4) orthogonal array corresponds to a fractional factorial design, interaction effects were not independently modeled. This statistical approach has been widely applied in food process optimization studies because it reduces the number of experimental trials while facilitating the identification of robust processing conditions in complex agroindustrial systems (Koyuncu *et al*., 2024; Patel *et al*., 2021).

4 Results

4.1 *Raw material characterization*

Table 4 presents the results of the physical analyses of the evaluated treatments. All experimental units were initially standardized using 40 cocoa pods per treatment according to the experimental design. However, natural variability in bean number and bean size among cocoa origins contributed to differences in total bean weight and seed index values. The highest total bean weights were observed in treatments using cocoa originating from low-altitude zones, whereas the highest seed index (g·bean^{-1}) values, defined as the average dry weight per bean calculated from 300 fermented and dried cocoa beans, were observed in treatments using cocoa from mid-altitude zones (101–200 m a.s.l.), specifically from the Garrapata site, Ricaurte Parish, Chone Canton. These results suggest that cocoa from mid-altitude zones presented larger average dry bean mass and greater cotyledon development, characteristics potentially associated with improved nib yield and processing performance.

The seed index values ranged from 1.12 g to 1.36 g among treatments, indicating variability in the average mass of dry cocoa beans. Treatments T5 and T6 registered the highest seed index values (1.36 and 1.31 g, respectively), reflecting larger average bean mass; however, these treatments exhibited lower total bean weights, likely associated with differences in bean number per pod rather than post-harvest processing conditions alone. In contrast, T1 and T2 achieved the highest total bean weights despite presenting intermediate seed index values. Bean moisture content ranged from 7.5% to 7.9%, remaining within the maximum limit established by NTE INEN 176:2021 (8%), indicating adequate drying conditions. This relatively uniform moisture behavior suggests that drying conditions remained stable among treatments. Higher shell percentages were observed in beans from mid-altitude zones, a characteristic that may negatively affect nib yield and chocolate processing efficiency.

Cut-test evaluation showed that T1 (83%), T2 (76%), and T8 (73%) presented the highest proportions of well-fermented beans. In contrast, violet beans, indicative of incomplete fermentation, were more frequent in T4 (31%) and T6 (27%). Moldy beans were more common in T8 (20%) and T9 (18%), whereas only T3 presented a considerable proportion of slaty beans (15%) (Table 5).

4.2 *Physicochemical analysis of chocolate*

The Taguchi analysis showed that cocoa origin (Factor A) and fermentation time (Factor B) exerted important effects on the physicochemical properties of chocolate (Figures 1 and 2). Main effects plots (Figure 1) revealed clear response variations among factor levels, while the Taguchi-based contribution analysis (Figure 2) quantified the relative influence of each factor on the evaluated variables. For pH, Factor A contributed the most (61.94%), followed by Factor B (20.23%), indicating that cocoa origin and fermentation time were the main sources of variability for this response. The average pH was 5.59 (SD = 0.02), whereas the predicted optimal combination A3B3C1D1 reached 6.02. This increase may influence physicochemical stability and sensory perception in fine-flavor chocolate formulations.

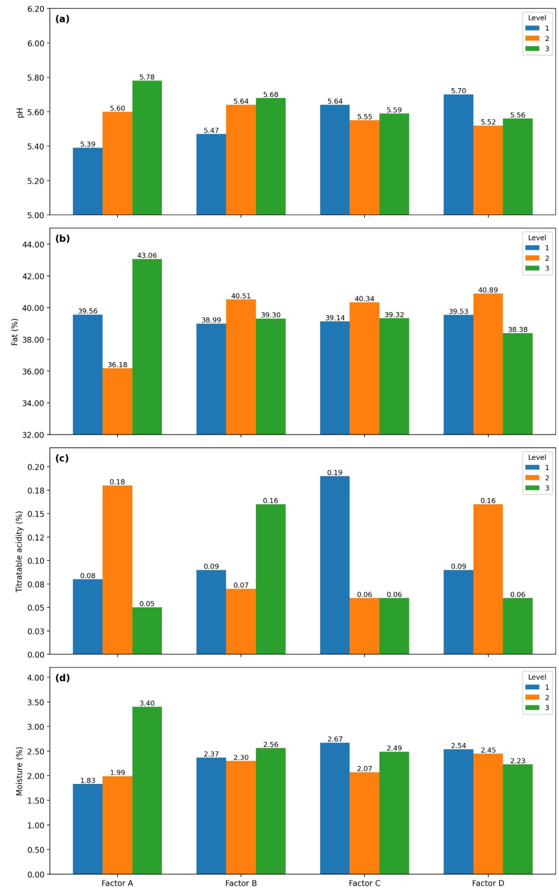

Figure 1. Main effects of the evaluated factors on the physicochemical properties of chocolate obtained from fine-flavor cocoa using a Taguchi L9 (3^4) experimental design. Subfigures show the influence of factors A (altitudinal origin), B (fermentation time), C (turning intervals during fermentation), and D (roasting temperature) on: (a) pH, (b) fat content (%), (c) titratable acidity (%), and (d) moisture content (%). Levels 1, 2, and 3 correspond to the three experimental levels defined for each factor. Values represent mean responses for each factor level.

Table 4. Physical analyses of cocoa beans according to treatments.

Treatments	Total bean weight (g)	Seed index (g·bean^{-1})	Bean moisture (%)	Testa (%)	Cotyledon (%)
T1 (A1B1C1D1)	1590	1.27	7.9	12.05	87.50
T2 (A1B2C2D3)	1480	1.27	7.9	10.11	89.89
T3 (A1B3C3D2)	1480	1.20	7.8	13.08	86.92
T4 (A2B1C2D3)	1120	1.28	7.9	16.51	83.17
T5 (A2B2C3D1)	1050	1.36	7.8	13.20	86.80
T6 (A2B3C1D2)	1050	1.31	7.7	16.83	83.49
T7 (A3B1C3D2)	1037	1.12	7.5	14.28	85.72
T8 (A3B2C1D3)	1048	1.13	7.6	15.63	84.37
T9 (A3B3C2D1)	1060	1.15	7.7	9.38	90.62

Note: Analyses were performed on cocoa beans that underwent standard post-harvest processing.

Table 5. Fermentation criteria of the treatments according to NTE INEN 176:2021.

Bean classification	Criterion	T1 (%)	T2 (%)	T3 (%)	T4 (%)	T5 (%)	T6 (%)	T7 (%)	T8 (%)	T9 (%)
Well-fermented beans	Brown color, brittle shell, chocolate aroma	83	76	71	66	72	70	72	73	72
Violet beans	Purple color, incomplete fermentation	16	22	11	31	21	27	12	7	10
Slaty beans	Grayish color, insufficient fermentation	1	1	15	0	0	0	0	0	0
Moldy beans	Presence of fungi	0	1	3	3	7	3	16	20	18
Total	—	100	100	100	100	100	100	100	100	100

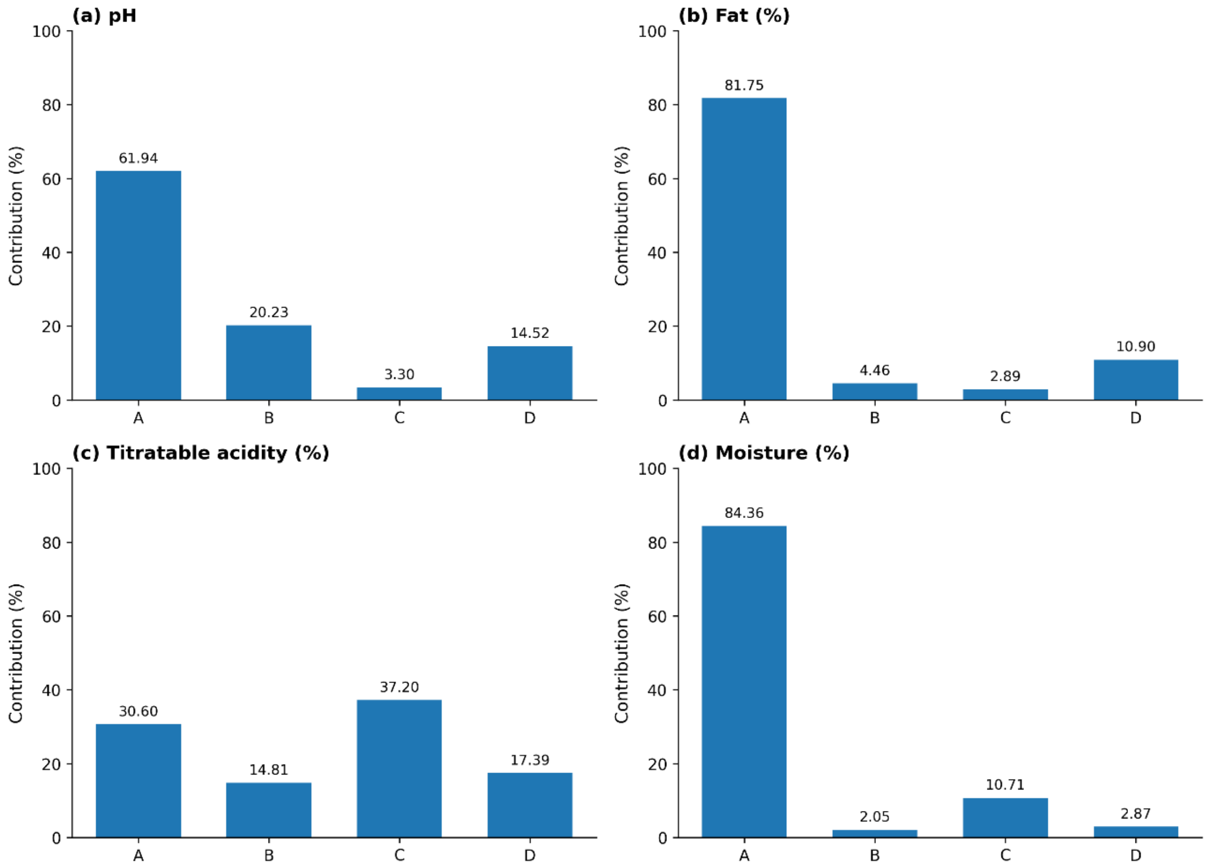

Figure 2. Relative contribution of Taguchi factors to the physicochemical responses of chocolate formulations determined through Taguchi-based contribution analysis: (a) pH, (b) fat content, (c) titratable acidity, and (d) moisture content.

Fat content followed a "larger-the-better" response trend and reached its maximum under the A3B2C2D2 combination. Contribution analysis showed that Factor A accounted for 81.75% of the observed variability, suggesting a strong influence of cocoa origin on differences in fat content among treatments. In contrast, titratable acidity was mainly affected by Factor C (37.20%), indicating that fermentation turning intervals contributed substantially to acidity variability. Lower acidity values were observed under treatment combinations involving cocoa from medium-altitude areas and extended fermentation periods, suggesting that both cocoa origin and post-harvest conditions influenced the final acidity response. Moisture content was also predominantly influenced by Factor A (84.36%), with lower values associated with cocoa from low-altitude zones, suggesting differences in moisture retention among cocoa origins.

Overall, the low standard deviation values observed among treatments (0.00–0.04) suggest that the Taguchi L9 orthogonal design provided acceptable control of response variability while allowing the identification of robust factor-level combinations under different raw material conditions.

4.3 Sensory analysis of chocolate

Integrative sensory analysis showed that treatments T1, T3, and T9 achieved the highest overall sensory quality (Figure 3), sharing processing conditions associated with balanced aromatic expression.

Table 6. One-way ANOVA results for sensory descriptors evaluated in chocolate samples.

Sensory descriptor	Df treatment	Df residual	F value	p value	Significance
Cocoa flavor	8	18	2.250	0.073	ns
Acidity	8	18	2.371	0.061	ns
Bitterness	8	18	2.344	0.064	ns
Astringency	8	18	4.249	0.005	**
Fresh fruit notes	8	18	1.229	0.338	ns
Brown fruit notes	8	18	1.648	0.180	ns
Vegetal notes	8	18	1.363	0.277	ns
Floral notes	8	18	0.781	0.625	ns
Woody notes	8	18	1.175	0.366	ns
Spicy notes	8	18	1.672	0.174	ns
Nutty notes	8	18	0.976	0.485	ns

Note: ns = non-significant ($p > 0.05$); ** = significant at $p < 0.01$. One-way ANOVA was performed considering treatment as the fixed factor using the mean sensory scores obtained from the trained panel for each experimental replicate.

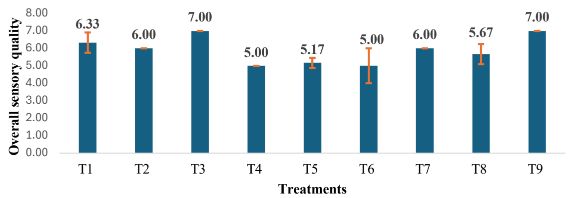

Figure 3. Global sensory quality scores assigned by trained panelists to chocolate samples obtained from the nine experimental treatments (T1–T9). Sensory evaluation was conducted using a quantitative descriptive analysis (QDA) approach based on the Cocoa of Excellence protocol. Global sensory quality scores correspond to the independent overall quality assessment assigned by trained panelists considering the integrated perception of flavor quality, aromatic balance, and absence of defects. Values represent mean scores, and error bars indicate standard deviation.

These treatments combined cocoa from high- or low-altitude regions, longer fermentation times (6 days), turning protocols involving extended anaerobic phases, and moderate roasting temperatures (130–135 °C). Sensory evaluation revealed relatively stable cocoa flavor, acidity, bitterness, and fresh fruit perceptions among treatments, whereas astringency showed significant differences according to ANOVA analysis ($p < 0.05$) (Table 6) Treatments T3 and T9 presented the highest astringency values, while T7 exhibited the lowest intensity for this descriptor. In contrast, treatments with lower overall sensory quality (T5 and T6) were associated with reduced fruit expression and less favorable aromatic balance. Roasting temperature mainly modulated caramelized and roasted sensory perceptions; however, higher temperatures (140 °C) alone did not guarantee improved sensory quality when fermentation-related defects were present. Defect attributes such as excessive acidity, smoky notes, moldy characteristics, burnt flavors, earthy notes, rancidity, excessive bitterness, excessive astringency, green or underfermented flavors, and other atypical off-flavors associated with inadequate fermentation, drying, roasting, or storage conditions were not detected with significant intensity among the evaluated chocolate formulations. Overall, the results suggest that superior sensory profiles were associated with the combined effects of cocoa origin, extended fermentation, appropriate turning strategies, and moderate roasting conditions that preserved delicate aromatic characteristics.

5 Discussions

5.1 Raw material characterization

Physicochemical analysis of cocoa beans. The seed index, an indicator of the average size and weight of beans, was primarily determined by genetic origin (Factor A). Although previous studies have reported that cocoa from high altitude zones tend to develop larger beans due to altitude adaptations such as lower temperatures and atmospheric pressures, the current study has shown that genotypes from low altitude zones had higher total bean weights, while those from medium altitude regions had the highest seed index values. This behavior is consistent with the conclusions of Vera-Chang *et al.* (2024), who reported that adapted genotypes to warm microclimates tend to accumulate higher dry matter and fat content, directly affecting cocoa's physicochemical quality. Fermentation time (factor B) and rotation frequency (factor C) showed a secondary effect on the seed index, but influenced the internal structure of the beans and shell adhesion. The rotation promoted mucilage degradation and facilitated cleaner shell separation, while extended fermentation increased the proportion of well-fermented beans and reduced defects such as violet and moldy beans (Quevedo *et al*., 2018). Natural drying allowed the moisture content to be within the optimal range (6–8%), as NTE INEN 176:2021. Standardized drying conditions have minimized the influence of genetic origin and fermentation on final moisture content, as reported by Barrientos *et al*. (2019) and Vera-Chang *et al*. (2024). However, higher shell percentages have been observed in cocoa from high-altitude areas, which can be attributed to genetic differences and slower drying rates that inhibit complete shell separation (Barrientos *et al*., 2019). Overall, these results confirm that genetic origin is the dominant factor influencing the characteristics of physical beans, while fermentation management and drying control are essential to ensure hygienic and technological quality.

5.1.1 Physicochemical analysis of chocolate

The observed pH behavior agrees with previous studies, which report that cocoa origin and fermentation duration strongly influence acidity-related characteristics in fine-flavor chocolate. Cartas *et al*. (2024) reported lower acidity values in cocoa associated with environmental and post-harvest conditions that affect acid retention during fermentation, whereas Moussoyi *et al*. (2024) suggested that altitude and local environmental conditions may contribute to differences in acidity behavior during cocoa processing. In the present study, the higher pH values observed under prolonged fermentation conditions (6 days) are consistent with González *et al*. (2024) and Calvo *et al*. (2021), who reported lower acidity responses during extended fermentation periods. Likewise, moderate roasting temperatures (130–135 °C) have been associated with reduced acidity perception and improved sensory acceptability in chocolate (Peña *et al*., 2022; Awaliyah *et al*., 2023). The pH values obtained in the best-performing treatments remained within the range commonly reported for well-processed fine-flavor chocolates.

The acidity behavior observed in this study also aligns with previous reports emphasizing the importance of fermentation management in shaping acidity-related responses. Santander *et al*. (2025) and Quintana-Fuentes *et al*. (2025) described that fermentation conditions and turning strategies can substantially modify acidity levels in cocoa beans and derived chocolate products. Similarly, Calvo *et al*. (2021) reported that turning frequency influences the evolution of acidity during cocoa fermentation. In the present study, lower acidity values were associated with extended fermentation combined with specific turning intervals, whereas intermediate turning conditions showed comparatively higher acidity responses. Roasting temperature showed a comparatively lower influence on acidity variability, although slightly lower acidity values were observed at higher roasting temperatures, consistent with observations reported by Moussoyi *et al*. (2024) and Awaliyah *et al*. (2023). The inverse relationship between pH and titratable acidity is also consistent with previous studies reporting acidity reduction under controlled fermentation and roasting conditions (Santander *et al*., 2025).

Moisture values obtained in the evaluated treatments were consistent with previous reports describing the influence of cocoa origin and thermal processing on residual moisture levels in chocolate. Ackah and Dompey (2021) and Erazo *et al*. (2023) reported that environmental conditions in cocoa production zones can influence moisture behavior during roasting and drying. In the present study, lower moisture values were associated with low-altitude cocoa subjected to higher roasting temperatures (140 °C), in agreement with Awaliyah *et al*. (2023), who observed progressive moisture reduction at increasing roasting temperatures. Despite the observed variability among treatments, all chocolate formulations remained within moisture ranges commonly considered acceptable for chocolate preservation (Santander *et al*., 2025).

Fat content variability was mainly associated with cocoa origin, consistent with previous reports linking lipid content differences to environmental and genetic factors. Silva *et al*. (2024) and Oliva *et al*. (2021) reported that cocoa produced under different environmental conditions may exhibit variability in fat accumulation and cocoa butter content. In the present study, chocolates produced from high-altitude cocoa showed the highest fat values, whereas fermentation time exerted a secondary influence. Mougang *et al*. (2024) similarly described the relative stability of fat content under prolonged fermentation conditions. Roasting temperature had a limited influence on total fat variability, consistent with reports that roasting temperatures below 150 °C generally have minimal effects on total cocoa fat content (Peña *et al*., 2022; Awaliyah *et al*., 2023). Overall, the results support the importance of cocoa origin as one of the main factors affecting fat variability under the evaluated processing conditions.

5.1.2 Sensory analysis of chocolate

The observed sensory differences confirm that the altitudinal origin of cocoa influences the sensory-related characteristics (Engeseth, 2018). Extensive fermentation and controlled rotation promoted the development of floral and fruit notes, while irregular processing increased the occurrence of sensory defects (Marseglia *et al*., 2020; Quintana-Fuentes *et al*., 2025). Longer intervals between turning influenced sensory responses associated with fermentation conditions (Hirko *et al*., 2023). In terms of roasting, moderate temperatures (130–135 °C) maintained fruit notes and modified roasting-related sensory characteristics, while higher roasting temperatures intensified roasting flavors without correcting the defects generated during fermentation (Torres-Moreno *et al*., 2012; Peña *et al*., 2022). The presence of atypical flavors in some treatments is consistent with Quelal *et al*., (2023), which associated these defects with errors during fermentation, drying or storage. Overall, the results suggest that sensory quality is influenced by the interaction between cocoa origin, fermentation management and roasting temperature (Anita-Sari *et al*., 2023).

Although the present study allowed the identification of robust fermentation and roasting conditions associated with improved physicochemical and sensory responses, some limitations should be acknowledged. The study did not include microbial characterization, fermentation temperature kinetics, quantification of aroma precursors, ecological diversity indicators, or physicochemical characterization of cocoa mucilage, including pH, reducing sugars, and titratable acidity. These parameters may influence microbial activity, fermentation dynamics, and variability associated with cocoa origin. Therefore, interpretations were restricted to the physicochemical and sensory parameters experimentally evaluated under the conditions of this study. Future studies should integrate mucilage characterization together with fermentation kinetics and microbial profiling to improve the understanding of cocoa quality development.

Conclusions

The physicochemical quality of chocolate was strongly influenced by the interaction between cocoa origin and post-harvest processing conditions. Taguchi analysis showed that cocoa origin was one of the main factors affecting pH, fat content, and moisture variability, whereas fermentation turning intervals exerted a greater influence on titratable acidity. High-altitude cocoa combined with prolonged fermentation and moderate roasting conditions was associated with more favorable pH values (6.02) and higher fat content (42.50%), characteristics commonly associated with balanced physicochemical properties and acceptable sensory quality in fine-flavor chocolate.

Fermentation and roasting conditions also influenced acidity and moisture responses. The combinations A2B3C1D2 and A1B2C2D3 were associated with lower acidity (0.11%) and moisture values (1.20%), respectively, suggesting that controlled processing conditions contributed to reduced response variability and improved product preservation conditions. Overall, the Taguchi L9 design allowed the identification of factor-level combinations associated with improved physicochemical quality under the evaluated experimental conditions.

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