



# Modulating fine flavor cocoa attributes: Impact of seed-to-bean transformation under controlled conditions on metabolite, volatile and sensory profiles

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## ABSTRACT

Fine-flavored chocolates are distinguished by their complex and distinct flavor profiles, which includes notes such as floral, fruity, nutty, and spicy. This study sought to modulate the flavor development of chocolates by establishing controlled processing conditions during the transformation from seed to bean in a laboratory setting, to produce superior quality chocolates. Our experimental setup comprised two varying temperature levels (30 °C and 45 °C) and three organic acids (OAs: acetic, lactic, and citric acids) at concentrations of 1–30 g/L to adjust the pH of the transformation system. Our study focused on how these conditions affect the development of distinct flavor profiles in chocolate bars, emphasizing the enhancement of fine-flavor notes. Flavor development was monitored through the untargeted metabolomics of cocoa beans and analyzing the volatile compounds and sensory profiles of the resultant chocolates. This study revealed that OA concentration markedly influenced metabolite formation, particularly affecting peptides, volatile organic compounds, and flavor notes. Chocolates derived from seeds processed with 30 g/L acid solutions demonstrated enhanced fruitiness and acidity, whereas those processed with 1 g/L acid solutions exhibited pronounced nuttiness and cocoa taste attributes but lower acidity. These findings underscore the significance of meticulously managing flavor development processes to produce fine-flavored chocolates with unique aromatic profiles. Crucially, variables in the controlling process, such as temperature and pH, are essential for fine-tuning flavor attributes, enabling the correlation and identification of key quality biomarkers to elucidate flavor development pathways.

## 1. Introduction

An amazing flavor from cocoa beans is what the chocolate industry is looking for and it is the cornerstone of research in the fields of post-harvest processes and cocoa quality. However, achieving this goal goes beyond creating innovative products with outstanding flavor attributes. In addition, it involves understanding the intricate process of converting

cocoa seeds to beans, which is crucial for developing flavor precursors that are vital in establishing the fine-flavor profiles that distinguish high-quality chocolate. Factors such as humidity, sunlight exposure, soil characteristics, time and temperature, among others, have been argued to play pivotal roles in the transformation (Kongor et al., 2016). However, elucidating the chemical and physical transformations that occur during this conversion process is central to this understanding as they

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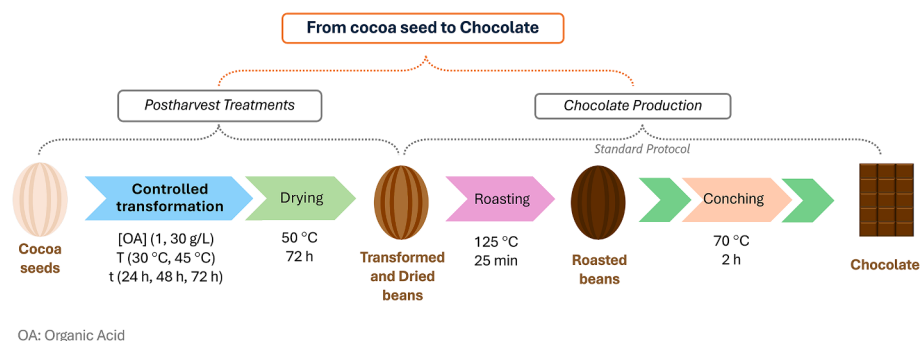


Fig. 1. Schematic illustration of the cocoa seeds processing. [OA] Organic acid concentration, T is temperature, and t is time.

directly influence flavor precursor development.

Recent advancements in processing cocoa seeds have led to the development of several methods aimed at enhancing bean quality using diverse analytical techniques to elucidate the dynamics of flavor formation during the processing stages (Herrera-Rocha et al., 2023). Traditional spontaneous fermentation, a critical step in flavor development, is challenging owing to its reliance on variable environmental factors, including microbial populations and climatic conditions. This variability has prompted the exploration of controlled processing techniques as alternatives to traditional methods, offering enhanced flavor development (Gutiérrez-Ríos et al., 2022; Santander et al., 2021; Schlüter et al., 2022).

In light of these challenges, the controlled postharvest processing of cocoa seeds has emerged as a strategic approach to mitigate the uncertainties associated with spontaneous fermentation. By precisely regulating physicochemical parameters, this methodology enables the targeted modulation of endogenous enzyme activities, catalyzing a cascade of biochemical reactions that yield critical flavor precursors. The process typically involves the immersion of cocoa seeds in an organic acid solution under specific conditions of temperature and agitation, allowing for the meticulous monitoring of chemical transformations (Becerra et al., 2022, 2023; John et al., 2019; Kadow et al., 2015; Santander et al., 2021). The impact of various process variables, such as organic acid (OA) type and concentration, temperature, and mechanical agitation on the key cocoa quality attributes (pH, reducing sugar, peptide content, bioactive compound content, color) were documented (Becerra et al., 2022, 2023; Eyamo Evina et al., 2016; John et al., 2019).

To achieve a predictable and consistent flavor profile in chocolate, the controlled transformation process focuses on the development of desirable flavor notes through the precise manipulation of key processing parameters (Santander et al., 2020). Previous studies (Eyamo Evina et al., 2016; John et al., 2016, 2019), have confirmed that transforming cocoa beans under controlled conditions can effectively influence the formation of flavor precursor compounds. More recently, Santander et al. (2021) revealed that fine flavor attributes could be obtained when the transformation was conducted using a fixed concentration of 30 g/L and a temperature ramp, further demonstrating the potential of precise control in developing desirable flavor characteristics in chocolate. Building on these findings, this study sought to further explore the impact of the variables associated with controlled transformation of cocoa seeds, specifically temperature and organic acid concentration, on the development of fine-flavor characteristics in chocolate. Through an integrative examination of the metabolomic profile of the cocoa beans, volatile aroma composition of the beans and chocolates, and a sensory evaluation of the final chocolate product, this study aimed to identify the optimal conditions for achieving high-quality chocolate with superior flavor profiles, thereby contributing to the broader understanding of flavor formation mechanisms in chocolate production.

Table 1

Trial nomenclatures for the controlled transformation of cocoa seeds. OA represents organic acid.

| Trial |                |                |                | Temperature<br>(° C) | OA<br>Concentration<br>(g/L) | Processing<br>time<br>(h) |
|-------|----------------|----------------|----------------|----------------------|------------------------------|---------------------------|
|       | Acetic<br>acid | Lactic<br>acid | Citric<br>acid |                      |                              |                           |
| A01   |                | L01            | C01            | 45                   | 30                           | 24                        |
| A02   |                | L02            | C02            | 45                   | 30                           | 48                        |
| A03   |                | L03            | C03            | 45                   | 30                           | 72                        |
| A04   |                | L04            | C04            | 45                   | 1                            | 24                        |
| A05   |                | L05            | C05            | 45                   | 1                            | 48                        |
| A06   |                | L06            | C06            | 45                   | 1                            | 72                        |
| A07   |                | L07            | C07            | 30                   | 30                           | 24                        |
| A08   |                | L08            | C08            | 30                   | 30                           | 48                        |
| A09   |                | L09            | C09            | 30                   | 30                           | 72                        |
| A10   |                | L10            | C10            | 30                   | 1                            | 24                        |
| A11   |                | L11            | C11            | 30                   | 1                            | 48                        |
| A12   |                | L12            | C12            | 30                   | 1                            | 72                        |

## 2. Material and methods

### 2.1. Chemicals and reagents

Lactic acid (98 %) and citric acid ( $\geq 99.5$  %) were supplied by Sigma Aldrich (Bornem, Belgium). Acetic acid (100 %, analytical grade) and formic acid ( $\geq 98$  %) were provided by Applichem Panreac (Darmstadt, Germany). Acetonitrile (HPLC grade) was purchased from Scharlau (Barcelona, Spain). *n*-hexane and acetone were obtained from Merck (Darmstadt, Germany). Aqueous solutions were prepared using Milli-Q water (18.2 mΩ) (Millipore, Bedford, MA, USA). PVDF filters were purchased from Merck (Darmstadt, Germany).

### 2.2. Transformation process from cocoa seed to chocolate

The overall transformation process from cocoa seed to chocolate is represented in Fig. 1. Each transformation stage is described below.

#### 2.2.1. Experimental design

A mixed  $2^2 \times 3$  experimental design was employed to investigate the main effects of process variables during the controlled transformation from cocoa seed to bean on the response variable “global quality” in the sensory analysis of chocolate. Specifically, the  $2^2$  component of the design refers to two factors (process variable)—temperature (30 and 45 °C) and organic acid concentration (1 and 30 g/L)—each with two levels. The  $\times 3$  component refers to the processing time, which is considered at three levels (24, 48, and 72 h). The organic acids (OA) included in the study were acetic acid (AA), lactic acid (LA), and citric acid (CA). This design resulted in 12 treatments for each type of acid, totaling 36 treatments. The statistical analysis employed the Ordinary Least Squares (OLS) method to fit the model (Global quality  $\sim$  Temperature  $\times$  Concentration  $\times$  Time), and the significance of each term was

evaluated using F-tests. Subsequently, a three-way analysis of Variance (ANOVA) was conducted to assess the significance of the main effects and their interactions. This analysis was performed individually for each organic acid following the experimental protocol, using R with the “stats” library. The trials and their corresponding nomenclature based on the experimental design are presented in Table 1

### 2.2.2. Seed-to-bean controlled transformation

The controlled transformation process of cocoa seeds was conducted following the procedure of Becerra et al., (2022). TCS01 pods were disinfected using a sodium hypochlorite solution (3 %w/w), sprayed with ethanol, and opened using a sterile knife. The seeds were rinsed in 70 % ethanol to remove the remaining microorganisms. Cocoa seeds (180 g) were placed in 500 mL conical flasks containing 300 mL of OA solution at concentrations defined in Table 1. The conical flasks were placed in incubator shakers at a constant stirring rate (100 rpm) and controlled temperature (Table 1). The transformed cocoa seeds were sampled at 24, 48, and 72 h.

### 2.2.3. Drying

The transformed cocoa seeds were dried via forced convection with hot air at 50 °C for 72 h to analyze their metabolomic, sensory, and volatile aroma compound profiles

### 2.2.4. Chocolate bar production

The CIRAD (Centre de Coopération Internationale en Recherche Agronomique Pour le Développement) protocol was applied (Santander et al., 2021). Chocolate bars with 70 % cocoa solids were produced. The transformed and dried cocoa beans were roasted by forced convection with hot air in an oven (Memmert UF 110, Büchenbach, Germany) at 125 °C for 25 min, and the roasted cocoa nibs were subsequently cracked in limprimita cocoa breaker (Capco Cocoabreaker240-1, London, UK) and deshelled using a cocoa winnower machine (Capco Cocoawinnower, London, UK). The cocoa nibs were grinded by using a mortar & pestle-alumina grinder (Capco Mortarmill1SS, London, UK) during 60 min. Sugar was added after 30 min of grinding. The chocolate mass was refined twice using a three-roll refiner (Exakt 80S, Schleswig-Holstein, Germany). The refined chocolate mass was conched for 2 h at 70 °C with 5 % cocoa butter and 1 % soy lecithin in a longitudinal conche with a rotating roller (ZumWald LR01, Erlenbach, Switzerland). The seed method was used for tempering. The tempering stage comprised heating the chocolate above 42.0 °C, cooling it to 32.0 °C, and subsequently to 29.0 °C. After reaching 29.0 °C, the temperature was increased to 31.0 °C, and finally, it was heated to 32.5 °C before being molded. The 90 g chocolate bars were stored at 12 °C before analysis.

### 2.3. Sensory analysis of chocolate

A descriptive sensory analysis was performed using the consensus profile method ISO 13299:2016 (2016) in the CIRAD sensory analysis laboratory under controlled conditions using a trained expert panel. Nine trained judges blindly tasted the chocolate samples. The tasting was performed in duplicate. All judges possess extensive experience in the sensory evaluation of dark chocolate. Prior to the analysis, training sessions were carried out to calibrate the panel. The panel consisted of 4 women and 5 men between 40 and 60 years. The descriptive method was developed using an agreed tasting card and vocabulary, which include the sensory attributes to describe the perceptions of the samples. Sixteen descriptors were selected to describe the taste of the chocolate samples: sweetness, acidity, bitterness, astringency, cocoa aroma, cocoa taste, fruitiness, nuttiness, florality, spices, roasted, aroma intensity, alcoholic, vegetal, and animal. Global quality was also considered. The attribute description format used in the descriptive sensory analysis performed by the CIRAD Sensory Panel is presented in Table S1 of Supplementary Material.

A tasting plan was employed where all judges evaluated the samples

based on a set of standardized attributes, ensuring a comprehensive and consistent assessment. The 36 chocolate samples, corresponding to the 36 treatments from the experimental design, were coded with three random letters and then randomized using the statistical software XLSTAT 2020 software (Addinsoft, Paris, France) to determine the analysis order across nine sessions. To ensure proper sample randomization, a Latin Square design was employed to manage the presentation sequence. Four chocolate samples were evaluated in each session. The chocolates (5 g) were served on identical plates, each pre-labeled with the sample code, and tested at  $20 \pm 1$  °C. The samples were presented successively, one by one, in a monadic sequence presentation order. The judges scored the chocolate samples using a 10-point scale with 0 denoting not perceivable and 10 denoting very intense regarding the sensory attributes defined initially. Filtered water and salt-free toasts were provided for neutralization between different samples.

### 2.4. Analysis of volatile organic compounds in transformed and dried beans and chocolate

The volatile organic compounds (VOCs) of the transformed and dried beans and chocolates were sampled using headspace solid-phase microextraction (HS-SPME), as previously described by Assi-Clair et al., (2019). Fifty grams of sample (transformed and dried beans or chocolate) was used for each analysis. The transformed and dried beans were cracked and deshelled in the equipment previously described to obtain small pieces of cocoa nibs, while the chocolate was manually broken into small pieces. Both sample types, were frozen in liquid nitrogen and ground in a laboratory mill (Moulinex AR1108, Ecully, France). Two grams of the chocolate/cocoa bean powder were accurately weighed and supplemented with 100 µL of 1-butanol (600 mg/L) and they were transferred into a 10-mL screw- magnetic cap headspace vials. The 1-butanol was used as an internal standard to make a relative quantification of the identified volatiles.

A 50/30-µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, Supelco) fiber was used, previously conditioned in the chromatograph injector at 270 °C for 60 min. The fiber was exposed to the headspace of chocolate/cocoa nibs sample and internal standard 1-butanol at 50 °C for 45 min.

VOC analysis was conducted using an Agilent 6890 N gas chromatograph (GC) (Agilent Technologies, Santa Clara, CA, USA) with an automatic sampler and coupled with a mass spectrometer (Hewlett Packard, Model 5973 N Palo Alto, CA, USA). A DB HEAVY-WAX capillary column (60 m length, 0.25 mm internal diameter, and 0.25 µm film thickness) (Agilent Technologies, Santa Clara, CA, USA) was used. The automatic splitless injection temperature was set at 250 °C. Initially, the GC oven temperature was 40 °C for 5 min, followed by a gradual increase to 140 °C at a rate of 2 °C/min, and then a further increase at a rate of 10 °C/min until reaching 250 °C. High-purity hydrogen was used as the carrier gas at a 1.5 ml/min flow rate. The mass detector used was a quadrupole equipped with an electron impact ionization system operating at 70 eV, and the ion source temperature was 230 °C.

VOCs were tentatively identified via comparison with the retention index in public and internal databases (CIRAD aromatic database) and by probability-based matching of their mass spectra with those obtained from the NIST library of mass spectra. Peak areas were used for relative quantification of well identified compounds using the Mass Hunter software V 11.1 (Agilent Technologies, Santa Clara, CA, USA) with 1-butanol as the internal standard. For each chocolate/cocoa bean sample, isolation, separation, identification and semi-quantification of the aroma volatiles were performed in triplicate and samples were randomized prior to analysis. The semi-quantitative concentration of each volatile compound was expressed as micrograms of the internal standard 1-butanol equivalents per gram of chocolate/cocoa nibs calculated according to the Equation (1):

$$q_i(\mu\text{g/g}) = \frac{60 \cdot A_i}{A_{\text{but}} \cdot m_i} \quad (1)$$

where  $q_i$  is the concentration of volatile compound  $i$ ,  $A_i$  is the area of compound  $i$ ,  $A_{\text{but}}$  is the area of 1-butanol (internal standard), 60 is the content of 1-butanol in 100  $\mu\text{L}$  of a test sample in  $\mu\text{g/L}$ , and  $m_i$  is the mass of the sample introduced into the vial in g.

To evaluate the contribution of a volatile compound to the overall flavor profile of the chocolates, odor activity values (OAVs) were calculated using odor threshold values (OTV) documented in the literature about oil media ((van Gemert, 2011)). OAVs were calculated as follows:

$$\text{OAV} = \frac{q_i}{\text{OTV}_i} \quad (2)$$

where  $q_i$  ( $\mu\text{g/g}$ ) is the VOC content, and  $\text{OTV}_i$  ( $\mu\text{g/g}$ ) is the aroma threshold of the compound  $i$  in oil media.

## 2.5. Untargeted metabolomic analysis

### 2.5.1. Cocoa extracts

Metabolites were extracted following the methodology described by Mayorga-Gross et al., (2016) and John et al., (2019), with some modifications. Specifically, an ultrasonic bath was used both during the defatting process and in the ultrasound-assisted extraction step. First, dried beans were deshelled and ground in a coffee grinder (KitchenAid, Benton Harbor, MI, USA). Next, the ground samples were defatted using  $n$ -hexane in an ultrasonic bath for 15 min. This defatting procedure was repeated twice to produce ground cocoa powder. Next, 150 mg of cocoa powder was subjected to ultrasound-assisted extraction using 5 mL of acetone–water–formic acid (70:29.5:0.5) for 15 min. Then, the extract was vortexed for 3 min and centrifuged at 6000 rpm for 10 min. This extraction procedure was repeated two more times. Finally, the combined supernatants were dried. The extract was redissolved in 3 % acetonitrile (ACN) and filtered using a 0.22- $\mu\text{m}$  polyvinylidene difluoride (PVDF) membrane

### 2.5.2. Instrumental analysis

The samples were analyzed using a UPLC-ESI-QTOF-MS system (Acquity, Waters, Milford, Massachusetts, USA) equipped with a CSH C<sub>18</sub> column (130 Å, 1.7  $\mu\text{m}$  particle size, 2.1 mm  $\times$  100 mm, Waters) coupled to a Vanguard CSH C<sub>18</sub> precolumn (130 Å, 1.7  $\mu\text{m}$  particle size, 2.1 mm  $\times$  5 mm, Waters). An elution gradient was employed using water (solvent A) and acetonitrile (solvent B), both adjusted with 0.1 % formic acid. Elution began with 0 % B and progressed as follows: (time in min; B concentration in percentage): (7; 10); (22; 95); (22.1; 0), followed by a re-equilibration stage (26; 0). The flow rate was set at 0.4 mL/min, with a total analysis time of 26 min. The column temperature was maintained at 30 °C. ESI-MS/MS analyses were conducted in positive ionization mode with a full scan range from 100 to 1500 DA in continuous mode. The ESI-QTOF-MS system operated under the following conditions: Capillary voltage of 0.4 kV, sampling cone voltage of 40 V, and nitrogen used as the desolvation gas at a flow rate of 1000 L/h. Collision energy was set at 6 eV, and the scan time was 0.5 s. The instrument was calibrated using leucine enkephalin (556.2771 Da) with an infusion period of 10 s and a flow rate of 12  $\mu\text{L}/\text{min}$ . For quality control, two pooled QC samples, prepared by combining aliquots from study samples, were analyzed every 10 samples. This quality control practice helped to monitor and ensure the accuracy and reproducibility of the analytical results (Broeckling et al., 2023)

### 2.5.3. Data processing

Raw files were collected in continuous mode and centered using MassLynx software V3.1 SCN 639 (Waters Inc., Milford, Massachusetts, USA). The data were processed using Progenesis QI software (Waters Inc.), and the chromatographic peaks were selected and aligned. Next,

the effects of the drift on the quality control baseline were corrected using the Metadrift tool (Thonusin et al., 2017). For this, the data were normalized using the LOESS method with an alpha of 0.5, and the ions were filtered with a relative standard deviation of  $>30$  % in the quality control samples. Data tidying was complemented by removing the masses of the ions with zero value in 90 % of the samples. Then, the zero values were replaced with the minimum value detected to represent the baseline signal

### 2.5.4. Metabolite annotation

Once the discriminating metabolites were identified using multivariate analysis, MS/MS fragmentation was performed using low, medium, and high energies (10–65 eV). The metabolites were identified using the Theobroma cacao library in the Plant metabolic network database (<https://plantcyc.org/>) by comparison of the MS/MS reported spectra or in silico fragmentation using the software SIRIUS (Jena, Germany). According to Schymanski et al. (2014), the probable structure was annotated using MS/MS fragments through library matching and experimental data published elsewhere (D'Souza et al., 2018) with a high confidence of identification (level 2). Moreover, multi-data network analysis was performed using the sensory, volatile, and metabolomics data to correlate and explain metabolic behaviour.

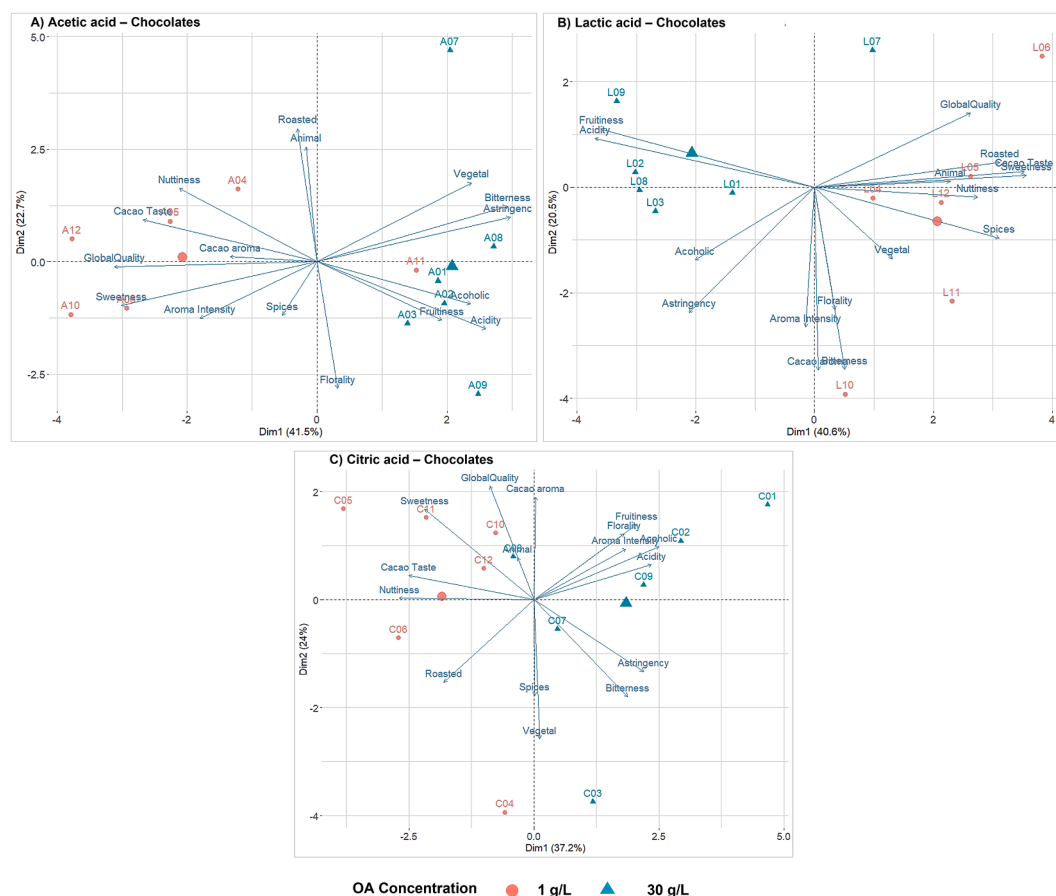
## 2.6. Statistical analysis

### 2.6.1. Sensory data

The data were tabulated and analyzed using XLSTAT 2020 (Addinsoft, Paris, France). Analysis of Variance (ANOVA) and principal Component analysis (PCA) were performed to study sensory traits of chocolates and to obtain the flavor profiles. The consistency of the sensory panel, assessed by their capacity to discern differences between chocolate samples, along with the repeatability of assessments and inter-panelist agreement (reproducibility), was evaluated using Cronbach's alpha coefficient (CAC). CAC values were used to identify panelists whose evaluations significantly deviated from the group, warranting their exclusion from the dataset. CAC values greater than 0.70 were deemed acceptable, indicating panelists who consistently provided similar results in terms of flavor profiles (Pinto et al., 2014). A regularized logistic regression model was implemented separately for each acid using L2 ("ridge") regularization with the "liblinear" library and 1000 iterations. The dataset was divided into the dependent variable "concentration" and sensory attributes as independent variables. Additionally, concentration was transformed into a binary variable where 1 indicated high concentration (30 g/L OA) and 0 indicated low concentration (1 g/L OA). Subsequently, the dataset was split into 70 % for training and 30 % for testing using stratified random sampling (Paakkunainen et al., 2007). The model was then executed, and accuracy, confusion matrices, and classification metrics were computed to evaluate model performance. For validation, the Bootstrap method with 1000 resampling iterations was employed to calculate mean values, standard errors, and confidence intervals. Z-scores and p-values were also determined for each coefficient.

### 2.6.2. VOCs data

Significant differences in the volatile component profiles of the transformed and dried beans and chocolate samples were tested using one-way analysis of variance (ANOVA) and principal component analysis (PCA) using R software (Vienna, Austria). The hierarchical clustering analysis (HCA) heatmap of volatile compounds was generated using MetaboAnalyst 5.0. The correlation network was visualized using Gephi (Version 0.10, London, UK). Moreover, an Orthogonal Partial Least Squares Analysis-Discriminant analysis (OPLS-DA) was applied to the chocolate volatiles data to compare the two concentrations (1 and 30 g/L) when the three organic acids were used. A variable importance in projection (VIP) score  $> 1$  was considered to OAs. Model quality indicators such as the fit parameter, prediction parameter, and level of



**Fig. 2.** PCA-score plot from the flavor profiles of chocolate samples produced from cocoa beans transformed with 1 g/L (red circles) and 30 g/L (blue triangles) of A) acetic acid, B) lactic acid, and C) citric acid. OA represents organic acid.

overfitting of the data through the permutation test were obtained. The RStudio software was used to perform this statistical analysis

### 2.6.3. Metabolomics data

Multivariate analysis was performed using MetaboAnalyst 5.0 (Pang et al., 2022). Quantile normalization, logarithmic transformation, and Pareto scaling were applied to the data. An Orthogonal Partial Least Squares Analysis-Discriminant analysis (OPLS-DA) was performed to identify discriminating metabolites according to the evaluated process variables (temperature, concentration, and time) for each OA. Then,  $R^2X$ ,  $R^2Y$ , and  $Q^2$  were measured, and cross-validation tests were performed to evaluate the performance of the model. Finally, the 20 features with the highest variable importance in projection (VIP) were selected to be identified.

## 3. Results and discussion

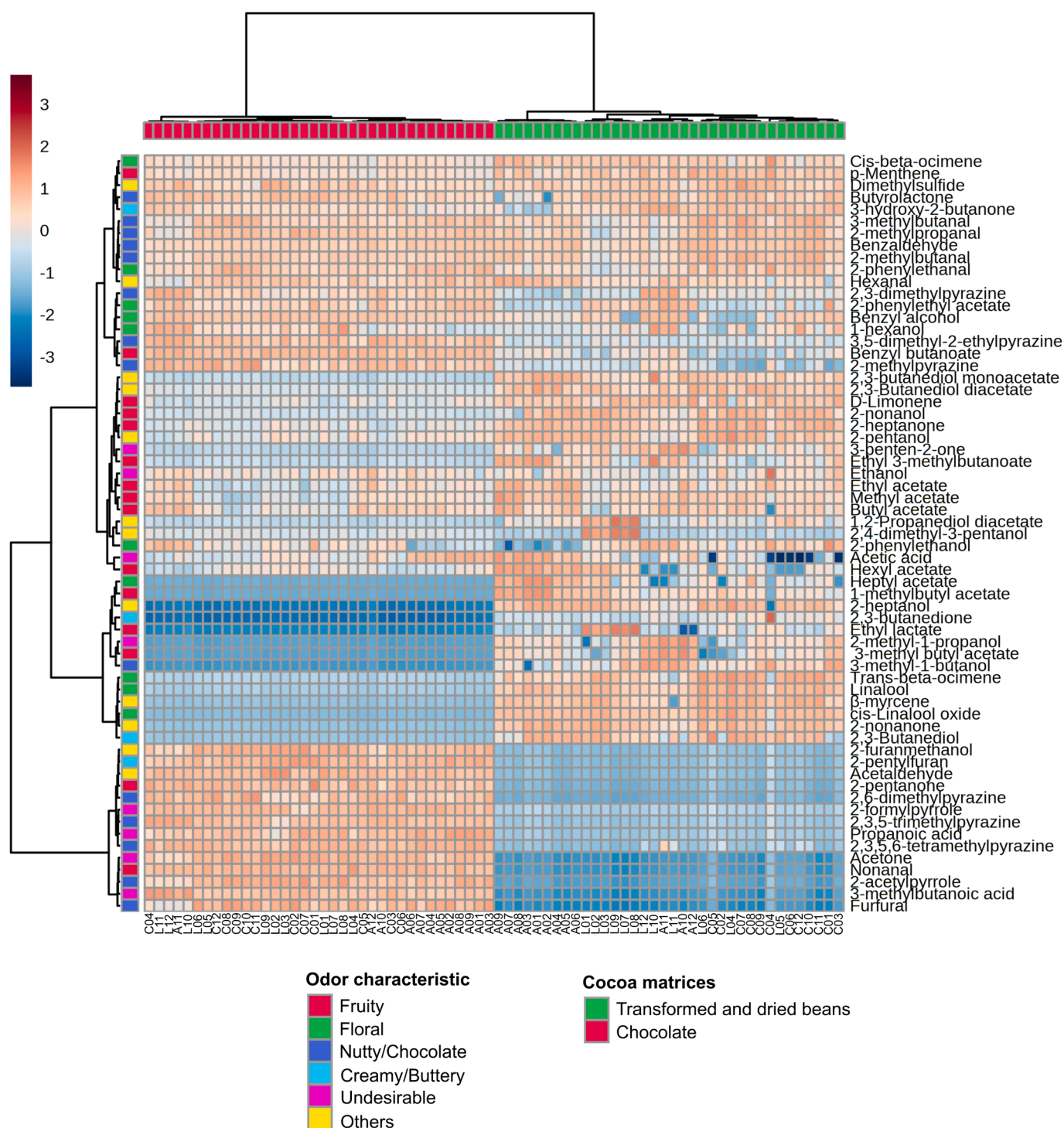
### 3.1. Sensory evaluation of chocolates

The sensory analysis of chocolates derived from the controlled transformation of cocoa seeds involved the evaluation of basic attributes such as sweetness, acidity, bitterness, and astringency; fine attributes such as cocoa aroma, cocoa taste, aroma intensity, fruitiness, nuttiness, florality, and spiciness; and undesirable attributes that included vegetal, animal, and alcoholic notes. Global quality was also considered. The average score for each attribute is presented in Table S2, and the chocolate flavor profile is shown using radial plots in Figure S1. It is highlighted that chocolates exhibited typical traits of fine-flavor cocoa, with relatively high perceptions of fruity, nutty, floral, and spicy aromatic notes.

Cronbach's Alpha (CAC) was employed to assess the reliability of the sensory evaluation results provided by the judges. Remarkably, all judges achieved CAC values close to 1.0 (Supplementary Table S3). This high CAC value indicates strong internal consistency within the sensory panel, demonstrating close alignment in the evaluation of flavor profiles among the nine panelists. Consequently, this consensus among the judges in analyzing the sensory attributes of chocolate samples confirms that the panel was composed of highly specialized and experienced judges, thus ensuring the reliability of the results.

The Global Quality descriptor, used in the sensory analysis of the chocolates, served as the response variable in the experimental design. This study aimed to investigate the main effects of temperature, concentration, and processing time—factors employed in the controlled transformation from cocoa seed to bean—on this descriptor. The results from the Ordinary Least Squares (OLS) method used to fit the model are provided in the supplementary material (Table S4). The findings indicate that for acetic acid, significant differences were found in concentration ( $p < 0.001$ ) and in the interaction between temperature and time ( $p < 0.01$ ). Similarly, for citric acid, significant effects on global quality were observed for concentration ( $p < 0.05$ ), as well as in the interactions between temperature and time, and concentration and time ( $p < 0.05$ ). In contrast, for lactic acid, significant differences were found for temperature and the concentration–time interaction. The concentration variable was close to the threshold of significance ( $p = 0.05$ ).

PCA was performed to identify patterns and relations among the different sensory attributes of the chocolate samples. The PCA biplot explains 64.2 %, 61.2 %, and 61.1 % of the variations in the first two dimensions for chocolates derived from AA, LA, and CA, respectively. The score plots for each group of samples according to the acid used (Fig. 2) reveal the grouping of the samples and closeness to sensory



**Fig. 3.** Heatmap and hierarchical clustering analysis (HCA) clustering analysis of 63 volatile compounds in the transformed and dried beans and chocolates.

attribute vectors based on the concentration of the OA applied in the controlled transformation. The other process variables of the controlled transformation, i.e., the OA type used, temperature, and time, did not reveal a pattern that could explain the sensory profile behavior of chocolates. Hence, the significance of OA concentration used in the controlled transformation of cocoa seeds for producing chocolates with desirable organoleptic features is emphasized.

These findings were validated using a logistic regression model (Supplementary Table S5). The results demonstrate that the logistic regression model performs effectively and exhibits robust classification capabilities based on sensory attributes and the concentration of organic

acids in chocolate. This enabled the classification of sensory attributes in chocolate based on the OA concentration used during the transformation of cocoa seeds. Chocolates produced from cocoa seeds subjected to controlled transformation at 30 g/L exhibited higher scores for fruitiness and acidity notes, regardless of the specific acid employed. However, samples from the L01–L03 and L07–L09 treatments received the highest acidity scores. This can be primarily attributed to the sour and acidic tastes of lactic acid in foods (Da Conceicao Neta et al., 2007). Due to its low volatility (< 0.1 hPa), lactic acid remains residual within the seed during controlled transformation, with thermal processes proving insufficient for its evaporation. Similar outcomes were observed in

treatments using citric acid. Astringency and bitterness descriptors also exhibited elevated values in these treatments, potentially masking the development of aroma notes crucial for achieving high-quality chocolate. In contrast, the chocolates obtained from cocoa seeds transformed using OA solutions at 1 g/L stood out for their higher scores in nuttiness, aroma intensity, cacao aroma and cocoa taste, contributing to the global quality of the chocolates.

In general, the scores of descriptors, such as bitterness and astringency, gradually decreased as the processing time advanced. These are typically attributed to the presence of methylxanthines and flavonoids in the cocoa seed (McClure et al., 2022), which migrate from the seed to the incubation solution during the controlled transformation. Furthermore, subsequent stages in chocolate production, such as drying and roasting, contribute to the degradation of these compounds, reducing the astringency and bitterness of the chocolates. By contrast, the acidity and fruitiness notes increased over time in most of the treatments evaluated. This trend is in line with the diffusion phenomenon of OAs from the incubation solution into the cocoa bean. As a result, the sensory profiles of the chocolates were perceived as more acidic and fruitier with the progress of the transformation process. Furthermore, attributes such as roastiness, sweetness, cocoa aroma, and cocoa taste remained relatively constant throughout the transformation process, suggesting that the processing time does not significantly influence them.

Notably, the chocolate with the highest global quality was A10, derived from cocoa seeds transformed using acetic acid at a concentration of 1 g/L at a temperature of 30 °C for 24 h. This treatment exhibited excellent scores for cocoa taste, sweetness, and nuttiness and low scores for bitterness and astringency. These findings are in concordance with balanced flavor profiles typically associated with high-quality chocolates. The global quality value of the chocolate from treatment A10 does not present significant differences with treatment A12 conducted under the same values of the operating variables but for 72 h. However, in terms of process efficiency, it is more convenient to conduct the controlled transformation of cocoa beans for a shorter time. Therefore, considering the sensory quality of chocolate, 24 h is an acceptable processing time for the development of its desirable sensory notes, such as sweetness, nuttiness, cocoa taste, and aroma intensity.

These sensory analysis results are in line with those of a previous study regarding chocolates produced using cocoa beans transformed under controlled conditions and spontaneous fermentation processes (Santander et al., 2021). Similar scores were observed for sensory descriptors related to fine-flavor and overall quality. Santander et al. (2021) observed that fruitiness, florality, and nuttiness were more pronounced when cocoa was processed under controlled transformation using AA and LA compared with that in cocoa that underwent spontaneous fermentation. This suggests that the transformation process proposed in our study has produced cocoa beans of comparable sensory quality to those produced through traditional fermentation methods. Moreover, the observed changes in the descriptors show the dynamic nature of flavor formation during the transformation time of the cocoa beans. Furthermore, it highlights the importance of carefully controlling the transformation parameters to achieve the desired chocolate flavor quality.

### 3.2. Volatile compounds in transformed and dried beans and chocolates

The composition of the volatile compounds of transformed and dried beans and chocolate was analyzed using GC-MS. A total of 63 volatile compounds were identified and quantified in this study, including alcohols, aldehydes, ketones, esters, carboxylic acids, lactones, terpenes, pyrazines, pyrroles, and furans. Based on their reported aroma descriptor, the volatile compounds were classified into fruity (16), floral (9), chocolate/nutty (14), buttery/creamy (3), undesirable (8), and other (13) (Tables S6–11). To assess the relative importance of some volatile compounds in terms of their aroma contribution to chocolates, their OAVs were calculated (Tables S12–14). A heatmap was

constructed using the normalized data to visualize the concentration of the identified compounds in the samples (Fig. 3). The main findings for each aroma descriptor are presented below. It was possible to identify specific patterns in the formation and loss of volatile compounds owing to the processing stages during the transition of beans to chocolate.

#### 3.2.1. Fruity volatiles

In the transformed and dried beans, the presence of alcohols and ketones such as 2,3-butanediol, 2-heptanol, and 2-nonanol, which have been associated with citrus, banana, and coconut notes, is highlighted (Hinne et al., 2019; Owusu et al., 2012). Alcohols have been previously identified in fermented, roasted beans and chocolates (Bastos et al., 2019) and have been highlighted as key odor compounds to describe the fruity notes of foods (Valle-Epquín et al., 2020). These compounds were found in higher concentrations, particularly in transformed beans derived from the transformation process using AA at 45 °C (A01–A06) and LA at 45 °C (L01–L06). These results suggest that this high-temperature level, as an operating variable in the controlled transformation of cocoa seeds, plays a significant role in forming flavor precursors. These precursors, in turn, contribute to generating alcohols during the subsequent drying and roasting stages, an effect that appears to be independent of the concentration of OAs in the controlled transformation process.

The fruitiness of chocolate is considerably influenced by acetate esters (Meersman et al., 2016). Esters such as methyl acetate, ethyl acetate, butyl acetate, and hexyl acetate, which are associated with tropical fruit notes, were also identified in all transformed and dried bean samples and strongly contributed to the aroma of the chocolates (OAV > 1). However, they were found in higher concentrations in the samples subjected to controlled transformation with AA (A01–A12). This trend was also observed for ethyl lactate, which is associated with fruity and sweet aromas, and it is present in higher concentrations in the samples treated with LA at 30 g/L (L01–L03, L07–L09). Another volatile, 3-methylbutyl acetate (isoamyl acetate), which is linked to banana notes, was found in higher concentrations in the L10–L12 and A10–A12 treatments (30 °C and 1 g/L). Therefore, mild controlled transformation conditions may enhance the formation of isoamyl acetate.

Herein, the high presence of alcohols, as previously described, and the OAs used in the incubation solution create favorable conditions for esterification. These findings suggest that the OAs diffusing into the cocoa seeds participate in the metabolic pathways leading to the formation of specific volatile compounds, including esters. Furthermore, reportedly, a higher concentration of sugars in the reaction medium promotes the production of acetate esters (Saerens et al., 2008). Therefore, based on our previous findings, it can be inferred that acetic acid treatments facilitate an increased presence of reducing sugars in the seeds, supporting this hypothesis (Becerra et al., 2022).

The content of previously described volatiles was substantially reduced in chocolates, and some were undetected. These drastic reductions in volatile contents during chocolate processing have been previously reported in the literature (Tuenter et al., 2020). The loss of the volatile fraction was primarily influenced by temperature and long conching time. However, the contents of compounds associated with citrus and sweet fruit notes, such as nonanal, 2-pentanone, and benzyl butanoate, increased in all the samples without following a trend associated with the conditions of the controlled transformation process. The increase in the content of these compounds can be attributed to factors related to the transformation process. For instance, additional Maillard reactions that did not occur during cocoa roasting may have been favored. Furthermore, the temperature and time conditions used in the latest stages of chocolate processing can promote the formation of these compounds. For example, nonanal is typically formed through the enzymatic oxidation of oleic acid by lipoxygenase, an enzyme present in cocoa beans. This reaction can occur at moderate temperatures (25 to 40 °C) in the presence of oxygen, which is abundant during the conching stage of cocoa processing (Schwab et al., 2008). This enzymatic pathway

might not be significantly active during high-temperature roasting but can proceed during other stages of chocolate processing where conditions are more favorable. Thus, the combination of prolonged exposure to suitable temperatures and oxidative conditions during stages such as conching can enhance the formation of nonanal, contributing to the fruity and citrus aroma notes in the final chocolate product.

### 3.2.2. Floral volatiles

Twelve floral volatiles were detected in the transformed and dried beans, including terpenes, esters, alcohols, and aldehydes. Terpenes such as linalool,  $\beta$ -myrcene, and *trans*- $\beta$ -ocimene were detected in higher concentrations, which are known to contribute to the floral, herbal, and spicy aroma notes that enhance the fine aroma characteristics of cocoa (Owusu et al., 2012). These compounds are formed through the terpenoid pathway, using carbohydrates as precursors (Rottiers et al., 2019). They have been identified in cocoa pulp and seeds, as well as in roasted beans and chocolate in lower concentrations (Hinneht et al., 2019). However, the concentrations of these compounds in the chocolate samples were relatively low, indicating their susceptibility to transformation processes during the transformation from beans to chocolate.

Conversely, compounds such as 1-hexanol, benzyl alcohol, and 2-phenylethyl acetate were detected in higher concentrations in the chocolates than in cocoa beans. They contribute to the herbal, jasmine, rose, and honey aroma notes in the chocolates. Previous studies by Hinneht et al. (2019) and Magagna et al. (2017) have also reported the presence of these compounds and their association with these specific aroma characteristics in cocoa products.

In the L10–L12 treatments (30 °C, 1 g/L LA), 2-phenylethanol was notably present in the cocoa beans and chocolates. This compound is related to a rose-like aroma and pleasant, sweet notes, making it a recognized aroma marker for the floral characteristics in chocolate (Hinneht et al., 2019). Furthermore, 2-phenylethanol exhibited a strong correlation with the processing time of the controlled transformation, with the highest content observed in chocolate (27.39  $\mu$ g/g) when cocoa seeds were transformed for 72 h (L12).

### 3.2.3. Nutty/chocolate volatiles

The presence of volatile compounds associated with nutty and chocolate aromas in the transformed and dried beans and chocolates was mainly attributed to pyrazines, aldehydes, and pyrroles. These compounds play a significant role in defining the typical aroma of cocoa and chocolate, and their formation occurs through Strecker degradation and Maillard reactions (Liu et al., 2017). During the processing from beans to chocolate, pyrazine concentrations increased. Likewise when discriminating volatiles were obtained after comparing both the applied concentrations (Figure S2 and Table S15). Pyrazines linked to nutty and cocoa aromas, such as 2,3,5-trimethyl pyrazine, 2,6-dimethyl pyrazine, 2,3-dimethyl pyrazine, and 3,5-dimethyl-2-ethylpyrazine, were present in higher intensity in the 1 g/L chocolates. However, pyrazine concentration was lower in the samples subjected to controlled transformation with CA than those treated with the other two OAs. The initial step in forming pyrazines involves the reaction between carbonyl and amine groups, forming a Schiff base. Cocoa seeds treated with CA further decrease the internal pH of the seeds to values below 5.0 (Table S16). This highly acidic environment can reduce the activity of the amino group, thereby limiting the production of pyrazines (Caporaso et al., 2018). Nevertheless, the general contribution of pyrazines to the overall flavor was considered negligible because their OAV was < 1.

In contrast to the effects on pyrazines, the processing stages involved in chocolate production did not significantly affect aldehydes such as 2-methylbutanal, 3-methylbutanal, 3-methylpropanal, and benzaldehyde, which are known for their malty, almond, and honey-like aromatic notes (Calva-Estrada et al., 2020). These aldehydes exhibited high OAV values (OAV > 1), indicating their strong contribution to the aroma of chocolates. These results suggest that the reactions responsible for forming these Strecker aldehydes continue during conching and that additional

reaction pathways will develop (Tran et al., 2016).

### 3.2.4. Creamy/buttery volatiles

Butyrolactone, 3-hydroxy-2-butanone, and 2,3-butanedione are volatile compounds associated with buttery and creamy notes which positively affect the aromatic quality of cocoa, as reported by Kouassi et al. (2022). Butyrolactone and 3-hydroxy-2-butanone were detected in beans and chocolate, whereas 2,3-butanedione was not detected in the chocolate samples. Specifically, 3-hydroxy-2-butanone and 2,3-butanedione have been recognized as markers of the controlled transformation process, as their presence is sensitive to the operational variables employed during chocolate production (Magagna et al., 2017). Indeed, the presence of these compounds in the samples could be used to indicate the effect of the operation variables on the desirable flavor attributes through the controlled transformation process.

### 3.2.5. Undesirable volatiles

Higher levels of short-chain carboxylic acids, including acetic acid, propanoic acid, and 3-methylbutanoic acid, were observed in the chocolate samples and not in the beans, except for AA. These compounds are responsible for the undesirable rancid, sour, and sweaty notes in the chocolate (Kouassi et al., 2022; Magagna et al., 2017). As expected, the chocolates from cocoa seeds transformed with acetic acid exhibited a higher acetic acid concentration, mainly when treatment at 30 g/L was performed. Consequently, higher OAVs for acetic acid were obtained in the chocolates of these trials, indicating the significant contribution of AA to the overall aroma.

In addition, a positive correlation was observed between the acetic acid content and the time of the controlled transformation process. This correlation can be attributed to the mass transfer process during the controlled transformation of cocoa seeds, as a longer duration of controlled transformation results in a higher amount of acid entering the seed (Becerra et al., 2022). It is important to note that the subsequent cocoa processing stages may not effectively eliminate acetic acid from the final chocolate product. However, these chocolate-making stages effectively decrease the concentration of other undesirable compounds, such as 3-penten-2-one and 2-methyl-1-propanol, which are associated with alcoholic and earthy notes. This highlights the need for careful management of the transformation process to prevent the accumulation of off-flavors in the chocolate and produce a high-quality product.

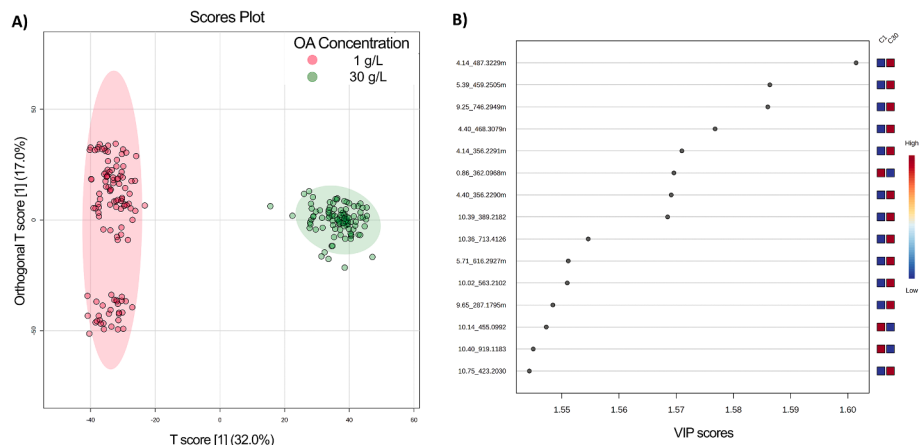
Noteably, the volatile compounds found in the controlled transformation of cocoa seeds have also been found in natural cocoa fermentation processes. This indicates that the controlled transformation process can accurately replicate and produce compounds that contribute to the desired flavor characteristics seen in traditional fermentation. By modulating the transformation process variables, cocoa farmers can produce high-quality cocoa with desirable flavor attributes.

## 3.3. UPLC–ESI–QTOF–MS metabolomic profiling of cocoa beans during controlled transformation

UPLC–ESI–QTOF–MS analysis was performed to evaluate the effect of the operating variables of the controlled transformation on the metabolites present in cocoa beans. After processing the raw files, 3885 ions were detected under full scan from 100 to 1500 Da in positive ionization mode (ESI + ). These data were used for subsequent chemometric analysis, including PCA and OPLS-DA.

### 3.3.1. Multivariate analysis

Unsupervised PCA was initially applied to observe distribution trends among all cocoa samples. General clustering trends were then analyzed considering four variables: OA, OA concentration, temperature, and time (Figure S3). The first two principal components explained 59.0 % of the information in the complete data matrix. However, the dispersion of the samples according to the type of OA, temperature, and



**Fig. 4.** A) OPLS-DA score plot of the cocoa samples obtained according to the experimental design. B) Variable Importance in Projection (VIP) plot of important features of fingerprinting of samples. The colored boxes on the right indicate the relative concentrations of the corresponding metabolite in each group under study. OA represents organic acid. C1 indicates 1 g/L concentration and C30 indicates 30 g/L concentration.

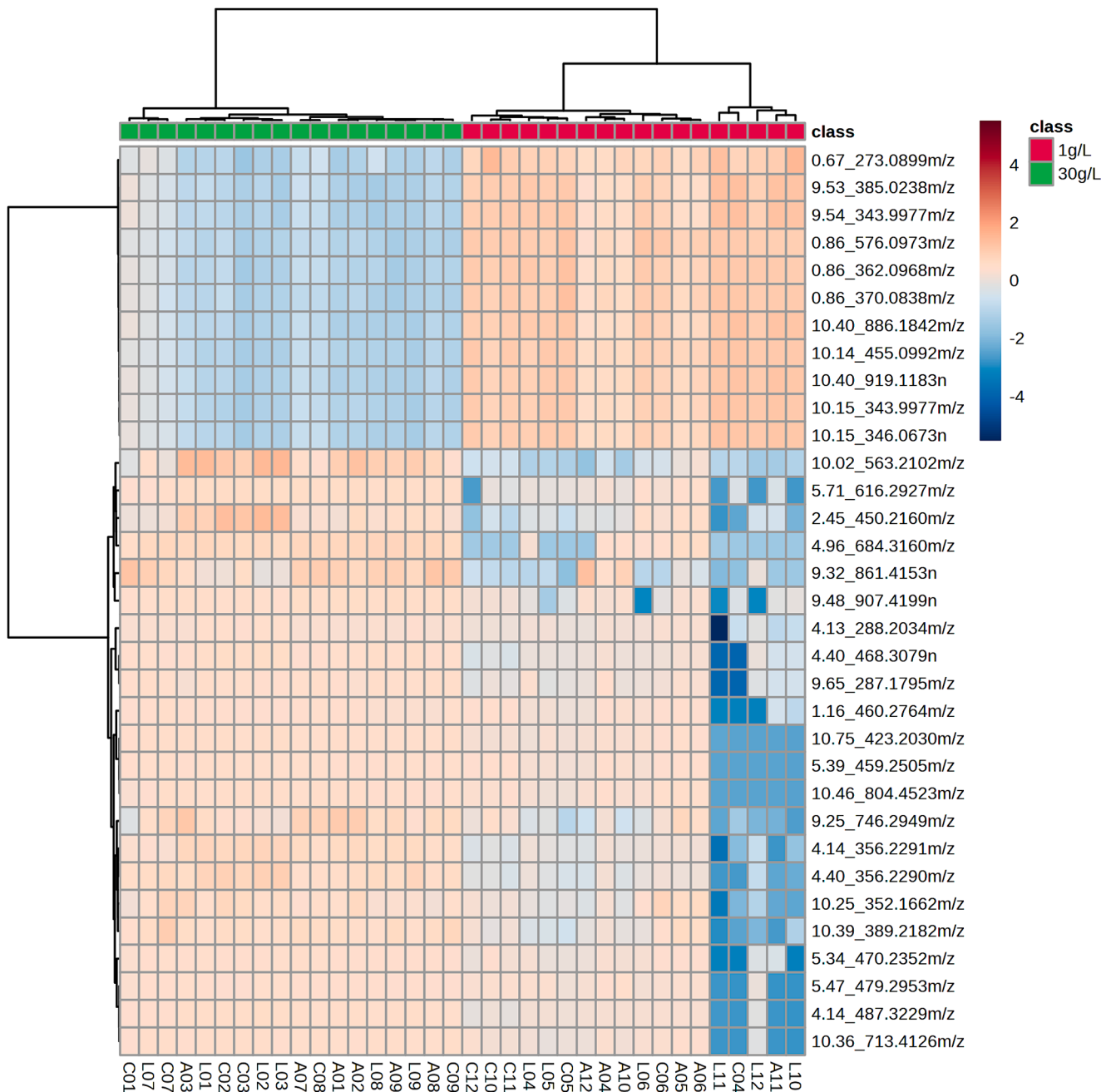
**Table 2**  
Overview of annotated candidate differential metabolites among different organic acid concentrations in the ESI + mode. RT is retention time, VIP is Variable Importance in Projection.

| Tentative identity | Formula                                                         | Observed m/z | Adduct               | Theoretical m/z | $\Delta$ m/z | RT<br>(min) | VIP value | p-value  | Fold change |
|--------------------|-----------------------------------------------------------------|--------------|----------------------|-----------------|--------------|-------------|-----------|----------|-------------|
|                    |                                                                 |              |                      |                 | (ppm)        |             |           |          |             |
| Unknown 1          | —                                                               | 273.0899     | —                    | —               | —            | 0.67        | 1.533     | 1.48E-61 | 4.674       |
| IR                 | C <sub>12</sub> H <sub>25</sub> N <sub>5</sub> O <sub>3</sub>   | 288.2034     | [M + H] <sup>+</sup> | 288.2030        | 1.3          | 4.13        | 1.511     | 1.71E-59 | 0.108       |
| Unknown 2          | —                                                               | 343.9977     | —                    | —               | —            | 9.54        | 1.540     | 5.49E-64 | 5.732       |
| Unknown 3          | —                                                               | 346.0673     | —                    | —               | —            | 10.15       | 1.535     | 1.83E-62 | 3.738       |
| WF                 | C <sub>20</sub> H <sub>21</sub> N <sub>3</sub> O <sub>3</sub>   | 352.1662     | [M + H] <sup>+</sup> | 352.1656        | 1.7          | 10.25       | 1.515     | 5.19E-59 | 0.155       |
| Unknown 4          | —                                                               | 356.2290     | —                    | —               | —            | 4.40        | 1.569     | 2.57E-69 | 0.074       |
| Unknown 5          | —                                                               | 362.0968     | —                    | —               | —            | 0.86        | 1.570     | 2.83E-69 | 4.003       |
| Unknown 6          | —                                                               | 370.0838     | —                    | —               | —            | 0.86        | 1.528     | 1.38E-63 | 5.547       |
| Unknown 7          | —                                                               | 385.0238     | —                    | —               | —            | 9.53        | 1.538     | 4.43E-63 | 4.887       |
| FKLNQGA            | C <sub>35</sub> H <sub>56</sub> N <sub>10</sub> O <sub>10</sub> | 389.2182     | [M + H] <sup>+</sup> | 389.2163        | 4.8          | 10.39       | 1.568     | 6.27E-69 | 0.140       |
| FAW                | C <sub>23</sub> H <sub>26</sub> N <sub>4</sub> O <sub>4</sub>   | 423.2030     | [M + H] <sup>+</sup> | 423.2027        | 0.7          | 10.75       | 1.544     | 2.19E-63 | 0.104       |
| Unknown 8          | —                                                               | 450.2160     | —                    | —               | —            | 2.45        | 1.536     | 2.13E-65 | 0.153       |
| Unknown 9          | —                                                               | 455.0992     | —                    | —               | —            | 10.14       | 1.547     | 5.41E-65 | 3.801       |
| Unknown 10         | —                                                               | 459.2505     | —                    | —               | —            | 5.39        | 1.586     | 2.76E-72 | 0.133       |
| Unknown 11         | —                                                               | 460.2764     | —                    | —               | —            | 1.16        | 1.535     | 3.60E-60 | 0.222       |
| Unknown 12         | —                                                               | 460.5664     | —                    | —               | —            | 10.40       | 1.545     | 5.27E-65 | 4.481       |
| Unknown 13         | —                                                               | 469.3124     | —                    | —               | —            | 4.40        | 1.577     | 2.31E-72 | 0.050       |
| Unknown 14         | —                                                               | 470.2352     | —                    | —               | —            | 5.34        | 1.535     | 1.45E-62 | 0.125       |
| Unknown 15         | —                                                               | 479.2953     | —                    | —               | —            | 5.47        | 1.520     | 1.82E-62 | 0.130       |
| KAGVL              | C <sub>22</sub> H <sub>42</sub> N <sub>6</sub> O <sub>6</sub>   | 487.3229     | [M + H] <sup>+</sup> | 487.3239        | 2.1          | 4.14        | 1.601     | 9.15E-78 | 0.030       |
| Unknown 16         | —                                                               | 563.2102     | —                    | —               | —            | 10.02       | 1.551     | 4.48E-65 | 0.074       |
| Unknown 17         | —                                                               | 576.0973     | —                    | —               | —            | 0.86        | 1.529     | 1.91E-62 | 3.324       |
| Unknown 18         | —                                                               | 616.2927     | —                    | —               | —            | 5.71        | 1.551     | 9.60E-66 | 0.045       |
| Unknown 19         | —                                                               | 684.316      | —                    | —               | —            | 4.96        | 1.519     | 2.36E-61 | 0.040       |
| Unknown 20         | —                                                               | 713.4126     | —                    | —               | —            | 10.36       | 1.555     | 6.75E-67 | 0.095       |
| Unknown 21         | —                                                               | 746.2949     | —                    | —               | —            | 9.25        | 1.586     | 3.87E-72 | 0.142       |
| Unknown 22         | —                                                               | 804.4523     | —                    | —               | —            | 10.46       | 1.522     | 6.20E-60 | 0.092       |
| Unknown 23         | —                                                               | 862.4226     | —                    | —               | —            | 9.32        | 1.530     | 5.69E-65 | 0.127       |
| Unknown 24         | —                                                               | 886.1842     | —                    | —               | —            | 10.40       | 1.536     | 4.93E-62 | 3.253       |
| Unknown 25         | —                                                               | 890.4166     | —                    | —               | —            | 9.48        | 1.529     | 3.78E-63 | 0.052       |
| Unknown 26         | —                                                               | 287.1795     | —                    | —               | —            | 9.65        | 1.548     | 3.36E-64 | 0.099       |

time (h) of the controlled transformation was significant, suggesting considerable variability among the samples. Consequently, these factors hindered the coherent grouping of the data. Conversely, the concentration of OA in the incubation solutions provided greater differentiation between the groups than the other variables. These findings underscore the notable influence of OA concentration as the main factor, underlining its critical role in modulating the chemical composition of seeds. However, these results indicate that the cocoa samples under the 36 experimental treatments could not be wholly distinguished through PCA.

Based on the PCA analysis results, the variable “OA concentration”

was selected to perform OPLS-DA. This model was constructed to maximize the differences between groups and identify chemical compounds acting as bioprocess markers responsible for the variation in the cocoa bean chemical composition due to OA concentration, as indicated by the value of the VIP (Gao et al., 2021). The OPLS-DA score plot output is presented in Fig. 4. The OPLS-DA score plot allows clear separation and differentiation between the cocoa samples ( $R^2X = 0.320$ ,  $R^2Y = 0.757$ , and  $Q^2 = 0.755$ ) according to the OA concentration used in the controlled transformation. Cross-validation and permutation tests (100 random permutations) were performed to verify the fit of the OPLS-DA model (Figure S4). The results indicate that the OPLS-DA model is



**Fig. 5.** Heatmap and hierarchical clustering analysis (HCA) analyses of differential metabolites obtained at different organic acid concentrations.

effective and has good predictive ability. Therefore, it can be used to explore the differences in the metabolic profile of seeds due to OA concentration and identify the metabolites that can be proposed as process biomarkers. The colored boxes on the right indicate the relative concentrations of the corresponding metabolite in each group under study.

### 3.3.2. Identification of candidate bioprocess markers associated with controlled transformation

Herein, metabolites that were considered process markers were defined using VIP and p values as standard criteria. Metabolites with a VIP value of  $> 1.5$  from OPLS-DA and p-value of  $< 0.05$  from Student's *t*-test were considered significant between the two OA concentrations (Ai et al., 2021). In addition, fold change was calculated to quantify the magnitude of metabolite change due to the two experimental conditions. A fold change of  $> 1$  indicated an upward trend, whereas a fold change

of  $< 1$  indicated a downward trend (Cui et al., 2022). Overall, 31 differential metabolites (10 upregulated and 22 downregulated) formed during the controlled transformation process and in response to the OA concentration used (Table 2). The annotated metabolites include peptides.

An HCA was conducted to assess the diversity in differential metabolites among the samples, and the results were visualized in a heatmap (Fig. 5). In the heatmap, the color gradient from blue to red represents the abundance of metabolite expression, ranging from low to high levels. The analysis revealed two distinct main groups based on the concentration of OAs, indicating variations in the intensity of differential markers between these groups. Fig. 5 displays the classification of 31 compounds, including 5 tentatively identified compounds and 26 unknown compounds, into 2 classes. The first group comprises 11 unidentified compounds found at higher levels in cocoa samples obtained from controlled transformations using 1 g/L of OA, regardless of the OA

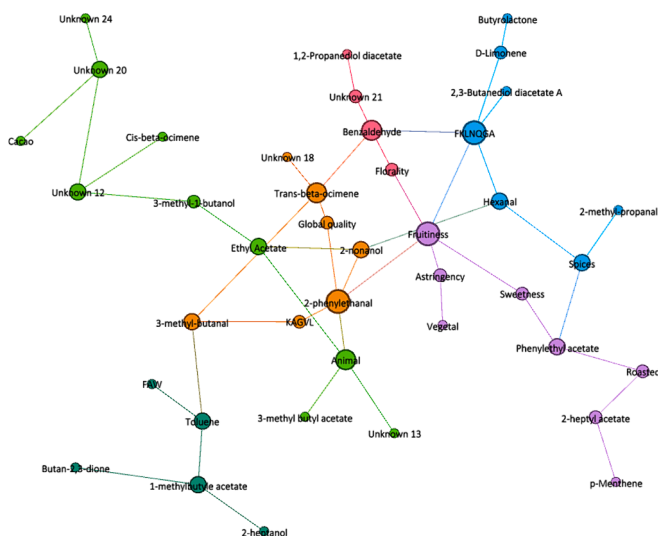


Fig. 6. Positive correlation network between metabolites, sensory attributes, and volatile compounds. Different colors represent different clusters.

used. By contrast, the other group, to which the six identified compounds belong, particularly peptides, exhibited higher levels in cocoa samples obtained from controlled transformations using 30 g/L of OA. However, it is important to note that the L10–L12 treatments displayed low levels of the described compounds.

A notable finding is the higher discriminating compound content, particularly peptides, at higher OA concentrations. This result suggests that the low pH (Table S16) resulting from these transformation conditions in cocoa seeds strongly influences the formation of peptides. The native enzymes present in the cocoa seeds play a vital role in transforming flavor precursors, and their activity is dependent on the physical and chemical conditions of the system, including temperature and pH. The reduction in pH increases the activity of aspartic endoprotease and carboxypeptidase enzymes, which break down proteins into peptides. This finding is favorable as it shows that peptides are essential for generating flavor compounds during subsequent processing stages.

### 3.4. Relations between metabolomics data, sensory attributes, and volatile compounds

To better understand the contribution of differential metabolites to the VOCs and sensory perception of chocolates, a correlation network was constructed (Fig. 6). The nodes of the network represent metabolites, volatile compounds, and sensory attributes, and edges indicate a positive correlation. A total of 41 nodes and 46 edges were obtained through network analysis.

In Fig. 6, positive correlations were identified among the specific metabolites, volatile compounds, and sensory descriptors of the chocolates. Some of these correlations align with the aroma characteristics previously described for the volatile compounds, highlighting the aroma precursor peptide with the sequence KAGVL, derived from albumin protein hydrolysis, which correlates with the compound 2-phenylethanal. This aldehyde has been previously linked to a pleasant honey note (Akoa et al., 2023) and, along with 3-methylbutanal, contributes to the distinctive cocoa flavor. Both aldehydes, in conjunction with *trans*-beta-cymene, are correlated with the fruity note and overall quality of the chocolates. This finding is consistent with those of previous studies (Counet et al., 2002; Kadow et al., 2013), indicating that these compounds are associated with fine aroma chocolates and are significant constituents of the cocoa flavor. By contrast, a direct correlation is observed between the peptide FKLNQGA, obtained through vicilin protein hydrolysis, and the perceived floral note in chocolates. It correlates with compounds such as limonene and hexanal, contributing to

the fruity and spicy notes, and benzaldehyde, which possesses aroma characteristics associated with sweet and almond notes.

While the correlation network analysis provides valuable information for identifying metabolites or volatile compounds that may influence the sensory descriptors of chocolate, it does not offer a complete understanding of the sensory profile. However, a higher concentration of key aromas positively impacted the overall characteristics of chocolates. In addition, it is important to note that the nonvolatile fraction of chocolate, which contains essential components such as methylxanthines, phenolic compounds, and sugars, contributes significantly to chocolate flavor in terms of sensory quality balance. Therefore, further research is needed to fully elucidate the contributions of the volatile and nonvolatile fractions to the flavor profile of chocolate.

The findings of this study provide valuable insights for the food industry, particularly in optimizing cacao processing to enhance chocolate quality. By identifying key processing parameters that influence flavor development, manufacturers can refine fermentation and post-harvest techniques to consistently achieve desired flavor profiles, thereby improving product quality and reducing variability. This research not only supports the production of premium chocolate but also aligns with industry trends toward process technification by potentially transforming post-harvest cacao processing from a spontaneous, artisanal practice into a standardized and scalable operation.

## 4. Conclusions

This study delineates the impact of controlled postharvest processing variables, specifically temperature, and pH modulation via OA solutions, on the metabolomic and volatile profiles of cocoa beans and the sensory attributes of the resulting chocolate.

We identified a marked influence of OA concentration during cocoa seed transformation on flavor profile development. High-concentration treatments (30 g/L) yielded chocolates with pronounced fruitiness and acidity, whereas lower-concentration treatments (1 g/L) produced chocolates with notable nuttiness, enhanced cocoa taste, and subdued acidity. Predominantly, peptides and various volatile compounds, including esters and aldehydes, were instrumental in defining the aroma and flavor spectrum of the chocolate. This study particularly highlighted the role of the type and concentration of OA in modulating the diversity and abundance of flavor-contributing esters, with AA treatments enhancing ester presence significantly.

The findings endorse the controlled transformation of cocoa seeds using OAs as an innovative strategy for crafting chocolates with fine-flavor attributes. This methodological advancement facilitates the induction of specific sensory profiles, laying a scientific foundation for producing chocolates that meet targeted flavor specifications. This strategy emerges as a powerful tool for enhancing the flavor of chocolate, pushing the frontier of flavor formation research and inviting further inquiry into optimizing chocolate production techniques.

Thus, the research highlights the importance of strategic adjustments in processing conditions, particularly temperature and pH, in influencing the flavor development of chocolate. Such modifications pave the way for the chocolate industry to achieve a consistent production of high-quality chocolates with the desired sensory attributes. Future studies should aim to delve deeper into the complex interplay between processing variables and flavor development with the ultimate goal of refining these transformation processes to fully exploit their potential in producing superior chocolates.

## CRedit authorship contribution statement

**Lili Dahiana Becerra:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Ruth Yolanda Ruiz-Pardo:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Fabrice Vailant:** Validation, Methodology, Data curation. **Martha Viviana**

**Zuluaga:** Writing – review & editing, Validation, Methodology, Data curation. **Renaud Boulanger:** Validation, Methodology, Formal analysis, Data curation. **Margareth Santander:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Sebastián Escobar:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2024.115109>.

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