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The University of Osaka

Doctoral Dissertation

**Evaluating the effect of different de-
pulping methods on cocoa beans' flavor
quality based on a metabolomics
approach**

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January 2026

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List of Abbreviations

(in alphabetical order)

ANOVA	Analysis of variance
DVB/CAR/PDMS	Divinylbenzene/Carboxen/Polydimethylsiloxane
EI	Electron Ionization
FAEE	Fatty Acid Ethyl Esters
FI	Fermentation Index
GC-MS	Gas chromatography–mass spectrometry
HS-SPME	Headspace Solid-Phase Microextraction
HSD	Tukey’s Honest Significant Difference
ICCO	International Cocoa Organization
ICCRI	Indonesian Coffee and Cocoa Research Institute
ISCQF	International Standards for the Assessment of Cocoa Quality and Flavor
MSTFA	N-methyl-N-trimethylsilyl-trifluoroacetamide
OPLS-DA	Orthogonal Partial Least Squared-Discriminant Analysis
PCA	Principal Component Analysis
PLS	Partial Least Squares
PLS-R	Partial Least Squares-Regression
QC	Quality Control
RI	Retention Index

RMSEcv	Root-Mean-Square-Error of Cross-validation
RMSEE	Root-Mean-Square-Error of Estimation
RSD	Relative Standard Deviation
VIP	Variable Importance in Projection

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Chapter 1

General introduction

1.1 The importance of flavor in cocoa products

Cocoa beans (*Theobroma cacao*) are a vital commodity used as the primary raw material in chocolate manufacturing. They are primarily cultivated in tropical countries, including the Ivory Coast, Ghana, Indonesia, Nigeria, Cameroon, Brazil, Ecuador, the Dominican Republic, and Malaysia (Okiyama et al., 2017). The processed cocoa beans are used to produce a wide range of cocoa-derived products, including chocolate liquor, cocoa powder, and cocoa butter. These ingredients are not only essential to chocolate manufacturing but are also widely utilized in cocoa beverages, ice cream, and baked goods, contributing to the signature cocoa flavor that defines their sensory appeal (Afoakwa et al., 2007).

From a commercial and industrial perspective, cocoa is classified into two types: bulk (or ordinary) cocoa, known for its basic taste; and fine-flavor cocoa (FFC), which is characterized by its unique flavor, such as fruity, flowery, woody, nutty, and spicy (Castro-Alayo et al., 2019). Bulk cocoa, which accounts for more than 90% of world production, is mainly derived from the Forastero variety. Forastero trees are renowned for their high yield and strong vigor; however, the beans often lack the complex flavor notes, resulting in lower market prices, averaging around USD 5.61 per kg (Velásquez-Reyes et al., 2023). By contrast, FFC is commonly derived from Criollo varieties, which are highly prized for their fruity, floral, herbal, woody, nutty, and caramel-like notes (Castro-Alayo et al., 2019). However, Criollo is extremely rare and nearly extinct due to

its high susceptibility to diseases and low yield, making it difficult to cultivate on a large scale. As a result, it accounts for less than 5% of global cocoa production (Putri et al., 2024). In addition to these, Trinitario, a hybrid of Criollo and Forastero, combines the disease resistance and productivity of Forastero with some of the aromatic qualities of Criollo. Depending on its flavor attributes, Trinitario may be classified as either bulk or fine cocoa (Putri et al., 2024).

FFC commands a higher price due to its superior sensory quality. It is often purchased at a premium, starting at about 20% more and sometimes reaching double or even triple the price of bulk cocoa beans, typically ranging from USD 5–10 per kg in commercial markets. Representing only about 5% of global cocoa production, FFC is nonetheless highly prized as a premium raw material for high-quality chocolate (Beckett et al., 2017). Meanwhile, the market for chocolate products, especially premium chocolate, has experienced significant growth, driven by rising consumer interest in distinctive and high-quality flavor experiences. The global premium chocolate market is projected to reach USD 46.12 billion by 2028 (Mordor Intelligence, 2023). This increasing demand highlights the importance of cocoa bean quality, which directly influences flavor and overall consumer acceptance (Santander et al., 2021).

Given the pivotal role of flavor in shaping product quality and consumer preference—particularly in the premium chocolate sector—there is growing interest in identifying and developing effective strategies to improve cocoa flavor (Campos et al., 2025). This is particularly crucial for bulk cocoa, which dominates global production but often fails to meet fine-flavor standards. By improving the flavor quality of bulk cocoa, farmers could gain access to premium markets, increase income opportunities, and

contribute to the sustainable growth of the chocolate industry. Therefore, exploring alternative methods to improve cocoa flavor is important not only for farmers striving for better livelihoods but also for the chocolate industry as a whole (Satama-Bermeo et al., 2025).

1.2 Cocoa postharvest process

The development of cocoa flavor is influenced by several factors, including genotype (Santander et al., 2021), origin (Marseglia et al., 2020), and the postharvest process (Rodriguez-Campos et al., 2011). Among these, postharvest handling plays the most significant role in determining the final flavor quality of cocoa. Even fine-flavor varieties, such as Criollo and Trinitario, can lose their distinctive attributes and become indistinguishable from bulk cocoa if post-harvest practices are poorly managed. Conversely, the flavor quality of bulk cocoa can be improved to meet fine-flavor standards through proper postharvest practices (Afoakwa et al., 2013; Escobar et al., 2021; Moreno-Rojas et al., 2023).

Flavor development primarily occurs during the postharvest process, which consists of two major stages: primary on-farm operations and agro-industrial processing. The on-farm stage begins with harvesting ripe cocoa pods, followed by pod breaking (also called pod opening) to extract the wet beans surrounded by mucilaginous pulp. These beans then undergo fermentation, where microbial activity transforms biochemical components into flavor precursors (Biehl et al., 1990; Santander Muñoz et al., 2019). After fermentation, the beans are dried, typically by sun-drying, to reduce moisture content and stabilize the product for further processing (Kongor et al., 2016). Following drying, the cocoa beans enter the agro-industrial phase. This stage starts with roasting, a critical step that enhances

and develops chocolate flavor through Maillard reactions and other thermal processes (Hinneht et al., 2020). After roasting, the beans are cracked and winnowed to separate the cocoa nibs from the shells. The nibs are then ground into cocoa liquor (also known as cocoa mass), which serves as the basis for various chocolate products [Click or tap here to enter text..](#) Subsequent processing of the cocoa liquor involves mixing with ingredients such as sugar, milk, and emulsifiers, followed by refining, conching, and tempering to produce finished chocolate products with the desired texture and flavor characteristics (Hinneht et al., 2019; Putri et al., 2024).

Among all postharvest steps, fermentation has been the most extensively studied because it is the most critical stage for flavor development, as it initiates the biochemical transformation of seed components into metabolites that serve as precursors of chocolate flavor (Biehl et al., 1990; Santander Muñoz et al., 2019). Recognized as a critical stage in flavor development, many studies have focused on optimizing fermentation through interventions such as starter culture addition, temperature regulation, extended or shortened durations, and mixing techniques (Díaz-Muñoz et al., 2021; Mougang et al., 2024). During this process, the cocoa pulp serves as the main substrate for microbial activity, supporting the growth of yeasts and bacteria that convert sugars and amino acids into key volatile and non-volatile compounds responsible for cocoa's complex sensory characteristics (Rottiers et al., 2019).

While much attention has been placed on modifying the fermentation conditions themselves, the role of the pulp—particularly its quantity and composition—remains a crucial yet often overlooked factor. The pulp forms a viscous layer around the beans, which may restrict oxygen flow and influence gas exchange, microbial succession, and

acid diffusion into the cotyledons (Streule et al., 2024). Therefore, excess pulp can disrupt the natural progression of microorganisms that drive the conversion of sugars and acids into flavor precursors, leading to uneven fermentation and higher acidity (Kongor et al., 2016). Conversely, too little pulp may reduce microbial activity and limit the production of key flavor precursors (Haruna et al., 2024a).

Careful management of pulp quantity is therefore essential. Managing pulp levels ensures proper aeration and oxygen diffusion, supporting efficient microbial activity and balanced fermentation (Kongor et al., 2016). It also promotes more uniform drying after fermentation by improving moisture distribution and airflow, helping to prevent mold contamination and off-flavor development (Streule et al., 2024). Thus, how the pulp is managed before fermentation begins plays a decisive role in guiding flavor development. This early-stage intervention may offer a valuable opportunity to control the fermentation process more effectively and improve flavor (Amanquah, 2013).

1.3 De-pulping process

Since pulp quantity plays a crucial role in shaping the formation of flavor compounds during fermentation, efforts have been made to control pulp levels prior to fermentation to improve cocoa flavor. One such approach is partial pulp removal, or de-pulping, which involves reducing the mucilaginous pulp surrounding cocoa beans before fermentation (López et al., 2022). Previous studies have indicated that reducing pulp content by 10–30% does not compromise the quality of the fermented and dried beans (Bangerter et al., 1993; Schwan & Wheals, 2004), and in some cases, it may even enhance flavor development.

Various de-pulping methods have been explored, including pod storage, mechanical de-pulping, and pre-drying (Afoakwa et al., 2014). Pod storage, the most studied technique (Hamdouche et al., 2019; Hinneh et al., 2018, 2019, 2020), involves storing harvested pods for 3–5 days to allow natural enzymatic breakdown of the pulp. However, to accelerate this process, faster alternatives such as pre-drying and mechanical de-pulping have gained attention (López et al., 2022). Pre-drying achieves pulp reduction within a day by spreading the beans in a single layer to allow for natural drying. This method has been shown to have potential not only in reducing defective beans (Gaviláñez Luna et al., 2018), but also in improving flavor by reducing bitterness and astringency (López et al., 2022). Studies have also reported that pre-drying enhanced volatile compounds in fine-flavor varieties (Moreno-Rojas et al., 2023) and improved fermentation efficiency, bean integrity, and overall quality (Haruna, et al., 2024b).

In addition, mechanical de-pulping methods offer ways to remove pulp within minutes using tools by physical rubbing, providing faster pulp reduction and potentially improving drying efficiency (Bangerter et al., 1993; Haruna et al., 2024a). However, this method carries the risk of bean bruising and enzymatic changes that may affect fermentation outcomes (Amanquah, 2013). Recent studies have reported mixed results: while some found an increase in bitterness (Marwati et al., 2024), others observed improvements in chocolate flavor and highlighted the potential for pulp valorization (Haruna et al., 2024a; Silveira et al., 2024).

Although various de-pulping methods are known to effectively reduce pulp content, they differ in principle and processing conditions, and it remains unclear whether these differences result in distinct effects on cocoa flavor. Moreover, despite the potential

benefits reported for pre-drying and mechanical de-pulping, no study has yet investigated how these treatments alter the chemical composition of cocoa beans or how such chemical changes correlate with flavor quality. Therefore, an approach that integrates metabolite profiling with sensory analysis is needed to clarify how different pulp reduction strategies influence cocoa quality and flavor development throughout the postharvest process.

1.4 Metabolomics approach

Understanding how different de-pulping methods affect cocoa quality and flavor requires a comprehensive analysis of the underlying chemical changes that occur during the postharvest and processing stages. Metabolomics provides a powerful tool to achieve this by enabling detailed profiling of chemical compositions that influence food quality and sensory characteristics (Putri & Fukusaki, 2014). In food research, metabolomics has been widely applied to assess how different processing treatments shape metabolite profiles (Jacobs et al., 2021), making it an especially valuable tool for assessing how different de-pulping strategies influence the biochemical and sensory outcomes of cocoa processing.

Previous studies have demonstrated that metabolomics is an effective approach for investigating the biochemical processes involved in the cocoa postharvest process. It has been used to monitor changes in both volatile and non-volatile compounds across key processing stages, including fermentation (Hanifah et al., 2022; Herrera-Rocha et al., 2021), drying (Moreno-Rojas et al., 2023; Rodriguez-Campos et al., 2011; Tejeda et al., 2023; Utrilla-Vázquez et al., 2020), roasting (Hinne et al., 2020; Marseglia et al., 2020), and chocolate production (Guzmán Penella et al., 2023; Llano et al., 2024). These studies

highlight the usefulness of metabolomics for tracking molecular transformations that contribute to cocoa flavor development (Herrera-Rocha et al., 2021; Silveira et al., 2024).

Among the analytical platforms used in metabolomics, gas chromatography–mass spectrometry (GC-MS) has been extensively applied in cocoa studies to detect a wide range of compounds and is well suited for examining metabolite profiles in cocoa beans (Afifah et al., 2024). GC-MS is commonly employed due to its robustness and high sensitivity (Putri et al., 2022). GC-MS-based metabolomics is particularly effective for profiling both non-volatile compounds—such as sugars, amino acids, organic acids, and flavanols—and volatile compounds, which together contribute to the sensory characteristics of cocoa (Putri et al., 2024). Additionally, metabolomics data can be correlated with sensory and physical analyses, enabling a more comprehensive understanding of how specific biochemical changes affect flavor perception (Escobar et al., 2021; Yue et al., 2024).

By linking metabolite profiles with sensory attributes, metabolomics enables a deeper understanding of how early-stage interventions, such as pulp removal, affect flavor development throughout the postharvest process. While previous studies on cocoa have applied metabolomics to individual processing stages—such as fermentation, drying, or roasting—none have comprehensively tracked how early interventions influence metabolite dynamics across all subsequent stages, particularly in relation to de-pulping. Through this approach, the study establishes the first metabolomic framework to reveal how different de-pulping methods, despite achieving similar pulp reduction, affect biochemical changes and flavor outcomes. This integrative approach provides new insight into how early pulp management influences cocoa quality throughout the

postharvest process, providing a basis for flavor optimization in cocoa postharvest handling.

1.5 Objective and Strategy

The objective of this study is to evaluate the impact of different de-pulping methods on metabolite changes that improve cocoa beans' flavor quality through the metabolomics approach. To achieve these objectives, the study employs two strategies: first, evaluating the impact of different de-pulping methods on cocoa liquor flavor modulation through metabolite profiling and sensory evaluation; and second, by investigating the key metabolites affected by different de-pulping methods along postharvest process to elucidate de-pulping process related metabolites. It is hypothesized that distinct de-pulping strategies will lead to specific alterations in key metabolites, which in turn will differentially affect the sensory characteristics of cocoa.

1.6 Thesis outline

This dissertation is organized into four chapters. **Chapter 1** introduces the importance of flavor in cocoa beans and explores the potential of the de-pulping process to improve cocoa flavor. It also discusses how metabolomics can address the existing research gaps.

Chapter 2 evaluates the effects of two different de-pulping methods—mechanical de-pulping and pre-drying—on cocoa liquor flavor and metabolite composition, combining metabolomics and sensory analysis. This chapter identifies how these de-pulping methods influence flavor and metabolite profiles across different bean types.

Building on these findings, **Chapter 3** delves deeper into how metabolite changes immediately after de-pulping affect the subsequent postharvest stages. This chapter elucidates how these initial biochemical shifts influence metabolite development throughout the postharvest process and ultimately shape the flavor of the end product.

Finally, **Chapter 4** presents the conclusions drawn from this study and offers perspectives for future research on optimizing cocoa flavor through improved postharvest practices.

Chapter 2

Evaluating the impact of different de-pulping methods on cocoa liquor through metabolite profiling and sensory evaluation

2.1. Introduction

De-pulping, or partial pulp removal, is an additional step in the cocoa postharvest process that reduces some of the pulp surrounding the beans after harvesting and before fermentation (Afoakwa et al., 2014; Streule et al., 2024). This process has shown potential to improve cocoa bean quality and is associated with improved flavor quality. Several studies have indicated that reducing pulp by approximately 25% does not compromise the quality of the fermented and dried beans (Amanquah, 2013; Bangerter et al., 1993; Marwati et al., 2024). Therefore, de-pulping is considered a promising alternative to improve cocoa flavor quality.

Pre-drying and mechanical de-pulping are the most employed method for de-pulping (Afoakwa et al., 2014). In pre-drying, cocoa beans are spread in a single layer and allowed to dry naturally for a day (López et al., 2022), while mechanical de-pulping accelerates pulp removal by physically scraping the beans against perforated stainless steel using specialized tools (Haruna et al., 2024a). Both methods have shown positive effects, such as reducing the occurrence of defective beans, decreasing bitterness and astringency, and improving fermentation efficiency and overall bean quality (Gavilánez Luna et al., 2018; Haruna et al., 2024a; López et al., 2022). Nevertheless, mechanical de-pulping has also been associated with increased bitterness in certain cases (Marwati et al.,

2024), although others have reported improved chocolate flavor and its potential for pulp by-product utilization (Silveira et al., 2024).

Although earlier studies have demonstrated the potential of de-pulping to improve cocoa flavor, few have directly compared these two methods under controlled pulp reduction levels. While both approaches can achieve similar degrees of pulp removal, their differing mechanisms may result in distinct flavor outcomes. Furthermore, most previous studies have not fully investigated the metabolite changes induced by de-pulping or how these changes influence flavor development. A comprehensive study integrating sensory analysis with metabolomic profiling is therefore needed to better understand how different de-pulping methods affect cocoa quality and flavor (Escobar et al., 2021).

Building upon the research hypothesis proposed in Chapter 1—that different de-pulping methods may distinctly affect flavor and be associated with specific changes in key metabolites—this chapter aims to evaluate the impact of pre-drying and mechanical de-pulping on cocoa liquor flavor and metabolite composition. By integrating GC-MS-based metabolomic profiling with sensory evaluation, this study seeks to identify key metabolites influenced by each de-pulping methods and clarify how these differences contribute to flavor modulation in cocoa liquor.

2.2 Materials and Methods

2.2.1 Vegetal materials

Two clones of *Theobroma cacao* varieties were used in this study: KW516 (Forastero) represents the bulk variety, and MCC02 (Trinitario) represents the fine-flavor variety from Indonesian Coffee and Cocoa Research Institute (ICCRI). These clones were

considered as bulk and fine cocoa based on sensory analysis conducted by ICCRI in 2022. For each clone, a single composite sample ($n = 1$) was prepared by pooling multiple pods and analyzed as a representative biological replicate. Healthy and ripe cocoa pods were harvested in April 2024 under the same cultivation management practices. The geographical location is approximately 8°15'26" south latitude and 113°36'46" east longitude, at an altitude of 45 m above sea level, with an average rainfall of 570 mm, and an average temperature of 28 °C.

2.2.2 Experimental design

This study evaluated pre-drying and mechanical de-pulping as alternative methods for reducing cocoa pulp, in comparison to the traditional fermentation process (control). An overview of the procedures is illustrated in **Fig. 2.1**. In the control, both bulk and fine cocoa beans were removed from the pods immediately after harvest and placed directly into fermentation boxes. For the pre-drying and mechanical de-pulping treatments, the beans underwent the respective processes before being placed into fermentation boxes. After fermentation, all samples followed the same postharvest steps, including drying, roasting, cracking, winnowing, and milling to produce cocoa liquor. The resulting cocoa liquor was then subjected to metabolomics analysis and sensory evaluation to assess changes in flavor profile and their correlation with metabolite composition.

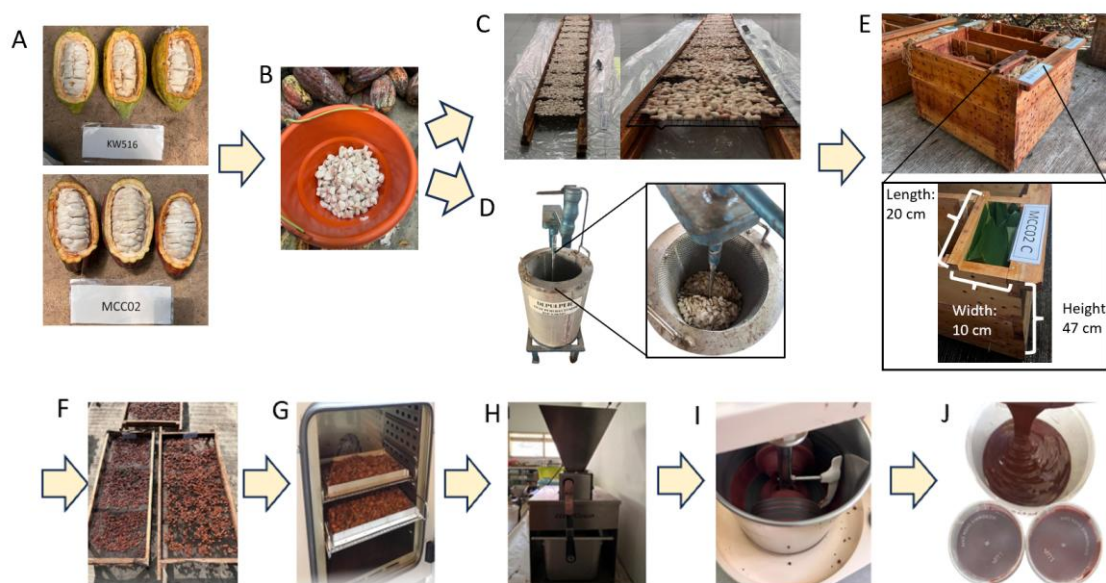


Fig 2.1. Preparation for de-pulping: A) Pod breaking of cocoa clones (KW516 and MCC02), B) Extraction of cocoa seeds, C) De-pulping by pre-drying, D) De-pulping by mechanical de-pulper, E) Fermentation, F) Sun drying, G) Roasting, I) Grinding, J) Cocoa liquor

2.2.3 Pod breaking and de-pulping processes

This study employed two de-pulping approaches, namely pre-drying and mechanical de-pulping, each of which was compared against the traditional (control) method. In the traditional (control) method, cocoa beans were removed from the pods immediately after harvest and placed directly into a fermentation box. In the pre-drying method, 15 kg of beans were spread in a single layer on a stainless-steel wire mesh (1 cm $\times$ 1 cm openings), which was elevated 8 cm above the floor to allow excess pulp to drain off, as illustrated in **Fig. 2.2**. The beans were left for 18 h at room temperature (20–25 °C) and 50%–70% relative humidity, following pod opening. This process resulted in a 25% reduction in pulp content.

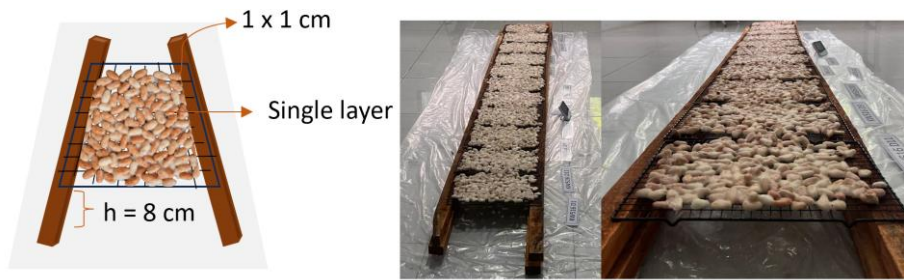


Fig 2.2. Design and actual application of the pre-drying

For mechanical de-pulping, 15 kg of beans were placed into a stainless-steel mechanical de-pulper after harvesting and removal from the pods. The de-pulper was a cylindrical device consisting of an outer body and a fixed inner chamber as depicted in **Fig 2.3**. The inner chamber had a diameter of 40 cm, while the outer cylinder measured 42 cm in diameter and 50 cm in height, creating a 2 cm gap between the two walls. The inner chamber was made of perforated stainless steel, allowing the removed pulp to pass through into the surrounding gap. Inside the chamber, the beans were stirred using a manually powered agitator, rotated manually by hand at around 60 rotations per minute. This stirring caused the beans to rub against the perforated surface, helping to separate the pulp. The process was carried out for approximately 15 minutes, until about 25% of the pulp was removed. After each treatment, the beans were placed into fermentation boxes.

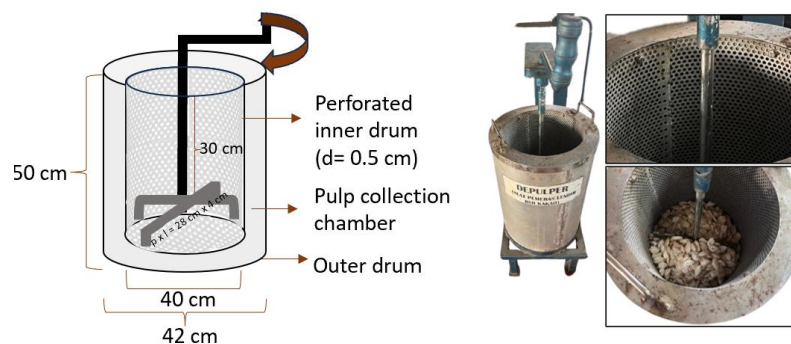


Fig 2.3. Design and actual application of the mechanical de-pulper

2.2.4 Postharvest process and sample preparation

Following the de-pulping treatments, each cocoa sample was transferred into identical fermentation boxes made from teak wood, a material traditionally favored in cocoa fermentation for its durability and stability under varying environmental conditions (Tunjung-Sari et al., 2021). The dimensions of the box are 20 cm × 10 cm × 47 cm (length × width × height) and feature 1 cm-diameter perforations spaced 3 cm apart to ensure proper aeration during fermentation, as illustrated in **Fig. 2.1E**. Fermentation was carried out with 10 kg of cocoa beans per clone in each box for a total of five days. On the second day, the beans were turned manually using a clean scoop by lifting and mixing the top and bottom layers within the same box to promote aeration and ensure uniform fermentation. To ensure consistency across different batches, all samples were processed identically during the postharvest processing.

After the fermentation process, the beans were spread in a single layer on bamboo trays for natural sun-drying over four days, with daily mixing to achieve a final moisture content below <8 %. After sun-drying, 500 g of the fermented beans were roasted according to the International Standard for the Assessment of Cocoa Quality and Flavor (ISCQF) (Cacao of Excellence, 2023), following the protocol detailed in **Table S1**, using an oven (Binder, Tuttlingen, Germany). The roasted beans were cracked using a manual cracker and winnowed with a basic winnower (Cocoa Town™, Alpharetta, USA) to obtain roasted nibs. The nibs were then ground in a melanger for 5 hours to produce cocoa liquor. The cocoa liquor was then packaged and stored at −30 °C for a maximum of 4 weeks prior to analysis.

2.2.5 Sensory evaluation

Sensory analysis was conducted in accordance with the guidelines of the International Standard for the Assessment of Cocoa Quality and Flavor (ICSQF). Cocoa liquor samples were assessed in a blind test by a panel of six trained ICCRI board members, consisting of two males and four females aged 30–50. The panelists had extensive experience and underwent regular structured training, including monthly refresher sessions to maintain their sensory skills. Prior to the evaluation, calibration exercises were conducted, during which panelists scored identical samples and discussed the results to ensure consistency in scoring criteria. For the test, approximately 2 g of cocoa liquor per sample was placed in a plastic cup and heated to 40 °C until the cocoa liquor fully melted. Samples were coded with alphabetical codes and presented in random order to reduce bias. Plain crackers and still water were provided to panelists for palate cleansing between samples. Sensory attributes were rated on a 10-point scale, ranging from 0 (not detectable) to 10 (very intense), according to the Cacao of Excellence (2023) guidelines. The evaluated flavor attributes were categorized as core attributes—cocoa, acidity, bitterness, and astringency—and complementary attributes, including floral, fresh fruit, browned fruit, spicy, woody, nutty, browned/roast, dirty/dusty, meaty/animal, putrid/over-fermented, smoky, moldy, other off-flavors, and overall global quality. Panelists performed evaluations individually and were encouraged to note any additional taste perceptions beyond the predefined attributes. To ensure reproducibility, the coefficient of variation (CV) was calculated for each attribute across panelists, providing a quantitative measure of consistency in scoring.

2.2.6 Sample preparation and gas chromatography-mass spectrometry (GC-MS) analysis for non-volatile compounds after derivatization

Before extraction, cocoa liquor samples were quenched in liquid nitrogen and homogenized using a multibead shocker (Yasui Kikai, Osaka, Japan). Metabolite extraction was performed with a solvent mixture of methanol, water, and chloroform (5:2:2, v/v/v), containing ribitol (0.1 mg/mL) as an internal standard. The extraction solvent was added to 2-mL tubes containing 5 mg of the homogenized sample. The extraction and derivatization procedures followed a protocol adapted from Hanifah et al. (2022). After derivatization, metabolite profiling was performed using a GC-MS-QP2010 Ultra system (Shimadzu, Kyoto, Japan) equipped with an InertCap 5MS/NP column (35 m × 0.18 mm I.D., 0.18 µm film thickness; GL Sciences, Tokyo, Japan). The mass spectrometer was tuned, and 1 µL of a derivatized sample was injected in split mode at a ratio of 25:1 v/v, with an injection temperature of 280°C, in a random order. Hydrogen was used as carrier gas, maintained at a linear velocity of 60 cm/s with a flow rate of 1.4 mL/min. The column temperature was initially set at 80 °C for 2 min, then increased to 330 °C at a rate of 10 °C/min and maintained for 6 min. The interface and ion source temperatures were set at 310 and 280 °C, respectively. Electron ionization (EI) was applied at 1.00 kV, and mass spectra were recorded across an m/z range of 85–500. Before the first sample injection, a standard mixture of alkanes (C9–C40) was injected to calculate the retention index (RI). Data processing and metabolite identification were performed according to Hanifah et al. (2022).

2.2.7 Headspace solid-phase microextraction (HS-SPME) arrow GC-MS analysis for volatile compounds

The HS-SPME arrow GC-MS analysis was performed using a modified version of a previously described procedure (Kitani et al., 2022; Moreno-Rojas et al., 2023; Velásquez-Reyes et al., 2023). A pooled Quality Control (QC) sample was prepared by combining 1 g of each sample into a 20 mL screw vial with a magnetic screw cap (Shimadzu, Japan). The SPME was performed automatically using a multifunctional autosampler AOC-6000 equipped with DVB/CAR/PDMS (20 mm x o. d. 1.5 mm, $df = 120\ \mu\text{m}$) (Shimadzu, Japan). For analysis, a total of 1.5 g of samples ($n = 3$) and the pooled QC were preconditioned at 250 °C for 15 min. The samples were then incubated at 60 °C for 30 min with shaking at 250 rpm. Extraction was performed by directly immersing the fibers in the sample vial at 60 °C for 30 min. After extraction, the fiber was inserted into the GC inlet, and the analyte was injected by thermal desorption at 250 °C for 2 min.

GC-MS analysis was carried out using a GC-MS-TQ8040 system (Shimadzu, Kyoto, Japan) equipped with an InertCap column (60 m $\times$ 0.32 mm I.D., 0.5 μm film thickness). The analytes released from the SPME fiber were introduced with a split ratio of 1:25. Helium was used as the carrier gas at a linear velocity of 40.7 cm/s and a flow rate of 2.5 mL/min. The column oven temperature was initially set at 40 °C for 3 min, then increased to 250 °C at a rate of 3 °C/min, and maintained at 250 °C for 10 min. The mass spectrometer was operated in the scan mode (m/z 24–350) with the ion source and interface temperature set to 250 °C. To determine retention indices (RIs) for identification, a standard of fatty acid ethyl esters (C2–C24) was injected at the beginning of the analysis.

Data processing followed the workflow outlined by Kitani et al. (2022). All data can be accessed in <https://repository.massbank.jp/MPST000141.0>.

2.2.8 Statistical analysis

The annotated metabolites were analyzed using multivariate statistical methods, including Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression, performed with SIMCA-P 13.0.1 using auto-scaling without data transformation (Umetrics, Umea, Sweden). PCA was applied to reveal patterns and groupings within the metabolite dataset, helping to characterize how differences in sensory scores or metabolite concentrations drive sample differentiation. PLS regression was employed to explore the potential relationships between metabolites (explanatory variables) and mean sensory scores (response variables), providing insights into how metabolites may influence sensory attributes. For each metabolite, variable importance in projection (VIP) scores and regression coefficients were calculated. Additionally, *t*-tests (RIKEN, Kanagawa, Japan) were conducted to identify metabolites that significantly differentiated the sample groups.

2.3 Results and Discussion

2.3.1 Effect of pre-drying on the sensory and metabolome profile of cocoa liquor

Pre-drying is one of the de-pulping methods that allows natural pulp drainage. This process may alter the microenvironment during fermentation and influence metabolite formation, shaping the flavor of the final cocoa liquor. Therefore, this study first evaluated the impact of pre-drying compared to the control (without de-pulping) on the sensory attributes of the final cocoa liquor. Analyzing cocoa liquor is important

because it can be used to directly assess the sensorial impact of the pre-drying process. By correlating these sensory attributes with metabolite changes, this study revealed how pre-drying influences the overall flavor and quality of the final product (Velásquez-Reyes et al., 2023).

Principal Component Analysis (PCA) was used to visualize the sensory profiles of cocoa liquor from pre-dried and control (**Fig. 2.4A**). The first two components accounted for 57.3% (PC1) and 31.8% (PC2) of the total variation. In this analysis, panelists identified 12 attributes (**Fig. 2.4B; Table S2**), with no off flavors detected in any of the samples. The PCA revealed that PC1 effectively separated the samples based on the pre-drying process, highlighting notable changes in the sensory profiles resulting from pre-drying. In bulk cocoa liquor, control samples were linked to higher levels of undesirable core attributes—bitterness and astringency. In contrast, the pre-dried bulk cocoa liquor exhibited more dominant complementary attributes, such as fresh fruits, and core attributes of acidic notes. These changes indicate that pre-drying positively altered the sensory profile by reducing undesirable bitterness and astringency in bulk cocoa and may enhance its overall quality. For fine cocoa, the control samples were characterized by floral, browned fruit, woody, nutty, and roasted notes. However, the pre-dried fine cocoa liquor shifted to an emphasis on spiciness, acidity, global quality, and cocoa attributes. These interesting changes demonstrate that pre-drying can alter the sensory profile of fine cocoa liquor without negatively impacting its taste. Overall, these findings demonstrate that the pre-drying treatment has a positive impact on the sensory profile of both bulk and fine cocoa varieties.

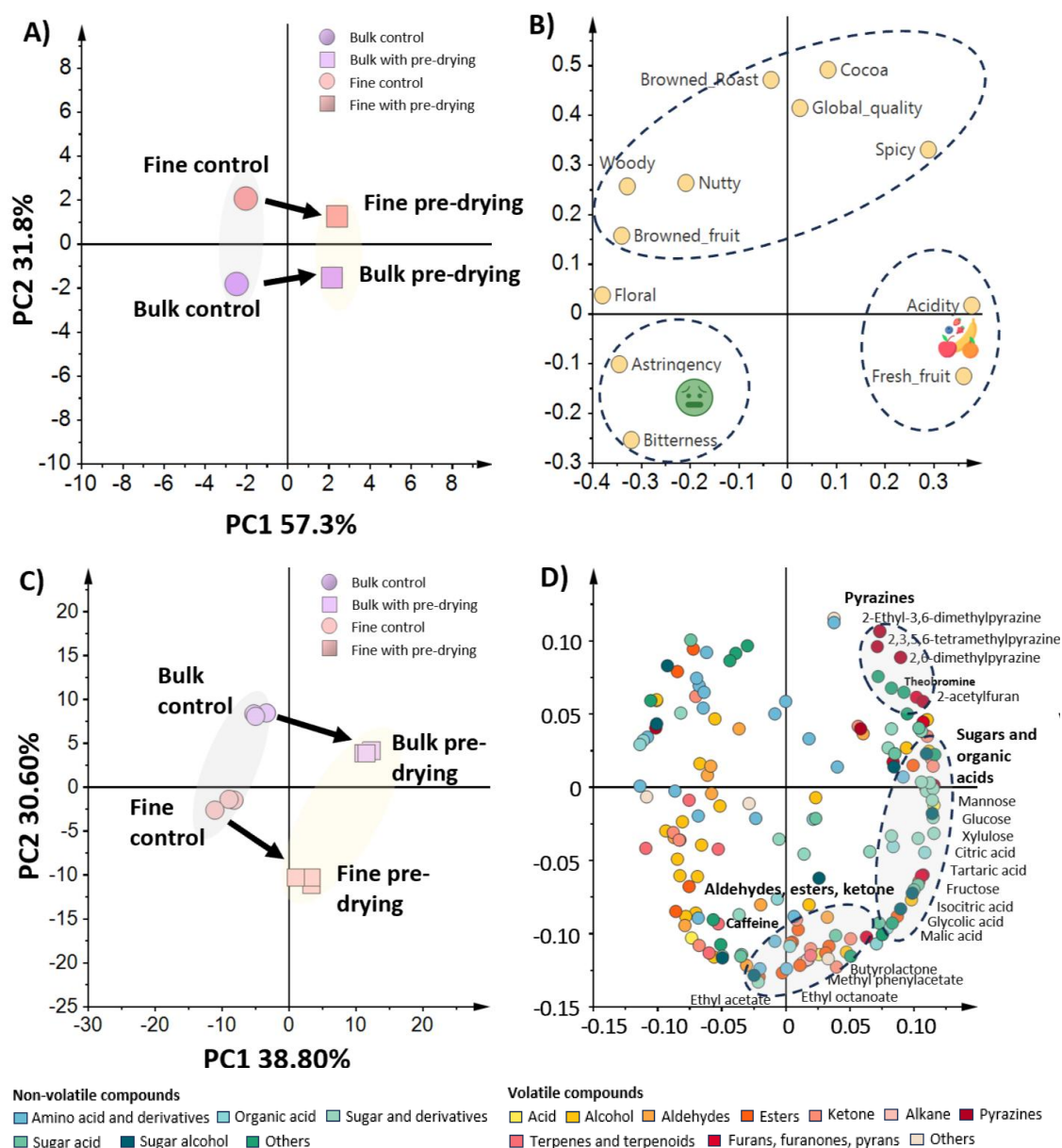


Fig. 2.4. Sensory evaluation of cocoa liquor from bulk and fine cocoa (control and pre-dried): (A) PCA score plot, (B) PCA loading plot. Scaling: auto, transformation: none, mean ($n = 3$). Metabolome profile (non-volatile and volatile) comparison of cocoa liquor from bulk and fine cocoa (control and pre-dried): (C) PCA score plot, (B) PCA loading plot. Scaling: auto, transformation: auto, replicate: $n = 3$.

Metabolomic analysis was further employed to highlight and evaluate the differences between cocoa liquors derived from pre-dried and non-pre-dried beans. Both non-volatile and volatile compounds were analyzed. As shown in the PCA score plot (**Fig. 2.4C**), PC1 effectively distinguished the samples based on the pre-drying treatment, reflecting the observed sensory differences. The key metabolites contributing to this separation are shown in **Fig. 2.4D** and **Table S3**.

The loading plot revealed that in pre-dried bulk cocoa, volatile compounds were improved, with higher levels of esters, ketones, pyrazines, and furans, including ethyl heptanoate, 3-methoxybutyl acetate, 2,3-butanedione, acetoin, 2,3,5-trimethylpyrazine, and 2-acetylfuran, compared to the bulk cocoa control. These desirable compounds potentially enhance cocoa quality, as ketones and furan are favorable for cocoa quality, esters are linked to fruity notes, and pyrazines indicate adequate fermentation (Moreno-Rojas et al., 2023). By contrast, fine cocoa liquor contained a wider range of volatile compounds than bulk cocoa, confirming the established understanding that fine-flavored cocoa varieties generally have more complex flavor profiles than bulk cocoa (Escobar et al., 2021; Putri et al., 2024). Both the fine cocoa control and pre-dried samples contained positive volatile compounds of different compositions. The fine cocoa control was characterized by 2-nonanone, benzaldehyde, linalool, and cis- β -ocimene, which contribute to nutty, fruity, and flowery notes (Guzmán Penella et al., 2023), whereas pre-dried fine cocoa had higher levels of methyl phenylacetate, ethyl acetate, ethyl octanoate, 2-methyl pyrazine, butyrolactone, γ -nonanolactone, and acetol, which are associated with cocoa and fruity notes (Hinne et al., 2018).

Notably, in terms of non-volatile metabolites, pre-dried cocoa beans exhibited higher levels of reducing sugars such as glucose and fructose, whereas control beans had higher sucrose. Sucrose is converted to glucose and fructose during fermentation by invertase in the cotyledons (Hansen et al., 1998; Misnawi et al., 2002). Higher sucrose levels have been associated with lower fermentation index in other studies, indicating suboptimal fermentation (Hinne et al., 2018; Rottiers et al., 2019). This correlation supports our findings, as we also observed that pre-dried cocoa beans had a higher fermentation index than control beans (**Fig. S1**), suggesting that pre-drying promotes better fermentation and contributes to improved flavor development. Additionally, the inherent differences between bulk and fine cocoa varieties were also confirmed in the final cocoa liquor. Key genotype markers—caffeine, were found higher in fine cocoa, and theobromine, and higher in bulk cocoa—aligned with their characteristic profiles in this study, supporting established knowledge (Borja Fajardo et al., 2022; Brunetto et al., 2007; Velásquez-Reyes et al., 2023).

In summary, pre-dried cocoa beans demonstrated notable improvements. This is the first study to demonstrate that pre-drying improves bulk cocoa until in the cocoa liquor, whereas previous research only reported such improvements in fermented beans of fine cocoa varieties (Moreno-Rojas et al., 2023). This finding is particularly significant, as it suggests that bulk cocoa can potentially achieve flavor characteristics associated with fine cocoa through controlled pre-drying treatments.

2.3.2. Effect of mechanical de-pulping on the sensory and metabolome profile of cocoa liquor

Mechanical de-pulping removes pulp rapidly, achieving the same reduction percentage as pre-drying but with distinct effects on flavor. To assess whether mechanical de-pulping had similar improvements, the sensory characteristics of cocoa liquor from mechanically de-pulped and non-de-pulped beans were analyzed using PCA. As shown in **Fig. 2.5A**, PC1 (70.10 %) captured the primary variation caused by mechanical de-pulping. Unlike pre-drying, mechanically de-pulped cocoa liquor exhibited more pronounced bitterness and astringency compared to the control in both bulk and fine cocoa. Sensory data (**Table S4**) showed significantly higher bitterness and astringency scores in de-pulped samples ($p < 0.05$), indicating that mechanical de-pulping consistently intensified these undesirable attributes across both cocoa types. These finding contrasts with (Haruna et al., 2024a), who reported that reducing pulp content by 10–30 % mechanically reduced bitterness and astringency, along with improved well-fermented beans. However, our results align with Marwati et al. (2024), who observed over-fermentation and increased bitterness from mechanical de-pulping, potentially due to increased fungal activity accelerating fermentation and causing off flavors. Bitterness in cocoa is often attributed to methylxanthines and polyphenols (Febrianto & Zhu, 2022), and the stronger bitter taste may indicate higher concentrations of monomeric or oligomeric polyphenols resulting from incomplete oxidation.

Cocoa flavor is highly complex and influenced by the composition of both non-volatile and volatile metabolites (Aprotosoie et al., 2016). To explain the observed sensory differences, principal component analysis (PCA) was performed on the

metabolite data using a dataset comprising 85 non-volatile and 85 volatile metabolites (**Tables S13 and S14**). A clear separation between mechanically de-pulped cocoa liquor and the control was observed in the PCA score plot (**Fig. 2.5C**). The loading plot (**Fig. 2.5D–Table S5**) indicated that both bulk and fine mechanically de-pulped cocoa liquor exhibited lower overall levels of non-volatile and volatile compounds compared to the control. Moreover, among the metabolites that were higher in mechanically de-pulped cocoa liquor, most were undesirable compounds, including butyric acid, butanoic acid, isobutyric acid, 1-phenylethyl alcohol, valeraldehyde, and 1-pentanol—compounds associated with rancid, green, acidic, pungent, bitter, and astringent flavors (Spada et al., 2022). Notably, compounds such as butanoic acid, butyric acid, and isobutyric acid are considered off-flavors that negatively affect cocoa aroma (Bonvehí, 2005; Velásquez-Reyes et al., 2023). The combination of lower desirable metabolites and higher undesirable compounds likely explains the more pronounced bitterness and astringency observed in sensory evaluations. This also clarifies why mechanically de-pulped cocoa liquor shows a different sensory profile compared to pre-dried cocoa liquor, as pre-dried samples have higher amounts of sugars, organic acids, and aromatic compounds like pyrazines, aldehydes, esters, and ketones that impart fruity, cocoa, and roasted notes (Guzmán Penella et al., 2023).

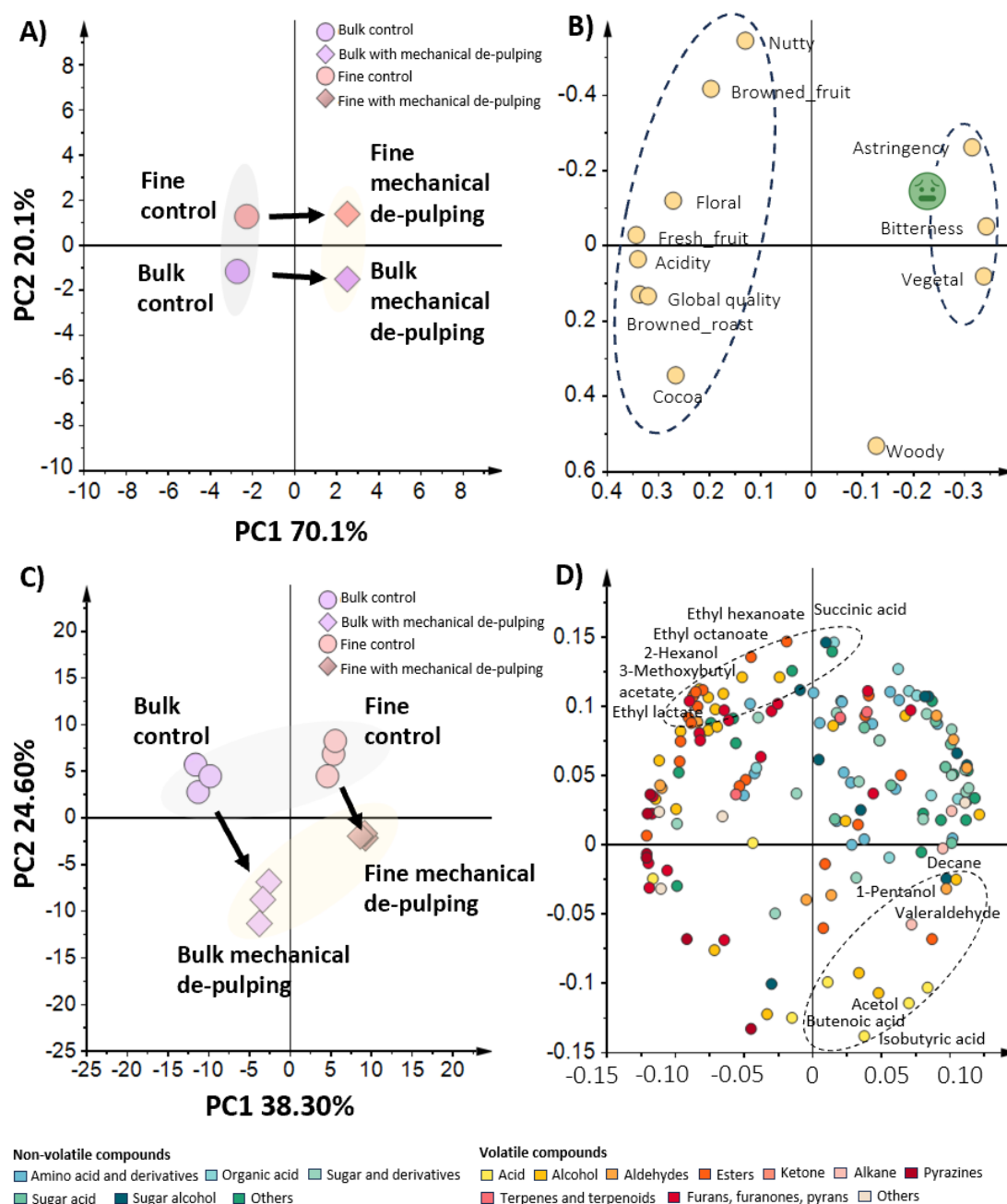


Fig. 2.5. Sensory evaluation of cocoa liquor from bulk and fine cocoa (control and mechanically de-pulped): (A) PCA score plot, (B) PCA loading plot. Scaling: auto, transformation: none, mean (n = 3). Metabolome profile (non-volatile and volatile) comparison of cocoa liquor from bulk and fine cocoa (control and mechanically de-pulped): (C) PCA score plot, (D) PCA loading plot. Scaling: auto, transformation: auto, replicate: n = 3.

In summary, mechanical de-pulping failed to improve flavor, as it increased undesirable attributes, namely bitterness and astringency. This contrasts sharply with the favorable sensory outcomes observed in pre-dried samples, despite both treatments reducing pulp by the same amount. These findings highlight that the de-pulping method has a critical influence on flavor development by shaping distinct metabolite profiles. This underscores the need for a deeper understanding of how de-pulping affects early metabolite changes and their impact throughout the subsequent cocoa postharvest process, which will be further explored in the next chapter.

2.3.3. Correlation between metabolites and sensory attributes in the improved pre-dried cocoa liquor

Since only pre-drying led to improved sensory quality, partial least squares regression (PLS-R) analysis was conducted to better understand the key metabolites contributing to these favorable attributes. Identifying these metabolites provides insight into the biochemical basis of flavor improvement and supports the development of more targeted postharvest strategies. The regression analysis assessed the contributions of (x variables, non-volatile = 83 and volatile compounds = 95 (detailed in **Tables S13** and **S14**) to the sensory attributes (y variables, n = 12) of the cocoa liquor from pre-dried and non-pre-dried beans. An overview of the correlation plot on axes t1 and t2 is shown in **Fig. 2.6**, while specific compounds with $VIP \geq 1$ that positively correlate with sensory attributes are detailed in **Table S6**. The resulting PLS-R model showed excellent fit and predictive ability: the proportion of variance explained in the metabolite data (R^2X) was 0.858, in the sensory data (R^2Y) was 0.997, and the predictive ability (Q^2) was 0.988, indicating that the model reliably captures the relationships between metabolite profiles

and sensory attributes. Model validation using a 200-permutation test further confirmed the reliability of the correlation, as the Q^2 and R^2 values of the permuted models were consistently lower than those of the actual dataset (**Fig. S2**) (Kim et al., 2023; Triba et al., 2015).

The correlation analysis identified several key metabolites associated with bitterness and astringency, which were dominant in bulk cocoa liquor but reduced following pre-drying. These undesirable attributes were linked to specific non-volatile compounds, including lysine, propionic acid, quinic acid, valine, epicatechin, catechin, mannitol, glucaric acid, and lactic acid. This finding aligns with previous reports indicating that polyphenols, particularly epicatechin and catechin, significantly contribute to the bitterness and astringency of cocoa products (Dagan-Wiener et al., 2019; Kim & Keeney, 1983; Stark et al., 2006). Additionally, lysine—an amino acid produced during carbohydrate fermentation—has a slightly bitter taste, potentially enhancing bitterness perception in cocoa (Marcus, 2019). Quinic acid, derived from chlorogenic acid during roasting, is also known to intensify bitter and astringent notes (Warmke & Belitz, 1993).

Conversely, the improved sensory attributes—fresh fruit and acidity—observed in pre-dried bulk cocoa liquor were associated with higher levels of esters, organic acids, and sugars. Esters, such as methyl phenylacetate and ethyl octanoate, are known for their fruity aromas (Colonges et al., 2022), along with organic acids—including citric acid, tartaric acid, and malic acid—and sugars such as fructose, trehalose, and glycerol, were positively associated with these attributes. These compounds appeared alongside a marked reduction in metabolites associated with bitterness and astringency, highlighting a shift toward a more desirable flavor profile. Importantly, Pearson’s correlation analysis

(Fig. S3) confirmed that acidity and fresh fruit attributes were strongly correlated, suggesting that the acidity perceived here reflects a desirable fruity and refreshing character, rather than an undesirable sourness. This is consistent with previous reports identifying citric, malic, and tartaric acids as contributors to fruity, floral, and acidic notes in cocoa (Jinap & Dimick, 1990; Kim et al., 2023; Velásquez-Reyes et al., 2023). Furthermore, sugars like fructose have also been shown to enhance fresh fruit notes in cocoa (Sari et al., 2023), supporting their role in improved sensory perception.

As previously noted, fine cocoa liquor is typically associated with a complex aromatic profile primarily shaped by volatile compounds (Utrilla-Vázquez et al., 2020). In this study, the control fine cocoa liquor exhibited pleasant sensory attributes, including floral, woody, browned fruit, nutty, and roasted notes. Floral, woody, and browned fruit notes were found to be correlated with volatile compounds, such as 2,3-butanediol, linalool, and 1-phenylethyl alcohol, which have floral and fruity aromas (Guzmán Penella et al., 2023; Velásquez-Reyes et al., 2023). Compounds such as isoamyl lactate, 1-methoxy-2-propyl acetate, and 2-methylpyrazine impart fruity, honey, chocolate, and cocoa notes, explaining their link to woody and browned fruit attributes. Nutty attributes were associated with octanol acetate, benzaldehyde, and 2-nonane, with benzaldehyde known for its almond and nutty attributes (Guzmán Penella et al., 2023). Additionally, the browned roast characteristics were linked to cis- β -ocimene, 1-phenyl ethyl alcohol, 2-nonanol, and 2-hexanol, which have warm, floral, herbal, flowery, sweet, and honey-like aromas that may contribute to the browned roast perception (Bastos et al., 2019). Altogether, these associations highlight the contribution of volatile compounds to the distinctive and complex aroma profile of fine cocoa.

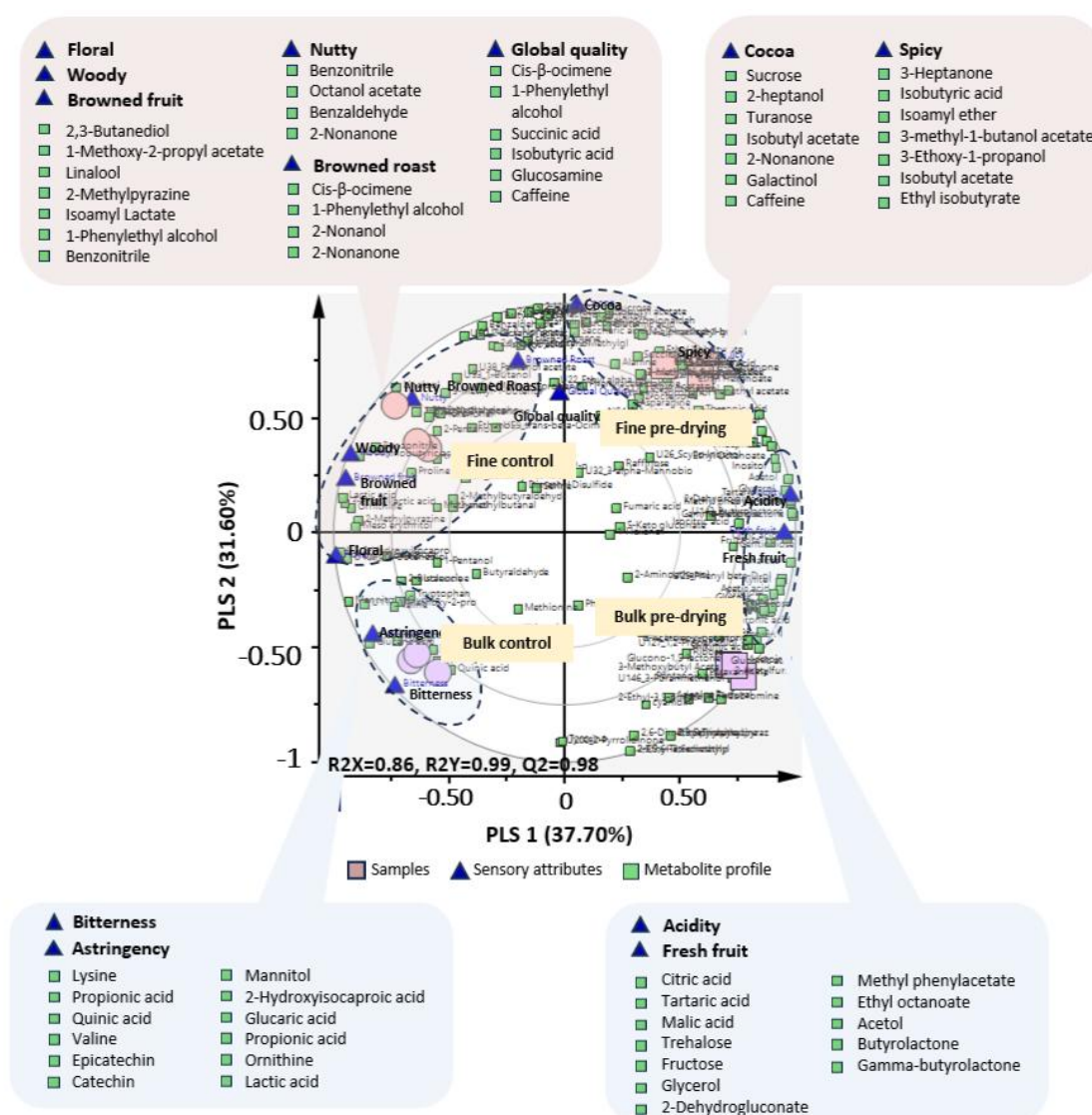


Fig. 2.6. PLS regression plot showing relationships between sensory attributes and metabolite profiles of control and cocoa liquor pretreated with pre-drying treatment ($R^2X = 0.858$, $R^2Y = 0.997$, $Q^2 = 0.988$). X-matrix: non-volatile and volatile compounds, Y-matrix: sensory attributes.

On the other hand, cocoa and spicy attributes—dominant in the liquor from pre-dried fine cocoa beans—were associated with a combination of non-volatile and volatile compounds. Cocoa attributes were linked to metabolites such as sucrose, 2-heptanol, turanose, isobutyl acetate, 2-nonanone, galactinol, and caffeine, each contributing sweet, fruity, or bitter notes (Velásquez-Reyes et al., 2023). Spicy attributes were associated

with compounds including 3-heptanone, isobutyric acid, isoamyl ether, 3-methyl-1-butanol acetate, 3-ethoxy-1-propanol, isobutyl acetate, and ethyl isobutyrate, which are known for their fruity, floral, and acidic aromas. However, due to the complexity of metabolite interactions and the inherent limitations of PLS regression analysis, some correlations may not fully explain the sensory attributes observed (Kitani et al., 2022).

2.3.4. Conclusion

The effects of both de-pulping methods were evaluated through sensory analysis and metabolomic profiling, revealing distinct differences in the metabolite composition of cocoa liquor. Despite achieving a comparable reduction in pulp, the two methods exerted markedly different impacts on flavor development. Pre-drying improved the cocoa liquor's sensory profile by enhancing fresh fruit and acidity attributes ($p < 0.05$) and reducing bitterness and astringency. In contrast, mechanical de-pulping failed to deliver similar results, instead increasing bitterness and astringency scores ($p < 0.05$). Partial Least Squares Regression (PLS-R) analysis linked 85 non-volatile and 85 volatile metabolites to key sensory attributes, identifying compounds such as organic acids (citric acid, malic acid, tartaric acid), sugars (fructose, trehalose), and esters (methyl phenylacetate, ethyl octanoate) as significant contributors to the desirable fresh fruit and acidity notes in pre-dried samples. Conversely, mechanical de-pulping resulted in elevated levels of undesirable metabolites—such as butyric acid, butanoic acid, 1-phenylethyl alcohol, and valeraldehyde—which corresponded with the increased perception of bitterness and astringency. These findings expand current knowledge by demonstrating, for the first time, the distinct effects of different de-pulping methods on cocoa flavor quality and their associated key metabolites. By integrating sensory

evaluation with metabolomic profiling, this study provides new insights into how early-stage postharvest modifications influence flavor formation in final cocoa liquor.

Chapter 3

Investigation of the effect of de-pulping on metabolite changes along the postharvest process to elucidate the de-pulping-related metabolites

3.1 Introduction

In the previous chapter, we demonstrated that different de-pulping methods resulted in distinct sensory profiles when compared to the traditional method without pulp reduction. Metabolomics analysis further revealed key differences in both volatile and non-volatile compounds present in the final cocoa liquor, which helped explain the observed variations in flavor. These findings indicate that such changes may originate early in the postharvest process—specifically, immediately after the de-pulping step, before fermentation begins. Since sugars, organic acids, and amino acids are known to serve as essential precursors during fermentation and subsequent stages, investigating how de-pulping affects these compounds during the process is important for understanding the mechanism behind the flavor modulation (Escobar et al., 2021).

Flavor development in cocoa is a complex, multi-stage process influenced by biochemical and microbial transformations that occur during postharvest handling (Aprotosoie et al., 2016; D. N. Putri et al., 2024). Each stage of the postharvest process, including fermentation, drying, roasting, and milling, plays a crucial role in determining the final flavor (Santander et al., 2021). During fermentation, microbial communities metabolize pulp sugars, generating heat and acids that diffuse into the beans, thereby activating enzymatic processes and contributing to the formation of key flavor precursors,

such as amino acids and reducing sugars (Calvo et al., 2021; Ordoñez-Araque et al., 2020; Streule et al., 2024). Drying then halts microbial activity while concentrating precursors and reducing moisture content. Roasting induces a series of thermal reactions, including Maillard and Strecker degradation, which produce volatile aroma compounds, while milling converts roasted nibs into cocoa liquor (Hinneht et al., 2019). Given that the postharvest process involves multiple stages, initial modifications to the raw beans—such as pulp removal—may be further influenced during subsequent stages as the beans progress through fermentation, drying, and roasting, ultimately contributing to flavor development in the final product.

Building on this hypothesis, this chapter investigates how different de-pulping methods alter metabolite profiles immediately after de-pulping and throughout the key postharvest stages. To answer this, cocoa beans were sampled before and after de-pulping, after fermentation, after roasting, and at the cocoa liquor stage. The samples were analyzed using gas chromatography–mass spectrometry (GC-MS) for non-volatile compounds following derivatization, and headspace solid-phase microextraction (HS-SPME-GC-MS) for volatile compound profiling. By tracking these changes, the study elucidates metabolites associated with de-pulping and their transformations during subsequent stages, providing insights into how early-stage interventions influence the final sensory characteristics of cocoa. Therefore, the objective of this chapter is to clarify how de-pulping modifies metabolite profiles and contributes to flavor development across the entire postharvest.

3.2 Materials and Methods

3.2.1 Vegetal materials

This study used two clones of *Theobroma cacao* varieties from the Indonesian Coffee and Cocoa Research Institute (ICCRI). KW516 (Forastero) represents the bulk variety, and MCC02 (Trinitario) represents the fine-flavor variety. These clones were planted in Jember, East Java, Indonesia, and harvested in April 2024. The geographical location is approximately 8°15'26" south latitude and 113°36'46" east longitude, at an altitude of 45 m above sea level, with an average rainfall of 570 mm, an average temperature of 28 °C, and under the same cultivation management.

3.2.2 Experimental design

To highlight differences between control and both types of de-pulped cocoa beans (pre-dried and mechanical de-pulped) in both bulk and fine cocoa varieties, metabolite profiling using GC-MS was conducted on sample from each stage of the cocoa postharvest process: raw beans (beans before fermentation) including before and after de-pulping process, fermented beans (raw beans after fermentation), roasted nibs (beans that were roasted, cracked, and winnowed), and cocoa liquor (roasted nibs that were ground). The same batches of beans were tracked across all stages to monitor metabolite changes throughout processing. The control, defined as raw beans not undergoing de-pulping, was compared with de-pulped beans in both bulk and fine cocoa varieties. Significant metabolite changes were analyzed to explore their influence from raw beans until the final cocoa liquor metabolome. All sample codes used for GC/MS analysis are listed in **Table 3.1**.

Table 3.1. List of sample codes used for GC–MS metabolite profiling

Cocoa types	Treatment	Postharvest stages	Sample Names
Bulk	Control	Raw beans	BC0
		Fermentation	BCF
		Roasting	BCR
		Liquor	BCL
	Pre-drying	Raw beans	BPR
		Fermentation	BPF
		Roasting	BPR
		Liquor	BPL
	Mechanical de-pulping	Raw beans	BMR
		Fermentation	BMF
		Roasting	BMR
		Liquor	BML
Fine	Control	Raw beans	FC0
		Fermentation	FCF
		Roasting	FCR
		Liquor	FCL
	Pre-drying	Raw beans	FPR
		Fermentation	FPF
		Roasting	FPR
		Liquor	FPL
	Mechanical de-pulping	Raw beans	FMR
		Fermentation	FMF
		Roasting	FMR
		Liquor	FML

3.2.3 Postharvest process and sample preparation

Cocoa pods were harvested and opened, and each 15 kg of raw beans was subjected to either pre-drying or mechanical de-pulping treatments. For the control samples (without de-pulping), raw cocoa beans were placed directly in the fermentation box without de-pulping. In the pre-drying process, beans were arranged in a single layer on a stainless-steel mesh (1 cm × 1 cm sieve) to allow the mucilage layer to dry. This process was conducted for 18 hours at a controlled room temperature of 25 °C and 50%–70% relative humidity — conditions typically used in laboratory environments. Pre-drying resulted in a 25% reduction of pulp relative to the total pulp-bean mass.

For mechanical de-pulping, 15 kg of freshly harvested beans were processed using a stainless-steel mechanical de-pulper. The de-pulper consisted of perforated drums that facilitated the separation of pulp from the beans. The pulp was scraped off by manually rotating the drum, and this was continued until a 25 % pulp reduction was achieved. After each treatment, beans were transferred into fermentation boxes. Raw bean samples were collected before and after both de-pulping treatments for pH analysis and were then sun-dried to a moisture content below 8% for subsequent GC-MS analysis. The dried raw beans were vacuum-sealed in plastic bags and stored at –30 °C.

Following the de-pulping treatments, all beans underwent the same postharvest process to ensure consistency across samples. Fermentation was carried out in identical teak wood boxes (20 cm × 23.5 cm × 47 cm) with perforated holes (1 cm diameter, spaced 3 cm apart). Each box contained a similar weight of 10 kg of cocoa beans per clone, which was maintained under similar environmental conditions with an average temperature of 28 °C and 85% relative humidity consistently monitored throughout the experiment. The

fermentation was conducted for five days, with manual turning performed on the second day to ensure homogenous fermentation. Temperature and pH data of the beans were collected every 24 h to monitor these processes (detailed in **Fig. S4**). After fermentation, the beans were spread in a single layer on a bamboo tray and naturally sun-dried for four days with daily mixing to achieve consistent moisture levels (<8%). Fermented beans were collected with vacuum-sealed packaging for fermentation index measurements and GC-MS analysis.

After sun-drying, 500 g of the fermented beans were roasted according to ISCQF standard (Cacao of Excellence, 2023) using a Binder oven (Tuttlingen, Germany), with roasting details provided in Table S1. The roasted beans were cracked using a manual cracker and winnowed using a basic winnower (Cocoa Town™, Alpharetta, USA) to obtain roasted nibs. A 50 g portion of roasted nibs was vacuum-packed for metabolomics analysis, while the remaining nibs were ground in a melanger for 5 hours to produce cocoa liquor. The cocoa liquor was stored at −30 °C in vacuum-sealed packaging.

3.2.4 Physicochemical measurement

The temperature and pH of the beans were monitored during fermentation. The average fermentation temperature was recorded every 24 h at the upper, middle, and bottom of the box, each spaced 20 cm apart, using a digital thermometer. A pH meter was used to measure the pH values of the pulp and beans. Samples of cocoa beans were taken from the top and bottom of the fermentation box, and the pulp and beans were separated. The cocoa beans were ground, and 5 g of cocoa bean powder ($n = 3$) was dissolved in 45 mL of aqueous solution, stirred for 1 min, and the pH was measured using a pH meter. The fermentation index (FI) was determined by mixing 0.5 g of ground cocoa beans

($n = 3$) with 50 mL of methanol and hydrochloric acid solution (97:3, v/v). The mixture was then vortexed and refrigerated for 12 h. The samples were filtered through a Whatman Paper No.1. The reddish/brownish supernatant was analyzed using a spectrophotometer (Peak Instruments, Shanghai, China). The FI was obtained by calculating the absorbance ratio at 460 and 530 nm ($FI = A_{460}/A_{530}$).

3.2.5 Metabolite extraction and gas chromatography-mass spectrometry (GC-MS) analysis for non-volatile compounds after derivatization

Before extraction, all the samples (raw beans, fermented beans, roasted nibs, and cocoa liquor) were quenched with liquid nitrogen and homogenized using a multi-bead shocker (Yasui Kikai, Osaka, Japan). Extraction was performed using a methanol:water:chloroform mixture (5:2:2, v/v/v) with ribitol (0.1 mg/mL) as an internal standard. This solvent mixture was added to 2-mL tubes containing 5 mg of the homogenized sample. Sample extraction and derivatization followed a protocol adapted from Hanifah et al. (2022). After derivatization, a GC-MS-QP2010 Ultra system (Shimadzu, Kyoto, Japan) fitted with an InertCap 5MS/NP column (35 m $\times$ 0.18 mm, I.D. 0.18 μ m; GL Sciences, Tokyo, Japan) was used for widely targeted metabolite profiling.

The mass spectrometer was tuned, and 1 μ L of the derivatized sample was injected in split mode (25:1 v/v) at 280 $^{\circ}$ C in a random order. Hydrogen was used as the carrier gas with a linear velocity of 60 cm/s and a flow rate of 1.4 mL/min. The column temperature was initially set at 80 $^{\circ}$ C for 2 min, then increased to 330 $^{\circ}$ C at a rate of 10 $^{\circ}$ C/min and maintained at the final temperature for 6 min. The interface and ion source temperatures were 310 and 280 $^{\circ}$ C, respectively. Electron ionization (EI) was performed at 1.00 kV, and mass spectra were recorded in the m/z range of 85–500. A standard alkane

mixture (C9–C40) was injected before sample analysis to determine the retention index (RI). Data processing and compound identification were conducted according to Hanifah et al. (2022).

3.2.6 Headspace solid-phase microextraction (HS-SPME) arrow GC-MS analysis for volatile compounds

HS-SPME arrow GC-MS analysis was performed based on a modified protocol adapted from Kitani et al. (2022), Moreno-Rojas et al. (2023), and Velásquez-Reyes et al. (2023). A pooled Quality Control (QC) was created by combining 1.5 g of each sample and placing it in a 20 mL screw vial with a magnetic screw cap (Shimadzu, Japan). The sample was prepared by combining 1 g of each sample type and placing the mixture into a 20 mL screw-cap vial with a magnetic cap (Shimadzu, Japan). Solid-phase microextraction (SPME) was conducted automatically using an AOC-6000 multifunctional autosampler equipped with a DVB/CAR/PDMS fiber (20 mm × 1.5 mm outer diameter, df = 120 µm; Shimadzu, Japan). For analysis, 1.5 g of each sample (n = 3) and the QC were preconditioned at 250 °C for 15 min, followed by incubation at 60 °C for 30 min with agitation at 250 rpm. Volatile extraction was performed by immersing the fiber directly into the sample vial at 60 °C for 30 min. After extraction, the fiber was transferred to the GC inlet, and analytes were thermally desorbed at 250 °C for 2 min.

Volatile analysis was performed using a GC-MS-TQ8040 system (Shimadzu, Kyoto, Japan) coupled with a capillary GC column (InertCap, 60 m × 0.32 mm × 0.5 µm film thickness). The desorbed analytes were introduced into the GC at a split ratio of 1:25. Helium was used as the carrier gas, with a linear velocity of 40.7 cm/s and a column flow rate of 2.5 mL/min. The column oven temperature was initially set at 40 °C (held for 3

min), then increased to 250 °C at a rate of 3 °C/min, and maintained at 250 °C for 10 min. The mass spectrometer operated in scan mode (m/z 24–350), with both the ion source and interface temperatures set at 250 °C. To calculate retention indices (RIs) for compound identification, a standard mixture of fatty acid ethyl esters (C2–C24) was injected prior to sample analysis. Data processing followed the workflow described by Kitani et al. (2022).

3.2.7 Statistical analysis

Multivariate statistical analyses of the annotated metabolites were conducted using SIMCA-P 13.0.1 (Umetrics, Umea, Sweden) for Principal Component Analysis (PCA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA), applying auto-scaling and auto-transformation to the data. Statistical significance was assessed using analytical tools developed by RIKEN (Kanagawa, Japan), with p -values < 0.05 considered significant. Data visualization, including volcano plots, bar charts, and radar charts, was carried out using Microsoft Excel, while heatmap generation was performed with MetaboAnalyst 6.0. Pathway enrichment analysis was also conducted using MetaboAnalyst 6.0 based on the *Arabidopsis thaliana* KEGG library, employing the Global Test and Relative-Betweenness Centrality algorithms. Pathway significance was expressed as $-\log_{10}(p)$, while pathway impact, reflecting the topological importance of each pathway, was calculated based on relative-betweenness centrality. Pathways with $p < 0.05$ were considered significantly enriched.

3.3 Results and Discussion

3.3.1 Analysis of differential metabolites in cocoa liquor from pre-drying and mechanical de-pulping process

In the previous chapter, the metabolite profile of pre-dried and mechanically de-pulped cocoa liquor was clearly distinct from that of the control, confirming that both treatments significantly impacted the metabolite composition. To further identify key metabolites that differentiate each treatment from the control, Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) combined with Variable Importance in the Projection (VIP) analysis was applied. Metabolites with a VIP score ≥ 1 were considered to have a significant influence on group separation (Amalia et al., 2023). While Principal Component Analysis (PCA) highlighted overall trends in metabolite variation, OPLS-DA provided a more precise identification of metabolites that strongly influenced the separation between groups (Lu et al., 2024).

As depicted in **Fig. 3.1A**, the metabolite profile of pre-dried cocoa liquor was clearly separated from the control, characterized by higher levels of malic acid, citric acid, isocitric acid, tartaric acid, methyl phenylacetate, ethyl octanoate, butyrolactone, and γ -butyrolactone (VIP ≥ 1) (**Fig. 3.1B–Table S7**), confirming their significant contribution to the observed differences. Similarly, mechanically de-pulped cocoa liquor exhibited a distinct metabolite pattern from the control, as illustrated in **Fig. 3.1C**. The loading plot (**Fig. 3.1D**) indicated increased levels of isobutyric acid, butanoic acid, butyric acid, nonanoic acid, prenol, and acetol (VIP ≥ 1) in the mechanically de-pulped samples (**Table S8**), aligning with the patterns identified in the PCA results.

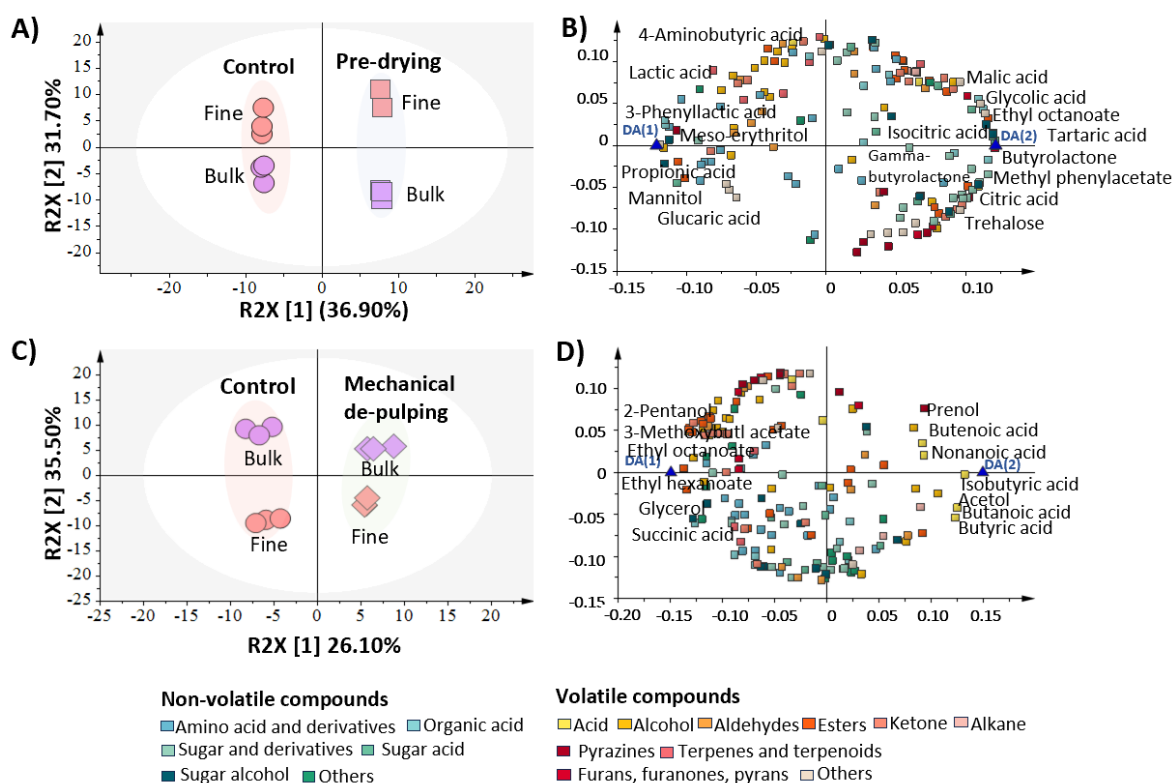


Fig. 3.1 Orthogonal partial least-squares discriminant (OPLS-DA) analysis results of non-volatile and volatile metabolites in cocoa liquor. (A) OPLS-DA score plot of cocoa liquor (control and pre-dried); (B) OPLS-DA loading plot of cocoa liquor (control and pre-dried); (C) OPLS-DA score plot of cocoa liquor (control and mechanically de-pulped); (D) OPLS-DA loading plot of cocoa liquor (control and mechanically de-pulped). Scaling: auto, transformation: auto, replicate: $n = 3$.

3.3.2 Analysis of differential metabolites in raw beans before and after pre-drying and mechanical de-pulping

Following the identification of key differential metabolites in cocoa liquor, we next investigated whether these differences were already present in the raw beans immediately after de-pulping. The differences in metabolite and sensory profiles of cocoa liquor across de-pulping treatments suggest that metabolic changes may begin immediately after de-pulping. Since de-pulping occurs before fermentation, investigating

its immediate impact on raw bean metabolites is necessary to determine whether these differences arise from early metabolic shifts or develop during subsequent processing.

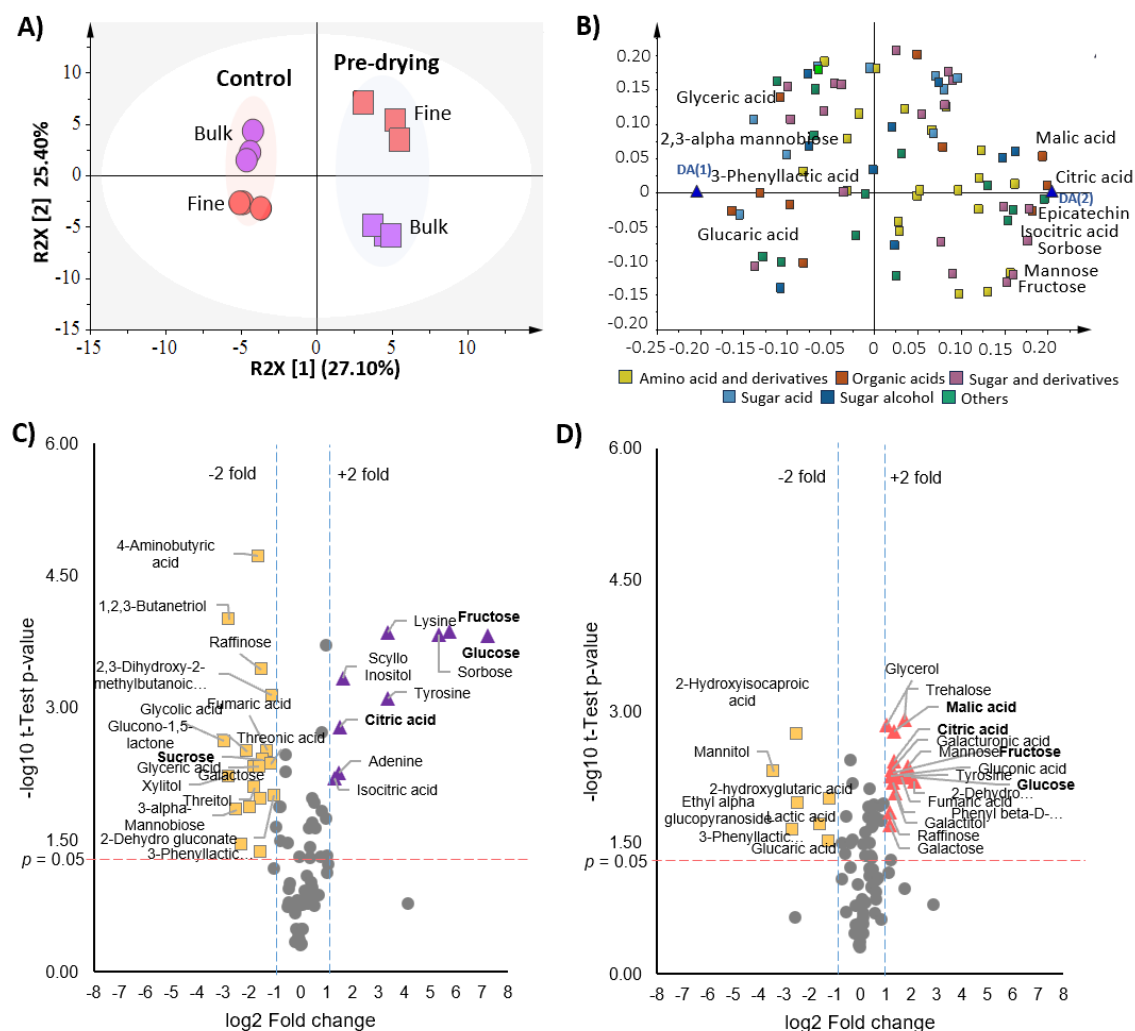


Fig. 3.2. Orthogonal partial least-squares discriminant (OPLS-DA) analysis results of non-volatile and volatile metabolites in raw beans before and after pre-drying treatment. (A) OPLS-DA score plot of raw beans (control and pre-dried); (B) OPLS-DA loading plot of raw beans (control and pre-dried). Significantly modulated metabolites after pre-drying treatment: (C) bulk cocoa (D) fine cocoa. A triangle indicates metabolites significantly increased ($p < 0.05$) with at least 2-fold change. A square indicates metabolites significantly decreased ($p < 0.05$) with at least 2-fold change. A circle indicates metabolites with a non-significant change ($p \geq 0.05$) and/or metabolites with less than 2-fold change.

To address this, OPLS-DA combined with VIP analysis was performed to identify key non-volatile metabolites in raw beans before and after de-pulping. As shown in **Fig. 3.2A**, pre-dried beans displayed a clear separation from the control. The corresponding loading plot (**Fig. 3.2B**) revealed that pre-dried beans contained higher levels of malic acid, citric acid, fructose, glucose, mannose, and sorbose — all with VIP scores ≥ 1 (**Table S9**). These metabolites, previously linked to acidity and fresh fruit attributes as shown in the previous chapter, suggest that pre-drying enhances compounds associated with these sensory qualities. Moreover, since these metabolites were also identified as key differentiators in cocoa liquor, this finding indicates that the effects of pre-drying persist throughout fermentation and roasting.

To further validate these findings, a volcano plot analysis combining ANOVA ($p < 0.05$) and fold-change analysis was conducted to highlight significantly altered metabolites (**Fig. 3.2C and D**). This analysis confirmed a more than twofold increase in several key metabolites that also exhibited VIP scores ≥ 1 , including fructose, glucose, citric acid, malic acid, and isocitric acid (**Table S11**). These results align with previous studies on pod storage — a pulp preconditioning method where beans are kept in their pods to reduce pulp content — which has been shown to promote the accumulation of flavor precursors, particularly reducing sugars (Afoakwa et al., 2011). The increased levels of organic acids in pre-dried beans may be attributed to the natural drying process during pre-drying, which lasts approximately one day. This extended drying time likely allows organic acids from the pulp to diffuse into the cotyledon (Afoakwa et al., 2013; Biehl et al., 1990; Schwan & Wheals, 2004), as supported by the observed decrease in pH of both the pulp and beans after pre-drying compared to before treatment (**Fig. S5A and B**). This diffusion not only directly raises the organic acid content but may also

initiate biochemical reactions within the bean, such as activating invertase, an enzyme that catalyzes the conversion of sucrose into glucose and fructose (Eskes et al., 2012; Vuyst & Leroy, 2020). Furthermore, the elevated malic and citric acids may contribute to acidity modulation during fermentation, potentially influencing fermentation dynamics (Calvo et al., 2021).

Consistent with these physicochemical changes, pathway enrichment analysis revealed that the TCA cycle, glyoxylate and dicarboxylate metabolism, and starch and sucrose metabolism were significantly affected (**Fig. S6A**). These pathways encompass the organic acids and sugars that increased after pre-drying, indicating that the observed metabolite shifts are biochemically interconnected within central carbon metabolism. The enrichment of these pathways reflects how diffusion-driven acid accumulation and enzyme-mediated sucrose breakdown contribute to a broader reorganization of metabolites associated with these networks. Overall, these findings suggest that pre-drying alters the substrate composition available for microbial activity and may reshape fermentation by affecting key metabolic pathways (Afoakwa et al., 2013; Kongor et al., 2016).

Mechanical de-pulping, in contrast to the trend observed with pre-drying, exhibited a distinctly different metabolic response (**Fig. 3.3A**). While pre-drying was associated with increased levels of sugars and organic acids, mechanical de-pulping led to higher concentrations of paraxanthine, catechin, epicatechin, and theobromine, whereas citric acid and isocitric acid remained more abundant in the control (**Fig. 3.3B**). These metabolites also showed VIP scores ≥ 1 (**Table S10**), underscoring their importance in distinguishing the metabolic effects of mechanical de-pulping. The volcano plot

analysis further confirmed a significant increase of these metabolites ($p < 0.05$) in mechanically de-pulped beans across both cocoa varieties (**Fig. 3.3C and D**), supporting their consistent association with mechanical de-pulping treatment.

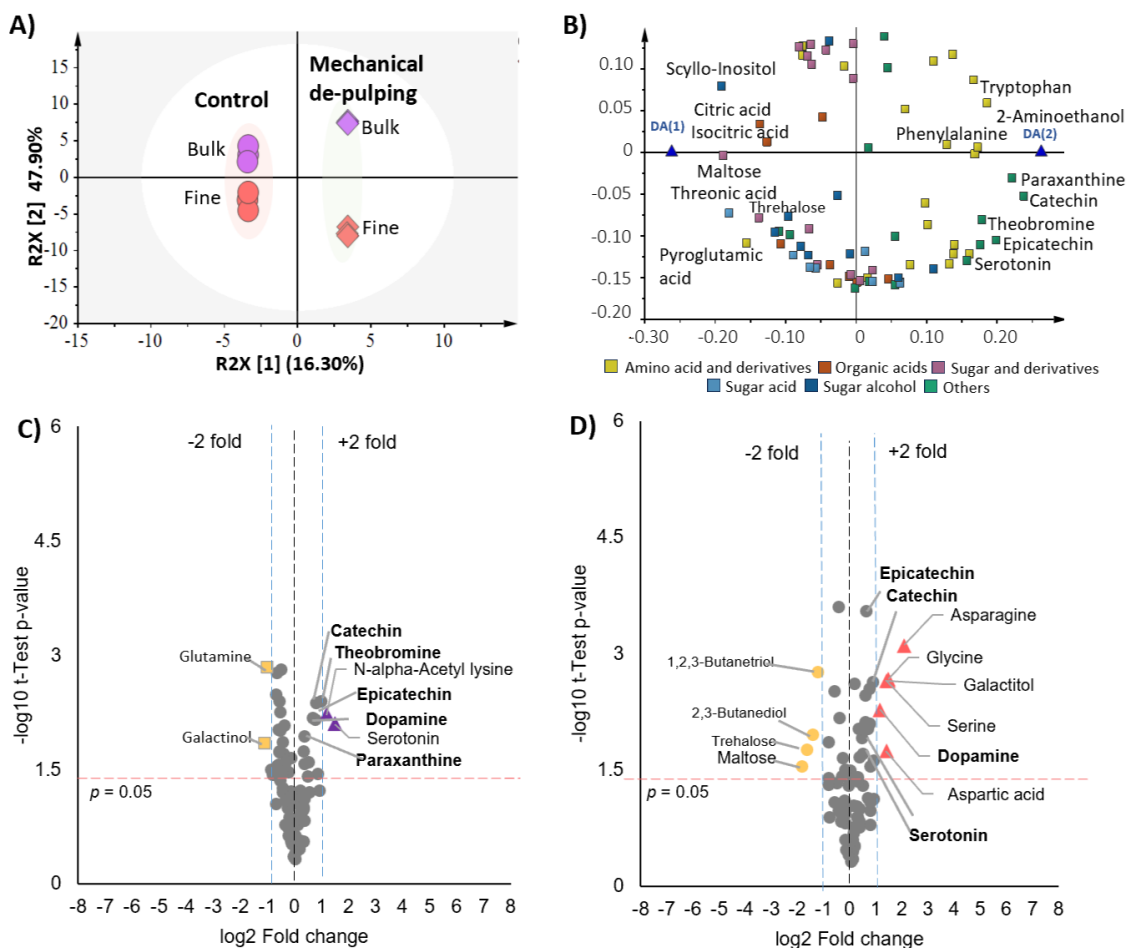


Fig. 3.3. Orthogonal partial least-squares discriminant (OPLS-DA) analysis results of non-volatile and volatile metabolites in raw beans before and after mechanical de-pulping treatment. (A) OPLS-DA score plot of raw beans (control and mechanically de-pulped); (B) OPLS-DA loading plot of raw beans (control and mechanically de-pulped). Significantly modulated metabolites after mechanical de-pulping treatment: (C) bulk cocoa (D) fine cocoa. A triangle indicates metabolites significantly increased ($p < 0.05$) with at least 2-fold change. A square indicates metabolites significantly decreased ($p < 0.05$) with at least 2-fold change. A circle indicates metabolites with a non-significant change ($p \geq 0.05$) and/or metabolites with less than 2-fold change.

The rapid removal of pulp during mechanical de-pulping likely limits the time available for biochemical changes to occur immediately after treatment, as suggested by the lack of significant pH differences observed in both the pulp and beans (**Fig. S5C and D**). This contrasts with the slower, gradual changes observed during pre-drying, such as the diffusion of acid into the bean. Consequently, mechanically de-pulped beans exhibit lower levels of key flavor precursors that play a crucial role in flavor development during subsequent postharvest stages, such as fermentation and roasting. At the same time, the increase in catechin and epicatechin—both bitter flavonoids— may result from stress responses triggered by tissue damage and dehydration during the scraping process inside the perforated drum. Such mechanical and dehydration stress is known to activate the LAR enzyme involved in flavonoid biosynthesis (Li et al., 2021; Liu et al., 2023). Additionally, flavonoid accumulation serves as a protective mechanism against oxidative stress caused by osmotic imbalance and reactive oxygen species, particularly in water-limited conditions (Huang et al., 2025). Moreover, several amino acids, including phenylalanine, were elevated in mechanically de-pulped beans, possibly due to protein hydrolysis and metabolic shifts induced by mechanical and dehydration stress (Huang et al., 2025; Suzuki et al., 2003; Wu et al., 2020).

Pathway enrichment analysis further supported these findings, indicating significant enrichment in flavonoid biosynthesis, phenylalanine, tyrosine, and tryptophan biosynthesis, and tyrosine metabolism (**Fig. S6B**). These pathways are closely related, as phenylalanine acts as a primary precursor for polyphenol and flavonoid synthesis. The enrichment of these aromatic amino acid-related pathways thus align with the observed accumulation of catechin and epicatechin, confirming that mechanical de-pulping stimulates metabolic routes linked to stress response and phenolic compound production.

This interpretation is consistent with previous reports showing that mechanical stress enhances flavonoid biosynthesis as a defense mechanism in plant tissues (Li et al., 2021; Liu et al., 2023). Furthermore, the enrichment of amino acid–related pathways, such as phenylalanine and tyrosine metabolism, supports the hypothesis that mechanical stress leads to partial protein degradation and redirection of nitrogen metabolism toward secondary metabolite synthesis. Collectively, these results demonstrate that mechanical de-pulping induces stress-associated biochemical responses that favor flavonoid accumulation rather than flavor precursor development, ultimately leading to a cocoa liquor with more pronounced bitterness and astringency. These findings suggest that improving the design or materials of de-pulping equipment to minimize tissue damage may help reduce adverse effects on cocoa quality and flavor in future applications.

Although both pre-drying and mechanical de-pulping altered the metabolite profiles in raw beans, only the metabolites influenced by pre-drying persisted in cocoa liquor. However, key metabolites that increased immediately after mechanical de-pulping were not identified as discriminant compounds in cocoa liquor. This indicates that subsequent postharvest steps—such as fermentation and roasting—further reshaped the metabolite composition. Consequently, tracking the changes of VIP metabolites from raw beans through to cocoa liquor is essential for understanding how each de-pulping treatment influences the entire postharvest process.

3.3.3 Dynamic changes of key differential metabolites during cocoa postharvest process

To further explore how mechanical de-pulping and pre-drying influence cocoa flavor, we tracked the dynamics of key differential metabolites across various postharvest stages: before de-pulping, after de-pulping, after fermentation, after roasting, and in the final cocoa liquor. Heatmaps of VIP metabolites from the OPLS-DA analysis highlighted shifts in metabolite profiles, providing a clear comparison between pre-drying and mechanical de-pulping (**Fig. 3.4**). These heatmaps revealed that the differences in metabolite profiles became apparent immediately after de-pulping and persisted throughout processing.

For non-volatile compounds (**Fig. 3.4A**), both bulk and fine pre-dried beans consistently showed elevated levels of sugars and sugar alcohols — including sorbose, mannose, xylulose, galactitol, glycerol, and xylitol. In contrast, mechanically de-pulped beans exhibited increased levels of bitterness- and astringency-associated metabolites such as dopamine, serotonin, epicatechin, catechin, and theobromine (Febrianto & Zhu, 2022). Organic acid differences were also evident across stages: pre-dried beans had higher levels of isocitric acid, citric acid, and malic acid compared to the control, whereas mechanically de-pulped beans showed lower concentrations of these acids compared to the control samples. Additionally, reducing sugars such as glucose and fructose were consistently higher in pre-dried beans but decreased in mechanically de-pulped beans, and this trend continued throughout fermentation and roasting. These differences likely originate from the contrasting levels of pulp removal, moisture exposure, and early biochemical conditions created by each de-pulping method.

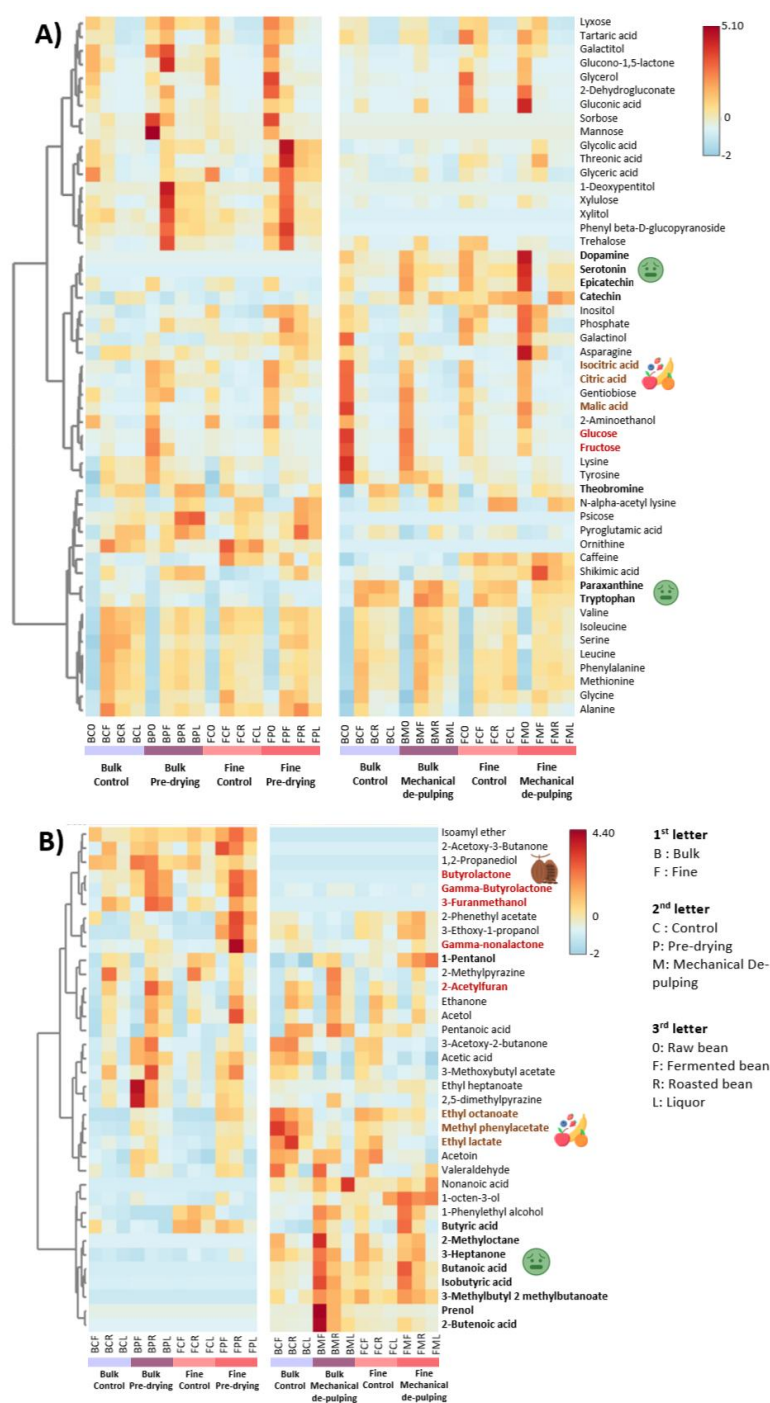


Fig. 3.4. Heat maps illustrating the dynamics of non-volatile and volatile metabolites during the post-harvest process. The metabolites shown are those with VIP ≥ 1 identified based on OPLS-DA in cocoa liquor and raw beans. (A) Heat maps of non-volatile metabolites with VIP ≥ 1 ; (B) Heat maps of volatile metabolites with VIP ≥ 1 . Sample codes are structured as follows: the first letter indicates cocoa type (B = bulk, F = fine), the second letter indicates treatment (C = control, P = pre-drying, M = mechanical de-pulping), and the final letter indicates postharvest stage (0 = raw beans, F = fermented beans, R = roasted nibs, L = cocoa liquor).

For volatile metabolites (**Fig. 3.4B**), pre-drying increased lactones, furans, furfurals, and esters— compounds known to have sweet, cocoa, and fruity aromas (Velásquez-Reyes et al., 2023). In contrast, mechanical de-pulping led to an increase in acids, alkanes, and alcohols, including butyric acid, 2-methyl octane, butanoic acid, isobutyric acid, prenol, and 1-octen-3-ol, which are associated with balsamic, green, alkane, acidic, and pungent aromas (Spada et al., 2022). These contrasting patterns were consistently observed from raw beans through to the final cocoa liquor, underscoring the distinct effects of each de-pulping method on cocoa flavor development during postharvest processing. Nevertheless, it should be noted that metabolite changes — and their impact on flavor — may also be influenced by factors such as microbial activity during fermentation, postharvest handling, and inherent varietal differences (Rodriguez-Campos et al., 2011; Santander et al., 2021).

3.3.3.1 Dynamics of fresh fruit and acid-related metabolites

To further illustrate significant differences across each processing stage, bar graphs were constructed, focusing on key metabolites linked to fresh fruit and acidity attributes—namely citric acid, malic acid, isocitric acid, methyl phenylacetate, and ethyl octanoate. As shown in **Fig. 3.5** shows that citric acid, malic acid, and isocitric acid significantly increased by more than 1.5-fold after pre-drying in raw beans (**Table S11**) and remained elevated in the final cocoa liquor, despite a decrease after roasting. This trend suggests that pre-drying not only induces an immediate increase in organic acids but also leads to chemical changes that persist through subsequent stages. The increase may be attributed to the diffusion of organic acids from the pulp into the bean during the pre-drying period, potentially facilitated by pulp-associated biochemical changes. Such

an increase is considered beneficial, as previous studies have demonstrated that the diffusion of organic acids into cocoa beans under controlled conditions enhances acidity and fruity flavors in cocoa liquor (Dahiana Becerra et al., 2024; Santander et al., 2021). In contrast, mechanical de-pulping led to a reduction in these metabolites immediately after treatment. This reduction continued during postharvest processing, particularly in bulk cocoa, while fine cocoa showed no significant decrease. As pulp removal occurs rapidly in this method, the biochemical transformations observed during pre-drying may not occur. Consequently, no notable increase in organic acids was observed, and cocoa liquor from mechanically de-pulped beans exhibited lower fresh fruit and acidity attributes.

Additionally, volatile compounds such as methyl phenylacetate and ethyl octanoate were significantly higher in pre-dried beans after fermentation compared to the control, whereas mechanically de-pulped beans contained lower levels. The increase in esters following pre-drying may be attributed to shifts in microbial composition induced by the pre-drying process, as many esters are microbial metabolites produced during fermentation (Aprotosoie et al., 2016). Aligning with this, previous studies have demonstrated that pulp reduction through pod storage prior to fermentation has also been associated with increased ester levels in the final chocolate (Hinneht et al., 2018). Moreover, lactic acid bacteria (LAB) can enzymatically convert alcohols and acids into esters (Chang et al., 2025), suggesting that microbial activity in pre-dried beans may have facilitated increased ester synthesis during fermentation, potentially enhancing the sensory quality of cocoa. Taken together, the persistence of increased organic acids and esters highlights how acidity and fresh fruit notes are developed in the final cocoa liquor.

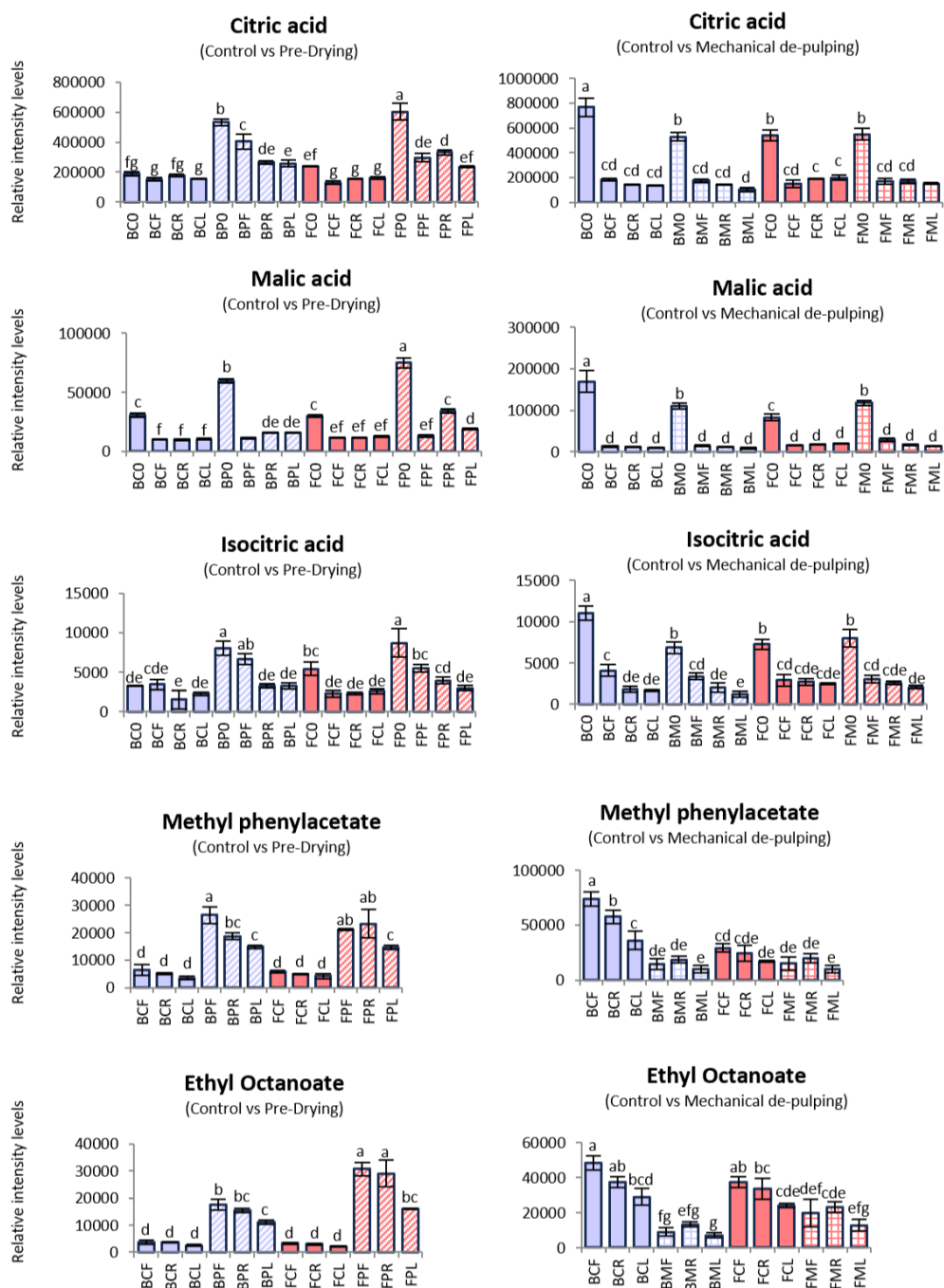


Fig. 3.5. Comparison of several fresh fruit and acid-related metabolite levels between control and de-pulping treatments in raw beans, fermented beans, roasted beans, and cocoa liquor. The vertical axis indicates relative intensity levels. Error bars represent ± 1 SD from the mean. Groups labeled with different letters are significantly different based on Tukey's adjustment ($p < 0.05$). Sample codes are structured as follows: the first letter indicates cocoa type (B = bulk, F = fine), the second letter indicates treatment (C = control, P = pre-drying, M = mechanical de-pulping), and the final letter indicates postharvest stage (0 = raw beans, F = fermented beans, R = roasted nibs, L = cocoa liquor).

3.3.3.2 Dynamics of cocoa-related metabolites

Following pre-drying, the levels of reducing sugars, such as glucose and fructose, increased by more than 2-fold (**Table S11**), whereas they decreased after mechanical de-pulping (**Fig. 3.6**). These sugars are essential precursors in the Maillard reaction and caramelization during roasting, processes that contribute to the formation of volatile compounds such as furans, furfurals, furanone, and pyrazines (Miralles-Garcia, 2008). Notably, in the pre-dried samples, elevated sugar levels persisted through fermentation, suggesting that these sugars were available for volatile compound formation during roasting but decrease after roasting process. In contrast, mechanically de-pulped beans showed reduced sugar levels from the beginning, which remained low throughout fermentation, likely limiting their contribution to volatile compound formation during roasting.

After roasting, the volatile profiles reflected these early differences in reducing sugars availability. Key aroma compounds — including 2-acetylfuran, furfural, γ -butyrolactone, γ -nonalactone, 2,3,5-trimethylpyrazine, and trimethylpyrazine — showed significant increases in the pre-dried samples but were absent or present at much lower levels in the mechanically de-pulped beans. These results suggested that higher reducing sugars may act as flavor precursors responsible for the Maillard reaction and caramelization for the generation of desirable volatile compounds such as pyrazines, furan, furfural, and pyranones (Miralles-Garcia, 2008). Consistent with these chemical findings, sensory analysis indicated that pre-dried beans developed a more complex aroma profile, enriched with cocoa and browned fruit notes, whereas mechanically de-pulped beans lacked these

desirable characteristics. These dynamics likely explain why pre-drying enhances the final cocoa liquor's aroma and flavor, while mechanical de-pulping does not.

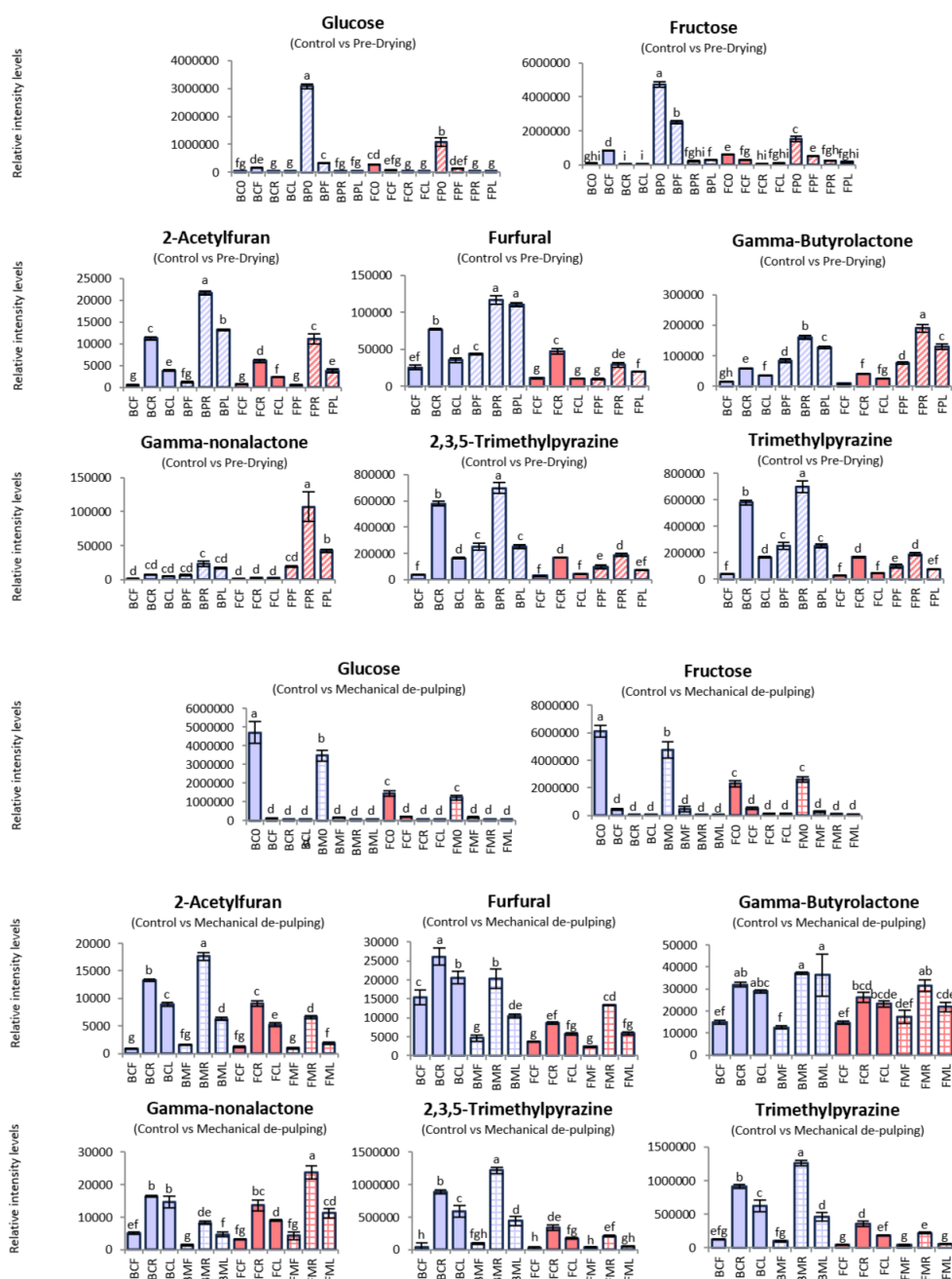


Fig. 3.6. Comparison of several cocoa-related metabolite levels between control and de-pulping treatments in raw beans, fermented beans, roasted beans, and cocoa liquor. The vertical axis indicates relative intensity levels. Error bars represent ± 1 SD from the mean. Groups labeled with different letters are significantly different based on Tukey's adjustment ($p < 0.05$). Sample codes are structured as follows: the first letter indicates cocoa type (B = bulk, F = fine), the second letter

indicates treatment (C = control, P = pre-drying, M = mechanical de-pulping), and the final letter indicates postharvest stage (0 = raw beans, F = fermented beans, R = roasted nibs, L = cocoa liquor).

3.3.3.3 Dynamics of bitter and astringent-related metabolites

Metabolites associated with bitterness and astringency were also analyzed (**Fig. 3.7**). Both epicatechin and catechin showed significant increases after pre-drying and mechanical de-pulping compared to the control. This was expected, as pulp reduction may induce dehydration stress, leading to an increase in flavonoids such as epicatechin and catechin (Liu et al., 2023). Interestingly, the increase was more pronounced in mechanically de-pulped beans, with both metabolites increased by more than 1.5-fold (**Table S12**), suggesting that this process exerts greater stress, resulting in higher levels of bitterness- and astringency-related metabolites. Moreover, the intensified bitterness and astringency in mechanically de-pulped cocoa may also be linked to elevated levels of undesirable volatile compounds, such as butyric acid and 1-pentanol, which increased during fermentation and persisted in the final cocoa liquor. Both compounds are known to negatively affect cocoa's aroma quality (Rodriguez-Campos et al., 2011), with butyric acid contributing to a hammy flavor defect and typically produced enzymatically during prolonged fermentation (Oberparleiter & Ziegler, 1997). The early increase of flavonoids and off-flavor volatiles in mechanically de-pulped beans, which persisted through to the final cocoa liquor, likely explains why this treatment produces higher bitterness and astringency, resulting in a less desirable flavor compared to pre-dried or control beans.

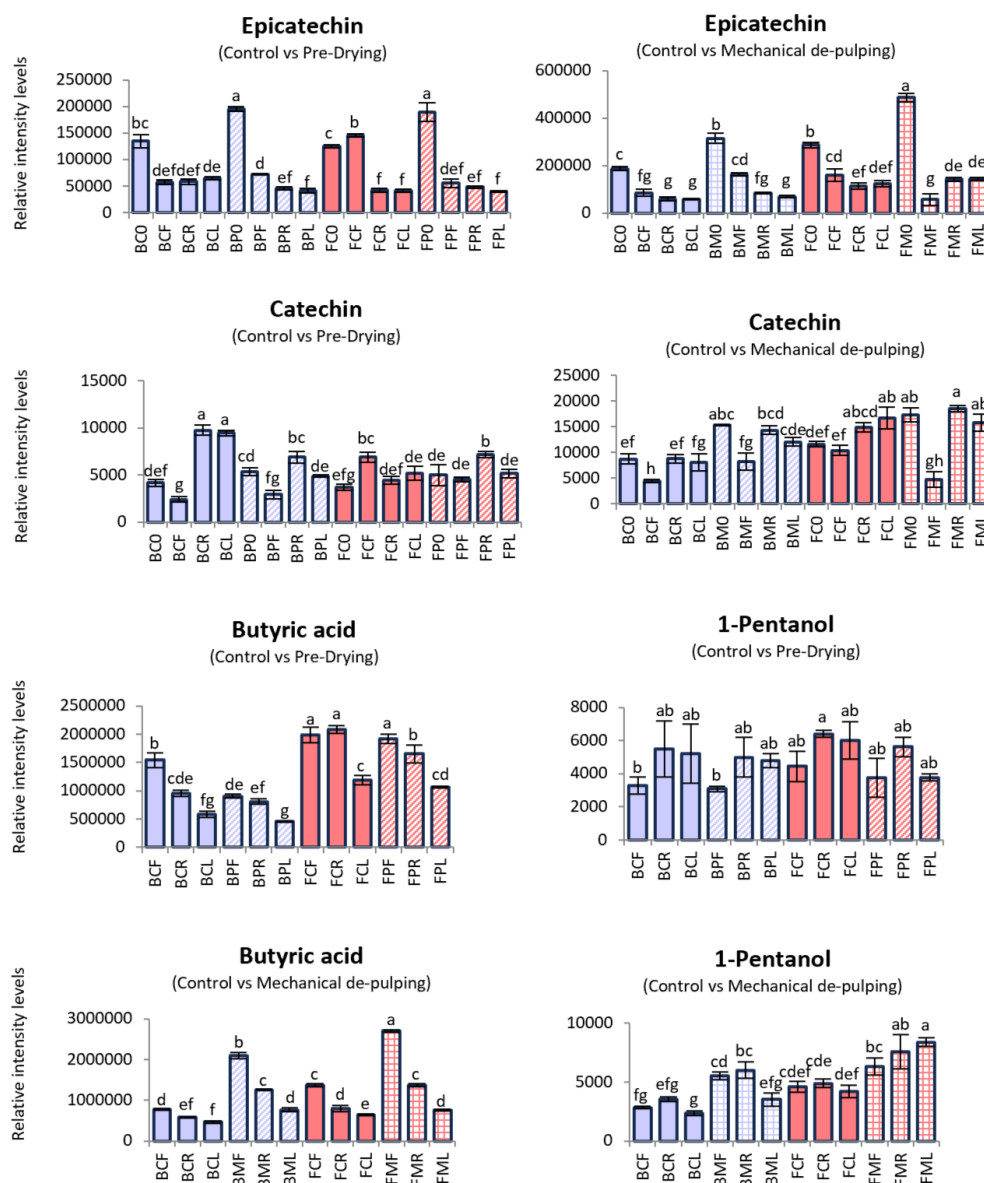


Fig. 3.7. Comparison of several bitter and astringent-related metabolite levels between control and de-pulping treatments in raw beans, fermented beans, roasted beans, and cocoa liquor. The vertical axis indicates relative intensity levels. Error bars represent ± 1 SD from the mean. Groups labeled with different letters are significantly different based on Tukey's adjustment ($p < 0.05$). Sample codes are structured as follows: the first letter indicates cocoa type (B = bulk, F = fine), the second letter indicates treatment (C = control, P = pre-drying, M = mechanical de-pulping), and the final letter indicates postharvest stage (0 = raw beans, F = fermented beans, R = roasted nibs, L = cocoa liquor).

3.3.4 Overall metabolite dynamics and potential quality markers

Altogether, the metabolite dynamics reveal distinct effects of de-pulping methods on cocoa quality. Although mechanical de-pulping effectively reduces pulp, it also induces stress on raw beans, leading to an increase in bitter and astringent-related metabolites such as epicatechin and catechin. The increase in these compounds correlates with the enhanced bitterness and astringency in the final cocoa liquor. Moreover, the rapid removal of pulp may reduce the availability of substrates for microbial fermentation, potentially disrupting microbial activity and favoring the formation of undesirable compounds, such as butyric acid and 1-pentanol (Marwati et al., 2024). In contrast, pre-drying not only reduces pulp but also increases the levels of key flavor precursors in raw beans, such as citric acid, malic acid, and isocitric acid. These organic acids, associated with fresh fruit and acidity attributes, remain elevated in the final cocoa liquor, reinforcing their role in shaping the flavor profile. Notably, malic acid and citric acid are characteristic markers of fine-flavored cocoa (Afifah et al., 2024) and Criollo-type cocoa (Velásquez-Reyes et al., 2023). This highlights the potential of pre-drying as a de-pulping method to enhance the quality of cocoa with fine-flavored characteristics.

Therefore, based on the observed metabolite dynamics, this study indicates that epicatechin and catechin have the potential to serve as early indicators of bitterness and astringency in raw beans immediately after de-pulping. Additionally, malic acid, citric acid, and isocitric acid may act as markers of de-pulping effectiveness, particularly in enhancing fresh fruit and acidity attributes. By monitoring these metabolites, it becomes possible to predict the effectiveness of de-pulping methods in enhancing the related sensory attributes. Optimizing de-pulping processes like pre-drying offers a practical and

cost-effective strategy for farmers to improve cocoa quality and add value to their products.

3.4 Conclusion

Overall, our results demonstrate that early postharvest treatments play a decisive role in shaping both the chemical composition and sensory quality of cocoa. Pre-drying significantly increased reducing sugars and organic acids (> 2-fold) in raw beans, potentially increasing precursors for Maillard reactions and promoting the formation of key aroma compounds—furans, pyranones, and pyrazines—during roasting. Furthermore, citric, malic, and isocitric acids remained elevated in the final cocoa liquor, correlating with enhanced fruity and acidic sensory attributes. In contrast, mechanical de-pulping increased levels of bitterness- and astringency-related compounds (epicatechin, catechin), likely due to physical stress on the beans. The rapid removal of pulp may have limited the early biochemical changes observed with pre-drying, potentially disrupting subsequent postharvest transformations and contributing to the formation of undesirable compounds such as butyric acid and 1-pentanol. By demonstrating that flavor precursors such as reducing sugars and fine-flavor markers like citric and malic acids can be enhanced early in the postharvest sequence through practical interventions such as pre-drying, this study reveals for the first time how initial biochemical modulation influences subsequent transformations that shape cocoa liquor quality. The identification of these compounds—along with epicatechin and catechin—as key quality markers provides a framework for evaluating the effectiveness of de-pulping treatments and guiding postharvest optimization toward improved and consistent cocoa flavor.

Chapter 4

Conclusion

The general objective of this research was to evaluate the impact of different de-pulping methods on metabolite changes that improve cocoa bean flavor quality, using a metabolomics approach. By integrating sensory evaluation with GC-MS-based metabolite profiling, this study has successfully evaluated how pre-drying and mechanical de-pulping modulate the chemical composition and sensory attributes of cocoa throughout the postharvest process.

From this study, despite achieving similar reductions in pulp, pre-drying and mechanical de-pulping exhibited markedly different effects on cocoa liquor flavor. Pre-drying significantly improved the sensory profile of cocoa liquor by reducing bitterness and astringency and enhancing fresh fruit and acidic notes ($p < 0.05$) in both bulk and fine cocoa varieties. In contrast, mechanical de-pulping failed to improve flavor and even increased bitterness and astringency scores ($p < 0.05$). Metabolomics analysis supported these findings by identifying metabolites associated with favorable attributes—such as organic acids, sugars, and esters—in the pre-dried samples. Meanwhile, higher levels of epicatechin, catechin, and quinic acid were found to correlate with bitterness and astringency, the undesirable traits. By integrating metabolite profiling with sensory data, this study provides, for the first time, a clear demonstration of the distinct effects of different de-pulping methods on cocoa flavor quality and identifies the key metabolites associated with these changes. These findings expand current knowledge by revealing specific metabolites that underpin flavor differences caused by the de-pulping process.

Given that cocoa postharvest processing involves multiple stages that collectively determine flavor, this study also investigated how early metabolite alterations induced by de-pulping persist or shift through fermentation, roasting, and grinding. Pre-drying significantly increased reducing sugars and organic acids (> 2 -fold) in raw beans, potentially increasing precursors for Maillard reactions and promoting the formation of key aroma compounds—furans, pyranones, and pyrazines—during roasting. Furthermore, citric, malic, and isocitric acids, which are fine-flavor markers, remained elevated in the final cocoa liquor, correlating with enhanced fruity and acidic sensory attributes.

In contrast, mechanical de-pulping increased levels of bitterness- and astringency-related compounds (epicatechin, catechin), likely due to physical stress on the beans. The rapid removal of pulp also may have limited the early biochemical changes observed with pre-drying, potentially disrupting subsequent postharvest transformations and contributing to the formation of undesirable compounds such as butyric acid and 1-pentanol. These findings provide, for the first time, a comprehensive understanding of how initial biochemical modulation through de-pulping influences subsequent postharvest transformations to shape the final cocoa flavor.

Overall, the metabolomics approach effectively revealed how different de-pulping methods modulate cocoa flavor by altering key metabolites throughout the postharvest process. Pre-drying emerges as a practical and effective method for improving cocoa flavor—particularly in bulk cocoa—while emphasizing the need for careful selection and optimization of de-pulping strategies. Furthermore, compounds such as citric, malic, and isocitric acids may serve as potential quality markers for improving desirable sensory traits, whereas epicatechin and catechin could act as early indicators of bitterness and

astringency. In the future, monitoring these metabolites can guide the optimization of de-pulping parameters, support quality control, and ensure consistent flavor development.

To further clarify the mechanisms underlying these metabolite changes, future research should also integrate microbial dynamics analysis, as shifts in microbial succession during fermentation are likely to play a central role in shaping flavor outcomes. Additionally, expanding this study across different cocoa varieties, growing regions, and processing environments will help validate the identified markers and broaden the applicability of these findings. Validation of the identified metabolite markers may also support the refinement of de-pulping strategies, including improvements in mechanical de-pulping designs to better align with desired flavor outcomes.

Ultimately, this study provides theoretical insight and practical strategies for improving and standardizing cocoa flavor through partial pulp removal. For the first time, this study integrates metabolomics and sensory evaluation to demonstrate how different de-pulping methods induce distinct early-stage metabolic shifts and how these changes are further modulated through fermentation, drying, roasting, and grinding to determine the final cocoa liquor flavor. In addition, the identification of potential metabolite markers enables early-stage monitoring of de-pulping effectiveness and offers a framework for improving flavor quality and consistency in cocoa production. These findings are especially significant for bulk cocoa, which accounts for more than 90% of global production, as the results demonstrate that its flavor quality can be improved through a simple and low-cost pre-drying treatment. By improving the quality of bulk cocoa, this approach has the potential to increase its economic value and market competitiveness, thereby contributing to improved farmer income and livelihood sustainability.

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List of Publication

Original Research Paper:

Ati, A. G., Sari, I. A., Firmanto, H., Malik, A., Fukusaki, E., & Putri, S. P. (2025). Effect of Pre-Drying on Flavor Modulation in Indonesian Cocoa Beans: A Metabolomics Study of Key Flavor Compounds and Sensory Profiles. *Food Bioscience*, 106056.

Ati, A. G., Sari, I. A., Firmanto, H., Malik, A., Fukusaki, E., & Putri, S. P. (2025). Metabolomics reveals the impact of different de-pulping methods on flavor modulation and their potential quality markers in Indonesian cocoa beans. *Food Bioscience*, 107007.

Conferences:

Ati, A. G., Sari, I. A., Firmanto, H., Malik, A., Fukusaki, E., & Putri, S. P. (2025). Enhancement of cocoa aroma through partial pulp removal by pre-drying in the postharvest process. Japan Society for Bioscience, Biotechnology, and Agrochemistry (JSBBA) Annual Meeting (2025). Poster presentation

Ati, A. G., Sari, I. A., Firmanto, H., Malik, A., Fukusaki, E., & Putri, S. P. (2025). Metabolomics highlights the distinct impacts of different partial pulp removal methods on cocoa flavor improvement. Metabolomics Society Annual Conference, Prague (2025). Poster presentation

Ati, A. G., Sari, I. A., Firmanto, H., Malik, A., Fukusaki, E., & Putri, S. P. (2025). Influence of Pre-drying on Cocoa Flavor Improvement: Tracking Flavor Precursors from

Beans to Liquor using Metabolomics. The Society of Biotechnology Japan (SBJ) 76th
Annual Meeting (2024). Oral presentation

Supplementary Figures and Tables

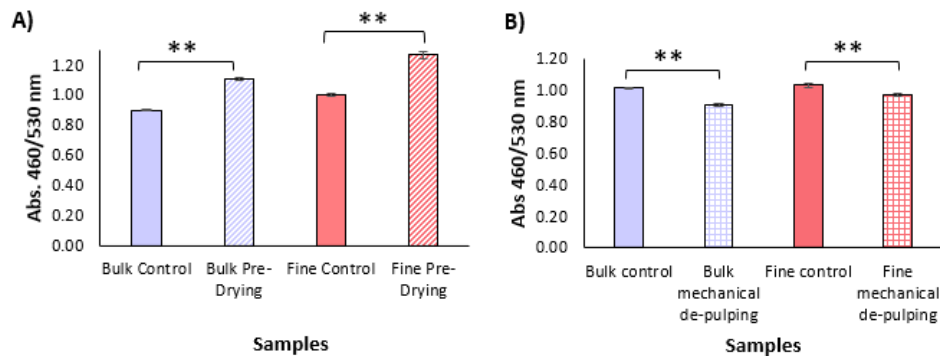


Fig. S1. Fermentation index: A) Pre-dried beans, B) Mechanical de-pulped beans. Asterisks indicate statistically significant differences based on t-test: *p < 0.05, **p < 0.01

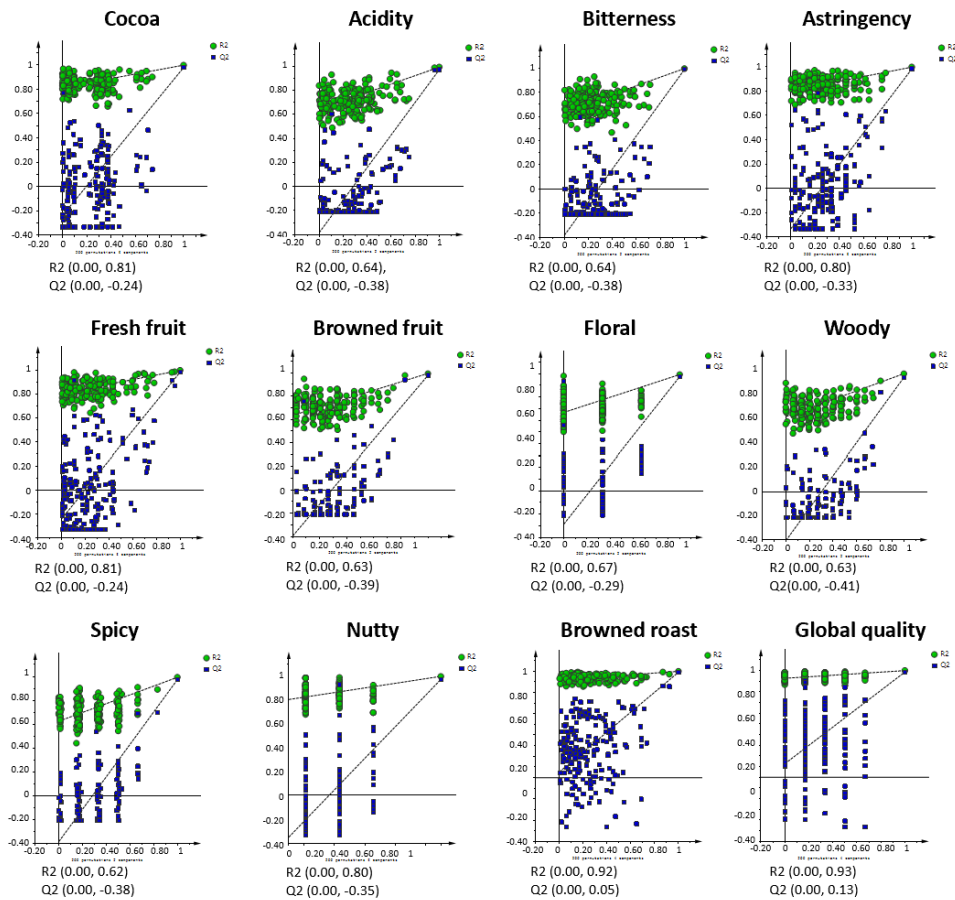


Fig. S2. Permutation test plot of PLS-regression model on the relationships between the metabolites and sensory attributes in cocoa liquor

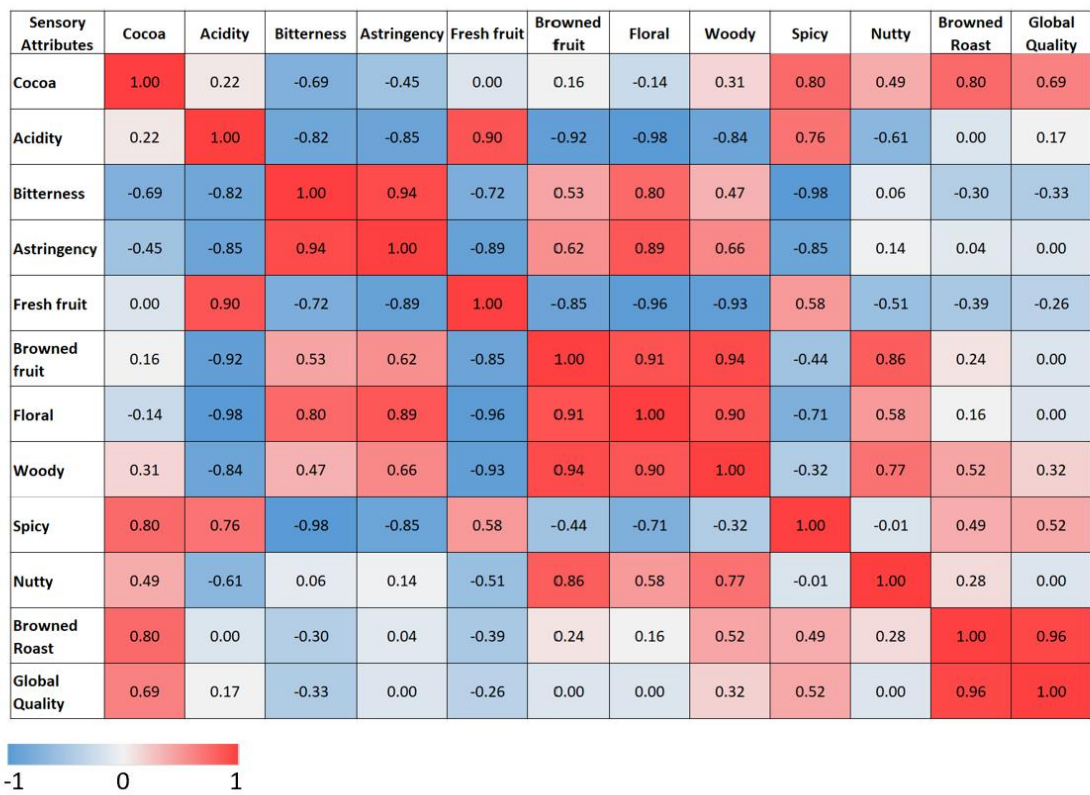


Fig. S3. Heatmap of the relationship between sensory attributes in cocoa liquor based on Pearson's correlation

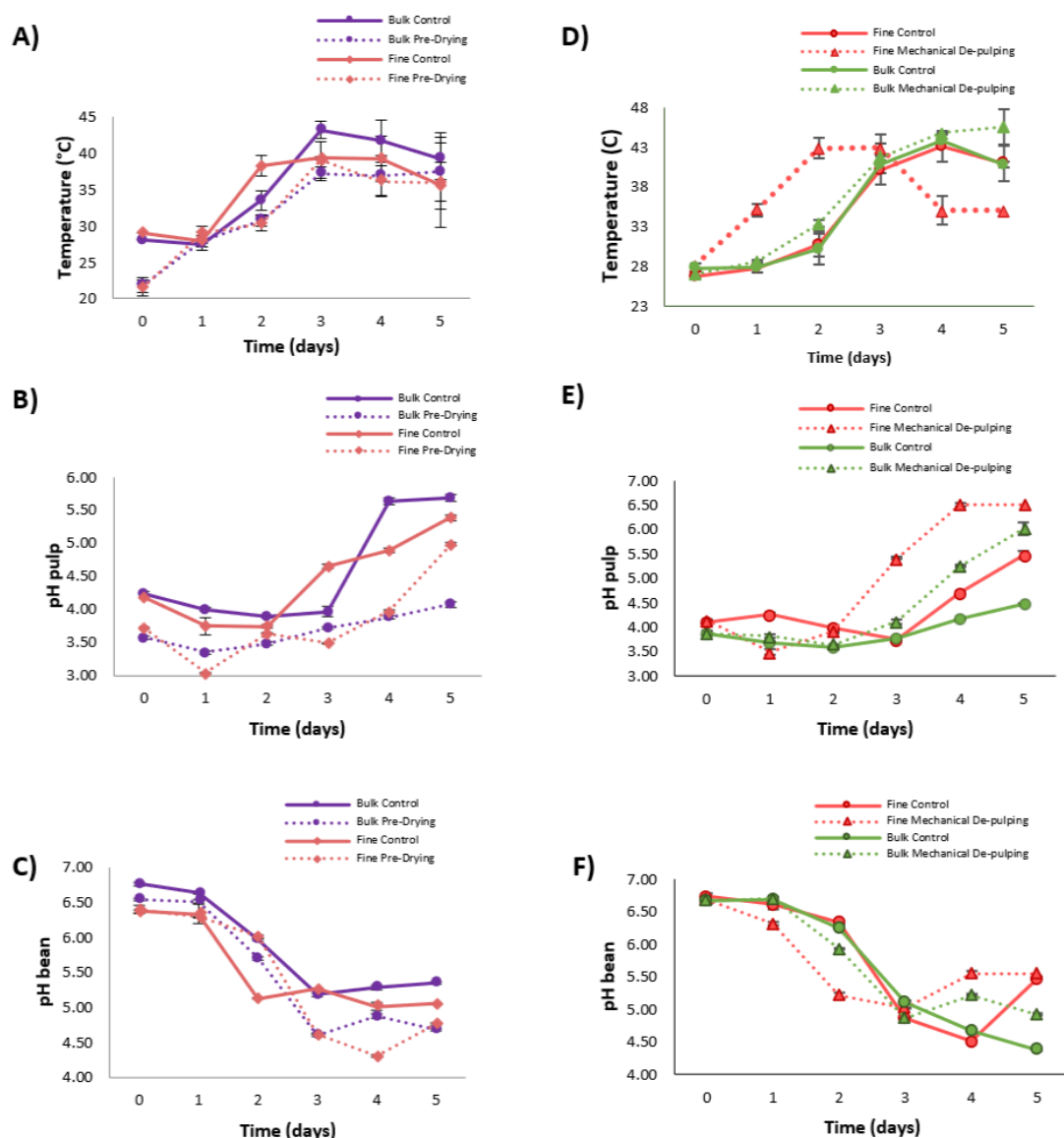


Fig. S4. A) Fermentation temperature of bulk and fine pre-dried cocoa compared to the control; B) Pulp pH of bulk and fine pre-dried cocoa compared to the control; C) Bean pH of bulk and fine pre-dried cocoa compared to the control; D) Fermentation temperature of bulk and fine mechanically de-pulped cocoa compared to the control.; E) Pulp pH of bulk and fine mechanically de-pulped cocoa compared to the control; F) Bean pH of bulk and fine mechanically de-pulped cocoa compared to the control.

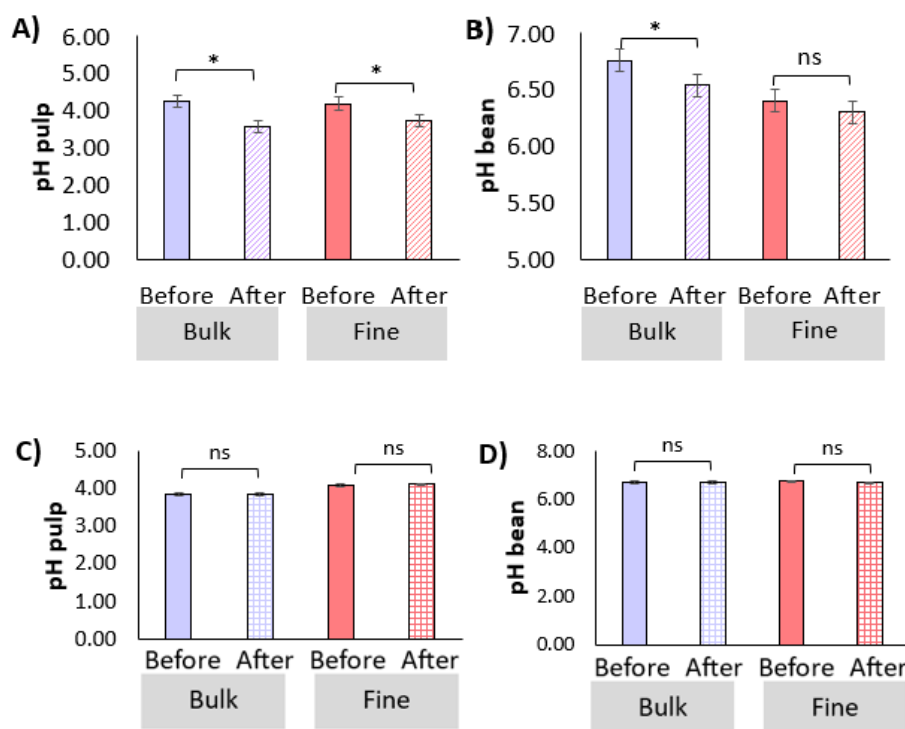


Fig. S5. Pulp and bean pH before and after de-pulping: A) Pulp pH before and after pre-drying; B) Bean pH before and after pre-drying; C) Pulp pH before and after mechanical de-pulping; D) Bean pH before and after mechanical de-pulping. Asterisks indicate statistically significant differences based on t-test: * $p < 0.05$, ** $p < 0.01$, ns: not significant

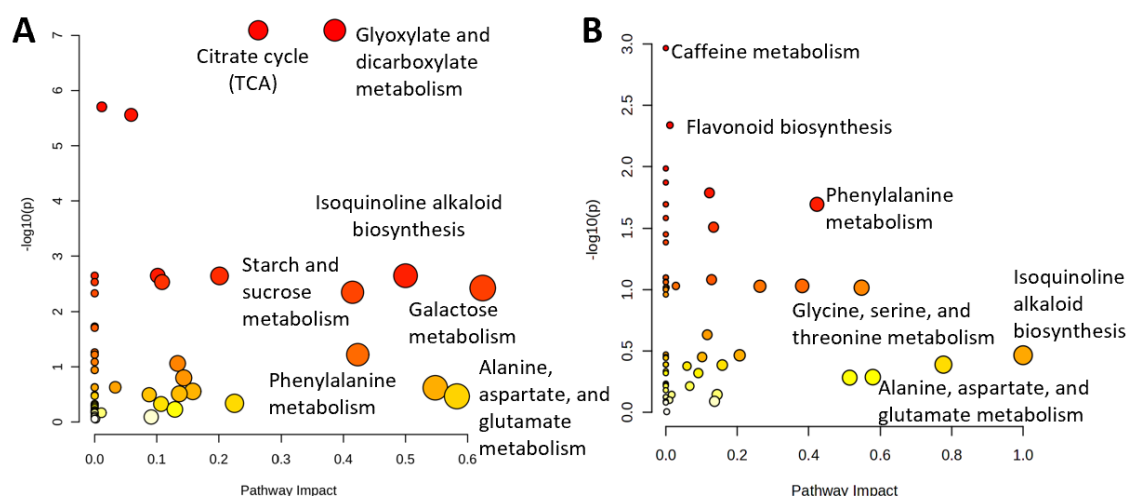


Fig S6. Pathway enrichment analysis of metabolites altered by de-pulping treatments in raw cocoa beans. (A) Pathways significantly affected by pre-drying treatment. The most impacted pathways include the citrate (TCA) cycle, glyoxylate and dicarboxylate metabolism, starch and sucrose metabolism, and amino acid-related pathways, indicating increased organic acids and sugars. (B) Pathways affected by mechanical de-pulping. Enrichment was observed in flavonoid biosynthesis, caffeine metabolism, and amino acid-related pathways, consistent with increased flavonoids and stress-related metabolites. Bubble size represents pathway impact (based on topology analysis), and color intensity indicates the significance level ($-\log_{10}(p)$).

Table S1. Adjustments in roasting temperature (°C) and time (minutes) by moisture content (%) and cacao bean size (bean count of 100g or average bean weight) (Cacao of Excellence, 2023).

Number	Sample	Bean count per 100 g	Moisture count (%)	Roasting temperature (°C)	Roasting Time (minute)
1	KW516 (Bulk control)	85	7.7	129	28
2	KW516 (Bulk pre-dried)	82	7.6	128	28
3	MCC02 (Fine control)	67	6.9	118	28
4	MCC02 (Fine pre-dried)	64	7.7	117	30
5	KW516 (Bulk control)	81	6.9	128	26
6	KW516 (Bulk Mechanical de-pulped)	87	6.9	129	25
7	MCC02 (Fine control)	73	7	118	27
8	MCC02 (Fine Mechanical de-pulped)	69	6.9	118	27

Table S2. Sensory score attributes assessed by 6 trained panellist (2024)

Sensory variable	Bulk control	Bulk pre-dried	Sensory variable	Fine control	Fine pre-dried
Cocoa	5.25 ± 0.80	5.33 ± 0.48	Cocoa	5.83 ± 0.69	5.92 ± 0.45
Acidity	1.08 ± 0.61	2.25 ± 0.56*	Acidity	1.33 ± 0.48	2.17 ± 0.69*
Bitterness	4.67 ± 0.39	4.42 ± 0.18	Bitterness	4.50 ± 0.28	4.17 ± 0.56
Astringency	4.50 ± 0.28	4.42 ± 0.34	Astringency	4.50 ± 0.28	4.33 ± 0.24
Fresh fruit	1.33 ± 0.48	2.00 ± 0.00*	Fresh fruit	1.00 ± 0.69	2.08 ± 0.60*
Browned fruit	1.50 ± 0.78	1.17 ± 0.97	Browned fruit	1.50 ± 0.53	1.33 ± 0.97
Floral	1.17 ± 0.81	0.83 ± 0.75	Floral	1.17 ± 0.69	0.83 ± 0.81
Woody	1.00 ± 0.69	0.67 ± 0.09	Woody	1.17 ± 0.69	0.83 ± 0.01
Spicy	0.00 ± 0.00	0.17 ± 0.00*	Spicy	0.17 ± 0.00	0.33 ± 0.00
Nutty	1.17 ± 0.75	1.00 ± 0.89	Nutty	1.17 ± 0.75	1.17 ± 0.95
Browned/Roast	4.83 ± 0.67	4.92 ± 0.57	Browned Roast	5.17 ± 0.55	5.00 ± 0.53
Global quality	6.58 ± 0.73	6.75 ± 0.56	Global quality	6.92 ± 0.18	6.75 ± 0.55

*Statistically significant different mean values ($p < 0.05$)

Table S3. Loading score of PCA results of cocoa liquor from bulk and fine cocoa (control and pre-dried)

Number	Metabolite and loading score			
	Metabolites	PC1	Metabolites	PC2
1	Shikimic acid	0.115891	2-Pyrrolidinone	0.115522
2	Acetic acid	0.11556	Tyrosine	0.112772
3	Trehalose	0.115519	2-Ethyl-3,6-dimethylpyrazine	0.106659
4	2,5-Dimethylpyrazine	0.115152	2,3,5,6-Tetramethylpyrazine	0.106568
5	Lyxose	0.114772	Glucaric acid	0.10059
6	Ethanone	0.114686	Quinic acid	0.0964459
7	Phenyl- β -D-glucopyranoside	0.114638	2,6-Dimethylpyrazine	0.0963489
8	Xylitol	0.114243	Isoamyl Lactate	0.0945973
9	Xylulose	0.113816	Lysine	0.0923408
10	3-Acetoxy-2-butanone	0.113595	Epicatechin	0.0916323
11	Mannose	0.112001	2,3,5-Trimethylpyrazine	0.0887201
12	1,2-Propanediol	0.111228	Trimethylpyrazine	0.0886987
13	3-Furanmethanol	0.110535	Catechin	0.0862775
14	Acetoin	0.110397	Mannitol	0.0829933
15	1-Deoxypentitol	0.109859	1-Methoxy-2-propyl acetate	0.0790494
16	Glucose	0.109747	cyanidin	0.0758402
17	Ethyl Lactate	0.10909	Valine	0.0748139
18	Pentanoic acid	0.108826	Glycine	0.0692906
19	Citric acid	0.108458	Adenine	0.0677867
20	2-Ethyl-3,5-dimethylpyrazine	0.107156	Tryptophan	0.0650573
21	2-Acetylfuran	0.107004	Theobromine	0.0647222
22	γ -Butyrolactone	0.106898	2-Butanone	0.0619438
23	Psicose	0.105907	Furfural	0.0617943
24	Butyrolactone	0.105739	2,3-Butanediol	0.059628
25	Glucono-1,5-lactone	0.105459	Propionic Acid	0.0592917
26	3-Methoxybutyl Acetate	0.104486	2-Hydroxyisocaproic acid	0.0590238
27	Methyl phenylacetate	0.103563	Leucine	0.0586178
28	Gluconic acid	0.103522	2-Ethyl-3,5-dimethylpyrazine	0.0584428
29	2-Dehydro gluconate	0.103099	Isoleucine	0.0541759
30	Fructose	0.102799	Galactose	0.0507597

31	Furfural	0.10192	Paraxanthine	0.0503594
32	Tartaric acid	0.0996365	Methionine	0.0502104
33	Ethyl heptanoate	0.0991199	1-Pentanol	0.0470579
34	Glycerol	0.0982791	3-Furanmethanol	0.0462646
35	Acetol	0.0977085	Pentanoic acid	0.0457083
36	Paraxanthine	0.0943419	2-Acetylfuran	0.0445664
37	1-Octen-3-ol	0.0942513	Meso Erythritol	0.0431285
38	Theobromine	0.0918196	2-Pentanone	0.0417969
39	Pyroglutamic acid	0.0915558	Gluconic acid	0.0404163
40	Trimethylpyrazine	0.0895851	2-Methylpyrazine	0.0402892
41	2,3,5-Trimethylpyrazine	0.0895657	Ribose	0.0401795
42	Inositol	0.0891485	3-Methoxybutyl Acetate	0.039943
43	Ethyl Octanoate	0.0867231	2,3-Butanedione	0.039938
44	Galacturonic acid	0.08515	Butyraldehyde	0.0398535
45	Galactitol	0.0840737	Glucono-1,5-lactone	0.0382981
46	4H-Pyran-4one	0.083644	Valeraldehyde	0.036835
47	Isocitric acid	0.0833645	Acetoin	0.0349641
48	Phosphate	0.0826706	Ornithine	0.0345079
49	Gentiobiose	0.0825386	Phenylalanine	0.0331646
50	Adenine	0.0825037	3-Phenyllactic acid	0.0321566
51	Ribose	0.0823479	Lactic acid	0.0294675
52	Sorbose	0.0797534	1-Octen-3-ol	0.026737
53	Glycolic acid	0.0791846	Sorbose	0.0267104
54	2,3-Dihydroxy-2-methylbutanoic acid	0.0748513	1,2-Propanediol	0.0249346
55	2,3,5,6-Tetramethylpyrazine	0.0732713	1-Deoxypentitol	0.0227612
56	2-Ethyl-3,6-dimethylpyrazine	0.0730739	Galacturonic acid	0.0227072
57	Glyceric acid	0.0723819	Shikimic acid	0.0222574
58	Cyanidin	0.0719536	3-Acetoxy-2-butanone	0.0198067
59	2,6-Dimethylpyrazine	0.071311	4H-Pyran-4one	0.0175257
60	Malic acid	0.0706796	Methanol	0.0159086
61	Melibiose	0.0654191	Ethyl heptanoate	0.0148298
62	γ -Nonalactone	0.0627066	3-Methylbutanal	0.0142383
63	Valeraldehyde	0.0605177	Ethanone	0.0141866
64	2,3-Butanedione	0.0585612	Galactitol	0.0137165
65	2-Pentanone	0.0563913	2-Aminoethanol	0.0136383
66	1,2,3-Butanetriol	0.0505919	2-Methylbutyraldehyde	0.00832561
67	2-Acetoxy-3-butanone	0.0504489	Pyroglutamic acid	0.00697885

68	3-Ethoxy-1-propanol	0.0469352	Psicose	0.00396397
69	2-Aminoethanol	0.0401346	Mannose	0.00290537
70	3-Heptanone	0.0393867	2,5-Dimethylpyrazine	0.00164284
71	Threonic acid	0.0390985	4-Aminobutyric acid	0.000966116
72	Tyrosine	0.0372565	Ethyl Lactate	-4.90E-05
73	2-Pyrrolidinone	0.0371687	Lyxose	-0.00099596
74	2-Phenethyl acetate	0.0334591	Glucose	-0.00234162
75	Isoamyl Ether	0.0326809	Proline	-0.00263936
76	Trans-2-Nonenal	0.0322291	3-Methyl-isovaleraldehyde	-0.00433191
77	Ethyl Hexanoate	0.0312182	Benzonitrile	-0.0064293
78	Scyllo Inositol	0.0260122	1-Hexanol	-0.00710789
79	Isobutyric Acid	0.0258328	Xylulose	-0.00862486
80	1-Hexanol	0.0234741	Trans-Linalool oxide	-0.00897814
81	5-Keto gluconate	0.0231637	Dimethyl Disulfide	-0.0109712
82	Fumaric acid	0.021205	Acetic acid	-0.0123755
83	β -Myrcene	0.019308	2-Methyl-3-buten-2-ol	-0.0128452
84	2,3-Pentanedion	0.0192755	Xylitol	-0.0180833
85	Phenylalanine	0.0182284	Threonine	-0.0195812
86	2-Hexanol	0.0179073	Phenyl- β -D-glucopyranoside	-0.0205322
87	Nonane	0.017468	5-Keto gluconate	-0.0212899
88	Dodecane	0.0169494	Serine	-0.0214301
89	Decane	0.0165914	Fumaric acid	-0.0216647
90	Heptane	0.016055	2-Pentanol	-0.0235743
91	Raffinose	0.0137938	1-Phenylethyl alcohol	-0.0298121
92	Octane	0.012768	3-Hexanone	-0.0311512
93	Ethyl Isobutyrate	0.0107701	Trehalose	-0.0315119
94	Ethyl Acetate	0.009629	Gentiobiose	-0.0334313
95	2-Octanone	0.00832267	Nonanal	-0.034331
96	Asparagine	0.00658996	Fructose	-0.0348275
97	2-Methylbutyl acetate	0.00469652	3- α -Mannobiose	-0.0355513
98	3-Methylbutyl acetate	0.00355639	2-Heptanone	-0.0358107
99	Succinic acid	0.00302058	3-Heptanone	-0.0362231
100	N- α -Acetyl lysine	0.00027365	Ethanol	-0.0396549
101	Leucine	-0.00017195	Isocitric acid	-0.0405991
102	3-Methyl-1-butanol acetate	-0.00289789	Linalool	-0.0417744
103	3- α -Mannobiose	-0.00555995	Trans- β -Ocimene	-0.0422503
104	2-Hydroxyglutaric acid	-0.00683613	Melibiose	-0.043855
105	Alanine	-0.00849248	Citric acid	-0.0444393
106	Methionine	-0.00864368	Raffinose	-0.045608
107	Phenylacetaldehyde	-0.0193948	3-Methyl-1-butanol	-0.049183
108	Glutamic acid	-0.020184	γ -Butyrolactone	-0.0595461
109	Isobutyl acetate	-0.0212668	Butyrolactone	-0.0602696
110	Sucrose	-0.0215677	1-Butanol	-0.0602817

111	Serine	-0.0233808	2-Methyl-1-propanol	-0.0606742
112	Threitol	-0.0249883	Scyllo Inositol	-0.06236
113	Dimethyl Disulfide	-0.0285856	Methyl phenylacetate	-0.0641995
114	Quinic acid	-0.0303102	2-Dehydro gluconate	-0.0668149
115	2-Phenylpropionaldehyde	-0.0304327	Pentanol acetate	-0.0675232
116	Glucosamine	-0.0344008	Tartaric acid	-0.0698905
117	Saccharic acid	-0.0350591	Glycerol	-0.0725686
118	Butyraldehyde	-0.0363074	2-Hydroxyglutaric acid	-0.076464
119	Ethyl- α - glucopyranoside	-0.0367297	Acetol	-0.0770809
120	Epicatechin	-0.0389665	2-Hexanol	-0.0800346
121	Catechin	-0.0435881	Phenylacetaldehyde	-0.0801575
122	Turanose	-0.0492555	Inositol	-0.0832579
123	Galactinol	-0.0495592	2-Octanol acetate	-0.0850215
124	Caffeine	-0.0508664	2-Methyl-1-butanol	-0.0858643
125	2-Methyl-3-buten-2-ol	-0.0517028	Ethyl- α glucopyranoside	-0.0868358
126	Cis- β -Ocimene	-0.0529916	Ethyl Octanoate	-0.0879103
127	Trans- β -Ocimene	-0.0530622	Asparagine	-0.0879852
128	1-Pentanol	-0.055218	2-Nonanol	-0.088383
129	2-Heptanol	-0.0558018	Trans-2-Nonenal	-0.0888208
130	3-Hydroxy-3-Methylglutaric acid	-0.0566698	Aspartic acid	-0.0891508
131	3-Methyl-isovaleraldehyde	-0.058241	2-Octanone	-0.0902595
132	3-Methylbutanal	-0.0587461	3-Hydroxy-3-Methylglutaric acid	-0.0905603
133	3-Carene	-0.0597009	Phosphate	-0.0929516
134	2-Methylbutyraldehyde	-0.0610433	Glyceric acid	-0.0929808
135	Lysine	-0.0619963	Cis- β -Ocimene	-0.0932895
136	Tryptophan	-0.0640735	Benzaldehyde	-0.0944719
137	Isoleucine	-0.0646206	Glycolic acid	-0.095891
138	Ethanol	-0.065977	Ethyl Acetate	-0.0972532
139	Methanol	-0.0669439	2,3-Dihydroxy-2-methylbutanoic acid	-0.100559
140	2-Nonanone	-0.0675481	Threonic acid	-0.100983
141	Glycine	-0.0679245	γ -Nonalactone	-0.102631
142	Threonine	-0.0680743	Butyric acid	-0.102872
143	Aspartic acid	-0.0684495	2-Acetoxy-3-butanone	-0.103494
144	2-Methyl-1-propanol	-0.0688399	Alanine	-0.104916
145	Valine	-0.0698148	2-Methylbutyl acetate	-0.105694
146	2-Butanone	-0.0706087	3-Methylbutyl acetate	-0.106191
147	2-Methyl-1-butanol	-0.0706231	Malic acid	-0.106878
148	Isoamyl Lactate	-0.0724225	Caffeine	-0.107215
149	Butyric acid	-0.0733773	2-Nonanone	-0.107808
150	Glucaric acid	-0.074356	Succinic acid	-0.108347

151	Pentanol acetate	-0.0756248	2-Phenethyl acetate	-0.108602
152	Trans-Linalool oxide	-0.0758181	2,3-Pentanedion	-0.110318
153	2-Nonanol	-0.0782752	3-Ethoxy-1-propanol	-0.112767
154	Benzaldehyde	-0.0790578	3-Carene	-0.113035
155	2-Pentanol	-0.0805971	Ethyl Hexanoate	-0.11326
156	Galactose	-0.082309	Isobutyric Acid	-0.114115
157	1-Butanol	-0.0824378	Glucosamine	-0.114189
158	2-Heptanone	-0.0829268	β-myrcene	-0.115091
159	2-Heptanone	-0.0830248	Saccharic acid	-0.115261
160	1-Methoxy-2-propyl acetate	-0.0847596	1,2,3-Butanetriol	-0.115391
161	3-Methyl-1-butanol	-0.0849126	Turanose	-0.115438
162	2-Octanol acetate	-0.086105	2-Heptanol	-0.116235
163	Proline	-0.0864473	Galactinol	-0.116384
164	Nonanal	-0.0874264	Isoamyl Ether	-0.117092
165	3-Hexanone	-0.0877667	Decane	-0.117388
166	Mannitol	-0.0922853	Nonane	-0.117474
167	1-Phenylethyl alcohol	-0.0935212	Dodecane	-0.117587
168	2,3-Butanediol	-0.100788	Heptane	-0.118359
169	Propionic Acid	-0.100904	Octane	-0.120011
170	Meso erythritol	-0.100979	2-Phenylpropionaldehyde	-0.121378
171	2-Methylpyrazine	-0.101618	Ethyl Isobutyrate	-0.121704
172	2-Hydroxyisocaproic acid	-0.105262	3-Heptanone	-0.122644
173	Ornithine	-0.107725	Glutamic acid	-0.123948
174	Benzonitrile	-0.108449	N-α-Acetyl lysine	-0.124134
175	Linalool	-0.10906	3-Methyl-1-butanol acetate	-0.126638
176	3-Phenyllactic acid	-0.111296	Threitol	-0.128472
177	Lactic acid	-0.113845	Isobutyl acetate	-0.129698
178	4-Aminobutyric acid	-0.114118	Sucrose	-0.132873

Table S4. Sensory score attributes of mechanically de-pulped cocoa liquor (bulk and fine) compared to the control, assessed by six trained panelists.

Sensory variable	Bulk control	Bulk mechanical de-pulped	Sensory variable	Fine control	Fine mechanical de-pulped
Cocoa	6.00 ± 0.00*	5.33 ± 0.26	Cocoa	5.42 ± 0.20	5.17 ± 0.26
Acidity	2.00 ± 0.00*	1.17 ± 0.41	Acidity	2.00 ± 0.00*	1.00 ± 0.00
Bitterness	4.50 ± 0.00	5.17 ± 0.26*	Bitterness	4.58 ± 0.38	5.25 ± 0.27*
Astringency	4.50 ± 0.00	5.00 ± 0.32*	Astringency	4.75 ± 0.27	5.25 ± 0.27*
Fresh fruit	2.00 ± 0.00*	1.00 ± 0.00	Fresh fruit	2.00 ± 0.00	1.00 ± 0.00

Browned fruit	1.00 ± 0.00	0.67 ± 0.52	Browned fruit	1.00 ± 0.00	1.00 ± 0.00
Floral	0.92 ± 0.49	0.50 ± 0.55	Floral	2.00 ± 0.00	0.00 ± 0.00
Woody	1.25 ± 0.42	1.83 ± 0.41*	Woody	1.08 ± 0.20	1.00 ± 0.00
Spicy	n.d.	n.d.	Spicy	n.d.	n.d.
Nutty	1.25 ± 0.42	1.17 ± 0.26*	Nutty	1.92 ± 0.20	1.50 ± 0.20
Browned Roast	5.42 ± 0.20*	5.08 ± 0.20	Browned Roast	5.33 ± 0.26*	5.00 ± 0.00
Global quality	7.47 ± 0.08*	6.50 ± 0.00	Global quality	7.00 ± 0.00*	6.53 ± 0.08

*Statistically significant different mean values ($p < 0.05$)

Table S5. Loading score of PCA results of cocoa liquor from bulk and fine cocoa (control and mechanically de-pulped)

Number	Metabolite and loading score			
	Metabolites	PC1	Metabolites	PC2
1	1-Octen-3-ol	0.121	Ethyl Hexanoate	0.147
2	Epicatechin	0.118	Succinic acid	0.146
3	Sucrose	0.114	Glycerol	0.146
4	Paeoniflorin	0.113	Phosphate	0.140
5	Galactinol	0.112	Ethyl Octanoate	0.136
6	Nonanal	0.112	Citric acid	0.127
7	Glutamic acid	0.112	Paraxanthine	0.126
8	Catechine	0.112	2-Methyl-3-buten-2-ol	0.121
9	Raffinose	0.112	2-Hexanol	0.121
10	Ethyl benzene	0.111	2-Pentanol	0.113
11	Glucose	0.111	3-Methoxybutyl Acetate	0.112
12	Fumaric acid	0.106	Mannitol	0.112
13	Galactitol	0.105	Isocitric acid	0.111
14	1-Pentanol	0.105	3-Hexanone	0.111
15	Shikimic acid	0.103	Glycine	0.111
16	Trans-2-Nonenal	0.102	1-Methoxy-2-propyl acetate	0.111
17	Glutamine	0.102	2-Aminoethanol	0.110
18	Octane	0.101	2-Methyl-1-butanol	0.109
19	2-Dehydro gluconate	0.101	Acetic acid	0.109
20	Fructose	0.101	Ethyl heptanoate	0.108
21	Gentiobiose	0.101	Malic acid	0.108
22	Saccharic acid	0.100	Ethyl Lactate	0.108
23	Valeraldehyde	0.097	Inositol	0.108
24	Threitol	0.097	Scyllo-Inositol	0.107
25	Gluconic acid	0.097	3-Methyl-1-butanol	0.107
26	Glucono-1,5-lactone	0.097	Methionine	0.104
27	Decane	0.094	3-Acetoxy-2-butanone	0.104
28	Serotonin	0.094	Alanine	0.104
29	N- α -Acetyl lysine	0.091	Caffeine	0.104

30	2-Phenylpropionaldehyde	0.090	Aspartic acid	0.103
31	Tartaric acid	0.089	3-Heptanone	0.102
32	Caffeine	0.087	Pyroglutamic acid	0.102
33	3-Methylbutyl methylbutanoate	2- 0.087	Propyl acetate	0.100
34	Glycolic acid	0.086	Ethanol	0.098
35	Inositol	0.084	Acetophenone	0.098
36	Butyric acid	0.083	2-Heptanone	0.097
37	Galactose	0.083	γ -Nonalactone	0.097
38	Scyllo-Inositol	0.082	Cis- β -Ocimene	0.096
39	Dopamine	0.080	Trehalose	0.095
40	Ribose	0.080	Galactose	0.095
41	1,2,3-Butanetriol	0.079	1-Hexanol	0.094
42	Malic acid	0.076	Ethyl-DL-2- Methylbutyrate	0.093
43	2-Methyloctane	0.072	2-Phenylpropionaldehyde	0.093
44	Acetophenone	0.071	Maltose	0.092
45	Butanoic acid	0.070	3-Carene	0.092
46	Isocitric acid	0.070	3-Methylbutyl acetate	0.092
47	1-Hexanol	0.067	Cyanidin	0.092
48	2-Phenethyl acetate	0.064	Leucine	0.091
49	Methionine	0.063	2-Pentanone	0.090
50	Citric acid	0.062	Tartaric acid	0.090
51	Threonine	0.060	Benzaldehyde	0.089
52	Threonic acid	0.057	Methyl phenylacetate	0.088
53	Glycine	0.056	Adenine	0.088
54	3-Phenyllactic acid	0.056	Tyrosine	0.088
55	Lysine	0.049	Phenylalanine	0.088
56	Xylulose	0.048	N- α -Acetyl lysine	0.087
57	Acetol	0.048	3-Ethoxy-1-propanol	0.086
58	2,3-Pentanedion	0.044	2-Propanol	0.085
59	Phenylalanine	0.043	Glyceric acid	0.085
60	Ethyl heptanoate	0.041	Fructose	0.084
61	3-Hexanone	0.040	2-Methyl-1-propanol	0.083
62	Cis- β -Ocimene	0.040	Methanol	0.082
63	Ethyl DL-2-Methylbutyrate	0.038	Acetoin	0.081
64	Asparagine	0.037	1-Propanol	0.079
65	Isobutyric Acid	0.037	Trans-2-Nonenal	0.077
66	Glyceric acid	0.037	Lysine	0.076
67	Erythritol	0.035	Xylulose	0.076
68	1-Phenylethyl alcohol	0.034	Ethanone	0.075
69	β -Lactose	0.033	Ethyl Acetate	0.075
70	Butyl acetate	0.033	Quinic acid	0.074
71	Turanose	0.032	Saccharic acid	0.073
72	Proline	0.028	Galactitol	0.067
73	Serine	0.028	4H-Pyran-4-one	0.064
74	1-Butanol	0.024	Meso erythritol	0.062
75	Isoleucine	0.023	2-Heptanol	0.061

76	Aspartic acid	0.021	Isobutyl acetate	0.061
77	Alanine	0.021	Galactinol	0.058
78	Leucine	0.020	Glucono-1,5-lactone	0.057
79	3-Carene	0.020	Gluconic acid	0.056
80	Trehalose	0.017	Lactic acid	0.056
81	Valine	0.017	Nonanal	0.056
82	Galacturonic acid	0.016	Glucose	0.054
83	Succinic acid	0.016	Paeoniflorin	0.054
84	3-Ethoxy-1-propanol	0.015	Isoleucine	0.053
85	Phosphate	0.014	Theobromine	0.053
86	Phenylacetaldehyde	0.013	Tryptophan	0.052
87	Nonanoic acid	0.011	Shikimic acid	0.051
88	Glycerol	0.009	Gentiobiose	0.051
89	Ethyl Isobutyrate	0.009	2-Phenethyl acetate	0.051
90	Methyl 2-methylbutanoate	0.008	Ethyl propanoate	0.047
91	Tyrosine	0.005	β -Lactose	0.046
92	Meso erythritol	0.005	Serine	0.046
93	2-Aminoethanol	0.001	3-Methylbutanal	0.043
94	Butyraldehyde	-0.005	Threonic acid	0.043
95	Mannitol	-0.009	Methyl acetate	0.042
96	Lyxose	-0.012	2-Methylbutyraldehyde	0.041
97	Paraxanthine	-0.015	Sucrose	0.041
98	2-Butenoic acid	-0.015	Threonine	0.041
99	Ethyl Hexanoate	-0.019	Raffinose	0.039
100	2-Methyl-3-buten-2-ol	-0.024	Lyxose	0.037
101	3-Heptanone	-0.025	2,3-Pentanedione	0.037
102	Ribulose	-0.028	2,6-Dimethylpyrazine	0.036
103	γ -Nonalactone	-0.030	Trans-Linalool oxide	0.036
104	Sorbitol	-0.030	Glycolic acid	0.036
105	Prenol	-0.033	4-Aminobutyric acid	0.036
106	4H-Pyran-4-one	-0.038	2-Acetylfuran	0.035
107	Maltose	-0.040	Epicatechin	0.034
108	Lactic acid	-0.041	2,3-Butanediol	0.033
109	Tryptophan	-0.042	Fumaric acid	0.033
110	Propionic Acid	-0.044	Glutamic acid	0.032
111	Ethyl Octanoate	-0.045	Ethyl benzene	0.031
112	2-Methylpyrazine	-0.045	2-Nonanol	0.026
113	Pyroglutamic acid	-0.046	Erythritol	0.025
114	Ethyl propanoate	-0.049	Octane	0.025
115	4-Aminobutyric acid	-0.050	Dimethyl Disulfide	0.024
116	2-Hexanol	-0.052	Dopamine	0.023
117	Methyl acetate	-0.053	2,3,5,6-Tetramethylpyrazine	0.022
118	Trans-Linalool oxide	-0.056	Furfural	0.022
119	Quinic acid	-0.056	1-Octen-3-ol	0.022
120	Cyanidin	-0.058	Dimethyl Sulfide	0.021
121	2-Pentanone	-0.061	Galacturonic acid	0.019

122	2-Heptanone	-0.064	Ribose	0.018
123	γ -Butyrolactone	-0.065	Catechin	0.018
124	2-Methyl-1-butanol	-0.065	Serotonin	0.018
125	Dimethyl Sulfide	-0.066	1-Butanol	0.018
126	2-Propanol	-0.070	Valine	0.017
127	Ethanol	-0.071	2- α -mannobiose	0.015
128	3-Methyl-3-buten-1-ol	-0.072	Butyl acetate	0.015
129	Adenine	-0.074	Vinyl n-Hexanoate	0.007
130	2-Methyl-1-propanol	-0.076	Glutamine	0.005
131	3-Methyl-1-butanol	-0.076	Asparagine	0.004
132	3-Methoxybutyl Acetate	-0.080	Propionic Acid	0.001
133	1-Propanol	-0.082	2-Dehydro gluconate	0.001
134	Ethanone	-0.082	Proline	0.000
135	2-Pentanol	-0.083	Decane	-0.003
136	Acetoin	-0.083	1,2,3-Butanetriol	-0.005
137	Benzaldehyde	-0.084	2-Ethyl-3,5-dimethylpyrazine	-0.007
138	Propyl acetate	-0.084	Trimethylpyrazine	-0.009
139	1-Methoxy-2-propyl acetate	-0.085	2,3,5-Trimethylpyrazine	-0.009
140	Acetic acid	-0.087	3-Phenyllactic acid	-0.009
141	Ethyl Lactate	-0.087	3-Methyl-2-butanone	-0.013
142	Methyl phenylacetate	-0.089	Ethyl Isobutyrate	-0.014
143	3-Acetoxy-2-butanone	-0.090	2,3-Butanedione	-0.018
144	3-Methylbutyl acetate	-0.090	Turanose	-0.023
145	2,5-Dimethylpyrazine	-0.092	Threitol	-0.024
146	Ethyl Acetate	-0.096	Pentanoic acid	-0.024
147	Methanol	-0.096	1-Pentanol	-0.025
148	Isobutyl acetate	-0.097	2,3-Butanediol	-0.029
149	Theobromine	-0.098	2-Nonanone	-0.031
150	2,3-Butanediol	-0.099	Benzonitrile	-0.031
151	2- α -mannobiose	-0.099	Valeraldehyde	-0.032
152	2-Nonanol	-0.100	Phenylacetaldehyde	-0.036
153	2,3-Butanedione	-0.106	Butyraldehyde	-0.039
154	2-Methylbutyraldehyde	-0.110	Ribulose	-0.049
155	Benzonitrile	-0.110	2-Methyloctane	-0.057
156	3-Methylbutanal	-0.111	Methyl 2-methylbutanoate	-0.060
157	Dimethyl Disulfide	-0.112	2,5-Dimethylpyrazine	-0.068
158	2-Heptanol	-0.112	3-Methylbutyl 2-methylbutanoate	-0.068
159	2,3-Butanediol	-0.114	γ -Butyrolactone	-0.069
160	Pentanoic acid	-0.116	3-Methyl-3-buten-1-ol	-0.076
161	2-Acetylfuran	-0.117	1-Phenylethyl alcohol	-0.092
162	Furfural	-0.117	Nonanoic acid	-0.099
163	2,6-Dimethylpyrazine	-0.118	Sorbitol	-0.100

164	2-Nonanone	-0.119	Butyric acid	-0.103
165	3-Methyl-2-butanone	-0.120	Acetol	-0.107
166	2,3,5,6-Tetramethylpyrazine	-0.120	Butanoic acid	-0.114
167	2-Ethyl-3,5-dimethylpyrazine	-0.121	Prenol	-0.122
168	2,3,5-Trimethylpyrazine	-0.121	2-Butenoic acid	-0.125
169	Trimethylpyrazine	-0.121	2-Methylpyrazine	-0.133
170	Vinyl n-Hexanoate	-0.121	Isobutyric Acid	-0.138

Table S6. Compounds with high VIP values (VIP > 1.0) and a positive coefficient from PLS regression analysis

Cocoa		Acidity		Bitterness		Astringency	
Metabolite name	VIP score	Metabolite name	VIP score	Metabolite name	VIP score	Metabolite name	VIP score
Sucrose	1.733	Citric acid	1.581	Glucaric acid	1.636	Mannitol	1.507
		Tartaric acid	1.574	Mannitol	1.554	2-Hydroxyisocaproic acid	1.499
2-Heptanol	1.728	Trehalose	1.568	Lysine	1.464	Glucaric acid	1.492
Turanose	1.718			Propionic Acid	1.348	Meso erythritol	1.450
Isobutyl acetate	1.717	Glycerol	1.560	Quinic acid	1.299	Propionic Acid	1.421
2-Nonanone	1.706	Phosphate	1.531			3-Phenyllactic acid	1.395
Glucosamine	1.699	Fructose	1.522	Valine	1.296	Lactic acid	1.381
Galactinol	1.692	Inositol	1.497	Epicatechin	1.292	Lysine	1.247
Caffeine	1.677	Malic acid	1.494	Catechin	1.255	Valine	1.176
Butyric acid	1.668	Acetic acid	1.491	Glycine	1.219		
		Glycolic acid	1.4656	Meso erythritol	1.189	Galactose	1.148
3-Carene	1.667		5				
Fresh fruit		Browned fruit		Floral		Woody	
Metabolite name	VIP score	Metabolite name	VIP score	Metabolite name	VIP score	Metabolite name	VIP score
Butyrolactone	1.607	2,3-Butanediol	1.470	2,3-Butanediol	1.580	Linalool	1.479
				1-Methoxy-2-propyl acetate	1.444	2-Methylpyrazine	1.395
Glycerol	1.606	Benzonitrile	1.377				
		1-Methoxy-2-propyl acetate	1.359	Isoamyl Lactate	1.376	1-Phenylethyl alcohol	1.388
γ-Butyrolactone	1.604	Linalool	1.284	Benzonitrile	1.238	2-Methylpyrazine	1.395
Tartaric acid	1.585						
		2-Methylpyrazine	1.267	2-Butanone	1.130	1-Phenylethyl alcohol	1.388
2-Dehydrogluconate	1.579						

Ethyl Octanoate	1.558	Isoamyl Lactate	1.196	Linalool	1.097	3-Hexanone	1.164
Methyl phenylacetate	1.553	1-Phenylethyl alcohol	1.043	1-Phenylethyl alcohol	1.011	Nonanal	1.158
Inositol	1.538					2-Octanol acetate	1.143
Acetol	1.517					2-Nonanol	1.088
Glycolic acid	1.505					2-Pentanol	1.085
Spicy		Nutty		Browned roast		Global quality	
Metabolite name	VIP score	Metabolite name	VIP score	Metabolite name	VIP score	Metabolite name	VIP score
3-Heptanone	1.694	Benzonitrile	1.276	Cis- β -Ocimene	1.879	Cis- β -Ocimene	1.921
Isobutyric Acid	1.644	2-Octanol acetate	1.180	1-Phenylethyl alcohol	1.693	1-Phenylethyl alcohol	1.664
Isoamyl Ether	1.566	Benzaldehyde	1.140	2-Nonanol	1.703	Succinic acid	1.812
3-Methyl-1-butanol acetate	1.528	2-Nonanone	1.086	2-Hexanol	1.017	Isobutyric Acid	1.661
3-Ethoxy-1-propanol	1.525	3-Carene	1.084	Trans- β -Ocimene	1.089	Glucosamine	1.681
Isobutyl acetate	1.521	2-Nonanol	1.059	2-Nonanone	1.577	Caffeine	1.694
Ethyl Isobutyrate	1.517	1-Butanol	1.043	Linalool	1.419	Sorbose	1.299
				Nonanal	1.318	Galactitol	1.323
				2-Octanol acetate	1.555	Fumaric acid	1.059
				Benzaldehyde	1.547	3-Hydroxy-3-Methylglutaric acid	1.556

Table S7. Loading score of OPLS-DA results, VIP score, and coefficient of control and pre-dried cocoa liquor

Number	Metabolites	Loading score		VIP score	Coefficient
		R2X[1]	R2X[2]		
1	2-Dehydro gluconate	0.121	0.006	1.643	-0.017
2	Butyrolactone	0.121	-0.001	1.640	-0.017
3	γ -Butyrolactone	0.121	-0.003	1.638	-0.016
4	Methyl phenylacetate	0.121	0.004	1.636	-0.017
5	Acetol	0.120	0.015	1.620	-0.016
6	Tartaric acid	0.120	0.011	1.617	-0.017

7	Glycerol	0.119	0.014	1.617	-0.017
8	Citric acid	0.116	-0.017	1.577	-0.016
9	Ethyl Octanoate	0.116	0.033	1.568	-0.017
10	Trehalose	0.116	-0.034	1.570	-0.014
11	Inositol	0.115	0.025	1.551	-0.015
12	Phosphate	0.114	0.038	1.539	-0.016
13	Glycolic acid	0.112	0.044	1.516	-0.016
14	2,3-Dihydroxy-2-methylbutanoic acid	0.110	0.050	1.493	-0.016
15	Phenyl- β -D-glucopyranoside	0.110	-0.044	1.488	-0.013
16	Acetic acid	0.109	-0.049	1.477	-0.014
17	Malic acid	0.109	0.056	1.471	-0.015
18	Xylitol	0.109	-0.046	1.469	-0.012
19	Xylulose	0.105	-0.052	1.426	-0.013
20	Glyceric acid	0.104	0.043	1.409	-0.015
21	Fructose	0.104	-0.028	1.414	-0.011
22	2,5-Dimethylpyrazine	0.102	-0.062	1.382	-0.012
23	Ethyl Lactate	0.102	-0.048	1.375	-0.016
24	Lyxose	0.101	-0.062	1.373	-0.011
25	γ -Nonalactone	0.101	0.059	1.364	-0.016
26	Mannose	0.098	-0.063	1.328	-0.011
27	Glucose	0.097	-0.058	1.322	-0.010
28	Ethanone	0.097	-0.071	1.315	-0.012
29	1,2,3-Butanetriol	0.096	0.077	1.295	-0.015
30	Shikimic acid	0.095	-0.077	1.294	-0.012
31	3-Acetoxy-2-butanone	0.094	-0.075	1.269	-0.011
32	Isocitric acid	0.092	-0.009	1.248	-0.012
33	3-Ethoxy-1-propanol	0.091	0.076	1.228	-0.014
34	Psicose	0.091	-0.062	1.236	-0.009
35	2-Acetoxy-3-butanone	0.091	0.067	1.226	-0.015
36	Gluconic acid	0.089	-0.082	1.208	-0.010
37	Gentiobiose	0.088	-0.012	1.193	-0.012
38	1-Deoxypentitol	0.088	-0.078	1.194	-0.010
39	1,2-Propanediol	0.088	-0.082	1.190	-0.009
40	2-Phenethyl acetate	0.086	0.065	1.165	-0.015
41	Acetoin	0.085	-0.085	1.156	-0.011
42	3-Heptanone	0.084	0.081	1.145	-0.011
43	Pyroglutamic acid	0.084	-0.044	1.134	-0.014
44	3-Methoxybutyl Acetate	0.081	-0.081	1.095	-0.012
45	Threonic acid	0.080	0.072	1.077	-0.013
46	3-Furanmethanol	0.079	-0.098	1.070	-0.008
47	Pentanoic acid	0.078	-0.095	1.061	-0.009
48	Ethyl heptanoate	0.077	-0.073	1.046	-0.005
49	Glucono-1,5-lactone	0.077	-0.090	1.046	-0.008
50	2-Acetylfuran	0.076	-0.096	1.031	-0.008
51	1-Octen-3-ol	0.076	-0.067	1.030	-0.011
52	Isoamyl Ether	0.074	0.076	1.000	-0.008
53	Ethyl Hexanoate	0.073	0.078	0.988	-0.009
54	2-Ethyl-3,5-Dimethylpyrazine	0.072	-0.104	0.979	-0.008
55	Trans-2-Nonenal	0.071	0.068	0.958	-0.014
56	β -myrcene	0.067	0.098	0.909	-0.012

57	4H-Pyran-4one	0.067	-0.059	0.916	-0.008
58	Galactitol	0.067	-0.061	0.911	-0.006
59	Isobutyric Acid	0.067	0.076	0.907	-0.007
60	Galacturonic acid	0.065	-0.066	0.883	-0.006
61	Furfural	0.065	-0.106	0.886	-0.007
62	Nonane	0.065	0.088	0.881	-0.006
63	Decane	0.065	0.089	0.874	-0.006
64	Dodecane	0.064	0.088	0.867	-0.006
65	Paraxanthine	0.064	-0.092	0.862	-0.007
66	Heptane	0.063	0.089	0.856	-0.006
67	Melibiose	0.060	-0.002	0.798	-0.012
68	2,3-Pentanedion	0.059	0.077	0.795	-0.006
69	Octane	0.058	0.089	0.779	-0.006
70	Ethyl Isobutyrate	0.056	0.091	0.757	-0.006
71	2-Methylbutyl acetate	0.055	0.101	0.745	-0.014
72	Theobromine	0.055	-0.103	0.742	-0.005
73	Sorbose	0.055	-0.074	0.750	-0.002
74	3-Methylbutyl acetate	0.054	0.102	0.734	-0.013
75	2-Octanone	0.053	0.087	0.718	-0.014
76	Ribose	0.052	-0.086	0.702	-0.001
77	N- α -Acetyl lysine	0.050	0.103	0.685	-0.007
78	Ethyl Acetate	0.050	0.080	0.684	-0.009
79	3-Methyl-1-butanol acetate	0.049	0.108	0.668	-0.008
80	Scyllo Inositol	0.047	0.035	0.629	-0.005
81	Asparagine	0.046	0.077	0.621	-0.009
82	Succinic acid	0.045	0.085	0.608	-0.004
83	Trimethylpyrazine	0.044	-0.120	0.599	-0.005
84	2,3,5-Trimethylpyrazine	0.044	-0.120	0.599	-0.005
85	Adenine	0.044	-0.103	0.595	-0.003
86	2-Hexanol	0.041	0.045	0.561	0.000
87	2,3-Butanedione	0.041	-0.055	0.555	-0.008
88	2-Pentanone	0.038	-0.055	0.518	-0.008
89	5-Keto gluconate	0.035	0.017	0.470	-0.009
90	Alanine	0.035	0.092	0.486	-0.006
91	Sucrose	0.035	0.123	0.477	-0.006
92	Valeraldehyde	0.035	-0.070	0.484	-0.001
93	Raffinose	0.034	0.035	0.462	-0.006
94	Glutamic acid	0.034	0.117	0.468	-0.007
95	Isobutyl acetate	0.033	0.119	0.452	-0.005
96	Threitol	0.033	0.126	0.444	-0.007
97	Cyanidin	0.031	-0.105	0.419	-0.001
98	2-Hydroxyglutaric acid	0.027	0.072	0.367	-0.006
99	Fumaric acid	0.026	0.004	0.375	-0.004
100	2,6-Dimethylpyrazine	0.026	-0.114	0.352	-0.004
101	2-Aminoethanol	0.025	-0.041	0.351	0.000
102	2,3,5,6-Tetramethylpyrazine	0.022	-0.127	0.300	-0.002
103	2-Ethyl-3,6-dimethylpyrazine	0.022	-0.127	0.297	-0.002
104	Phenylacetaldehyde	0.020	0.086	0.277	-0.007
105	2-Phenylpropionaldehyde	0.020	0.113	0.264	-0.002
106	1-Hexanol	0.018	-0.017	0.234	0.003
107	Saccharic acid	0.018	0.118	0.233	-0.004

108	3- α -Mannobiose	0.015	0.040	0.196	-0.005
109	Glucosamine	0.015	0.111	0.207	-0.002
110	Phenylalanine	0.009	-0.027	0.118	-0.005
111	Ethyl- α -glucopyranoside	0.008	0.102	0.101	-0.006
112	Turanose	0.002	0.121	0.033	-0.001
113	Galactinol	0.001	0.120	0.021	0.000
114	2-Heptanol	-0.001	0.128	0.019	-0.001
115	Caffeine	-0.004	0.112	0.049	0.001
116	3-Carene	-0.005	0.130	0.066	-0.002
117	Tyrosine	-0.009	-0.106	0.118	-0.001
118	Serine	-0.010	0.033	0.121	-0.002
119	2-Pyrrolidinone	-0.012	-0.112	0.161	0.001
120	Cis- β -Ocimene	-0.012	0.100	0.159	0.003
121	3-Hydroxy-3-Methylglutaric acid	-0.012	0.107	0.158	0.000
122	2-Nonanone	-0.017	0.125	0.225	0.001
123	Dimethyl Disulfide	-0.018	0.030	0.251	0.000
124	Leucine	-0.022	-0.045	0.281	0.000
125	Aspartic acid	-0.024	0.111	0.316	0.001
126	Butyric acid	-0.024	0.123	0.327	0.003
127	2-Methyl-1-butanol	-0.024	0.115	0.331	0.000
128	Methionine	-0.027	-0.036	0.357	0.002
129	2-Methyl-1-propanol	-0.030	0.100	0.406	-0.002
130	Trans- β -Ocimene	-0.031	0.062	0.402	0.003
131	Benzaldehyde	-0.031	0.122	0.422	0.002
132	2-Nonanol	-0.035	0.112	0.474	0.005
133	Pentanol acetate	-0.037	0.100	0.504	0.002
134	Butyraldehyde	-0.038	-0.001	0.499	0.000
135	2-Methyl-3-buten-2-ol	-0.041	0.058	0.542	0.002
136	2-Octanol acetate	-0.042	0.116	0.566	0.004
137	Ethanol	-0.044	0.064	0.595	0.006
138	1-Butanol	-0.044	0.102	0.605	0.002
139	3-Methyl-isovaleraldehyde	-0.046	0.041	0.627	0.003
140	Threonine	-0.048	0.061	0.632	0.001
141	3-Methyl-1-butanol	-0.052	0.093	0.702	0.003
142	3-Methylbutanal	-0.054	0.027	0.730	0.004
143	2-Heptanone	-0.056	0.078	0.763	0.005
144	2-Heptanone	-0.056	0.078	0.766	0.005
145	Trans-Linalool oxide	-0.059	0.055	0.800	0.004
146	Nonanal	-0.062	0.079	0.829	0.005
147	3-Hexanone	-0.064	0.075	0.863	0.006
148	Quinic acid	-0.064	-0.061	0.875	0.007
149	2-Methylbutyraldehyde	-0.066	0.015	0.905	0.005
150	Methanol	-0.067	0.021	0.905	0.004
151	2-Pentanol	-0.067	0.084	0.904	0.006
152	1-Pentanol	-0.069	-0.012	0.918	0.008
153	Epicatechin	-0.070	-0.052	0.949	0.007
154	Catechin	-0.072	-0.045	0.979	0.007
155	1-Phenylethyl alcohol	-0.075	0.066	1.013	0.012
156	Proline	-0.076	0.047	1.015	0.008
157	Isoleucine	-0.076	-0.006	1.029	0.006

158	Linalool	-0.081	0.090	1.096	0.010
159	Tryptophan	-0.083	-0.019	1.116	0.009
160	2-Butanone	-0.083	-0.006	1.133	0.007
161	Glycine	-0.087	-0.019	1.184	0.010
162	Valine	-0.090	-0.022	1.213	0.009
163	Lysine	-0.091	-0.041	1.235	0.010
164	Benzonitrile	-0.091	0.068	1.237	0.009
165	Galactose	-0.092	0.006	1.241	0.009
166	Isoamyl Lactate	-0.102	-0.038	1.379	0.012
167	4-Aminobutyric acid	-0.104	0.056	1.408	0.014
168	1-Methoxy-2-propyl acetate	-0.107	-0.019	1.448	0.013
169	Glucaric acid	-0.107	-0.045	1.449	0.013
170	2-Methylpyrazine	-0.107	0.019	1.454	0.014
171	Meso erythritol	-0.113	0.007	1.529	0.018
172	Ornithine	-0.113	0.022	1.525	0.016
173	3-Phenyllactic acid	-0.115	0.026	1.562	0.016
174	Lactic acid	-0.116	0.031	1.567	0.016
175	Mannitol	-0.116	-0.022	1.576	0.015
176	Propionic Acid	-0.117	-0.002	1.584	0.017
177	2,3-Butanediol	-0.117	-0.002	1.584	0.017
178	2-Hydroxyisocaproic acid	-0.120	0.002	1.622	0.016

Table S8. Loading score of OPLS-DA results, VIP score, and coefficient of control and mechanical de-pulped cocoa liquor

Number	Metabolites	Loading score		VIP score	Coefficient
		R2X[1]	R2X[2]		
1	Isobutyric acid	0.132	-0.002	1.774	-0.021
2	Butanoic acid	0.125	-0.041	1.682	-0.019
3	Butyric acid	0.123	-0.054	1.686	-0.019
4	Acetol	0.106	-0.025	1.418	-0.016
5	2-Methylpyrazine	0.094	0.077	1.252	-0.017
6	Nonanoic acid	0.093	0.021	1.316	-0.017
7	3-Methylbutyl methylbutanoate	0.092	-0.071	1.176	-0.011
8	2-Butenoic acid	0.091	0.035	1.119	-0.013
9	2-methyloctane	0.090	-0.040	1.327	-0.016
10	1-Phenylethyl alcohol	0.085	-0.019	1.111	-0.012
11	Prenol	0.083	0.054	1.024	-0.013
12	Valeraldehyde	0.078	-0.074	1.170	-0.013
13	1-Pentanol	0.076	-0.081	1.158	-0.012
14	Threitol	0.068	-0.080	1.002	-0.010
15	Methyl 2-methylbutanoate	0.055	0.010	0.838	-0.011
16	Decane	0.054	-0.075	0.890	-0.010
17	1,2,3-Butanetriol	0.054	-0.056	0.881	-0.010
18	2-Dehydro gluconate	0.051	-0.087	0.769	-0.007

19	Glutamine	0.047	-0.090	0.699	-0.006
20	Turanose	0.043	-0.013	0.673	-0.008
21	Ribulose	0.038	0.050	0.584	-0.009
22	Sorbitol	0.037	0.054	0.344	-0.004
23	Butyraldehyde	0.037	0.021	0.475	-0.006
24	1-octen-3-ol	0.033	-0.121	0.506	-0.003
25	Octane	0.031	-0.092	0.574	-0.005
26	γ -Butyrolactone	0.030	0.080	0.439	-0.008
27	Catechine	0.027	-0.117	0.434	-0.002
28	Ethyl benzene	0.025	-0.109	0.397	-0.002
29	3-Methyl-3-buten-1-ol	0.024	0.076	0.214	-0.004
30	Glutamic acid	0.024	-0.109	0.440	-0.003
31	Epicatechin	0.024	-0.118	0.408	-0.002
32	Phenylacetaldehyde	0.023	-0.020	0.275	-0.002
33	Ethyl Isobutyrate	0.023	0.004	0.299	-0.004
34	3-Phenyllactic acid	0.022	-0.059	0.400	-0.004
35	Fumaric acid	0.020	-0.104	0.365	-0.002
36	Butyl acetate	0.020	-0.013	0.418	-0.006
37	Serotonin	0.019	-0.099	0.275	0.000
38	Sucrose	0.017	-0.114	0.332	-0.001
39	Raffinose	0.016	-0.114	0.266	0.000
40	Glycolic acid	0.012	-0.082	0.352	-0.003
41	2,5-Dimethylpyrazine	0.012	0.096	0.081	-0.003
42	Ribose	0.010	-0.090	0.174	0.000
43	Proline	0.006	-0.029	0.230	-0.003
44	Shikimic acid	0.005	-0.105	0.139	0.001
45	Dopamine	0.004	-0.095	0.064	0.005
46	Glucose	0.004	-0.115	0.148	0.001
47	Asparagine	0.003	-0.044	0.141	-0.001
48	1-Butanol	0.003	-0.018	0.154	-0.002
49	Galactinol	-0.001	-0.121	0.061	0.002
50	Threonic acid	-0.001	-0.051	0.151	-0.002
51	Paeoniflorin	-0.002	-0.126	0.001	0.004
52	Gentiobiose	-0.003	-0.112	0.001	0.003
53	Propionic Acid	-0.004	0.062	0.104	-0.005
54	Galacturonic acid	-0.005	-0.015	0.054	0.001
55	Nonanal	-0.005	-0.128	0.074	0.005
56	Valine	-0.008	-0.016	0.158	-0.004
57	Gluconic acid	-0.009	-0.107	0.021	0.003
58	Glucono-1,5-lactone	-0.010	-0.108	0.027	0.003
59	Galactitol	-0.011	-0.114	0.025	0.003
60	2-Phenethyl acetate	-0.015	-0.074	0.186	0.004
61	Benzonitrile	-0.016	0.119	0.199	-0.002
62	Threonine	-0.018	-0.075	0.137	0.003
63	2,3-Pentanedion	-0.020	-0.059	0.180	0.003
64	Saccharic acid	-0.021	-0.115	0.216	0.006
65	β -Lactose	-0.023	-0.040	0.256	0.004
66	2,3-Butanediol	-0.025	0.093	0.396	0.002
67	Erythritol	-0.025	-0.061	0.459	0.009
68	2-Nonanone	-0.026	0.118	0.408	0.001

69	Trans-2-Nonenal	-0.028	-0.125	0.393	0.009
70	2,3-Butanedione	-0.033	0.103	0.481	0.003
71	Pentanoic acid	-0.033	0.111	0.520	0.003
72	Fructose	-0.034	-0.124	0.460	0.010
73	Serine	-0.037	-0.046	0.435	0.006
74	Tartaric acid	-0.038	-0.106	0.433	0.008
75	3-Methyl-2-butanone	-0.040	0.118	0.532	0.002
76	Xylulose	-0.041	-0.062	0.532	0.008
77	Lyxose	-0.042	-0.002	0.531	0.006
78	2-Phenylpropionaldehyde	-0.043	-0.112	0.529	0.009
79	Ethyl propanoate	-0.044	0.056	0.423	0.002
80	2-Ethyl-3,5-dimethylpyrazine	-0.044	0.119	0.590	0.003
81	Isoleucine	-0.045	-0.042	0.479	0.006
82	2,3,5-Trimethylpyrazine	-0.045	0.116	0.628	0.004
83	Trimethylpyrazine	-0.045	0.117	0.629	0.004
84	N- α -Acetyl lysine	-0.045	-0.119	0.613	0.011
85	Dimethyl Sulfide	-0.047	0.053	0.831	0.010
86	2- α -mannobiose	-0.049	0.100	0.550	0.002
87	Methyl acetate	-0.052	0.052	0.469	0.002
88	Lysine	-0.053	-0.073	0.624	0.009
89	Galactose	-0.053	-0.112	0.725	0.012
90	3-Ethoxy-1-propanol	-0.053	-0.019	0.591	0.006
91	1-Hexanol	-0.053	-0.091	0.674	0.011
92	Glyceric acid	-0.053	-0.054	0.672	0.009
93	Ethyl DL-2-Methylbutyrate	-0.055	-0.049	0.592	0.007
94	Caffeine	-0.055	-0.112	0.672	0.011
95	Vinyl n-Hexanoate	-0.056	0.115	0.790	0.006
96	4-Aminobutyric acid	-0.059	0.033	0.768	0.008
97	Inositol	-0.060	-0.112	0.773	0.012
98	Dimethyl Disulfide	-0.061	0.110	0.761	0.005
99	Meso erythritol	-0.063	-0.031	0.772	0.009
100	Scyllo-Inositol	-0.063	-0.112	0.836	0.013
101	Phenylalanine	-0.063	-0.068	0.731	0.010
102	Furfural	-0.063	0.113	0.824	0.006
103	2-Nonanol	-0.065	0.087	0.958	0.009
104	Acetophenone	-0.066	-0.109	0.958	0.016
105	Isocitric acid	-0.069	-0.099	0.923	0.014
106	Methionine	-0.069	-0.092	0.869	0.013
107	Malic acid	-0.069	-0.110	0.927	0.015
108	Trans-Linalool oxide	-0.069	0.026	1.006	0.012
109	2,3,5,6-Tetramethylpyrazine	-0.070	0.110	0.941	0.008
110	Tryptophan	-0.070	0.021	0.878	0.009
111	Ethyl heptanoate	-0.075	-0.066	0.962	0.013
112	2-Methylbutyraldehyde	-0.076	0.103	0.960	0.007
113	3-Hexanone	-0.079	-0.067	0.954	0.012
114	Leucine	-0.079	-0.050	0.939	0.012
115	3-Methylbutanal	-0.079	0.102	1.003	0.008
116	2,6-Dimethylpyrazine	-0.081	0.105	1.065	0.009
117	Glycine	-0.081	-0.093	1.085	0.016
118	2,3-Butanediol	-0.082	0.095	1.142	0.011

119	Cis- β -Ocimene	-0.082	-0.083	1.221	0.018
120	Lactic acid	-0.083	0.006	1.166	0.014
121	4H-Pyran-4-one	-0.084	0.004	1.180	0.014
122	γ -Nonalactone	-0.084	0.017	1.019	0.010
123	Tyrosine	-0.084	-0.037	1.047	0.013
124	2-Acetylfuran	-0.085	0.097	1.194	0.012
125	Isobutyl acetate	-0.086	0.085	1.167	0.011
126	Alanine	-0.088	-0.051	1.035	0.013
127	Citric acid	-0.088	-0.098	1.140	0.016
128	Theobromine	-0.089	0.077	1.249	0.013
129	3-Carene	-0.089	-0.064	1.338	0.019
130	Quinic acid	-0.091	0.031	1.212	0.013
131	Aspartic acid	-0.091	-0.057	1.130	0.014
132	Trehalose	-0.091	-0.060	1.271	0.017
133	2-Methyl-1-propanol	-0.093	0.064	1.155	0.011
134	2-Pentanone	-0.095	0.046	1.215	0.012
135	1-Propanol	-0.096	0.065	1.307	0.013
136	3-Heptanone	-0.097	0.001	1.183	0.013
137	2-Aminoethanol	-0.098	-0.031	1.204	0.014
138	2-Propanol	-0.099	0.049	1.234	0.012
139	2-Heptanone	-0.102	0.047	1.323	0.014
140	2-Heptanol	-0.104	0.087	1.449	0.015
141	Ethanol	-0.105	0.054	1.346	0.014
142	Adenine	-0.108	0.050	1.388	0.014
143	Methanol	-0.109	0.075	1.437	0.014
144	Maltose	-0.110	0.000	1.505	0.018
145	Ethanone	-0.110	0.047	1.542	0.017
146	Methyl phenylacetate	-0.111	0.065	1.454	0.015
147	3-Methylbutyl acetate	-0.113	0.068	1.517	0.016
148	2-Methyl-1-butanol	-0.113	0.044	1.408	0.014
149	Benzaldehyde	-0.114	0.056	1.461	0.015
150	Mannitol	-0.115	-0.037	1.643	0.022
151	Pyroglutamic acid	-0.115	0.011	1.490	0.017
152	Cyanidin	-0.116	0.019	1.587	0.018
153	Paraxanthine	-0.116	-0.016	1.397	0.015
154	Acetoin	-0.117	0.045	1.648	0.019
155	2-Methyl-3-buten-2-ol	-0.118	-0.011	1.523	0.018
156	Propyl acetate	-0.118	0.058	1.554	0.016
157	3-Methyl-1-butanol	-0.118	0.053	1.526	0.016
158	Phosphate	-0.120	-0.055	1.575	0.020
159	Ethyl Acetate	-0.120	0.062	1.593	0.017
160	Succinic acid	-0.126	-0.060	1.680	0.022
161	3-Acetoxy-2-butanone	-0.128	0.059	1.702	0.018
162	2-Hexanol	-0.128	0.018	1.677	0.019
163	Glycerol	-0.129	-0.055	1.746	0.023
164	3-Methoxybutyl Acetate	-0.129	0.049	1.725	0.019
165	Ethyl Lactate	-0.132	0.053	1.770	0.019
166	Acetic acid	-0.132	0.054	1.765	0.019
167	1-Methoxy-2-propyl acetate	-0.132	0.052	1.786	0.020
168	2-Pentanol	-0.133	0.050	1.781	0.019
169	Ethyl Hexanoate	-0.134	-0.020	1.776	0.021

170	Ethyl Octanoate	-0.137	0.006	1.829	0.021
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Table S9. Loading score of OPLS-DA results, VIP score, and coefficient of control and pre-dried raw cocoa beans

Number	Metabolites	Loading score		VIP score	Coefficient
		R2X[1]	R2X[2]		
1	Citric acid	0.208	0.069	1.875	-0.048
2	Malic acid	0.204	0.136	1.838	-0.052
3	Epicatechin	0.203	0.030	1.829	-0.043
4	Isocitric acid	0.184	-0.050	1.660	-0.031
5	Sorbose	0.184	-0.036	1.656	-0.036
6	Psicose	0.183	-0.014	1.643	-0.035
7	Mannose	0.172	-0.046	1.553	-0.037
8	Glucose	0.172	-0.051	1.547	-0.036
9	Tyrosine	0.169	-0.045	1.522	-0.036
10	Caffeine	0.168	0.085	1.524	-0.046
11	Galactitol	0.168	0.100	1.516	-0.040
12	Fructose	0.164	-0.059	1.481	-0.034
13	Methionine	0.161	-0.028	1.455	-0.026
14	Catechin	0.159	-0.070	1.440	-0.023
15	Galactinol	0.149	0.041	1.339	-0.029
16	Gentiobiose	0.143	-0.111	1.311	-0.015
17	Lysine	0.142	-0.082	1.282	-0.029
18	Paraxanthine	0.124	-0.088	1.137	-0.011
19	Ornithine	0.119	0.000	1.085	-0.017
20	Phenylalanine	0.119	-0.062	1.077	-0.016
21	Pyroglutamic acid	0.119	-0.019	1.075	-0.018
22	2-Aminoethanol	0.103	0.057	0.925	-0.026
23	Asparagine	0.100	-0.158	0.920	-0.011
24	Glucosamine	0.100	-0.053	0.904	-0.022
25	2-Dehydro gluconate	0.093	0.155	0.861	-0.023
26	5-Keto gluconate	0.092	0.294	0.893	-0.043
27	Phenyl-β-D-glucopyranoside	0.088	0.159	0.815	-0.023
28	Isoleucine	0.086	0.164	0.786	-0.027
29	Trehalose	0.085	0.189	0.787	-0.025
30	Succinic acid	0.079	0.077	0.719	-0.019
31	Melibiose	0.079	-0.080	0.716	-0.011
32	Lyxose	0.077	0.096	0.710	-0.016
33	Glycerol	0.073	0.162	0.678	-0.021
34	Glutamic acid	0.069	0.129	0.638	-0.022
35	Gluconic acid	0.066	0.145	0.626	-0.017
36	Tartaric acid	0.066	0.077	0.604	-0.015
37	Ribose	0.061	0.195	0.586	-0.027
38	Tryptophan	0.049	-0.040	0.454	-0.004
39	Serine	0.046	-0.041	0.426	-0.004
40	Fumaric acid	0.045	0.184	0.456	-0.015
41	Threonine	0.033	0.127	0.329	-0.012
42	Aspartic acid	0.031	-0.044	0.284	-0.005

43	Scyllo Inositol	0.029	-0.030	0.279	-0.008
44	Adenine	0.028	-0.105	0.283	-0.002
45	Leucine	0.024	-0.087	0.247	0.003
46	Xylulose	0.024	0.232	0.322	-0.017
47	Theobromine	0.018	-0.069	0.294	0.010
48	Inositol	0.016	0.080	0.180	-0.005
49	Meso erythritol	-0.005	0.008	0.068	0.003
50	Glycine	-0.010	0.091	0.240	0.006
51	Shikimic acid	-0.015	-0.047	0.159	0.009
52	Galacturonic acid	-0.017	0.103	0.259	0.005
53	Alanine	-0.028	0.031	0.304	0.011
54	Phosphate	-0.030	-0.153	0.336	0.020
55	N- α -Acetyl lysine	-0.039	-0.073	0.378	0.017
56	Turanose	-0.040	-0.035	0.367	0.012
57	Valine	-0.043	-0.020	0.426	0.018
58	Sucrose	-0.047	0.110	0.450	0.008
59	Galactose	-0.057	0.090	0.540	0.013
60	Quinic acid	-0.057	0.263	0.651	-0.016
61	1-Deoxypentitol	-0.065	0.223	0.678	-0.011
62	4-Aminobutyric acid	-0.066	0.149	0.627	0.010
63	Raffinose	-0.068	0.057	0.626	0.016
64	Threonic acid	-0.071	0.185	0.665	0.005
65	Xylitol	-0.074	0.126	0.691	0.013
66	Lactic acid	-0.079	-0.067	0.724	0.015
67	2,3-Dihydroxy-2-methylbutanoic acid	-0.082	0.057	0.758	0.021
68	Threitol	-0.088	0.105	0.814	0.018
69	Proline	-0.089	-0.016	0.802	0.022
70	3- α -Mannobiose	-0.101	0.109	0.915	0.014
71	Cyanidin	-0.102	-0.030	0.937	0.015
72	3-Hydroxy-3-Methylglutaric acid	-0.106	-0.078	0.956	0.029
73	Saccharic acid	-0.109	0.012	0.980	0.024
74	Mannitol	-0.110	-0.151	0.999	0.029
75	Glucono-1,5-lactone	-0.113	0.076	1.028	0.025
76	Glycolic acid	-0.121	0.053	1.100	0.028
77	1,2,3-Butanetriol	-0.122	0.117	1.110	0.022
78	2-Hydroxyisocaproic acid	-0.132	-0.114	1.196	0.032
79	2-Hydroxyglutaric acid	-0.140	-0.048	1.256	0.033
80	Ethyl- α -glucopyranoside	-0.146	-0.171	1.323	0.041
81	Glyceric acid	-0.156	-0.014	1.410	0.040
82	Glucaric acid	-0.168	-0.132	1.521	0.046
83	3-Phenyllactic acid	-0.171	-0.059	1.536	0.038

Table S10. Loading score of OPLS-DA results, VIP score, and coefficient of control and mechanically de-pulped raw cocoa beans

Number	Metabolites	Loading score	VIP score	Coefficient
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		R2X[1]	R2X[2]		
1	Catechin	0.238	-0.052	2.245	-0.108
2	Paraxanthine	0.221	-0.031	2.087	-0.045
3	Epicatechin	0.198	-0.104	1.868	-0.092
4	2-Aminoethanol	0.185	0.059	1.747	-0.057
5	Theobromine	0.177	-0.081	1.673	-0.032
6	Serotonin	0.176	-0.110	1.659	-0.065
7	Phenylalanine	0.172	0.007	1.627	0.001
8	Isoleucine	0.168	-0.001	1.585	-0.023
9	Tryptophan	0.166	0.088	1.575	-0.044
10	Alanine	0.160	-0.121	1.508	-0.057
11	Dopamine	0.156	-0.129	1.473	-0.070
12	Asparagine	0.138	-0.110	1.303	-0.049
13	Serine	0.138	-0.121	1.299	-0.034
14	N- α -Acetyl lysine	0.137	0.118	1.289	-0.110
15	Glycine	0.132	-0.133	1.243	-0.047
16	Valine	0.127	0.010	1.201	-0.014
17	Galactitol	0.109	-0.139	1.033	-0.041
18	Leucine	0.109	0.110	1.031	-0.050
19	Proline	0.101	-0.086	0.950	0.012
20	Threonine	0.097	-0.060	0.920	0.050
21	Glutamic acid	0.076	-0.134	0.718	-0.001
22	Aspartic acid	0.069	0.053	0.651	-0.001
23	Raffinose	0.062	-0.155	0.583	0.002
24	Glucono-1,5-lactone	0.061	-0.156	0.580	-0.036
25	Gluconic acid	0.061	-0.156	0.580	-0.036
26	Inositol	0.059	-0.150	0.559	-0.007
27	Caffeine	0.055	-0.100	0.520	-0.017
28	Paeoniflorin	0.054	-0.158	0.511	-0.008
29	Lactic acid	0.046	-0.152	0.435	-0.034
30	Adenine	0.044	0.101	0.419	-0.039
31	Cyanidin	0.039	0.139	0.366	-0.041
32	2-Dehydro gluconate	0.023	-0.154	0.215	-0.026
33	Ribulose	0.022	-0.141	0.208	-0.023
34	Shikimic acid	0.018	-0.154	0.167	0.011
35	Quinic acid	0.016	0.006	0.154	0.014
36	4-Aminobutyric acid	0.016	-0.150	0.148	0.009
37	Galacturonic acid	0.012	-0.119	0.113	-0.026
38	Xylulose	0.005	-0.153	0.045	-0.009
39	Succinic acid	0.001	-0.155	0.009	0.003
40	Phosphate	-0.002	-0.162	0.019	0.033
41	Gentiobiose	-0.005	0.089	0.039	-0.057
42	2- α -mannobiose	-0.005	0.131	0.048	-0.053
43	Ribose	-0.008	-0.146	0.071	-0.009

44	Mannitol	-0.009	-0.121	0.080	-0.002
45	Fumaric acid	-0.011	-0.147	0.102	0.029
46	Methionine	-0.018	0.103	0.168	-0.008
47	Glutamine	-0.026	-0.156	0.251	0.051
48	Erythritol	-0.027	-0.051	0.255	0.003
49	Glycolic acid	-0.037	-0.134	0.349	0.020
50	Sorbitol	-0.039	0.134	0.372	0.007
51	Fructose	-0.043	0.123	0.405	-0.002
52	Malic acid	-0.048	0.042	0.449	0.024
53	Sucrose	-0.056	-0.134	0.527	0.020
54	Saccharic acid	-0.058	-0.138	0.544	0.032
55	β-Lactose	-0.063	0.106	0.597	0.008
56	Glucose	-0.065	0.129	0.615	0.017
57	Tartaric acid	-0.067	-0.137	0.628	0.002
58	Lyxose	-0.068	-0.092	0.639	-0.032
59	Glycerol	-0.068	-0.123	0.646	0.011
60	Turanose	-0.069	0.116	0.654	0.027
61	Lysine	-0.077	0.128	0.724	0.005
62	Tyrosine	-0.077	0.117	0.725	0.019
63	Threitol	-0.079	-0.112	0.744	0.005
64	Galactose	-0.081	0.127	0.766	0.005
65	Glyceric acid	-0.090	-0.122	0.853	0.003
66	1,2,3-Butanetriol	-0.095	-0.099	0.899	0.062
67	Galactinol	-0.097	-0.076	0.913	0.085
68	3-Phenyllactic acid	-0.107	-0.109	1.012	0.017
69	2,3-Butanediol	-0.110	-0.094	1.038	0.043
70	Meso erythritol	-0.115	-0.094	1.087	0.001
71	Isocitric acid	-0.127	0.012	1.204	0.031
72	Citric acid	-0.137	0.034	1.297	0.039
73	Trehalose	-0.138	-0.078	1.308	0.036
74	Pyroglutamic acid	-0.156	-0.109	1.478	0.081
75	Threonic acid	-0.181	-0.072	1.713	0.052
76	Maltose	-0.190	-0.003	1.795	0.078
77	Scyllo-Inositol	-0.191	0.080	1.804	0.085

Table S11. List of metabolites in volcano plot comparing raw cocoa beans before and after pre-drying treatment on bulk and fine cocoa, which different significantly based on the t-test ($p < 0.05$)

A. Bulk cocoa

B. Fine cocoa

Significantly increase ($p < 0.05$)				
No	Metabolites	Fold Change (FC)	Log2FC	Minus log10 p value
1	Glucose	150.59	7.23	3.82
2	Mannose	110.02	6.78	6.98
3	Fructose	54.01	5.76	3.87
4	Sorbose	40.40	5.34	3.83
5	Tyrosine	10.20	3.35	3.11
6	Lysine	10.15	3.34	3.85
7	Scyllo Inositol	3.06	1.62	3.33
8	Citric acid	2.84	1.51	2.78
9	Adenine	2.79	1.48	2.26
10	Isocitric acid	2.47	1.30	2.20
11	Methionine	2.03	1.02	1.31
12	Malic acid	1.95	0.96	3.71
13	Lactic acid	1.94	0.95	1.74
14	Mannitol	1.77	0.82	1.82
15	Succinic acid	1.75	0.81	2.72
16	Psicose	1.70	0.77	1.30
17	Caffeine	1.49	0.57	1.63
18	Epicatechin	1.45	0.53	1.92
19	Phenylalanine	1.34	0.42	1.69
20	Turanose	1.34	0.42	1.97
21	Galactinol	1.29	0.37	1.42
22	Galactitol	1.26	0.34	1.63
Significantly decrease ($p < 0.05$)				
Number	Metabolites	Fold Change (FC)	Log2FC	Minus log10 p value
1	Theobromine	0.96	-0.06	1.30
2	Phenyl- β -D-glucopyranoside	0.70	-0.50	1.47
3	Glycerol	0.67	-0.57	2.27
4	Glycine	0.66	-0.60	2.46
5	Saccharic acid	0.58	-0.78	1.87
6	Glucaric acid	0.57	-0.80	1.49
7	Gluconic acid	0.56	-0.83	1.88
8	Xylulose	0.52	-0.95	1.65
9	2-Dehydrogluconate	0.49	-1.03	2.00
10	2,3-Dihydroxy-2-methylbutanoic acid	0.46	-1.10	3.14
11	Threonic acid	0.45	-1.15	2.36
12	Fumaric acid	0.40	-1.31	2.51
13	Sucrose	0.36	-1.46	2.41
14	Raffinose	0.35	-1.52	3.44
15	Galacturonic acid	0.34	-1.54	1.36
16	2-Hydroxyglutaric acid	0.34	-1.55	1.96

Significantly increase ($p < 0.05$)				
No	Metabolites	Fold Change (FC)	Log2FC	Minus log10 p value
1	2-Dehydrogluconate	4.31	2.11	2.20
2	Glucose	3.94	1.98	2.25
3	Mannose	3.65	1.87	2.37
4	Trehalose	3.35	1.74	2.90
5	Tyrosine	3.26	1.71	2.27
6	Fumaric acid	3.10	1.63	2.24
7	Galactitol	2.56	1.36	2.06
8	Malic acid	2.50	1.32	2.77
9	Citric acid	2.50	1.32	2.43
10	Phenyl- β -D-glucopyranoside	2.45	1.29	2.18
11	Fructose	2.43	1.28	2.29
12	Galacturonic acid	2.36	1.24	2.33
13	Gluconic acid	2.27	1.18	2.25
14	Galactose	2.24	1.17	1.70
15	Raffinose	2.20	1.14	1.84
16	Glycerol	2.05	1.04	2.84
17	Xylulose	1.85	0.89	1.94
18	Psicose	1.78	0.83	1.75
19	Lysine	1.70	0.76	1.92
20	Epicatechin	1.51	0.59	1.78
21	4-Aminobutyric acid	1.45	0.54	1.93
22	Threonic acid	1.44	0.52	1.79
23	Threitol	1.39	0.47	1.70
24				
25	Glycolic acid	1.38	0.46	1.33
26	Succinic acid	1.35	0.43	1.68
27	Glycine	1.33	0.41	1.35
28	Lyxose	1.33	0.41	1.89
29	Glutamic acid	1.29	0.37	1.50
30	Isoleucine	1.27	0.35	2.11
31	Sucrose	1.25	0.32	1.91
32	Tartaric acid	1.24	0.31	1.79
33	Caffeine	1.16	0.21	1.62
34	Inositol	1.14	0.19	1.35
35				
36	Glucono-1,5-lactone	1.07	0.09	1.48
Significantly decrease ($p < 0.05$)				
Number	Metabolites	Fold Change (FC)	Log2FC	Minus log10 p value
1	Glyceric acid	0.81	-0.31	1.53
2	Aspartic acid	0.81	-0.31	2.17
3	Saccharic acid	0.76	-0.39	1.49
4	Proline	0.76	-0.40	2.45

17	Glyceric acid	0.33	-1.61	2.33	5	Turanose	0.68	-0.55	1.80
18	4-Aminobutyric acid	0.32	-1.65	4.72	6	Adenine	0.66	-0.60	1.38
19	Threitol	0.29	-1.80	2.09	7	3-Hydroxy-3-Methylglutaric acid	0.60	-0.74	1.49
20	Galactose	0.29	-1.81	2.33	8	2-hydroxyglutaric acid	0.44	-1.19	1.99
21	Glucono-1,5-lactone	0.24	-2.08	2.51	9	Glucaric acid	0.42	-1.25	1.52
22	2,3-Butanediol	0.21	-2.28	3.99	10	Lactic acid	0.33	-1.60	1.70
23	3-Phenyllactic acid	0.20	-2.29	1.44	11	Ethyl- α -glucopyranoside	0.18	-2.48	1.95
24	3- α -Mannobiose	0.18	-2.49	1.84	12	Hydroxyisocaproic acid	0.18	-2.51	2.74
25	1,2,3- Butanetriol	0.14	-2.79	4.00	13	3-Phenyllactic acid	0.16	-2.68	1.65
26	Xylitol	0.14	-2.79	2.22	14	Mannitol	0.09	-3.43	2.31
27	Glycolic acid	0.13	-2.96	2.62	15	2,3-Butanediol	0.07	-3.82	3.43
Not significant					Not significant				
Number	Metabolites	Fold Change (FC)	Log2FC	Minus log10 <i>p</i> value	Number	Metabolites	Fold Change (FC)	Log2FC	Minus log10 <i>p</i> value
1	2-Aminoethanol	1.11	0.15	0.91	1	2-Aminoethanol	1.08	0.11	0.69
2	Alanine	0.74	-0.43	1.00	2	Alanine	1.09	0.13	0.58
3	Asparagine	2.04	1.03	1.12	3	Asparagine	0.88	-0.18	0.78
4	Aspartic acid	2.09	1.06	1.22	4	Leucine	0.93	-0.10	1.02
5	Glutamic acid	1.19	0.25	1.09	5	Methionine	1.53	0.61	1.07
6	Isoleucine	0.99	-0.01	0.32	6	N- α -Acetyl lysine	0.87	-0.20	0.57
7	Leucine	1.18	0.24	0.90	7	Ornithine	2.18	1.13	1.15
8	N- α -Acetyl lysine	1.00	0.00	0.31	8	Phenylalanine	1.09	0.12	0.64
9	Ornithine	1.63	0.71	0.88	9	Pyroglutamic acid	1.28	0.36	1.20
10	Proline	0.73	-0.46	0.94	10	Serine	1.00	0.00	0.31
11	Pyroglutamic acid	1.33	0.41	1.02	11	Threonine	1.29	0.37	1.12
12	Serine	1.45	0.54	0.75	12	Tryptophan	1.53	0.61	0.86
13	Threonine	0.85	-0.24	0.66	13	Valine	0.99	-0.01	0.37
14	Tryptophan	1.02	0.03	0.32	14	Isocitric acid	1.61	0.68	1.10
15	Valine	0.89	-0.17	0.76	15	Cyanidin	0.60	-0.73	1.09
16	3-Hydroxy-3-Methylglutaric acid	0.80	-0.32	0.76	16	Paraxanthine	1.47	0.55	0.69
17	Cyanidin	1.06	0.09	0.77	17	Phosphate	0.77	-0.38	1.22
18	Paraxanthine	1.27	0.34	0.82	18	Quinic acid	0.96	-0.07	0.33
19	Phosphate	1.24	0.31	0.86	19	Shikimic acid	0.94	-0.09	0.49
20	Quinic acid	0.71	-0.50	0.74	20	1,2,3-Butanetriol	1.08	0.11	0.47
21	Shikimic acid	1.03	0.05	0.48	21	2,3-Dihydroxy-2-methylbutanoic acid	1.16	0.21	0.82
22	2-Hydroxyisocaproic acid	0.93	-0.10	0.40	22	Theobromine	1.06	0.09	0.86
23	Catechin	1.29	0.37	1.30	23	Catechin	1.35	0.43	1.00
24	Gentiobiose	1.36	0.44	0.95	24	Gentiobiose	1.38	0.47	0.98
25	Glucosamine	17.43	4.12	0.78	25	Glucosamine	0.68	-0.57	0.71
26	Lyxose	0.98	-0.03	0.37	26	Melibiose	1.03	0.04	0.46
27	Melibiose	1.19	0.26	0.78	27	Ribose	3.35	1.74	0.97
28	Ribose	0.86	-0.22	0.34	28	Sorbose	7.31	2.87	0.79
29	Trehalose	0.48	-1.05	1.17	29	3- α -Mannobiose	1.75	0.80	0.63
30	Ethyl- α -glucopyranoside	0.90	-0.15	0.74	30	5-Keto gluconate	2.32	1.21	1.30

31	5-Keto gluconate	0.90	-0.16	0.49	31	Galactinol	1.39	0.47	1.20
32	Tartaric acid	1.13	0.17	0.90	32	Meso erythritol	0.95	-0.07	0.58
33	Inositol	0.90	-0.15	0.81	33	1-Deoxypentitol	0.85	-0.24	0.46
34	Meso erythritol	1.04	0.06	0.48	34	Scyllo Inositol	0.17	-2.57	0.65
35	1-Deoxypentitol	0.73	-0.45	0.78	35	Xylitol	1.54	0.63	0.94

Table S12. List of metabolites in volcano plot comparing raw cocoa beans before and after mechanical de-pulping treatment on bulk and fine cocoa, which different significantly based on the t-test ($p < 0.05$)

A. Bulk cocoa					B. Fine cocoa				
Significantly increase ($p < 0.05$)					Significantly increase ($p < 0.05$)				
No	Metabolites	Fold Change (FC)	Log2FC	Minus log10 <i>p</i> value	No	Metabolites	Fold Change (FC)	Log2FC	Minus log10 <i>p</i> value
1	Serotonin	2.85	1.51	2.09	1	Asparagine	4.23	2.08	3.11
2	N- α -Acetyl lysine	2.30	1.20	2.21	2	Glycine	2.76	1.47	2.66
3	Caffeine	1.98	0.98	2.39	3	Galactitol	2.75	1.46	2.65
4	Paraxanthine	1.82	0.87	1.44	4	Serine	2.60	1.38	2.65
5	Catechin	1.75	0.81	2.37	5	Aspartic acid	2.59	1.37	1.74
6	Epicatechin	1.68	0.75	2.14	6	Dopamine	2.22	1.15	2.28
7	Theobromine	1.61	0.68	2.18	7	Serotonin	1.88	0.91	1.62
8	2- α -mannobiose	1.45	0.53	1.59	8	Alanine	1.81	0.86	2.62
9	Galacturonic acid	1.38	0.47	1.39	9	Adenine	1.71	0.77	1.54
10	Dopamine	1.30	0.37	1.57	10	Epicatechin	1.70	0.76	2.55
11	Glucono-1,5-lactone	1.28	0.36	1.92	11	Glucono-1,5-lactone	1.66	0.73	2.10
12	Gluconic acid	1.25	0.32	1.73	12	Gluconic acid	1.66	0.73	2.10
Significantly decrease ($p < 0.05$)					13	Quinic acid	1.62	0.69	2.03
Nu mbe r	Metabolites	Fold Change (FC)	Log2FC	Minus log10 <i>p</i> value	14	Inositol	1.53	0.61	3.55
1	Glycine	0.90	-0.15	1.65	15	Catechin	1.49	0.58	2.46
2	Tartaric acid	0.85	-0.23	1.45	16	Shikimic acid	1.49	0.58	2.11
3	Threonic acid	0.79	-0.35	1.57	17	Malic acid	1.42	0.51	1.71
4	Fructose	0.78	-0.36	2.07	18	2-Aminoethanol	1.40	0.48	1.91
5	Maltose	0.75	-0.41	1.70	19	Raffinose	1.29	0.37	1.67
6	Glucose	0.74	-0.44	1.85	20	Paeoniflorin	1.28	0.36	2.03
7	Inositol	0.74	-0.44	1.45	21	Phosphate	1.23	0.30	1.36
8	Quinic acid	0.72	-0.48	1.46	22	Lactic acid	1.23	0.30	1.40
9	Asparagine	0.71	-0.50	2.81	23	Fructose	1.13	0.18	2.62
10	Lysine	0.69	-0.53	2.01	24	Succinic acid	1.06	0.09	1.42
11	Citric acid	0.69	-0.53	2.24	25	4-Aminobutyric acid	1.03	0.04	1.49
12	β -Lactose	0.69	-0.54	1.35	Significantly decrease ($p < 0.05$)				
13	Turanose	0.69	-0.54	1.38	Nu mbe r	Metabolites	Fold Change (FC)	Log2FC	Minus log10 <i>p</i> value
14	Trehalose	0.68	-0.55	2.40	1	Meso erythritol	0.88	-0.19	1.49

15	2-Dehydrogluconate	0.67	-0.57	1.74	2	Sucrose	0.88	-0.19	1.51
16	Galactose	0.67	-0.58	1.71	3	Glucose	0.84	-0.25	1.65
17	Pyroglutamic acid	0.66	-0.59	1.54	4	Saccharic acid	0.81	-0.31	1.42
18	Tyrosine	0.66	-0.61	2.02	5	Glycolic acid	0.78	-0.37	2.16
19	Malic acid	0.65	-0.62	1.53	6	Scyllo-Inositol	0.75	-0.42	3.60
20	Galactitol	0.65	-0.63	1.53	7	Threonic acid	0.71	-0.50	1.33
21	Phosphate	0.64	-0.64	2.76	8	Erythritol	0.67	-0.57	2.52
22	Isocitric acid	0.62	-0.68	2.47	9	2- α -mannobiose	0.57	-0.81	1.39
23	Fumaric acid	0.57	-0.81	1.42	10	Glycerol	0.57	-0.82	1.85
24	Shikimic acid	0.54	-0.89	1.50	11	Threitol	0.56	-0.83	1.32
25	Glutamine	0.50	-1.00	2.83	12	1,2,3-Butanetriol	0.42	-1.24	2.77
26	Galactinol	0.47	-1.09	1.84	13	2,3-Butanediol	0.37	-1.43	1.95
Not significant					14	Trehalose	0.33	-1.62	1.76
Nu mber	Metabolites	Fold Change (FC)	Log2FC	Minus log10 <i>p</i> value	Not significant				
1	2-Aminoethanol	1.11	0.16	1.04	15	Maltose	0.28	-1.83	1.54
2	Aminobutyric acid	1.09	0.13	0.76	Not significant				
3	Alanine	1.07	0.10	0.85	Nu mber	Metabolites	Fold Change (FC)	Log2FC	Minus log10 <i>p</i> value
4	Aspartic acid	0.81	-0.31	0.77	1	Glutamic acid	1.18	0.24	0.75
5	Glutamic acid	1.08	0.11	1.14	2	Glutamine	1.06	0.08	0.44
6	Isoleucine	1.24	0.31	0.96	3	Isoleucine	1.10	0.13	0.76
7	Leucine	1.16	0.22	1.22	4	Leucine	1.22	0.28	0.90
8	Methionine	0.88	-0.18	1.06	5	Lysine	0.66	-0.60	1.09
9	Phenylalanine	1.17	0.22	0.56	6	Methionine	1.08	0.11	0.34
10	Proline	1.25	0.32	0.99	7	N- α -Acetyl lysine	1.03	0.05	0.32
11	Serine	0.86	-0.22	0.74	8	Phenylalanine	1.65	0.72	1.14
12	Threonine	0.95	-0.07	0.51	9	Proline	1.13	0.17	0.52
13	Tryptophan	1.29	0.37	1.11	10	Pyroglutamic acid	0.96	-0.06	0.49
14	Valine	0.97	-0.05	0.60	11	Threonine	1.75	0.81	0.80
15	Glycolic acid	1.16	0.22	0.92	12	Tryptophan	1.20	0.26	0.89
16	Succinic acid	0.84	-0.25	0.98	13	Tyrosine	0.96	-0.06	1.29
17	3-Phenyllactic acid	0.62	-0.69	1.04	14	Valine	1.41	0.50	1.30
18	Lactic acid	1.14	0.18	0.44	15	Citric acid	1.02	0.03	0.59
19	Adenine	0.91	-0.14	0.54	16	Fumaric acid	1.32	0.40	0.84
20	Paeoniflorin	0.92	-0.11	0.65	17	Isocitric acid	1.10	0.14	0.69
21	Cyanidin	1.25	0.33	0.97	18	3-Phenyllactic acid	0.75	-0.42	0.97
22	2,3-Butanediol	0.94	-0.09	0.52	19	Caffeine	0.88	-0.19	0.93
23	1,2,3-Butanetriol	1.29	0.37	0.82	20	Paraxanthine	1.89	0.92	1.12
24	Gentiobiose	0.83	-0.26	0.74	21	Cyanidin	0.89	-0.16	0.82
25	Lyxose	1.03	0.04	0.40	22	Theobromine	1.70	0.77	0.97
26	Raffinose	1.00	-0.01	0.34	23	β -Lactose	1.12	0.16	1.04
27	Ribose	1.02	0.03	0.62	24	Galactose	0.59	-0.77	0.89
28	Ribulose	1.27	0.34	0.55	25	Gentiobiose	1.30	0.38	0.76
29	Sucrose	0.94	-0.08	0.71	26	Lyxose	0.81	-0.30	0.91
					27	Ribose	0.95	-0.07	1.03

30	Xylulose	0.86	-0.22	0.62	28	Ribulose	1.13	0.17	0.52
31	Glyceric acid	0.74	-0.44	1.19	29	Turanose	0.97	-0.05	0.40
32	Saccharic acid	0.90	-0.16	0.86	30	Xylulose	1.07	0.09	0.60
33	Glycerol	0.91	-0.13	1.21	31	2-Dehydrogluconate	1.20	0.26	1.03
34	Mannitol	1.89	0.92	1.22	32	Galacturonic acid	0.91	-0.13	0.47
35	Meso erythritol	0.87	-0.20	0.99	33	Glyceric acid	0.88	-0.19	0.60
36	Sorbitol	0.72	-0.46	1.25	34	Tartaric acid	0.85	-0.23	1.11
37	Threitol	1.01	0.01	0.32	35	Galactinol	1.29	0.36	1.00
38	Erythritol	1.50	0.58	1.19	36	Mannitol	0.79	-0.33	0.84
39	Scyllo Inositol	0.79	-0.34	1.09	37	Sorbitol	1.71	0.78	1.08

Table S13. Annotation of non-volatile compounds in raw cocoa beans, fermented beans, roasted beans, and cocoa liquor by GC-MS metabolomics

Alignment ID	Metabolite name	t _R (min) ^a	RI ^b	Quant mass (m/z)	Similarity (%)	RSD ^c	Library
29	2-Aminoethanol	7.348	1276.28	174.0873	99.5	11.8	in-house
65	4-Aminobutyric acid	10.792	1541.95	174.0941	99	9.3	in-house
12	Alanine	4.996	1109.29	116.1529	96.3	13.1	in-house
81	Asparagine	12.485	1687.05	116.154	96.8	6.6	in-house
63	Aspartic acid	10.725	1536.36	232.0605	98	11.3	in-house
74	Glutamic acid	11.893	1635.22	246.0914	98.5	12.8	in-house
96	Glutamine	13.598	1788.92	156.0753	91.7	6.8	in-house
38	Glycine	7.899	1316.42	174.092	99.6	13.7	in-house
34	Isoleucine	7.732	1303.96	158.1124	95.7	4.7	in-house
30	Leucine	7.429	1282.1	158.1057	93.4	8.0	in-house
134	Lysine	15.167	1941.86	174.0937	95.8	14.2	in-house
59	Methionine	10.649	1530.02	176.0672	89.6	13.2	in-house
164	N- α -Acetyl lysine	16.559	2086.6	174.0966	78.7	15.6	in-house
77	Phenylalanine	11.937	1639.13	218.0412	99.2	23.7	in-house
36	Proline	7.748	1305.11	142.1044	93.2	16.6	in-house
60	Pyroglutamic acid	10.67	1531.81	156.0667	95.4	10.4	in-house
44	Serine	8.684	1375.07	204.0733	98.9	9.8	in-house
46	Threonine	9.053	1402.78	218.0662	98.3	12.9	in-house
170	Tryptophan	17.957	2242.18	202.05	83.8	12.3	in-house
141	Tyrosine	15.327	1958.04	218.0368	96.3	9.4	in-house
22	Valine	6.633	1225.13	144.1169	97.6	12.2	in-house
105	Citric acid	14.199	1846.5	273.0714	98.1	8.5	in-house
42	Fumaric acid	8.386	1352.79	245.0263	89.3	8.6	in-house
10	Glycolic acid	4.597	1079.87	147.0778	97.1	13.1	in-house
106	Isocitric acid	14.208	1847.35	245.0662	85.2	10.0	in-house
55	Malic acid	10.339	1504.35	147.0756	99	8.6	in-house
39	Succinic acid	7.943	1319.73	147.0797	96.8	3.4	in-house
72	3-Phenyllactic acid	11.449	1596.55	193.0556	95.1	10.1	in-house

8	Lactic acid	4.409	1065.69	147.0786	99.8	17.7	in-house
116	Adenine	14.526	1878.12	264.0714	88.9	10.3	in-house
107	Caffeine	14.245	1850.95	194.026	95.3	9.2	in-house
166	Dopamine	16.697	2101.33	174.1017	90	11.5	in-house
190	Epicatechin	23.04	2899.91	368.1117	98.3	11.6	in-house
210	Paeoniflorin	26.832	3505.41	147.0429	78.6	12.2	in-house
152	Paraxanthine	15.687	1994.39	237.0267	72.1	14.9	in-house
31	Phosphate	7.468	1284.86	299.0427	99	4.4	in-house
117	Quinic acid	14.713	1896.17	345.1402	85.2	11.8	in-house
173	Serotonin	20.118	2502.47	174.0693	84.3	9.6	in-house
102	Shikimic acid	14.056	1832.64	204.0434	87.2	7.7	in-house
103	Cyanidin	14.078	1834.62	193.0038	85.3	16.6	in-house
4	2,3-Butanediol	4.045	1038.31	117.1483	96	4.9	NIST 20
192	Catechine	23.202	2923.92	368.1113	81	6.3	NIST 20
37	1,2,3-Butanetriol	7.786	1307.96	117.1387	90	21.7	NIST 20
113	Theobromine	14.426	1868.33	180.0318	84	9.6	NIST 20
185	β -Lactose	22.204	2780.81	217.0578	87.6	13.4	in-house
119	Fructose	14.87	1911.88	103.1	99.4	25.4	in-house
138	Galactose	15.31	1956.33	205.0588	98.5	24.4	in-house
191	Gentiobiose	23.051	2901.5	204.045	82.4	11.9	in-house
158	Glucono-1,5-lactone	16.17	2045.38	147.0776	98.5	15.5	in-house
133	Glucose	15.133	1938.39	205.0556	99.2	25.2	in-house
83	Lyxose	12.564	1694.09	103.0939	89.2	10.8	in-house
186	Maltose	22.459	2816.65	147.0833	82	11.7	in-house
209	Raffinose	26.83	3505	361.1222	98.6	14.7	in-house
87	Ribose	12.736	1709.65	103.1071	86.5	15.0	in-house
85	Ribulose	12.731	1709.07	205.0833	85.6	19.5	in-house
179	Sucrose	21.699	2710.81	361.1259	97.9	10.3	in-house
187	Trehalose	22.461	2816.95	361.121	98.2	14.4	in-house
188	Turanose	22.6	2836.88	217.0459	84.6	14.7	in-house
86	Xylulose	12.733	1709.35	147.08	94.2	18.5	in-house
203	2- α -mannobiose	24.906	3184.38	204.0435	80	5.6	NIST 20
132	2-Dehydro gluconate	15.074	1932.5	103.1	97.1	18.2	in-house
149	Galacturonic acid	15.592	1984.82	333.0878	94.7	15.6	in-house
157	Gluconic acid	16.169	2045.2	147.0722	93	21.3	in-house
41	Glyceric acid	8.288	1345.48	147.075	98.8	15.5	in-house
162	Saccharic acid	16.305	2059.69	147.0774	86.7	21.0	in-house
78	Tartaric acid	12.237	1665.37	147.0773	98.4	13.4	in-house
70	Threonic acid	11.322	1585.99	147.0833	93	13.1	in-house
199	Galactinol	24.199	3073.83	204.0524	97.2	12.2	in-house
150	Galactitol	15.603	1985.88	217.0424	93.1	8.4	in-house
32	Glycerol	7.511	1287.94	147.087	99.2	16.1	in-house
167	Inositol	16.973	2131.7	217.0518	99	18.0	in-house
146	Mannitol	15.464	1971.83	205.0546	99.4	6.6	in-house
61	Meso erythritol	10.678	1532.45	147.0756	99.4	22.3	in-house
145	Sorbitol	15.391	1964.62	147.0711	89.7	7.7	in-house

58	Threitol	10.583	1524.56	217.0491	96.1	14.1	in-house
62	Erythritol	10.685	1533.06	217.1	86	21.0	NIST 20
163	Scyllo-Inositol	16.4	2069.73	217.0449	87	5.8	NIST 20

^a Retention time in minute(s)

^b Retention indices (RI) are calculated using a standard alkane mixture (C9–C40)

^c Relative standard deviation (RSD)

Table S14. Annotation of volatile compounds in fermented beans, roasted beans, and cocoa liquor by HS-SPME GC-MS metabolomics

Alignment ID	Metabolite name	t _R (min) ^a	RI ^b	Quant mass (m/z)	Similarity (%)	RSD ^c	Library
256	Acetic acid	29.624	798.93	60	80.1	0	in-house
363	Butyric acid	38.617	998.32	60.05	90.1	0	in-house
310	Isobutyric Acid	34.518	907.4	43.09608	74.3	0	in-house
399	Pentanoic acid	43.323	1112.6	60.03455	80.4	1.442E-05	in-house
296	Propionic Acid	33.404	882.68	28.05	75.1	10.983353	in-house
360	Butanoic acid	38.624	998.48	41.05094	91	0	NIST 20
404	2-Butenoic acid	43.652	1120.61	100.0595	80	0	NIST 20
491	Nonanoic acid	56.075	1448.71	60.04166	85	0	NIST 20
130	1-Butanol	15.214	498.4	56.075	92.1	0	in-house
211	1-Hexanol	24.941	702.24	56.08158	77.4	0	in-house
251	1-Octen-3-ol	29.284	791.91	57.05	81.1	0	in-house
s	1-Pentanol	20.176	603.87	55.07187	92.7	0	in-house
435	1-Phenylethyl alcohol	46.692	1194.52	79.08334	71.8	0	in-house
94	1-Propanol	10.715	402.66	31.04821	86.5	0	in-house
190	2-Heptanol	23.345	669.3	45.05	90.4	0	in-house
111	2-Methyl-1-propanol	12.83	447.67	43.09167	99.1	0	in-house
95	2-Methyl-3-buten-2-ol	10.716	402.68	71.0625	96.7	9.365E-06	in-house
118	2-Pentanol	14.09	474.47	45.05119	99.2	0	in-house
222	3-Ethoxy-1-propanol	26.093	726.02	31.05	88.6	10.943108	in-house
150	3-Methyl-1-butanol	18.14	560.65	55.07758	92.7	1.489E-14	in-house
168	3-Methyl-3-buten-1-ol	20.576	612.12	68.075	85.5	1.586E-14	in-house
185	Acetol	23.036	662.9	43.04904	95	0	in-house
148	2-Methyl-1-butanol	18.114	560.1	57.1	90.7	1.648E-14	in-house
46	Ethanol	7.136	269.89	31.05	79.4	1.977E-14	in-house
35	Methanol	6.177	233.74	31.05	76.4	0	in-house
191	Prenol	23.623	675.03	71.05	91.6	0	in-house
151	2-Hexanol	18.725	573.1	45.06667	92.2	1.592E-05	in-house
43	2-Propanol	6.893	260.72	45.05208	81.9	0	in-house
284	2-Nonanol	32.249	857.07	43.06667	81	0	NIST 20
325	2,3-Butanediol	35.76	934.93	45.05	91	0	NIST 20
38	2-Methylbutyraldehyde	6.555	248.01	57.1	83.2	1.458E-14	in-house
364	Phenylpropionaldehyde	38.871	1004.33	105.05	82.5	0	in-house
41	3-Methylbutanal	6.68	252.69	44.05	83.4	0	in-house
291	Benzaldehyde	33.177	877.66	106.0513	83.4	1.797E-14	in-house
21	Butyraldehyde	4.495	179.54	43.06618	87.8	7.15E-06	in-house
236	Nonanal	27	744.75	57.08026	85.1	0	in-house
353	Phenylacetaldehyde	38.064	986.04	91.06273	70.2	1.901E-14	in-house
306	Trans-2-Nonenal	33.899	893.66	56.09091	81.1	0	in-house

64	Valeraldehyde	8.637	326.43	44.04839	83.9	1.898E-14	in-house
18	2-Methyloctane	4.138	170.27	43.07647	92.8	2.007E-14	in-house
47	Decane	7.25	274.16	57.1	89	1.445E-14	NIST 20
67	Octane	8.765	331.25	57.1	86	0	NIST 20
433	2-Phenethyl acetate	46.961	1201.19	104.1	73.9	0	in-house
216	3-Methoxybutyl Acetate	25.386	711.43	43.04118	83.5	6.018E-06	in-house
181	3-Methylbutyl 2- methylbutanoate	22.193	645.5	70.1	92.6	0	in-house
123	3-Methylbutyl acetate	14.351	480.02	43.05	98.4	0	in-house
107	Butyl acetate	12.187	433.98	43.0475	97.9	0	in-house
34	Ethyl Acetate	5.92	224.07	43.05	80.1	0	in-house
100	Ethyl DL-2- Methylbutyrate	11.299	415.1	57.09615	98.7	1.055E-05	in-house
195	Ethyl heptanoate	24.106	685	88.0625	81.7	0	in-house
158	Ethyl Hexanoate	19.476	589.08	88.06136	91.7	0	in-house
207	Ethyl Lactate	24.692	697.1	45.05143	88.2	0	in-house
243	Ethyl Octanoate	28.652	778.86	88.05673	82.2	0	in-house
56	Ethyl propanoate	7.859	297.1	57.05667	88.2	0	in-house
79	Isobutyl acetate	9.758	368.65	43.05	94.2	0	in-house
14	Methyl acetate	3.538	154.65	43.05	96	0	in-house
401	Methyl phenylacetate	43.371	1113.76	91.05882	74.5	0	in-house
161	Vinyl n-Hexanoate	19.666	593.12	71.07273	83.9	1.705E-14	in-house
58	Ethyl Isobutyrate	8.066	304.9	43.075	96	1.525E-14	in-house
76	2-Methylbutanoate	9.476	358.04	57.1	84.3	0	in-house
61	Propyl acetate	8.441	319.04	43.045	88.8	0	in-house
313	1-Methoxy-2-propyl acetate	34.791	913.46	43.05	86		NIST 20
287	2-Acetylfuran	32.314	858.51	95.01833	74.1	9.21E-06	in-house
266	Furfural	30.619	820.91	96.03	81.3	0	in-house
477	γ -Nonalactone	53.503	1375.91	85.03659	70.2	0	in-house
351	γ -Butyrolactone	37.704	978.07	28.05	78.5	0	in-house
512	4H-Pyran-4-one	59.694	1554.24	43.05	84	0	NIST 20
65	2,3-Butanedione	8.671	327.69	43.05	84	24.252969	in-house
140	2-Heptanone	17.211	540.88	43.05	96.7	1.981E-14	in-house
233	2-Nonanone	26.778	740.18	58.051	85.7	1.571E-14	in-house
62	2-Pentanone	8.561	323.55	43.05139	88.5	0	in-house
227	3-Acetoxy-2-butanone	26.654	737.61	43.05	83.9	0	in-house
134	3-Heptanone	15.715	509.05	57.09038	84.7	3.583E-05	in-house
101	3-Hexanone	11.312	415.36	71.104	82.6	0	in-house
36	3-Methyl-2-butanone	6.272	237.32	43.04364	79.3	0	in-house
359	Acetophenone	38.421	993.96	105.05	73	0	in-house
103	2,3-Pentanedion	11.796	425.67	43.048	95.5	0	in-house
177	Acetoin	22.146	644.54	45.05	93.4	0	in-house
460	Ethanone	50.13	1285.81	94.05	88	0	NIST 20
334	Benzonitrile	36.614	953.89	103.0583	76.8	0	in-house
108	Dimethyl Disulfide	12.389	438.28	94.00385	95.3	0	in-house
9	Dimethyl Sulfide	1.872	111.33	62.02941	91.4	0	in-house
126	Ethyl benzene	14.721	487.89	91.075	90.6	0	in-house
268	2,3,5,6- Tetramethylpyrazine	30.67	822.04	54.05	85.9	2.018E-14	in-house
237	2,3,5- Trimethylpyrazine	27.602	757.17	42.05	75.7	0	in-house
196	2,5-Dimethylpyrazine	24.185	686.64	108.0933	97.4	0	in-house
215	2,6-Dimethylpyrazine	25.064	704.78	108.0971	85.2	0	in-house

260	2-Ethyl-3,5-Dimethylpyrazine	30.07	808.74	135.1037	97.9	0	in-house
173	2-Methylpyrazine	21.356	628.23	94.06765	93.4	0	in-house
238	Trimethylpyrazine	27.615	757.45	122.1	90.8	0	in-house
137	3-Carene	16.14	518.09	93.09375	80.4	0	in-house
159	Cis- β -Ocimene	19.509	589.78	93.07941	87.1	0	in-house
262	Trans-Linalool oxide	30.325	814.4	59.06704	76.5	9.773E-06	in-house

^a Retention time in minute(s)

^b Retention indices (RI) are calculated using a standard Fatty Acid Ethyl Ester mixture (C2–C24)

^c Relative standard deviation (RSD)