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REVIEW

Polyphenols and theobromine in cacao (*Theobroma cacao*): Compositional changes across variety, growing region, fermentation, drying and roasting

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Abstract

In recent years, cacao and its derivatives have gained significant attention due to their potential health benefits. The primary bioactive compounds in cacao are polyphenols and methylxanthines, predominantly represented by theobromine. Their concentrations vary widely, influenced by cacao variety, growth region, and postharvest processing. Fermentation typically leads to a marked decrease in polyphenols and theobromine, with further reductions during drying and roasting. This review aims to consolidate current knowledge on how these factors affect compound levels, providing insights crucial for optimizing practices to enhance the health benefits and quality of cacao products. Literature consistently shows that cacao properties are shaped by genetics, environmental conditions, and processing stages. Moreover, the unique polyphenol and theobromine profiles can serve as distinctive fingerprints to differentiate cacao origins. Understanding these dynamics is essential for improving both nutritional value and industrial applications.

Keywords: cacao chemical composition; polyphenolic compounds; cacao varieties; cacao methylxanthines; postharvest operations, phenolic content, microbial fermentation.

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1. Introduction

Cacao (*Theobroma cacao*) is a fruit tree native to South America that belongs to the Malvaceae family, and it is currently cultivated throughout the equator region around the world. The seeds of the cacao fruit known as cacao beans are the cornerstone for the manufacture of chocolate (Barišić et al., 2023; Osorio-Guarín et al., 2017). Furthermore, cacao beans are processed into widely consumed products such as cacao powder (commonly known as cocoa) and cacao butter, which have applications in the cosmetic, pharmaceutical, and food industries (Joel et al., 2013; Wang et al., 2020).

In recent years, several studies have elucidated the potential health and nutritional benefits of cacao by-products (D'Souza et al., 2017; Soares & Oliveira, 2022; Todorovic et al., 2017). The predominant bioactive compounds in cacao and its derivatives are polyphenols, which are secondary metabolites responsible for the bitterness and astringency of cacao beans (Flores, 2019). These polyphenols

significantly influence the organoleptic qualities of cacao, including taste (Pedan et al., 2016).

Polyphenols comprise several compounds, including flavonoids, tannins, phenolic acids, and various polymerized derivatives (Williamson, 2017). The major polyphenols found in cacao are procyanidins (58%), catechins also known as flavan-3-ols (37%) and anthocyanins (4%) (Maldonado & Figueroa, 2023). Flavanol-rich cacao and its processed products have been reported to possess neuroprotective properties and enhance cognition, as these compounds can cross the blood-brain barrier (Nehlig, 2013). Additionally, recent research has demonstrated anti-diabetic and anti-obesity activities of cacao compounds (Crichton et al., 2017; Rabadan-Chávez et al., 2016). Moreover, cacao polyphenols may influence gut microbial dynamics by promoting beneficial bacterial taxa and inhibiting pathogenic species, suggesting a potential prebiotic property (Gibson et al., 2017). Consequently, cacao is regarded by some authors

as a functional food, due to the presence of bioactive compounds that could benefit human health (Ackar et al., 2013; Jaćimović et al., 2022).

While research has focused on the health influences of polyphenolic compounds in cacao and its by-products, it is also important to note that cacao and its derivatives are rich in methylxanthines, which are secondary plant metabolites derived from purine nucleotides (Hiroshi et al., 2011). Theobromine and caffeine constitute the total alkaloid composition in cacao, with theobromine being the most abundant and caffeine present in smaller quantities (Bartella et al., 2019; Goya et al., 2022). Theobromine, together with polyphenols, has been credited with various health benefits, including the prevention of diabetes, neurodegenerative, and cardiovascular diseases (Jean-Marie et al., 2021; Pagliari et al., 2022). Low amounts of theobromine also promote serotonin release in the brain, suggesting potential antidepressant properties (de Mejia & Ramirez-Mares, 2014; Scapagnini et al., 2012). For these reasons, the present review will also focus on the theobromine composition of cacao.

The diversity of cacao varieties, geographical location, growth conditions, and postharvest activities can influence the content of polyphenol compounds, theobromine, and quality properties of cacao beans (Oracz et al., 2015a; Santander Muñoz et al., 2020). Cacao postharvest treatments include fermentation, drying, and industrial procedures such as roasting (Rawel et al., 2019). Throughout these processes, polyphenolic constituents and theobromine in cacao seeds undergo numerous transformations, including polymerization, hydrolysis, and reactions with proteins (Rojas et al., 2022).

Despite extensive research, the full impact of postharvest processing on bioactive compound profiles in cacao varieties remains unclear. This review aims to consolidate current knowledge on how cacao variety and postharvest processing affect polyphenols and theobromine. By examining changes in these compounds during different processing stages, the review provides insights crucial for optimizing practices to enhance the health benefits and quality of cacao products.

2. Polyphenols and theobromine in cacao

Polyphenols are secondary metabolites formed in plants via the phenylpropanoid and acetate/mevalonate pathways (Rojas et al., 2015). In cacao beans, polyphenols are stored within the pigment cells of the cotyledons, which are known as polyphenol-storage cells. These cells are white or purple colored depending on their anthocyanin content (Soares & Oliveira, 2022).

The predominant polyphenols found in cacao are the procyanidins, also known as flavan-3-ols which include monomers (catechin and epicatechin) and various long chain polymers (Jean-Marie et al., 2021). Epicatechin is the major flavan-3-ol monomer found in the cacao beans and constitutes the foundation of proanthocyanidins, which impart cacao its astringent and bitter taste (Agudelo et al., 2022). Anthocyanins confer the typical red violet pigmentation observed in cacao beans and are comprised by leucoanthocyanins (L1, L2, L3, L4), cyanidin-3- α -L-arabinoside, and cyanidin-3- β -D-galactoside (Aprotosoai et al., 2016).

Flavonols based on quercetin, such as quercetin-3-O-galactosid and quercetin-3-O-arabinoside along with other flavonols like kaempferol-3-O-hexoside and kaempferol-3-O-rutinoside have been identified in different cacao varieties (D'Souza et al., 2017). Additionally, polyphenolic constituents serve as defense mechanisms of the plant, particularly in cacao beans, to pathogens and stress conditions (Kouam et al., 2022).

Methylxanthines are alkaloid purines found in various plant orders. In *Theobroma cacao*, the predominant alkaloids are theobromine and caffeine, with theobromine being the most abundant (Tušek et al., 2024). A study by Zheng et al. (2004) suggests that the synthesis and accumulation of alkaloids in *T. cacao* occurs in the seeds within the fruits. In recent years, theobromine has gained notoriety because of its potential health benefits (Zhang et al., 2024). The chemical structure of some important cacao polyphenols and theobromine are displayed in Figure 1.

3. Changes in cacao composition due to variety and growing region

From a morphogenetic perspective, there are four primary types of cacao varieties: Criollo, Forastero, Trinitario, and Nacional, each possessing distinctive chemical characteristics and sensory qualities (Oracz et al., 2015a; Żyżelewicz et al., 2018). The fine grade cacao varieties, Criollo and Nacional, are cultivated in Ecuador, Venezuela, and Mexico (Perez et al., 2021). Authors like Di Mattia et al. (2013) have evidenced that the polyphenol and theobromine content in beans is linked to the cacao variety among other factors.

A recent investigation by Borja Fajardo et al. (2022) into Tolima, Colombia cacao demonstrated significant variation in antioxidant activity, total polyphenol content, and methylxanthine ratios across four different genotypes, underscoring the regional and genetic influences on bioactive compound profiles.

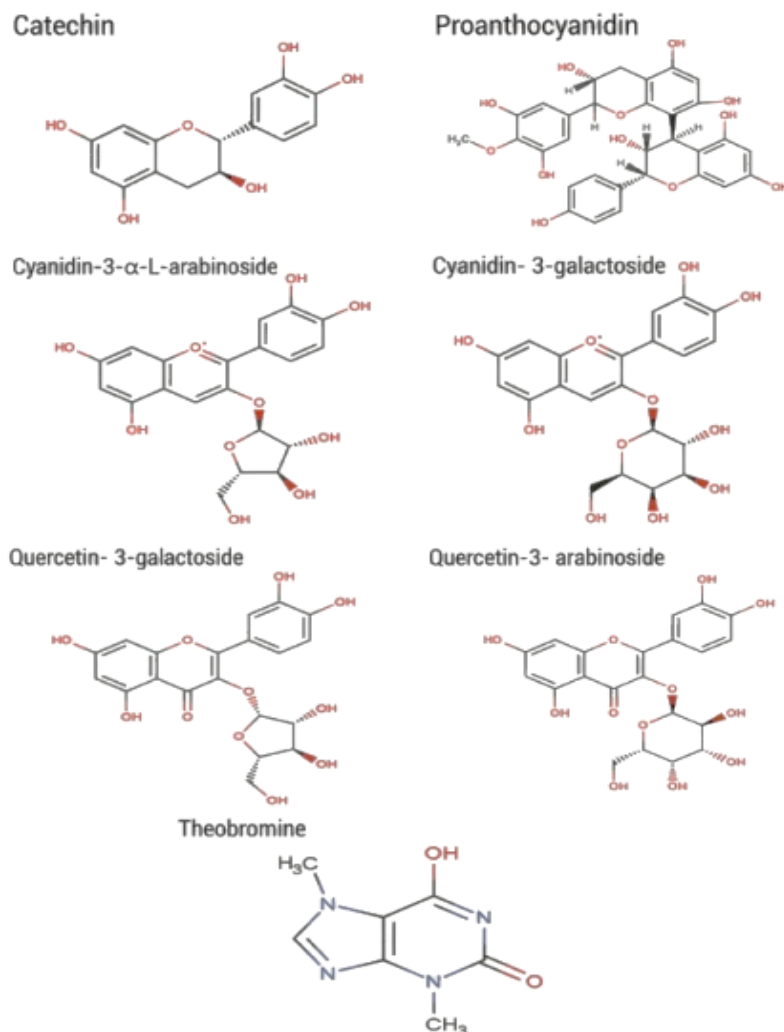


Figure 1. Chemical structure of: catechin, proanthocyanidin, cyanidin-3-α-L-arabinoside, cyanidin-3-galactoside, quercetin-3-galactoside, quercetin-3-arabinoside and theobromine. The chemical structures of the polyphenols and theobromine were retrieved from the Human Metabolome Database (<https://hmdb.ca/>).

Criollo beans are characterized by their caramel notes, low astringency, reduced bitterness, and milder cacao flavor (Lachenaud & Motamayor, 2017). The low astringency of Criollo beans could be attributed to fewer polyphenol levels in comparison to other cacao varieties (Jalil & Ismail, 2008). Criollo seeds have a pale purple color, which could be caused by an anthocyanin inhibitor gene (Kongor et al., 2016). Indeed, Elwers et al. (2009) compared seed samples from different varieties and found no anthocyanins in Criollo seeds. Furthermore, Criollo cacao beans have two-thirds of the polyphenol constituents observed in Forastero cacao beans (Oracz et al., 2015b).

Moreover, a recent study by Lavorgna et al. (2021) further highlights the nutraceutical potential of Criollo beans: extracts from Indonesian Criollo showed stronger radical-scavenging (ABTS) activity ($EC_{50} \sim 73 \mu\text{g/mL}$) compared to Peruvian varieties, exhibit-

ing antimutagenic and cytotoxic activities in cancer cell lines, and containing significant phenylprope-noyl-amino acids and procyanidin compounds. Forastero cacao trees are predominantly grown in Ghana, Nigeria, Cameroon, and Côte d'Ivoire, and are believed to be tolerant to diseases. are considered "bulk cacao," making up most of the global production. Despite their good quality and intense cacao flavor, Forastero beans have fewer fine chocolate notes compared to the Criollo variety (Beckett, 2009). The seeds of Forastero cacao are astringent and purple-colored due to the presence of anthocyanins (Goya et al., 2022). According to Loureiro et al. (2017) Amazon Forastero cacao exhibits an epicatechin content of $43.27 \pm 0.44 \text{ g/kg}$, similar to the 43.6 g/kg catechin content in cacao beans described by Febrianto & Zhu (2019a).

In a study by de Barros Kobi et al. (2024) of cacao kernels grown under different farming systems, the

CCN51 cultivar consistently showed higher epicatechin and catechin levels, especially under cabruca (shade) conditions. Conversely, full sun systems resulted in lower total phenolics and antioxidant properties, though theobromine and caffeine levels remain unaffected by farming system or variety.

Tritinario cacao is an aromatic variety that represents about 5–6% of the world's cacao production. Tritinario cacao beans are used for high-quality or premium chocolates, often commanding higher prices than Forastero cacao (Utrilla-Vázquez et al., 2020). Tritinario cacao trees are a hybrid species of Criollo and Forastero and types, resulting in beans that are less aromatic but have higher yields and resistance to phytopathogens (Ascrizzi et al., 2017). Raw cacao beans are characterized by high levels of methylxanthines and polyphenols which impart bitterness and astringency (Aprotosoie et al., 2016; Kongor et al., 2016). Rich polyphenolic content has been reported in fresh Tritinario cacao beans. For instance, Schlüter et al. (2022) found that unfermented Tritinario cacao samples contained an elevated epicatechin content of 38.9 mg/g fat-free dry matter (ffdm) and a lower but significant catechin content of 1.67 mg/g ffm. Additionally, De Taeye et al. (2016) detected anthocyanins, cyanidin-3-arabinoside, and cyanidin-3-galactoside in concentrations ranging between 2850 and 3112 mg/kg in two Tritinario clones, higher than in fermented beans.

The Nacional cacao tree is renowned for its Arriba flavor, making it a preferred choice for chocolate manufacturers (Colonges et al., 2021). The distinctive organoleptic properties of Nacional cacao are due to its phytochemical constituents, including polyphenols such as flavonoids, anthocyanins, flavones, phenols, hydroxylated stilbene derivatives, and phenolic acids (Oracz et al., 2015a). Higher concentrations of flavonoids and anthocyanins are observed in Arriba Nacional cacao beans from the Amazonian and Andean regions, suggesting that abiotic factors, particularly soil nutrients, significantly influence the phytochemical composition, antioxidant properties, and sensory quality of cacao (Mihai et al., 2022).

Recent findings from Amazonas-Peru observed by Cortez et al. (2024) show that the CCN-51 variety has the highest total polyphenol content (TPC) at 19 mg GAE/g and significant theobromine content at 20 mg/g. Fine Aroma Cacao (FAC) also stands out with a TPC of 16 mg GAE/g and a theobromine content of 21.93 ± 2.04 mg/g. These variations highlight the significant impact of both the variety and growing region on the chemical composition of cacao beans.

The variation in theobromine content of cacao beans depends on the cacao variety (Aprotosoie et al., 2016). For instance, in a study by Febrianto & Zhu (2019a) genetic variations in the methylxanthine composition were assessed among twenty-six cacao bean genotypes. The findings indicated that a low theobromine to caffeine ratio was typically found in fine-flavor cacao beans, while bulk cacao exhibited higher concentrations of both theobromine and caffeine. Loureiro et al. (2017) reviewed the theobromine and caffeine ratio, finding ranges of 15-10 (Forastero), 10-5 (Tritinario), and 2-1 (Criollo).

A study conducted by Samaniego et al. (2020) assessed the theobromine content and other characteristics of Nacional x Tritinario cacao beans from various regions of Ecuador, each with distinct climatic conditions. The results revealed a notable variability in the theobromine content across different regions. These findings demonstrate that the production area plays a crucial role in influencing the theobromine content, which may have a direct impact on the quality attributes of cacao beans.

The main findings of the previously reviewed reports regarding cacao polyphenol and theobromine composition depending on variety and growing region are detailed in Table 1.

4. Cacao postharvest practices

The genetics of cacao beans significantly influence postharvest operations. For instance, Criollo cacao beans typically require shorter processing periods, particularly for fermentation, compared to Tritinario and Forastero cacao beans. The varying fermentation periods are correlated with differences in polyphenol content. Furthermore, the diverse cacao processing operations not only heavily influence the organoleptic characteristics of cacao and its derivatives but also affect the polyphenol and theobromine fractions (De Vuyst & Weckx, 2016). The main cacao postharvest operations are discussed below.

Fermentation

Raw cacao bean fermentation is the initial stage in cacao processing, characterized by a spontaneous microbial fermentation of the mucilaginous pulp covering the beans. The primary objective of fermenting fresh cacao beans is to remove the pulp and promote the color and flavor development of fermented dry cacao beans (De Vuyst & Weckx, 2016). Cacao fermentation involves a succession of microbial activities from three groups: yeasts, lactic acid bacteria, and acetic acid bacteria (Schwan et al., 2014).

Table 1
Polyphenol and theobromine content of different cacao varieties from various growing regions

Variety	Growing region	Catechin (mg/g)	Epicatechin (mg/g)	Procyanidins (mg/g)	Anthocyanins (mg/g)	Total Polyphenols (mg/g) *	Theobromine (mg/g)	Reference
UTLP02	Tolima, Colombia	NR	NR	NR	NR	44.51± 0.90	6.02 ± 0.04	(Borja Fajardo et al., 2022)
UTLM02	Tolima, Colombia	NR	NR	NR	NR	77.96 ± 3.94	7.12 ± 0.15	
CCN-51	Tolima, Colombia	NR	NR	NR	NR	95.41 ± 2.50	5.35 ± 0.09	
Forastero	Indonesia	1.73	43.6	6.75 _c	449.6 _d 865.3 _d	NR	24.9	(Febrianto & Zhu, 2019b)
Nacional	Ecuador, Guayas (Pacific coast)	4.82 ± 1.34	5.42 ± 2.30	3.19 ± 1.16 _e	NR	47.40 ± 6.20	1.59 ± 0.20	(Samaniego et al., 2020)
	Ecuador, Los Rios (Pacific coast)	6.03 ± 1.83	7.78 ± 4.84	2.91 ± 1.54 _e	NR	43.45 ± 8.56	1.52 ± 0.21	
	Ecuador, Sucumbios (Amazon)	7.40 ± 1.56	3.45 ± 1.13	1.44 ± 0.59 _e	NR	42.75 ± 8.19	2.15 ± 0.11	
	Ecuador, Napo (Amazon)	7.66 ± 1.00	8.94 ± 2.21	5.17 ± 1.80 _e	NR	71.66 ± 3.94	2.18 ± 0.09	
Nacional	Ecuador, coastal region	568 ± 5	NR	NR	22.80 ± 0.95	2.29	15	(Mihai et al., 2022)
	Ecuador, Amazonian region	723 ± 126	NR	NR	22.23 ± 1.50	2.97	25.4	
	Ecuador, Andean region	515 ± 33	NR	NR	21.28 ± 0.59	2.46	25.1	
FAC (Fine aroma cacao)	Amazonas, Peru	~ 0.10	~ 0.48	NR	NR	~16	21.93 ± 2.04	(Cortez et al., 2024)
CCN-51	Amazonas, Peru	~ 0.12	0.6	NR	NR	~19	~20	
TSH-565	Amazonas, Peru	0.18	~ 0.51	NR	NR	~ 11	~ 18	
PS1319	Bahia, Brazil (cabruca)	NR	NR	NR	90.41 ± 21.47	257.46 ± 46.50	135659.67 ± 12481.85 ^f	(de Barros Kobi et al., 2024)

Cyanidin 3-o-b-D-galactoside results
Cyanidin 3-o-a-L-arabinoside results
Proanthocyanidin B type dimer (1) results
Results reported as eq/kg db
Procyanidin B2 results
Results expressed as area of the extracted chromatogram (EIC).
*Results reported as gallic acid equivalents (GAE) on a dry weight basis.
NR: not reported

Microbial metabolism in the mucilaginous pulp generates various compounds, such as alcohols and acids, and increases the temperature. Consequently, the bean undergoes several chemical alterations that are essential for the formation of chocolate flavor (Romero-Cortes et al., 2013).

During fermentation, anthocyanins are hydrolyzed into anthocyanidins and sugars, namely arabinose and galactose. Additionally, sugars polymerize with catechins to produce tannins, while anthocyanins tend to fade. Hence, anthocyanin content is commonly used as an indicator of cacao bean fermentation (Nazaruddin et al., 2006). It is well established that fermentation decreases the polyphenolic content in cacao beans. Polyphenol oxidase converts polyphenols to quinones, which form complexes with proteins and peptides (Camu et al., 2008). This transformation impacts the flavor by reducing bitterness and astringency, which are associated with higher polyphenol content. Figure 2 illustrates the main biochemical reactions and compound transformations that occur during cacao fermentation, particularly focusing on polyphenol and theobromine dynamics.

Fermentation also causes a 20% loss in theobromine content, reducing the bitter taste of cacao beans (Sanchez-Capa et al., 2022). This reduction is attributed to the exudation of the beans during fermentation, promoted by the increased temperature (Aprotosoie et al., 2016) and (Krähmer et al., 2015). Polyphenol and theobromine loss during fermentation of raw beans from Trinitario, Forastero, Criollo and Nacional Arriba varieties was evidenced by Caligiani et al. (2014), where epicatechin and theobromine levels decreased in fermented beans compared to unfermented, slaty beans. Additionally, Nacional Arriba samples exhibited higher carbohydrate, epicatechin, and methylxanthine content, compatible with shorter fermentation periods.

Research carried out by Albertini et al. (2015), demonstrated a significant reduction in polyphenol content and antioxidant capacity within the first 48

hours of fermenting Nacional cacao beans, with less significant changes over the subsequent days. Furthermore, Calvo et al. (2021) reported that theobromine content initially increased and then decreased after 72 hours of fermentation in Trinitario beans. This initial increase may be due to the diffusion of theobromine from the mucilaginous pulp into the bean at the onset of fermentation Goya et al. (2022), a trend also observed by Aprotosoie et al. (2016).

Fermentation techniques influence the polyphenol content of cacao beans. For example, Sanchez-Capa et al. (2022) observed decreased polyphenols using a wooden box fermenter due to genetic material. Nacional Arriba cacao polyphenol composition ranged between 57.23 and 79.18 mgGAE/gDW, while the “Super árbol” Trinitarian cacao type reported 48.46 to 55.54 mgGAE/gDW.

Fermentation time varies: two to three days for Criollo varieties and five to ten days for Forastero and Trinitario varieties (Lima et al., 2011). Prolonged fermentation times contribute to a decrease in the polyphenol content of fermented beans. For instance, do Carmo Brito et al. (2017) showed a 31% reduction in total phenolic content and a 79% decrease in total anthocyanins after a seven-day fermentation period for Forastero beans. Furthermore, Febrianto & Zhu (2020) suggested a 72-hour fermentation as optimal for retaining theobromine and polyphenols while producing Sulawesi 1 (Trinitario) beans with good organoleptic qualities. The bioactive compound content in cacao beans is linked to the degree of fermentation. The fermentation index (FI) is a tool indicating fermentation level through spectrophotometric analysis (Caporaso et al., 2018). In the study of Febrianto & Zhu (2019b) a significant correlation between the fermentation index and the concentrations of major flavan-3-ols derivatives was evidenced while an ample variation in theobromine and caffeine content was found in cacao beans with similar FI.

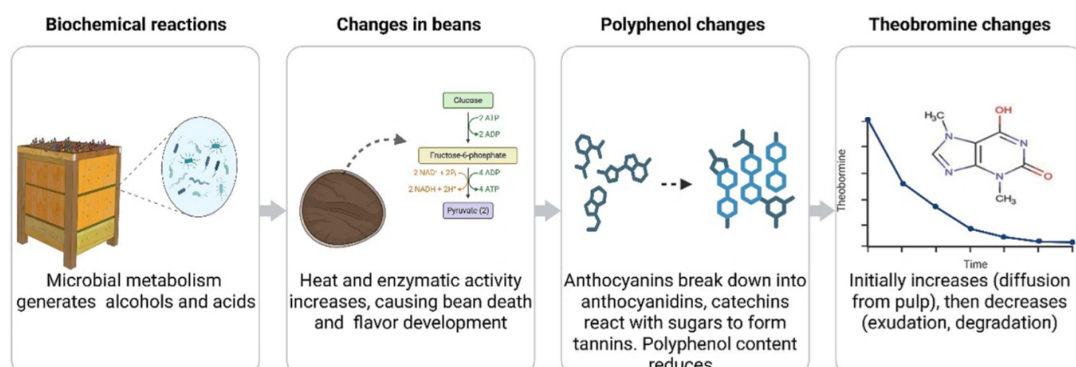


Figure 2. Polyphenol and theobromine changes during cacao fermentation.

According to **Racine et al. (2019)** variable FI trends can be observed in different cacao varieties. For example, Criollo beans, with low anthocyanin levels, do not exhibit typical FI trends seen in Trinitario and Forastero varieties.

Interesting findings on the influence of starter cultures on polyphenol and theobromine content in fermented beans have been reported. In this regard, **Chagas Junior et al. (2021)** found that inoculating *Saccharomyces cerevisiae* and *Pichia kudriavzevii* in Forastero fermentation resulted in higher phenolic compounds and theobromine concentrations. The inoculation likely facilitated the release of theobromine from the bean shell. Similarly, **Sandhya et al. (2016)** demonstrated that the inoculation of *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and *Acetobacter aceti* during Forastero fermentation decreased anthocyanin and polyphenol content. Theobromine composition varied between 0.3 and 6.4 mg/g, depending on inoculum concentrations.

Literature suggests that microbial starter cultures, in addition to variety and other factors, influence polyphenolic and theobromine content in fermented beans. However, understanding the contribution of inoculated strains to microbial dynamics and their effect on bioactive compounds requires fine-scale monitoring, metagenomic sequencing techniques, and novel chromatography and detection systems.

5. Drying

Drying is an indispensable operation that strongly influences the final quality of cacao beans and their processed products (**Castellanos et al., 2018**). The main objective of this activity is to decrease the moisture of the beans under 7–8 wt% (on a wet basis) (**Jinap et al., 2002**).

According to **Dzelagha et al. (2020)** there are five drying techniques that are used in the cacao sector, namely open sun, solar, oven, microwave, and freeze drying. Each drying method alters the physical structure of cacao beans and degrades various chemical compounds, including polyphenols and theobromine (**Alean et al., 2016; Deus et al., 2018**). Oxidative degradation of polyphenolic constituents transforms these chemical structures first into quinones and subsequently into melanin, mediated by the enzymatic activity of polyphenol oxidase (**Alean et al., 2016; Teh et al., 2016**). Additionally, theobromine primarily diffuses into the testa of the grain, causing a decrease in theobromine concentration and subsequently reducing the bitterness and astringency of cacao beans (**Chagas Junior et al., 2021; Febrianto & Zhu, 2020**).

The influence of drying techniques on antioxidant capacity, polyphenol, and theobromine content in Forastero cacao beans was evaluated by **Deus et al. (2018)**. This research utilized four solar dryers: one with a stainless-steel platform, another with a wooden platform and an artificial heat source, a traditional dryer with a wooden platform and direct sunlight exposure, and a dryer with a stainless-steel platform and a mobile plastic roof. Results showed that drying reduced the catechin content from 0.04 mg/g to 0.02 mg/g and theobromine from 19.44 mg/g to 11.71 mg/g. The traditional drying method preserved the most antioxidant activity, theobromine, and polyphenols. The authors explained that in traditional drying, cacao beans are exposed to heat, but the maximum temperature is cooler compared to other methods, leading to fewer structural changes and less impact on chemical and enzymatic reactions.

Increasing the drying temperature might contribute to conserving bioactive compounds in Amazonian Forastero cacao beans. This behavior was evidenced by **Herman et al. (2018)**, where the polyphenolic composition of cacao beans increased less at drying air temperatures of 30 – 40 °C compared to higher temperatures of 50 – 60 °C. This increase is attributed to the polyphenol oxidase losing its ability to catalyze polyphenol oxidation at unfavorable temperature conditions, typically around 35 °C.

Romanens et al. (2018) compared the response of various Trinitario hybrids in a lab-scale fermentation (LS-F) to on-farm fermentation (OF-F). They analyzed microbial dynamics, physico-chemical parameters, and the final dried bean quality. Results showed that proanthocyanidin content was higher in dried cacao beans from OF-F due to a lower pH of the cotyledon. Conversely, theobromine content remained relatively stable throughout fermentation and drying at LS-F, possibly due to the mild effect of sun drying temperatures on cacao bioactive constituents.

The effect of the different drying temperatures on the polyphenol content of the CCN51 hybrid of the Trinitario coca variety was evaluated by **Alean et al. (2016)**. Results showed the least degradation of polyphenols at 40 °C, with a polyphenolic content of 3329.76 mg gallic acid/100 g of dried fruit, representing a 45% reduction. Conversely, the highest polyphenol degradation occurred at 60 °C. The authors concluded that polyphenol degradation is influenced by temperature, moisture, and drying times. However, a more in-depth analysis including methylxanthines would provide a comprehensive view of the fate of bioactive compounds in response to the drying temperatures studied.

Several drying methods for Criollo cacao pod husks were investigated by Nieto-Figueroa et al. (2020) including microwave, forced-air drying, and forced air drying-extrusion. Results indicated that microwave-dried cacao had the highest flavonoid content, followed by forced-air drying and forced-air drying-extrusion. This finding can be attributed to the quick drying rate of microwaves, reducing polyphenol degradation. Moreover, theobromine content was higher with forced-air drying extrusion compared to microwave drying. The degree of bioactive compound degradation during cacao bean drying is linked to the volatility of these compounds and their synergy with water due to polarity. Hence, water content during drying permits polyphenols and theobromine to dissolve and diffuse to the surface (Alean et al., 2016). Recent studies have explored innovative drying methods for cacao by-products such as pod husk and bean shell that could optimize the retention of bioactive compounds, particularly polyphenols and theobromine. For instance, Ramos-Escudero et al. (2023) found that vacuum drying retained higher concentrations of polyphenols and theobromine compared to conventional methods. Minimizing

thermal exposure during drying helps safeguard these valuable bioactive compounds. Building on these insights, Cortez et al. (2024) traced the behavior of both phenolic compounds, catechin, epicatechin, gallic acid, caffeic acid, and alkaloids theobromine, caffeine, theophylline, across postharvest stages (fresh, fermented, dried, roasted, and cocoa paste) in three cocoa varieties from Amazonas, Peru. They found that except for roasting, phenolic levels increased during drying, fermentation, and refining, likely due to enhanced extractability resulting from bean breakdown, while theobromine consistently decreased through fermentation, drying, and roasting. These findings highlight that optimizing drying parameters (e.g., moderate temperatures, controlled thermal exposure) is key to preserving cacao’s bioactive value while still achieving moisture targets. Figure 3 summarizes the main cacao bean drying methods and their respective effects on polyphenol and theobromine composition. The main results of the previously mentioned reports about cacao drying effects on polyphenols and theobromine content are detailed in Table 2.

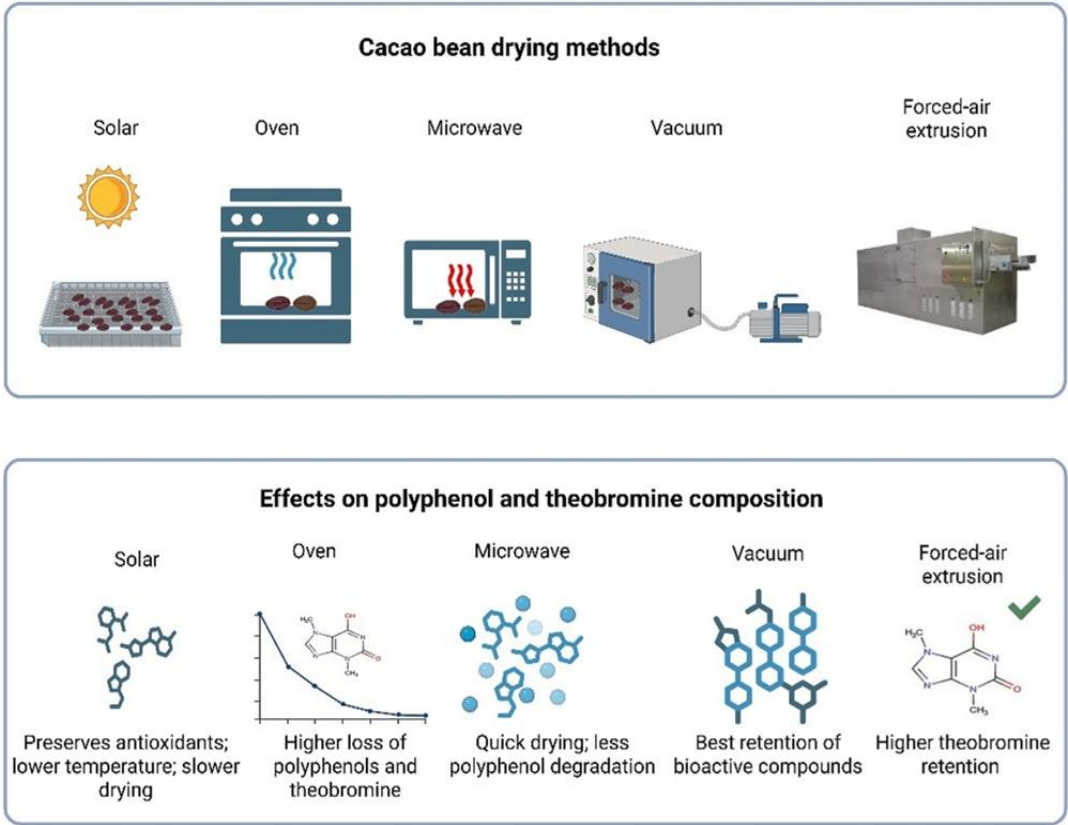


Figure 3. Cacao bean drying methods and their effects on polyphenol and theobromine composition.

Table 2
Polyphenol and theobromine content of dried cacao beans

Variety	Fermented or unfermented	Fermentation method	Drying method	Drying temperature	Catechin (mg/g)	Epicatechin (mg/g)	Procyanidins (mg/g)	Total Polyphenols (mg/g) *	Theobromine (mg/g)	Reference
Forastero	Fermented	Natural fermentation	DP	NR	0.02 ± 0.00	0.90 ± 0.05	NR	NR	11.14 ± 0.59	(Deus et al., 2018)
			AD	60 °C	0.021± 0.00	0.635 ± 0.00	NR	NR	10.12 ± 0.58	
			TD	NR	0.037 ± 0.00	1.037 ± 0.02	NR	NR	14.96 ± 0.55	
			MD	NR	0.020 ± 0.00	0.756 ± 0.03	NR	NR	10.63 ± 0.09	
Trinitario	Fermented	Laboratory incubator, plastic pots	Sun drying	NR	NR	~0.90	~0.80	NR	15.7 ± 1.24	(Romanens et al., 2018)
		Wooden box			NR	~9.00	~3.50	NR	15.7 ± 0.89	
Criollo	Unfermented	NA	Microwave	NR	23.1 ± 1.4 _a		NR	27.3 ± 2.0	8.1 _b	(Nieto-Figueroa et al., 2020)
		NA	Forced air drying	45 °C	16.5 ± 0.6 _a		NR	26.7 ± 1.6	44.7 _b	
		NA	Forced air Drying plus, extrusion	60 °C - 160 °C	15.8 ± 0.3 _a		NR	24.8 ± 1.1	24.1 _b	
Fine Aroma Cocoa (FAC)	Fermented	Wooden crates	Solar dome dryer	30 °C	~ 0.40	~ 0.90	NR ~ 22		~16	(Cortez et al., 2024)

Results reported as [mg (+)- catechin/g]

Results reported as µg/g

* Results reported as gallic acid equivalents (GAE) on a dry weight basis.

NR: not reported

NA: not applicable

DP: Dryer with stainless steel platform and plastic roof with UV protection,

AD: Artificial dryer using wooden platform with artificial heat source

TD: Traditional dryer in barge with wooden platform and direct sun light,

MD: Mixed dryer with stainless steel platform and mobile plastic roof with UV protection

6. Roasting

Roasting is a pivotal postharvest process that greatly influences the quality of cacao beans and their derived products. Typically performed at temperatures ranging from 100 - 150 °C, the roasting process impacts polyphenol levels, which are further influenced by roasting time (Oracz & Nebesny, 2016). For example, Spizzirri et al. (2019) demonstrated that Forastero cacao beans roasted with a forced air flow oven at lower temperatures (95 °C for 60 minutes) retain higher concentrations of theobromine, catechin, epicatechin, procyanidins, and total polyphenols compared to higher temperatures (110 °C and 125 °C) and shorter roasting times, which significantly diminish these beneficial compounds.

Another study by Giltekin-Özgüven et al. (2016) investigated the effect of roasting, grinding, and alkalization on the stability of total phenolics, antioxidant capacity, and procyanidins in Forastero cacao beans from Ghana.

The study found that roasting and alkalization significantly reduced polyphenols (65% and 87%, respectively) and antioxidant capacity. Interestingly, grinding after roasting increased polyphenols and antioxidant capacity due to the gradual diffusion of phenolic compounds from the cell wall breakage.

Several studies have explored the effects of roasting temperatures on the polyphenol composition of cacao beans. In this context, Ioannone et al. (2015) found that flavanol and total proanthocyanidin loss increased with roasting temperatures in Criollo cacao beans. Djikeng et al. (2018) reported that traditional roasting (200 - 220 °C) significantly reduced the polyphenol content and antioxidant activity of Trinitario beans. Fernández-Romero et al. (2020) observed that roasting Criollo beans at 200°C for 50 minutes resulted in total degradation of epicatechin and a 92.29% reduction in TPC. According to Oracz & Nebesny (2016) high temperature and oxygen contact during roasting are the main factors for polyphenolic degradation. Hence, alternative procedures such as vacuum roasting and superheated steam roasting have been proposed to conserve flavonoids in cacao beans (Zzaman et al., 2014).

Oracz et al. (2015a) studied the effect of different roasting conditions on individual flavan-3-ols, anthocyanins, and flavanols in five cacao varieties. The study found that Forastero beans contained the highest levels of these compounds, while Trinitario beans had the lowest polyphenol content. Roasting significantly affected the profile and levels of

polyphenolic compounds across the studied varieties.

Some reports have shown that certain roasting temperatures can preserve or even increase the content of bioactive compounds in cacao beans. The study by Lemarcq et al. (2020) determined that roasting various cacao hybrids from Ecuador at 130 °C for 30 minutes did not affect the contents of epicatechin, procyanidin B2, and theobromine. Stanley et al. (2018) found that roasting Trinitario beans at ≥150°C increased the levels of catechin and proanthocyanidin hexamers and heptamers. As explained by Dorta et al. (2012); Suazo et al. (2014) this increment could be attributed to the degradation of cell structures during heat treatment, resulting in the release of bound polyphenols. Moreover, according to De Taeye et al. (2017) roasting beans at 120°C for 30 minutes or 90°C for one hour prevents strong degradation of flavan-3-ols in Forastero, Trinitario, and Criollo varieties.

Recent quantitative modeling work by McClure et al. (2021) further refined the understanding of roasting effects by evaluating eight roast profiles across three cacao origins. Using response-surface modeling, they found that roasting consistently decreased epicatechin and procyanidin B2 while increasing catechin, with slight increases in theobromine and caffeine attributed to moisture loss. Complementing this, Hermund et al. (2025) investigated the fate of flavonoids and theobromine at 100 and 150 °C for up to 20 minutes, showing that a profile of 150 °C for 15 minutes preserved high flavonoid levels while reducing theobromine content, achieving a desirable balance between bioactive retention and sensory quality.

In general, elevated processing temperatures and prolonged processing times reduce the polyphenolic content in cacao beans (Hurst et al., 2011). These degradations are attributed to enzymatic oxidations during manufacturing processes (Giacometti et al., 2015). High roasting temperatures also affect theobromine composition. For example, Zapata Bustamante et al. (2015) studied the influence of roasting on bioactive compounds and antioxidant activity in five Trinitario cacao clones and found that roasting at 180°C reduced theobromine levels.

Colonges et al. (2022) found that roasting increased the polyphenol and theobromine content in Nacional cacao beans. Roasted beans had higher levels of epicatechin, various procyanidins, and theobromine compared to non-roasted beans. In

this research, a genome-wide association study (GWAS) identified 20 regions linked to bitterness and astringency, 53 areas related to nonvolatile compounds, and 81 associated genes.

In conclusion, roasting plays a pivotal role in determining the polyphenol and theobromine content in cacao beans, with lower temperatures preserving more beneficial compounds. These insights emphasize the need to optimize roasting conditions to maximize the health benefits and sensory qualities of cacao products. Future research should focus on the development of novel roasting methods that balance flavor and nutrient preservation. A visual summary of how roasting temperature and time influence polyphenol and theobromine levels in cacao beans is presented in **Figure 4**.

The main results of the previously discussed reports about cacao roasting effects on polyphenols and theobromine are detailed in **Table 3**.

7. Current and future challenges

Research on cacao processing and how different cacao varieties affect the levels of polyphenols and theobromine faces several challenges. One major issue is the inconsistency in cacao bean quality, which is impacted by climate change, pests, and diseases. These factors can dramatically alter the concentration and makeup of polyphenols and theobromine. Furthermore, the lack of standardized processing techniques leads to variations in the final product. The complexity of fermentation and drying also makes it challenging to maintain consistent levels of beneficial compounds.

Looking ahead, a key challenge will be to develop processing methods that are both sustainable and efficient, ensuring high-quality cacao beans with optimal levels of polyphenols and theobromine. Emerging technologies like precision agriculture and improved fermentation control are expected to play a pivotal role in overcoming these obstacles. Additionally, breeding cacao varieties that are more resilient to environmental stresses and offer higher levels of beneficial compounds is an important area of ongoing research. For these innovations to be sustainable, it's crucial that they are economically viable for smallholder farmers. Understanding how cacao variety influences the composition of polyphenols and theobromine is vital for enhancing the health benefits of cacao products. Different varieties naturally contain varying amounts of these compounds, with genetics, growing conditions, and post-harvest processing all playing a role.

Research aims to pinpoint the best cacao varieties and processing methods to maximize the content of these bioactive compounds, ultimately improving the nutritional value and health benefits of cacao. This knowledge could also pave the way for developing new cacao-based products with specific health benefits.

Figure 5 summarizes the key current and future challenges in cacao research, including variability among cacao varieties, complexity in fermentation and drying, the need for precision agriculture, and breeding for resilience and compound optimization.

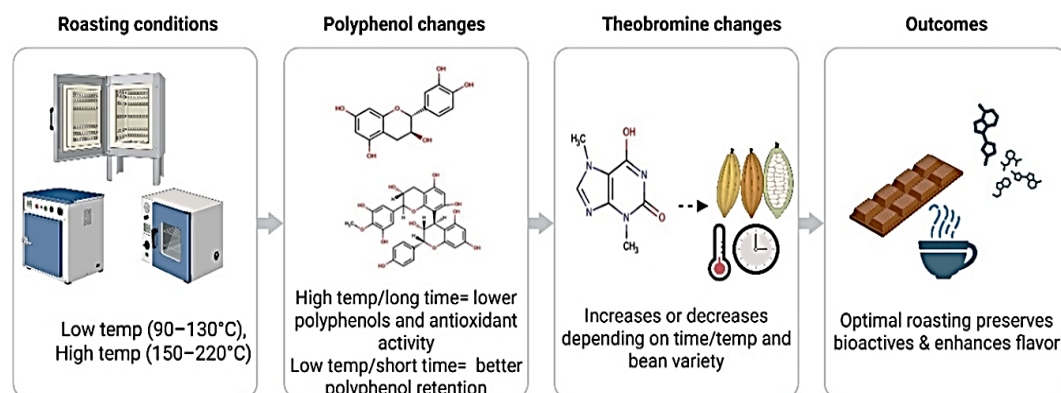


Figure 4. Polyphenol and theobromine changes during roasting.

Table 3
Polyphenol and theobromine content of roasted cacao beans

Variety	Roasting method	Roasting temperature	Roasting time	Catechin (mg/g)	Epicatechin (mg/g)	Procyanidins (mg/g)	Total Polyphenols (mg/g) *	Theobromine (mg/g)	Reference
Forastero	Forced airflow oven	95°C	60 min	0.15	1.08	2.47	5.16	8.49	(Spizzirri et al., 2019)
		110°C	30 min	0.10	0.58	1.17	2.72	7.68	
		125°C	20 min	0.14	0.73	1.01	2.37	7.14	
	DTRCB	200-220 °C	5 min	NR	NR	NR	~75.00	NR	(Djikeng et al., 2018)
	DORCB	180°C	5 min	NR	NR	NR	~80.00	NR	
	DTRCB	200-220 °C	10 min	NR	NR	NR	~60.00	NR	
	DORCB	180°C	10 min	NR	NR	NR	~75.00	NR	
		120°C	NR	2.83 ± 0.09	0.30 ± 0.02	2.33 ± 0.02a	NR	NR	
		135°C	NR	2.80 ± 0.07	0.27 ± 0.04	2.09 ± 0.05a	NR	NR	
		150°C	NR	2.22 ± 0.08	0.40 ± 0.02	1.65 ± 0.05a	NR	NR	
Modern Nacional	NR	NR	NR	NR	~4.00	~2.00	NR	~1.70	(Colonges et al., 2022)
Amazonian Nacional	NR	NR	NR	NR	~1.00	~1.50	NR	NR	
Ghanian cacao	Forced air convection oven	151 °C	54 min	0.39	0.72	0.25	NR	12.1	(McClure et al., 2021)
Nicaraguan cacao	Cofee roaster	150 °C	28 min	~12 °	~ 90 °	~12 °	~ 140 °	~23 °	(Hermund et al., 2025)

Procyanidin B2 results
Proanthocyanidin P1 results
Expressed as µg/mL of extract
* Results reported as gallic acid equivalents (GAE) on a dry weight basis.
NR: not reported,
DTRCB: Dried and traditionally roasted cacao beans, DORCB: Dried and oven roasted cacao beans

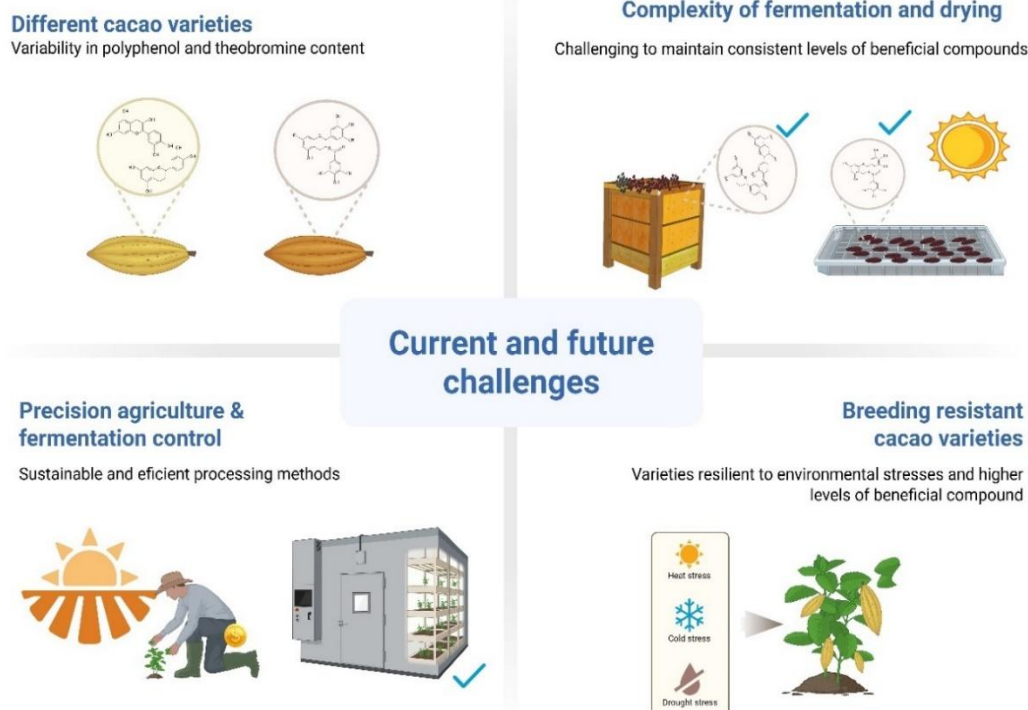


Figure 5 Current and future challenges.

8. Conclusion

Cacao beans and its derived products have a potential protective effect on human health due to the presence of bioactive compounds, such as polyphenols and methylxanthines. The major polyphenolic constituents of cacao beans are procyanidins, flavan-3-ols and anthocyanins. In addition, the most abundant methylxanthine found in cacao beans is theobromine.

Moreover, literature review showed that the content of polyphenolic compounds and theobromine of Nacional, Criollo, Forastero and Trinitario cacao varieties is highly variable. Hence, genetics strongly influence the polyphenol composition of cacao. The present investigation evidenced that the polyphenol and theobromine content found in different cacao varieties diminish throughout the various processing operations that the beans undertake after they are harvested to produce its finished products.

In this context, fermentation was found to be a critical stage for polyphenol and theobromine degradation, due to the numerous chemical and physical changes that beans undergo during this process. Moreover, as reviewed in various reports, the thermal treatments that include drying and roasting alter the composition of the polyphenol and theobromine fractions of cacao beans.

However, this composition variation highly depends on the specific temperatures and processing times allocated to the beans. Considering that cacao properties are highly dependent on their genetic structure and their processing circumstances; the chemical constituents such as polyphenols and theobromine might be utilized as a distinctive fingerprint to differentiate cacao from different varieties.

The findings reviewed in the current work can serve as reference for actors within the cacao sector as they contribute to increase the knowledge of the effects of varieties and processing activities on the levels of bioactive compounds in cacao. This knowledge can be applied by the cacao industry to optimize postharvest processes and enhance the bioactive compound content in the final products. Future studies should focus on refining these processes, such as exploring the precise impact of different fermentation, drying, and roasting conditions on the preservation of polyphenols and theobromine. Additionally, research on developing new technologies and methods for cacao processing can further improve the quality and health benefits of cacao products.

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Conflict of interest

The authors declare no conflict of interest

Ethical statement

Not applicable

Author contributions

J. Cevallos-Cevallos and J. Ruales- Nájera: Conceptualization, investigation, writing original draft, supervision, J. Tigriero-Vaca: investigation, writing, review and editing, visualization

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