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Effect of Fermentation on the bioactive compound of cocoa Beans: a systematic review and meta-analysis approach

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Several studies have found the effect of cocoa bean fermentation on bioactive compounds through various fermentation characteristics; however, these studies have yet to adopt the meta-analysis approach. The effect of cocoa bean fermentation on six bioactive compounds: epicatechin, catechin, total phenolic content (TPC), anthocyanins, theobromine, and caffeine, were systematically reviewed. This study aims to determine which variables that can affect the loss of bioactive compounds during fermentation so that their decrease can be reduced. The Cochrane Library, Medline, Science Direct, and Google Scholar databases were used to search the literature through November 2021. Twenty-nine studies were systematically reviewed, and 15 could be processed for meta-analysis. The results of the random effect model (REM) showed that cocoa bean fermentation significantly ($p < 0.05$) reduced the levels of catechins, epicatechins, TPC, anthocyanins, theobromine, and caffeine. The study results explain the concept that fermentation can reduce the number of bioactive compounds in cocoa beans (although there is an increase in some cases). However, with certain genotype varieties, pod storage treatment and the addition of starter cultures are considered to be able to maintain a specific concentration of bioactive compounds.

1. Introduction

The scientific name *Theobroma cacao L.* (Gr. Theo-God; Broma-drink) refers to cocoa. Since chocolate is delicious, it is considered the food of the gods (Hernandez et al., 2017; Lima et al., 2011; Montagna et al., 2019). In addition, chocolate is liked by people of all ages and has a good effect on health. Cocoa consumption can suppress premature aging, oxidative stress, blood pressure regulation, and atherosclerosis due to its biologically active phenolic compounds (Andujar et al., 2012; Katz et al., 2011; Latif, 2013; Oracz et al., 2015).

Cocoa contains high concentrations of phenolic compounds, especially epicatechin, catechin, procyanidins, and their oligomer derivatives (Brito et.al., 2017; Evina et.al., 2016). Cocoa has high levels of flavonoids, more than tea, red wine, blueberries, cranberries, and other types of fruit (Brito et.al., 2017; Dang and Nguyen, 2019; Terahara, 2015). Furthermore, chocolate is also rich in procyanidin flavonoids, comparable to the levels in apples, which are rich in procyanidins. Methylxanthines in the form of theobromine, a bitter alkaloid, and caffeine are nitrogen-rich compounds in cocoa that function as central nervous system stimulants, diuretics, and smooth muscle relaxants (Latif, 2013; Bauer et al., 2011).

The cocoa beans are processed into edible chocolate products. The process includes fermentation, roasting, conching, and tempering (Barisic et al., 2019). Fermentation is an essential part of processing cocoa beans into chocolate, further contributing to flavor precursors' production. The flavor is converted into flavor in the roasting process, increasing the sensory attributes for consumer acceptance. Fermentation mainly aims to produce chocolate flavor precursors (Aprotosoaie et al., 2015).

Generally, cocoa bean fermentation is carried out spontaneously in large quantities using the heap, box, basket, tray, barrel, or platform method. Generally, fermentation lasts about 2–10 days (Lefeber et al., 2010; Pereira et al., 2012; Ho et al., 2014; Miguel et al., 2017; Vuyst & Weckx, 2016). Many studies have been carried out to analyze the growing microbes, isolate them, and use them as fermented cultures. Another research study on cocoa bean fermentation involved the use of laboratory-scale and small-scale to facilitate the formulation process and further research (Romanens et al., 2019; Serra et al., 2019; Pereira et al., 2020). However, several studies have stated that the bioactive compounds also decrease as the fermentation time increases. The decrease in bioactive compounds is caused by microbial metabolic activity in the pulp and seeds (Caporaso et al., 2018; Caprioli et al., 2016; Ho et al., 2014).

Cocoa beans consist of pulp and kernel or cotyledons with dry weight percentages of 10–14% and 86–90%, respectively (Afoakwa et al., 2013; Nazaruddin et al., 2006). Pulp is the structure that covers the beans in liquid pectin. Sugar, pectin, and other polysaccharides comprise relatively high pulp constituents. Although its texture is thick, it tastes sweet and slightly acidic and can be consumed immediately (Afoakwa et al., 2013; Crafact et al., 2013). Under anaerobic conditions, yeast produces ethanol (Ho et.al., 2014; Yao et.al., 2014).

Meanwhile, under aerobic conditions, acetic acid bacteria produce acetic acid. These metabolites have contributed to inhibiting seed germination, supporting the chemical changes that occur in the cotyledons (Vuyst & Leroy, 2020). Under aerobic conditions, acetic acid bacteria further lower the pH of cocoa beans. Cotyledons or cocoa beans are composed of two-thirds water and fat in equal proportions. The remainder, in small concentrations, consists of starch, sugar, phenolic compounds, and many other components. During fermentation, microbial metabolic activity in cocoa dregs can generate heat, and metabolites can kill cocoa beans (Afoakwa et al., 2013; Crafact et al., 2013). The extensive research on cocoa bean fermentation is majorly focused on the analysis of the product's physicochemical and functional properties and sensory attributes. Therefore, a meta-analysis approach aims to determine fermentation's effect on bioactive compounds' content. Meanwhile, within the scope of a systematic review, which variables can maintain the decrease in these bioactive compounds can be analyzed.

2. Materials and Methods

The steps in this systematic review and meta-analysis study refer to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines (Moher et al., 2015).

2.1 Data Search and Strategy

Online databases such as Cochrane Library, Medline, Science Direct, and Google Scholar were used for systematic reference searches. Keywords were used to identify potentially relevant studies published up to November 2021. The keywords in the search terms consist of: Cocoa OR Cocoa bean* OR Theobroma* OR "Theobroma cacao OR Chocolate OR Cocoa liquor OR pulp AND microbial OR bacteria OR yeast OR lactic acid bacteria OR acetic acid bacteria OR starter culture* OR solid-state fermentation* OR metabolite* OR starter OR fermented AND anthocyanin OR total phenolic content OR methylxanthine OR theobromine OR caffeine OR catechin OR epicatechin OR functional properties* OR antioxidant activity* OR bioactive phenolic compound*. All the papers from the four databases were exported to the Mendeley reference manager software for further accurate screening by two investigators (NK, LC).

2.2 Inclusion criteria

Studies were considered for the initial identification process if they fulfilled the following categories: 1. original articles published in English; 2. studies that used the cocoa fermentation process (with or without culture addition); 3. studies that produced at least one

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of the characteristic of functional properties; 4. especially in a meta-analysis, there are adequate data to show relevant values such as [mean (standard deviation)] or graphs that can be converted into data using data extraction software. The excluded articles were poster abstracts, proceedings, case reports, editorials, and reviews (Ahn & Kang, 2018). This study did not limit the use of fermentation methods and types of starter cultures.

2.3 Data extraction and analysis

Two reviewers (NK, LC) reviewed information from each study. The data summarized are the first author's name, year of publication, author's country, fermentation time, fermentation method, seed weight, cultivar, starter culture, and other information. NK and LC carried out data extraction. The software used is Review Manager (RevMan) (Computer program, Version 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Quantitative data in mean ± standard deviation (SD) will be compiled to estimate the combined effect by meta-analysis. The REM was used based on the diversity of the data obtained. The p-value and I2 statistic could determine heterogeneity between studies. I2 values with numbers 0-30%, <30% to 60%, and more than 60% indicate low, medium, and high heterogeneity values, respectively. The significance value was indicated by a two-tailed test and p value <0.05.

Table 1. Characteristics of Included Studies

References	Country	Fermentation Time (hour)	Fermentation Method / Vessel	Weight Cocoa Bean (kg)	Cultivar	Starter Culture
De-Brito et.al., 2001	Brazil	144	Wooden boxes	250	Forastero, Trinitario, Criollo	No adding
Brito et.al., 2017	Brazil	168	Wooden boxes	45	Forastero	No adding
Caprioli et.al., 2016	Italy	120	Heap	1	Trinitario	No adding
Cruz et.al., 2015	Brazil	120	Wooden box	400	Forastero	No adding
Dang et.al., 2019	Vietnam	168	Wooden box	75	TD3 genotype	No adding
Estrada et.al., 2020	Mexico	-	-	-	Criollo, Trinitario, Nacional	-
Evina et.al., 2016	Belgium	120	Wooden box	50	Trinitario	No adding
Fang et.al., 2020	China	168	Wooden boxes	50	-	No adding
Hernández et.al., 2018	Spain	144	Wooden box	-	Different genotipe	No adding
Hurst et.al., 2011	USA	120	-	-	Forastero	No adding
Junior et.al., 2021	Brazil	168	Wooden boxes	-	Forastero	*Saccharomyces cerevisiae* and *Pichia kudriavzevii*
Kadow et.al., 2015	Germany	120	Incubation medium	-	-	No adding
Lefeber et.al., 2012	Belgium	96	heap	150	Forastero	-
Lessa et.al., 2017	Brazil	168	-	-	-	*P. roqueforti*
Melo et.al., 2020	Brazil	144	Wooden box	40	-	No adding
Misnawi et.al., 2003	Malaysia	120	Wodden box	200	F1 hybrids (GC7 vs. SCA6/SCA12)	No adding
Nazaruddin et.al., 2006	Malaysia	120	-	-	mixed-hybrids	No adding
Payne et.al., 2010	USA	120	-	-	Forastero	No adding
Papalexandratou et.al., 2011	Belgium	100	Fermentation box, heap	96	Nacional, Trinitario	No adding
Peláez et.al., 2016	Peru	120	Fermentation box	200	Criollo	No adding

Continue Table 1. Characteristics of Included Studies

References	Country	Fermentation Time (hour)	Fermentation Method / Vessel	Weight Cocoa Bean (kg)	Cultivar	Starter Culture
Sandhya et.al., 2016	India	168	Fermentation box	10	Forastero	Yeast:LAB:AAB (1:1:1) each 10-60% (w/v)
Saunshia et.al., 2018	India	168	Wooden box	10	Forastero	three different types of microbial cultures
Servent et.al., 2018	France	144	Nets	0.7	Trinitario, Criollo	No adding
Sunoj et.al., 2016	India	144	Heap	150	Mixed F1 progeny variety	No adding
Yao et.al., 2014	Cote d'Ivoire	144	Heap	100	Forastero, Trinitario, Criollo	No adding

3. Results

3.1 Search Results

A total of 6,156 articles were obtained from keywords assigned to the database. Removal of duplicates and title/ abstract screening resulted in 171 articles, which two reviewers reviewed. Seventy-nine papers were read and reviewed. Based on the fully understood text, there were 25 articles used in the systematic review, and 16 were included in the meta-analysis data (Table 1). The PRISMA flow chart has presented the complete results from the initial search process to the final screening (Fig.1).

3.2 Overview of included studies

The 25 papers included in the systematic review are summarized in Table 2. Articles were published between 2003–2021, of which six studies were conducted in Brazil, three in Belgium, three in India, two in the USA, and two in Malaysia. Each country contributed to one study: Spain, Vietnam, Mexico, Peru, Italy, China, Germany, France, and Cote d'Ivoire.

3.3 Findings from the meta-analysis

The results of the forest plot (Figure 2. a-f) show several findings, including the most significant decrease in epicatechin and TPC compounds. Meanwhile, the compounds of catechin, anthocyanin, theobromine, and caffeine did not decrease much.

Epicatechin. Nine studies with 32 subjects contributed to determining the effect of cocoa fermentation on epicatechin. The pooled estimate from the REM showed that cocoa fermentation significantly decreased epicatechin (SMD: -12.33; 95% CI, -13.78 to -10.89; $p < 0.00001$). There was high heterogeneity and significant effect between studies ($I2 = 100\%$; $p < 0.00001$).
Catechin. Ten studies with 33 subjects contributed to determining the effects of cocoa fermentation on catechin. The pooled estimate from the REM showed that cocoa fermentation significantly decreased catechins (SMD: -0.75; 95% CI, -0.83 to -0.68; $p < 0.00001$). There was high heterogeneity and significant effect between studies ($I2 = 100\%$; $p < 0.00001$).
Total Phenolic Content. Five studies with 12 subjects contributed to determining the effects of cocoa fermentation on total phenolic content. The pooled estimate from the REM showed that cocoa fermentation significantly decreased the total phenolic content (SMD: -19.32; 95% CI, -30.52 to -8.12; $p < 0.00001$). There was high heterogeneity and significant effect between studies ($I2 = 100\%$; $p < 0.00001$).
Anthocyanin. Four studies with 13 subjects contributed to determining the effects of cocoa fermentation on anthocyanin. The pooled estimate from the REM showed that cocoa fermentation significantly decreased anthocyanin (SMD: -0.30; 95% CI, -0.45 to -0.15; $p < 0.00001$). There was high heterogeneity and significant effect between studies ($I2 = 100\%$; $p < 0.00001$).

Theobromine. Eleven studies with 34 subjects contributed to determining the effects of cocoa fermen-

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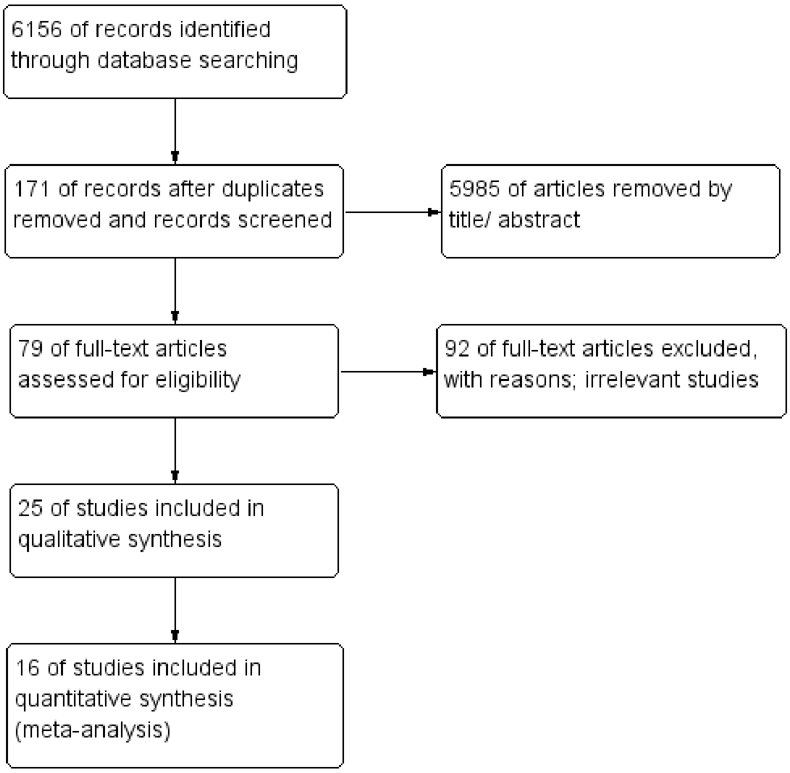

Figure 1. Flow Chart of the Study Screening Process

tation on theobromine. The pooled estimate from the REM showed that cocoa fermentation significantly decreased theobromine levels (SMD: -1.57; 95% CI, -2.16 to -0.98; $p < 0.00001$). There was high heterogeneity and significant effect between studies (I2 = 100%; $p < 0.00001$).

Caffeine. Ten studies with 34 subjects contributed to determining the effects of cocoa fermentation on theobromine. The pooled estimate from the REM showed that cocoa fermentation significantly decreased caffeine (SMD: -1.05; 95 % CI, -1.35 to -0.78; $p < 0.00001$). There was high heterogeneity and significant effect between studies (I2 = 100%; $p < 0.00001$).

Figure 3. Funnel Plot of the effect of cocoa fermentation on (a) Epicatechin, (b) Catechin, (c) Total Phenolic Content, (d) Anthocyanin, (e) Theobromine, and (f) Caffeine.

4. Discussion

In general, research on cocoa bean fermentation is carried out in vivo by using large-scale cocoa beans in wooden boxes. It is performed using different seed variants, including forastero, criollo, trinitario, or other genetic variants. Fermentation time is 120–168 hours. All studies show a decreased profile of bioactive compounds in fermented cocoa beans. However, there are also results that indicate an increase in these compounds. For example, studies using starter cultures such as Saccharomyces cerevisiae and Pichia kudriavzevii were able to improve functional properties compared to controls. Even the combined culture of the two was able to increase methylxanthine and phenolic and phenylethylamine compounds (Othman et al., 2010). Meanwhile, in another study, it was stated that the anthocyanin content seemed to increase, but it was not significant (Junior et al., 2021; Emmanuel et al., 2012). However, an increase in theobromine was found after fermentation. Yet, not much is explained as to what happens when this increase occurs (Melo et al., 2020). However, an increase in theobromine was found after fermentation. Yet, not much is explained as to what happens when this increase occurs. However, research assumes that an increase in theobromine and caffeine occurs when the cocoa pods are ripened first (Dang et al., 2019).

Table 2. Profile of bioactive compounds in each study

References	Epicatechin	Catechin	TPC	Anthocyanin	Caffeine	Theobromine
Brito et al., 2017			-	-		
Cruz et al., 2015	+-	+-			+-	+-
Dang et al., 2019		+-				+-
Estrada et al., 2020	+					
Evina et al., 2015	-					
Hernández et.al., 2017	-					
Junior et.al., 2021	+		-			
Kadow et.al., 2015					-	+-
Lessa et.al., 2017			-	+		
Melo et al., 2020	-	-	-		-	-
Misnawi et al., 2003	-					
Nazaruddin et.al., 2006	-					
Payne et al., 2010	-	-				
Peláez et.al., 2016	-	-			-	-
Sandhya et al., 2016				-	-	-
Saunshia et.al., 2018	-	-	-		-	-

*All treatments showed the value of bioactive compounds with a profile: (-) decreased; (+) increased; (+-) both

4.1 Findings from the meta-analysis

Based on the results of meta-analysis studies, cocoa bean fermentation causes a decrease in bioactive compounds. Several variables that are often used in cocoa bean fermentation research include pod storage time, fermentation time, cultivar type, the addition of starter culture, etc. Bioactive compounds have good benefits for health. However, in cocoa beans, through fermentation, some of them are degraded, along with increased flavor. Fermentation is mandatory in producing high-quality products. The decomposition or reduction of these compounds cannot be denied throughout the fermentation process. However, with the analysis of the fermentation variable, it is expected that the concentration of bioactive compounds can be reduced to smaller amounts.

4.2 Epicatechin

Epicatechin is a flavonoid compound that acts as one of the main antioxidants and is a procyanidin monomer (Katz et al., 2011; Afoakwa et al., 2013; Othman et al., 2010). Flavonoids are strong antioxidants in the main polyphenols class (Othman et al., 2010). Antioxidants are chemical substances that can significantly prevent the oxidation of substrates even though they are only present in relatively low concentrations in the body (Lobo et al., 2010).

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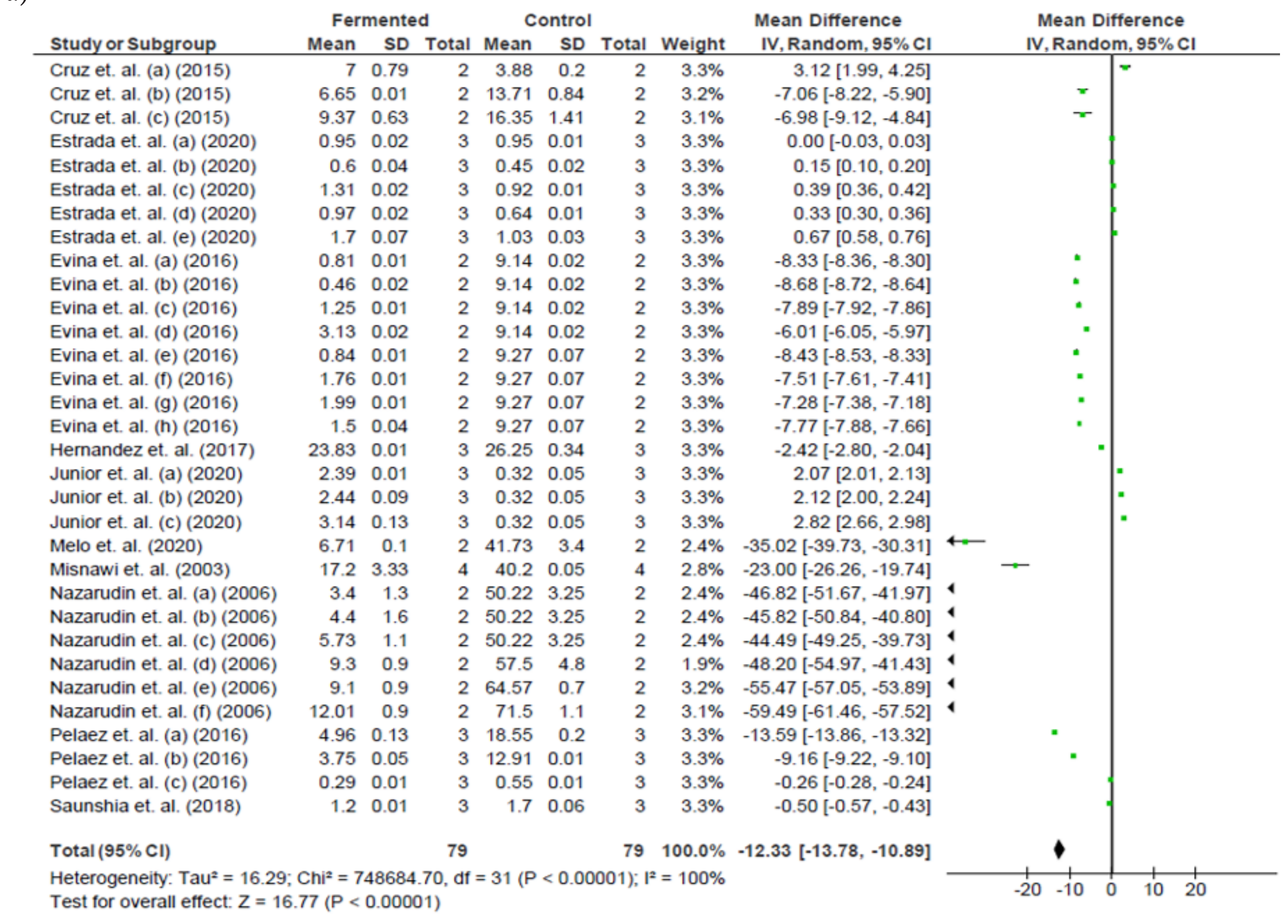
a)



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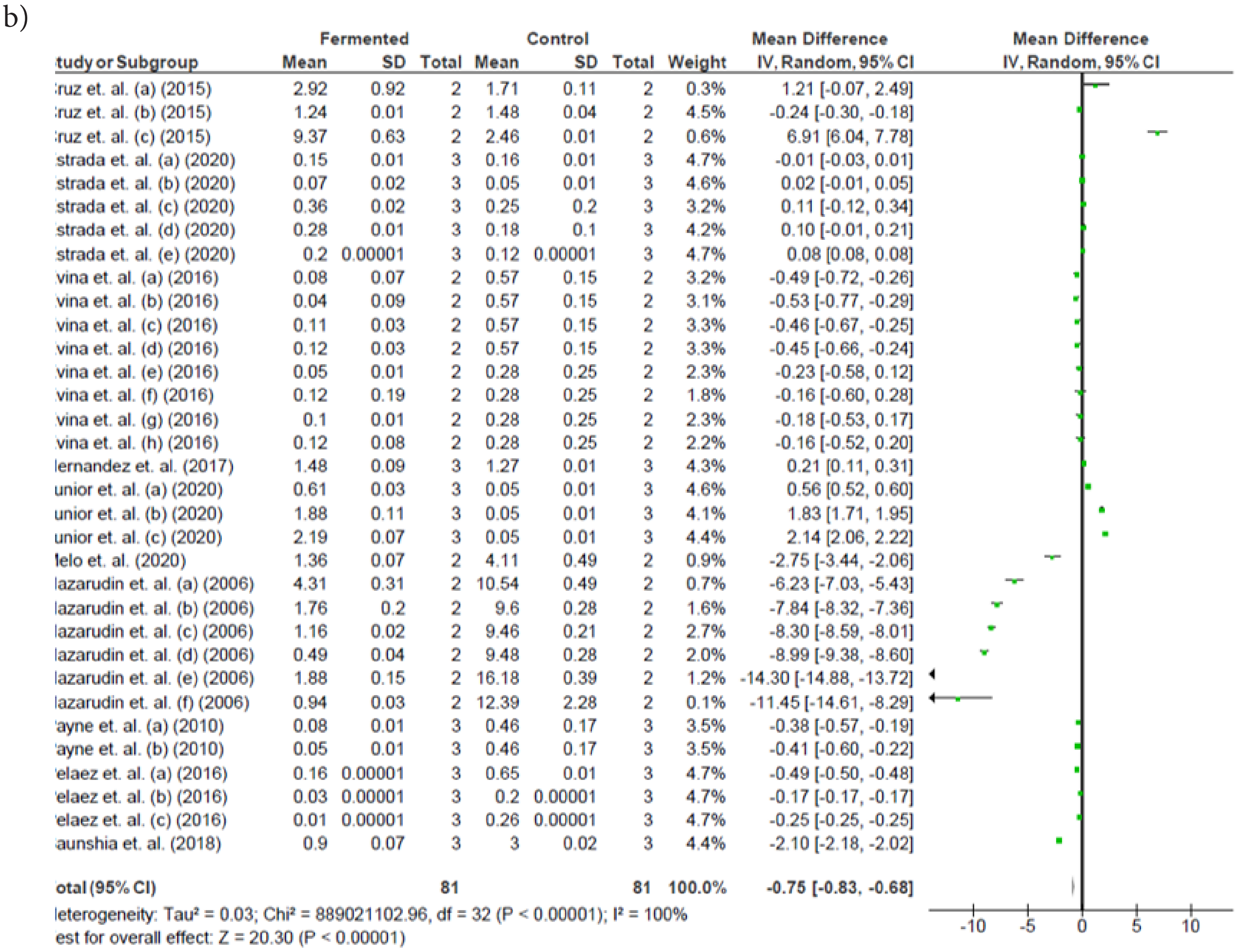
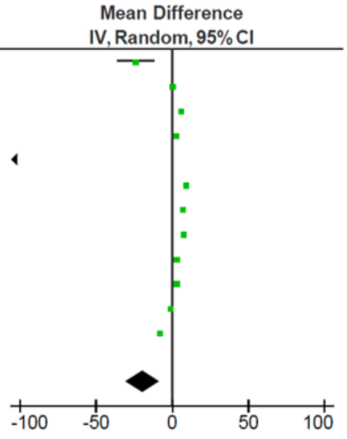


Figure 2. Forest Plot of the effect of cocoa fermentation on (a) Epicatechin, (b) Catechin, (c) Total Phenolic Content, (d) Anthocyanin, (e) Theobromine, and (f) Caffeine.

Study or Subgroup	Fermented Mean	Fermented SD	Fermented Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Brito et al. (2017)	53.26	10.72	3	77.31	2.25	3	7.6%	-24.05 [-36.44, -11.66]
Junior et. al. (a) (2020)	26.59	1.1	3	26.35	0.9	3	8.4%	0.24 [-1.37, 1.85]
Junior et. al. (b) (2020)	32.25	0.4	3	26.35	0.9	3	8.4%	5.90 [4.79, 7.01]
Junior et. al. (c) (2020)	29.05	0.5	3	26.35	0.9	3	8.4%	2.70 [1.53, 3.87]
Melo et. al. (2020)	154.96	3.03	2	395.15	0.9	2	8.3%	-240.19 [-244.57, -235.81]
Sandhya et. al. (a) (2015)	34	0.2	3	25	0.8	3	8.4%	9.00 [8.07, 9.93]
Sandhya et. al. (b) (2015)	32	0.2	3	25	0.8	3	8.4%	7.00 [6.07, 7.93]
Sandhya et. al. (c) (2015)	32.5	0.5	3	25	0.8	3	8.4%	7.50 [6.43, 8.57]
Sandhya et. al. (d) (2015)	28	0.2	3	25	0.8	3	8.4%	3.00 [2.07, 3.93]
Sandhya et. al. (e) (2015)	28	0.1	3	25	0.8	3	8.4%	3.00 [2.09, 3.91]
Sandhya et. al. (f) (2015)	24	0.8	3	25	0.8	3	8.4%	-1.00 [-2.28, 0.28]
Saunshia et. al. (2018)	25	0.5	3	33	0.5	3	8.4%	-8.00 [-8.80, -7.20]
Total (95% CI)			**35**			**35**	**100.0%**	**-19.32 [-30.52, -8.12]**

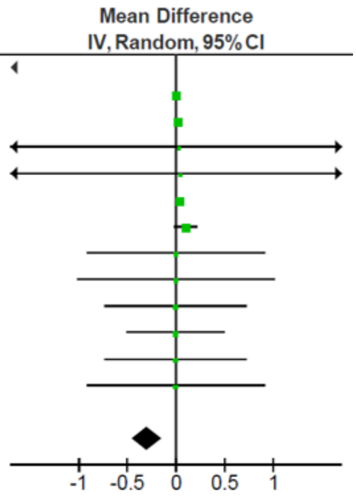
Heterogeneity: Tau² = 388.00; Chi² = 12854.66, df = 11 (P < 0.00001); I² = 100%
Test for overall effect: Z = 3.38 (P = 0.0007)



c)

Study or Subgroup	Fermented Mean	Fermented SD	Fermented Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Brito et al. (2017)	0.63	0.05	3	3.01	0.15	3	13.8%	-2.38 [-2.56, -2.20]
Estrada et. al. (a) (2020)	0.08	0.01	3	0.08	0.01	3	17.1%	0.00 [-0.02, 0.02]
Estrada et. al. (b) (2020)	0.06	0.01	3	0.04	0.01	3	17.1%	0.02 [0.00, 0.04]
Estrada et. al. (c) (2020)	0.08	1	3	0.05	2	3	0.3%	0.03 [-2.50, 2.56]
Estrada et. al. (d) (2020)	0.12	0.01	3	0.08	7	3	0.0%	0.04 [-7.88, 7.96]
Estrada et. al. (e) (2020)	0.11	0.03	3	0.07	0.01	3	16.9%	0.04 [0.00, 0.08]
Lessa et. al. (2018)	0.5	0.1	3	0.4	0.03	3	15.5%	0.10 [-0.02, 0.22]
Sandhya et. al. (a) (2015)	0.01	0.7	3	0.011	0.4	3	2.3%	-0.00 [-0.91, 0.91]
Sandhya et. al. (b) (2015)	0.0067	0.8	3	0.011	0.4	3	2.0%	-0.00 [-1.02, 1.01]
Sandhya et. al. (c) (2015)	0.0055	0.5	3	0.011	0.4	3	3.4%	-0.01 [-0.73, 0.72]
Sandhya et. al. (d) (2015)	0.005	0.2	3	0.011	0.4	3	5.8%	-0.01 [-0.51, 0.50]
Sandhya et. al. (e) (2015)	0.0052	0.5	3	0.011	0.4	3	3.4%	-0.01 [-0.73, 0.72]
Sandhya et. al. (f) (2015)	0.0047	0.7	3	0.011	0.4	3	2.3%	-0.01 [-0.92, 0.91]
Total (95% CI)			**39**			**39**	**100.0%**	**-0.30 [-0.45, -0.15]**

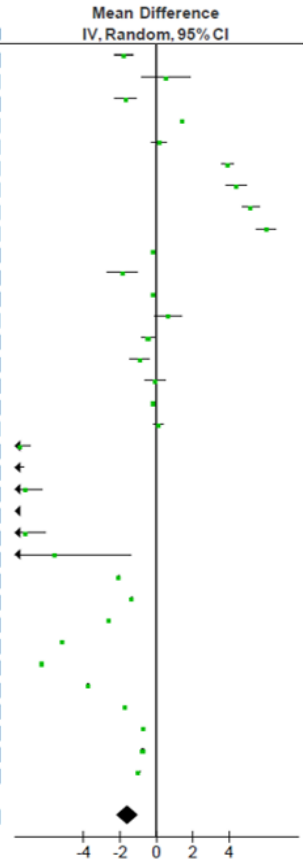
Heterogeneity: Tau² = 0.03; Chi² = 692.50, df = 12 (P < 0.00001); I² = 98%
Test for overall effect: Z = 3.94 (P < 0.0001)



d)

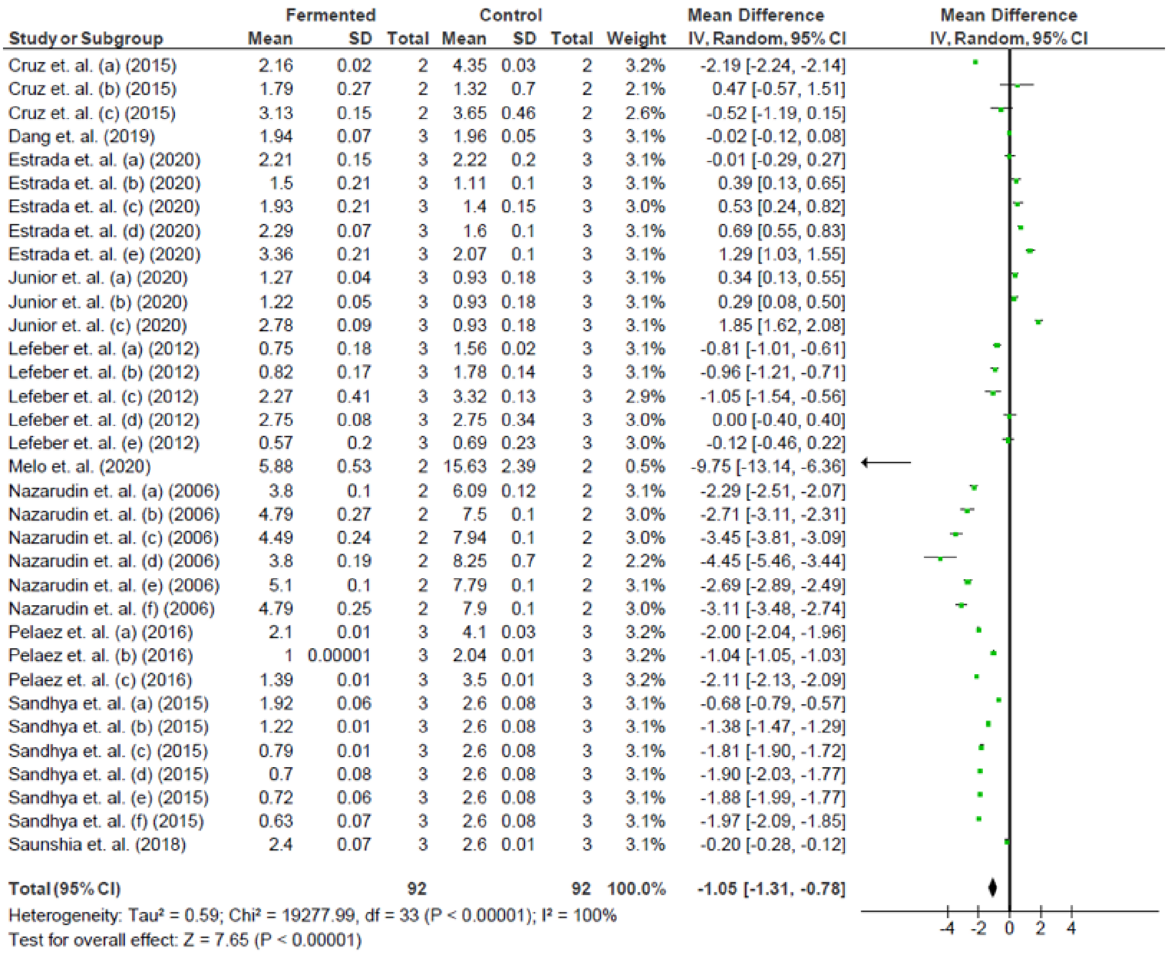
Study or Subgroup	Fermented Mean	Fermented SD	Fermented Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Cruz et. al. (a) (2015)	6.18	0.36	2	7.96	0.05	2	3.0%	-1.78 [-2.28, -1.28]
Cruz et. al. (b) (2015)	5.97	0.48	2	5.45	0.85	2	2.6%	0.52 [-0.83, 1.87]
Cruz et. al. (c) (2015)	4.81	0.21	2	6.5	0.36	2	3.0%	-1.69 [-2.27, -1.11]
Dang et. al. (2019)	10.88	0.03	3	9.48	0.01	3	3.1%	1.40 [1.36, 1.44]
Estrada et. al. (a) (2020)	12.16	0.29	3	12.02	0.25	3	3.0%	0.14 [-0.29, 0.57]
Estrada et. al. (b) (2020)	14.95	0.2	3	11.03	0.2	3	3.0%	3.92 [3.60, 4.24]
Estrada et. al. (c) (2020)	14.67	0.43	3	10.28	0.3	3	3.0%	4.39 [3.80, 4.98]
Estrada et. al. (d) (2020)	15.03	0.35	3	9.84	0.24	3	3.0%	5.19 [4.71, 5.67]
Estrada et. al. (e) (2020)	15.24	0.43	3	9.19	0.2	3	3.0%	6.05 [5.51, 6.59]
Hernandez et. al. (2017)	21.79	0.07	3	21.97	0.03	3	3.1%	-0.18 [-0.27, -0.09]
Junior et. al. (a) (2020)	5.53	0.74	3	7.4	0.02	3	2.9%	-1.87 [-2.71, -1.03]
Junior et. al. (b) (2020)	7.19	0.01	3	7.4	0.02	3	3.1%	-0.21 [-0.24, -0.18]
Junior et. al. (c) (2020)	8.05	0.64	3	7.4	0.02	3	2.9%	0.65 [-0.07, 1.37]
Lefeber et. al. (a) (2012)	1.96	0.21	3	2.41	0.23	3	3.0%	-0.45 [-0.80, -0.10]
Lefeber et. al. (b) (2012)	3	0.17	3	3.92	0.44	3	3.0%	-0.92 [-1.45, -0.39]
Lefeber et. al. (c) (2012)	2.03	0.39	3	2.11	0.33	3	3.0%	-0.08 [-0.66, 0.50]
Lefeber et. al. (d) (2012)	1.12	0.01	3	1.32	0.06	3	3.1%	-0.20 [-0.27, -0.13]
Lefeber et. al. (e) (2012)	1.9	0.17	3	1.79	0.19	3	3.0%	0.11 [-0.18, 0.40]
Melo et. al. (2020)	9.79	0.22	2	17.29	0.33	2	3.0%	-7.50 [-8.05, -6.95]
Nazarudin et. al. (a) (2006)	13.41	0.3	2	21.32	0.3	2	3.0%	-7.91 [-8.50, -7.32]
Nazarudin et. al. (b) (2006)	14.87	0.6	2	22.09	0.34	2	2.8%	-7.22 [-8.18, -6.26]
Nazarudin et. al. (c) (2006)	13.8	0.3	2	24.18	0.4	2	2.9%	-10.38 [-11.07, -9.69]
Nazarudin et. al. (d) (2006)	18.1	0.7	2	25.3	0.4	2	2.8%	-7.20 [-8.32, -6.08]
Nazarudin et. al. (e) (2006)	21.1	2.2	2	26.7	2.1	2	1.2%	-5.60 [-9.82, -1.38]
Pelaez et. al. (a) (2016)	12.41	0.01	3	14.49	0.00001	3	3.1%	-2.08 [-2.09, -2.07]
Pelaez et. al. (b) (2016)	9.52	0.02	3	10.88	0.01	3	3.1%	-1.36 [-1.39, -1.33]
Pelaez et. al. (c) (2016)	10.63	0.04	3	13.24	0.07	3	3.1%	-2.61 [-2.70, -2.52]
Sandhya et. al. (a) (2015)	2.2	0.08	3	7.4	0.04	3	3.1%	-5.20 [-5.30, -5.10]
Sandhya et. al. (b) (2015)	1.07	0.01	3	7.4	0.04	3	3.1%	-6.33 [-6.38, -6.28]
Sandhya et. al. (c) (2015)	3.64	0.02	3	7.4	0.04	3	3.1%	-3.76 [-3.81, -3.71]
Sandhya et. al. (d) (2015)	5.66	0.02	3	7.4	0.04	3	3.1%	-1.74 [-1.79, -1.69]
Sandhya et. al. (e) (2015)	6.65	0.06	3	7.4	0.04	3	3.1%	-0.75 [-0.83, -0.67]
Sandhya et. al. (f) (2015)	6.64	0.04	3	7.4	0.04	3	3.1%	-0.76 [-0.82, -0.70]
Saunshia et. al. (2018)	7	0.07	3	8	0.05	3	3.1%	-1.00 [-1.10, -0.90]
Total (95% CI)			**93**			**93**	**100.0%**	**-1.57 [-2.16, -0.98]**

Heterogeneity: Tau² = 2.96; Chi² = 106139.57, df = 33 (P < 0.00001); I² = 100%
Test for overall effect: Z = 5.20 (P < 0.00001)



e)

Figure 2. Forest Plot of the effect of cocoa fermentation on (a) Epicatechin, (b) Catechin, (c) Total Phenolic Content, (d) Anthocyanin, (e) Theobromine, and (f) Caffeine.

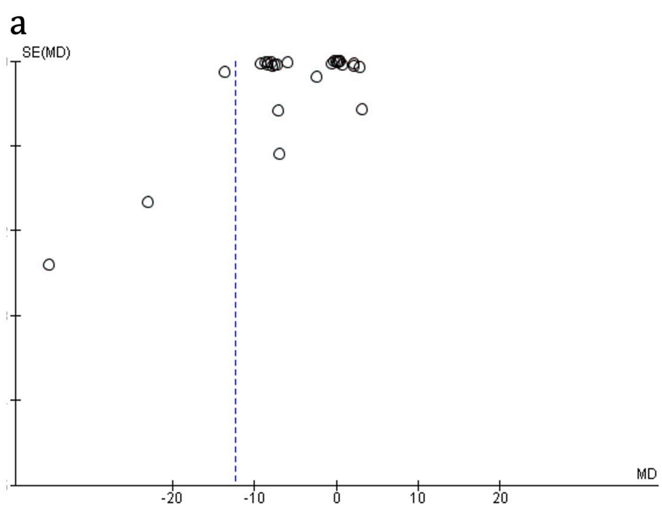


Study or Subgroup	Fermented Mean	Fermented SD	Fermented Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Cruz et. al. (a) (2015)	2.16	0.02	2	4.35	0.03	2	3.2%	-2.19 [-2.24, -2.14]
Cruz et. al. (b) (2015)	1.79	0.27	2	1.32	0.7	2	2.1%	0.47 [-0.57, 1.51]
Cruz et. al. (c) (2015)	3.13	0.15	2	3.65	0.46	2	2.6%	-0.52 [-1.19, 0.15]
Dang et. al. (2019)	1.94	0.07	3	1.96	0.05	3	3.1%	-0.02 [-0.12, 0.08]
Estrada et. al. (a) (2020)	2.21	0.15	3	2.22	0.2	3	3.1%	-0.01 [-0.29, 0.27]
Estrada et. al. (b) (2020)	1.5	0.21	3	1.11	0.1	3	3.1%	0.39 [0.13, 0.65]
Estrada et. al. (c) (2020)	1.93	0.21	3	1.4	0.15	3	3.0%	0.53 [0.24, 0.82]
Estrada et. al. (d) (2020)	2.29	0.07	3	1.6	0.1	3	3.1%	0.69 [0.55, 0.83]
Estrada et. al. (e) (2020)	3.36	0.21	3	2.07	0.1	3	3.1%	1.29 [1.03, 1.55]
Junior et. al. (a) (2020)	1.27	0.04	3	0.93	0.18	3	3.1%	0.34 [0.13, 0.55]
Junior et. al. (b) (2020)	1.22	0.05	3	0.93	0.18	3	3.1%	0.29 [0.08, 0.50]
Junior et. al. (c) (2020)	2.78	0.09	3	0.93	0.18	3	3.1%	1.85 [1.62, 2.08]
Lefeber et. al. (a) (2012)	0.75	0.18	3	1.56	0.02	3	3.1%	-0.81 [-1.01, -0.61]
Lefeber et. al. (b) (2012)	0.82	0.17	3	1.78	0.14	3	3.1%	-0.96 [-1.21, -0.71]
Lefeber et. al. (c) (2012)	2.27	0.41	3	3.32	0.13	3	2.9%	-1.05 [-1.54, -0.56]
Lefeber et. al. (d) (2012)	2.75	0.08	3	2.75	0.34	3	3.0%	0.00 [-0.40, 0.40]
Lefeber et. al. (e) (2012)	0.57	0.2	3	0.69	0.23	3	3.0%	-0.12 [-0.46, 0.22]
Melo et. al. (2020)	5.88	0.53	2	15.63	2.39	2	0.5%	-9.75 [-13.14, -6.36]
Nazarudin et. al. (a) (2006)	3.8	0.1	2	6.09	0.12	2	3.1%	-2.29 [-2.51, -2.07]
Nazarudin et. al. (b) (2006)	4.79	0.27	2	7.5	0.1	2	3.0%	-2.71 [-3.11, -2.31]
Nazarudin et. al. (c) (2006)	4.49	0.24	2	7.94	0.1	2	3.0%	-3.45 [-3.81, -3.09]
Nazarudin et. al. (d) (2006)	3.8	0.19	2	8.25	0.7	2	2.2%	-4.45 [-5.46, -3.44]
Nazarudin et. al. (e) (2006)	5.1	0.1	2	7.79	0.1	2	3.1%	-2.69 [-2.89, -2.49]
Nazarudin et. al. (f) (2006)	4.79	0.25	2	7.9	0.1	2	3.0%	-3.11 [-3.48, -2.74]
Pelaez et. al. (a) (2016)	2.1	0.01	3	4.1	0.03	3	3.2%	-2.00 [-2.04, -1.96]
Pelaez et. al. (b) (2016)	1	0.00001	3	2.04	0.01	3	3.2%	-1.04 [-1.05, -1.03]
Pelaez et. al. (c) (2016)	1.39	0.01	3	3.5	0.01	3	3.2%	-2.11 [-2.13, -2.09]
Sandhya et. al. (a) (2015)	1.92	0.06	3	2.6	0.08	3	3.1%	-0.68 [-0.79, -0.57]
Sandhya et. al. (b) (2015)	1.22	0.01	3	2.6	0.08	3	3.1%	-1.38 [-1.47, -1.29]
Sandhya et. al. (c) (2015)	0.79	0.01	3	2.6	0.08	3	3.1%	-1.81 [-1.90, -1.72]
Sandhya et. al. (d) (2015)	0.7	0.08	3	2.6	0.08	3	3.1%	-1.90 [-2.03, -1.77]
Sandhya et. al. (e) (2015)	0.72	0.06	3	2.6	0.08	3	3.1%	-1.88 [-1.99, -1.77]
Sandhya et. al. (f) (2015)	0.63	0.07	3	2.6	0.08	3	3.1%	-1.97 [-2.09, -1.85]
Saunshia et. al. (2018)	2.4	0.07	3	2.6	0.01	3	3.1%	-0.20 [-0.28, -0.12]
Total (95% CI)			**92**			**92**	**100.0%**	**-1.05 [-1.31, -0.78]**

Heterogeneity: Tau² = 0.59; Chi² = 19277.99, df = 33 (P < 0.00001); I² = 100%
Test for overall effect: Z = 7.65 (P < 0.00001)

f)

Figure 2. Forest Plot of the effect of cocoa fermentation on (a) Epicatechin, (b) Catechin, (c) Total Phenolic Content, (d) Anthocyanin, (e) Theobromine, and (f) Caffeine.



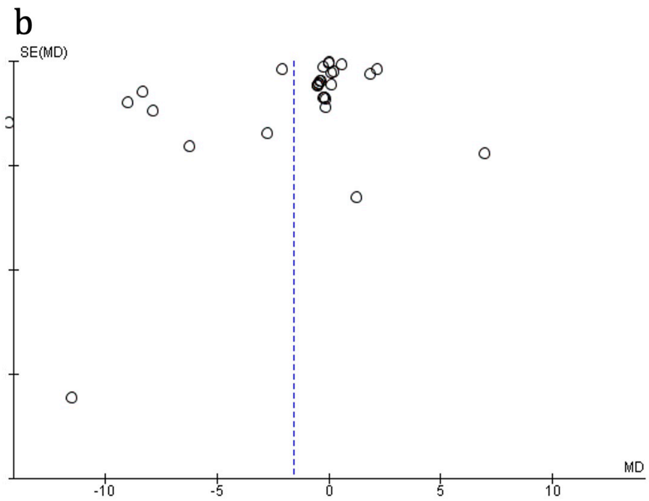


Figure 3. Funnel Plot of the effect of cocoa fermentation on (a) Epicatechin, (b) Catechin, (c) Total Phenolic Content, (d) Anthocyanin, (e) Theobromine, and (f) Caffeine.

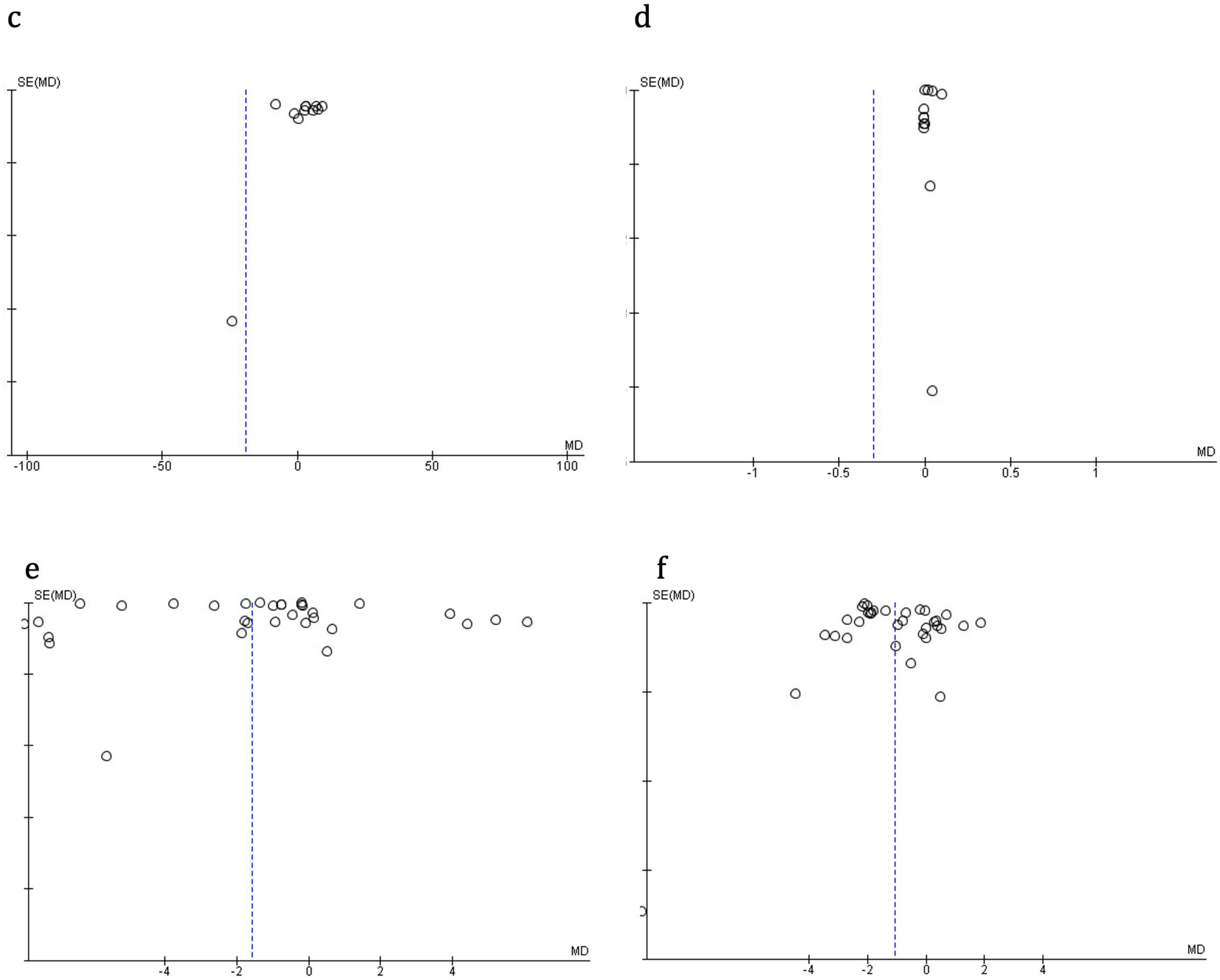


Figure 3. Funnel Plot of the effect of cocoa fermentation on (a) Epicatechin, (b) Catechin, (c) Total Phenolic Content, (d) Anthocyanin, (e) Theobromine, and (f) Caffeine.

The meta-analysis study (Figure 2.a) showed decreased epicatechin in fermented cocoa beans. This proved that epicatechin is degraded during the fermentation process. This process reduces the polyphenol content through oxidation or exudation (Misnawi et al., 2003). An indicator that can be recognized as a result of this process is a change in the color of the seeds from purple to brown (Nazaruddin et al., 2006). The epicatechin content varies in each variety of cocoa. Many studies have shown a decrease in epicatechin in the beans after the fermentation stage. Fermentation causes hydrolysis of tannins and procyanidins and converts them into monomeric epicatechin compounds, reducing proanthocyanidin levels (Nazaruddin et al., 2006). In specific genotype variants, epicatechin content varies; for example, the RIM105, MO31, and MO33 genotypes have higher concentrations of theobromine, epicatechin, and catechins than other genotypes tested (Hernández et al., 2018).

The research showed an increase in epicatechin after fermentation due to the presence of microorganisms that can hydrolyze phenolic complexes into free and also soluble phenols, and there has been an increase in the number of these compounds (Junior et al., 2021). Yeast species such as Pichia kudriavzevii are known to facilitate the release of this compound, thereby leading to the measurement of a high epicatechin content (Ooi et al., 2020). However, in general, epicatechin levels will decrease during the fermentation process.

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The funnel plot results show publication bias (Figure 3. a).

4.3 Catechin

Cocoa is rich in polyphenols, especially flavan-3-ol or catechins, particularly with high content of flavanol monomers such as epicatechin and catechin) as well as oligomers (procyanidins). The main flavan-3-ol or catechin in cocoa beans consists of (−)-epicatechin as well as (+)-catechin, (+)-gallocatechin, and (−)-epigallocatechin (Andujar et al., 2012; Aprotosoaie et al., 2015). Thus, bioactive compounds have been reported that can reduce lipid peroxyl radicals and inhibit lipid peroxidation (Othman et al., 2010).

Figure 2.b reflects the observation that cocoa fermentation can reduce the content of catechin compounds. The funnel plot graph shows no publication bias (Figure 3.b). The decrease in the content of these compounds can occur due to enzymatic oxidation reactions with microorganism activity in the early stages of fermentation; then, these compounds diffuse out of the seeds so that the decrease in compounds in the seeds can be measured (Albertini et al., 2015). In addition, several studies have shown that pulpal preconditioning contributes to a reduction in the amount of (−)-epicatechin and (+)-catechin, theobromine, and caffeine. It was stated that preconditioning the pulp for 15 days was the optimum condition for reducing the number of these compounds (Nazaruddin et al., 2006).

Each variety of cocoa beans contains a different amount of catechin compounds. Differences in the profile and concentration of these compounds can be influenced by genetics and the environment (Katz et al., 2011). The amount of the type of (+)-catechin contained in cocoa beans tends to decrease less than for epicatechin. It can even be said that the amount is almost constant during the fermentation process (Cruz et al., 2015).

In addition, reducing pulp volume, for instance, during pulp washing or using a depulper machine, contributes to accelerating the diffusion of catechins. Pressing the seeds can also cause the loss of some of these catechin compounds. This reduction in catechins reduces the astringency level in cocoa beans (Nazaruddin et al., 2006; Lessa et al., 2017).

4.4 Total Phenolic Content

The results of the meta-analysis study revealed that the fermentation process could cause a decrease in the total phenol content (Figure 2. c), suggesting a publication bias (Figure 3.d). The formation of polyphenols increased with the level of fruit maturity. There are three main categories of polyphenols in cocoa beans: catechins or flavan-3-ols, proanthocyanidins, and anthocyanins (Oracz et.al., 2015). Polyphenols in cocoa beans are primarily stored in the pigment cells of cotyledons and cocoa leaves (Osman et al., 2004). This pigment is closely related to the number of anthocyanins, namely polyphenol storage pigment cells, which range from white to dark purple (Nazaruddin et al., 2006). In measuring antioxidant activity, phenolic compounds such as alkaloids, flavonoids, and terpenoids are often detected. Several studies have stated that there is a correlation between polyphenol measurements and antioxidant activity (Lessa et al., 2017; Fang et al., 2020).

Each study has cocoa cultivars with varying characteristics that affect the amount of each phenolic compound (Figure 2. c). The polyphenol content decreased gradually due to the fermentation process. The presence of microbial activity in the pulp supports this. Various studies on cocoa bean fermentation have observed a decrease in total polyphenols with increasing inoculum concentration. The results showed that high inoculum levels decreased the duration of fermentation by accelerating the loss of anthocyanins and polyphenols. Even though there was a decrease in polyphenol content or total phenol content, this compound still has considerable potential to act as a high antioxidant. However, decreasing the phenolic content can increase the taste precursors. The production of flavor precursors and the low residual amount of individual raw cocoa phenolic compounds can be controlled by the effects of heat and the acidity formed (Kadow et al., 2015; Bastos et al., 2019; Moreira et al., 2018; Bortolini et al., 2016). The decrease in phenolic compounds was detected as polymer formation due to the oxidation reaction. This condition is a stress response from the reduced available nutrients during the fermentation period30. The amount of polyphenols usually decreases during controlled and natural fermentation (Afoakwa et al., 2013; Calvo et al., 2021; Pérez et al., 2018; Qin et al., 2017; Rottiers et al., 2019).

It was found that the presence of polyphenol oxidase (PPO) activity caused the degradation of polyphenols, which correlated with the enzymatic browning reaction and resulted in brown cocoa beans. In one study, measurements were made by looking at the cut test and fermentation index. The fermentation index indicates the completeness of the cocoa bean fermentation process. This is related to the change in seed color from purple to brown due to the diffusion of polyphenols, followed by oxidation and reduction with other cellular compounds (Hernández et al., 2017; Kresnowati & Febriami, 2015). In addition, polyphenol oxidase enzymes contribute to the formation of synergistic complexes with polyphenols, peptides, and other proteins by converting polyphenolic compounds and anthocyanins to quinones during fermentation. This process causes the reduction of phenolic compounds (Shahidi & Ambigaipalan, 2015). The polyphenol oxidase enzyme works optimally at a specific temperature and pH, generally on the third day of fermentation, with temperatures between 42–45°C (Caporaso et al., 2018; Ho et al., 2014).

4.5 Anthocyanin

The forest plots (Figure 2.d) show that the fermentation significantly decreased anthocyanins. Anthocyanins can be lost from cocoa beans through microbial activity and polyphenol oxidase enzyme activity. In the fermentation process, the anthocyanins in cocoa beans can be hydrolyzed faster by increasing the inoculum levels of starter microbes. The microbial inoculum and the anthocyanin released were higher than the natural and fermented inoculum. The literature review showed that anthocyanins decreased very quickly during fermentation.

In addition, it was found that during the fermentation process, anthocyanins can be hydrolyzed by glycosidases to anthocyanidins and produce cotyledon brightness (Oracz et al., 2015). The decrease in anthocyanin content is considered an index of quality degree during cocoa bean fermentation. Anthocyanins are compounds that give the characteristic purple color. The change in color of the seeds from purple to brown indicates successful fermentation (Brito et al., 2017; Ramos et al., 2014). Furthermore, during drying, polyphenols were reduced enzymatically catalyzed by polyphenol oxidase and diffused out of the seeds. The anthocyanin value has been used as an index representing the degree of fermentation of cocoa beans (Saunshia et al., 2018). The funnel plot graph reflects a publication bias (Figure 3.d).

4.6 Theobromine

Methylxanthines, such as theobromine, theophylline, and caffeine, are another group of bioactive compounds found in cocoa beans. Theobromine is the dominant alkaloid in cocoa, and its presence is influenced by the origin and maturity of cocoa and the fermentation process (Montagna et al., 2019). However, the presence of theobromine, along with other methylxanthine compounds and phenolic compounds, is considered to affect the characteristics of cocoa beans, namely the presence of sensory attributes such as bitter and astringent taste (Miguel et al., 2017).
Figure 2. e shows how the cocoa bean fermentation process negatively affects the concentration of the theobromine compounds. The content of methylxanthines (theobromine and caffeine) generally increases over the first few days of fermentation and then gradually decreases (Peláez et al., 2016). High theobromine content was found in samples and natural fermentation starters. This means that microbial metabolic activity can accelerate the decrease in theobromine. Another indication of a decrease in theobromine is its conversion to tannins or evaporation. The diffusion of these compounds caused the decrease in theobromine and caffeine in fermented cocoa cotyledons during fermentation. Meanwhile, if there is a large enough increase during fermentation, it is not in the cocoa beans but in the husks. The results of the funnel plot study reflect no publication bias (Figure 3. e). Based on cultivar variations, there was no significant difference in the theobromine content of each type of cocoa bean (Nazaruddin et al., 2006).

The values found for these methylxanthines can be discussed from two perspectives: first, from a technological aspect since these compounds, along with polyphenols, are responsible for the bitterness and astringency of the resulting chocolate (Miguel et al., 2017). Unlike caffeine, theobromine has no stimula-

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tory effect because the action of theobromine in the central nervous system is very mild or almost non-existent. However, it can act as a vasodilator, muscle relaxant, and diuretic as well as lower blood pressure (Peláez et al., 2016).

4.7 Caffeine.

Figure 2. f shows that the decrease in caffeine occurred significantly after fermentation; however, the value was relatively small, as was the decrease in theobromine and anthocyanins. Funnel plots show similar results to other studies, reflecting no publication bias (Figure 3. f). The results of several studies showed that higher caffeine content was found in fresh cocoa beans. Another study showed that the Trinitario variety had higher caffeine content than the Criollo and Forastero varieties. The decrease in alkaloid concentration was probably caused by compounds that diffuse out of the seeds when the seeds die.

The riper the cocoa pods, the higher the alkaloid concentration. Because these alkaloid compounds exist with phenolic compounds stored in the same storage cell (Dang et al., 2019), they can co-evolve with the phenolics during maturation.

Sensory changes due to changes in chemical compounds can be identified by the appearance of a brown color gradually; the bitter taste slowly disappears, further changing the texture. The characteristic astringent taste of raw cocoa is mainly due to the phenolic compounds. Raw cocoa with a low fermentation rate still has an unacceptable sensory attribute: a strong, unpleasant taste. The content of epicatechins, catechins, total polyphenols, and total flavonoids was influenced by the clones' fermentation process and type. The content is higher in number if the cocoa beans are not fermented (Nazaruddin et al., 2006).

From existing references, cocoa bean fermentation research appears to be done conventionally. However, research has now been developed for the laboratory scale. Small-scale research saves research raw materials by adjusting the fermentation process according to its natural conditions. This meta-analysis research has limitations: the variables used are uniform, and the variables considered in very few studies have only obtained meaningful results.

5. Conclusion

This study revealed that cocoa fermentation, in general, reduced epicatechin, catechin, total phenol levels, anthocyanins, theobromine, and caffeine levels. There was no difference in the results regarding the decrease in bioactive compounds based on cocoa pod cultivar variables, fermentation time, and the number of beans. However, the genotype of the cross of cocoa pods and the addition of starter cultures such as yeast found higher concentrations of bioactive compounds. It was found that these bioactive compounds generally decreased due to the metabolic activity of microorganisms and the increase in temperature during fermentation and other processing. Correlation with the fermentation index and cut test, changes in sensory attributes such as reduced bitterness and astringent taste, and changes in seed color from purple to brown. In general, epicatechin compounds and TPC underwent a more significant reduction than catechins, anthocyanins, theobromine, and caffeine compounds.

Conflict of Interest

All authors declare no conflict of interest. LC screened literature, tabulated data, analyzed, and wrote manuscripts. AR, NA, and WD discussed the results and commented on the manuscript.

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