JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

FROM GASTRIC TO COLON: HOW MILK MATRIX AFFECTS THE STABILITY AND RELEASE OF COCOA PHENOLICS

Dwi Indah Permata Sari[1,2], *Endang Prangdimurti**[1], *Dias Indrasti*

Address(es): Prof. Dr. Ir. Endang Prangdimurti, M.Si,
[1] IPB University (Bogor Agricultural University), Faculty of Agricultural Technology, Department of Food Science and Technology, Bogor, Indonesia, +622518622642.
[2] Sriwijaya University, Faculty of Agriculture, Department of Agricultural Technology, Palembang, Indonesia.

*Corresponding author: prangdimurti@apps.ipb.ac.id

https://doi.org/10.55251/jmbfs.12260

ARTICLE INFO

Received 14. 1. 2025
Revised 11. 11. 2025
Accepted 13. 11. 2025
Published 1. 12. 2025

Regular article

OPEN ACCESS

ABSTRACT

This study evaluated in vitro bioaccessibility of phenolic compounds in cocoa beverages formulated with different milk matrices, such as full cream milk (CF), skim milk (CS), and whey protein powder (CW), compared to a control without milk. Samples underwent sequential gastric (pepsin, pH 3) and intestinal (pancreatin and bile salts, pH 7) simulated digestion following INFOGEST protocol. Quantification of total phenolic and flavonoid was using spectrophotometric assays, and compound-specific profiling was achieved by UHPLC-HRMS. Results showed that although incorporation of dairy matrices led to an initial decline in total phenolic content of undigested sample, it enhanced phenolic retention during simulated digestion. Significant increase of phenolic bioaccessibility was observed in the intestinal phase, with CS formulation achieving the highest phenolic bioaccessibility index (129.71 ± 5.99 %). Total flavonoid content declined progressively during digestion in all samples. UHPLC-HRMS analysis identified 10 phenolic compounds in undigested CS sample, but only two of those phenolics still detected in intestinal phase. Hydrolysis of insoluble fractions that resulted from intestinal digestion released additional bound phenolics, suggesting potential colonic release and absorption. These findings highlight the role of milk matrices, particularly skim milk, in improving phenolic bioaccessibility of cocoa beverages. However, the use of a static in vitro digestion model represents a limitation, as it cannot fully replicate dynamic human gastrointestinal condition. Future studies should explore in vivo validation, apply dynamic digestion models, and assess the metabolic fate and biological activity of released phenolics following colonic fermentation.

Keywords: bioaccessibility, cocoa, *in-vitro* digestion, phenolic, UHPLC-HRMS

INTRODUCTION

In recent years, consumer interest in functional foods and beverages has surged, prompting innovations in ingredient formulations that enhance health benefits. Cocoa-based beverages, widely appreciated for their rich flavor and sensory appeal, offer more than just taste—they contain bioactive phenolic compounds associated with various health-promoting effects. Cocoa bean contain approximately 12-18% phenolic compounds, with 95% consisting of flavanol monomers (catechin and epicatechin) and procyanidin oligomers (dimers and decamers), which contribute to various bioactivities **(Melo *et al.*, 2020)**. Research by **Lee *et al.* (2003)** further highlights cocoa powder's high flavonoid content, demonstrating a greater antioxidant capacity compared to tea and red wine.

Despite its health benefits, cocoa beverages are rarely consumed in their pure form due to the inherent bitterness of cocoa beans. To enhance palatability, ingredients such as sugar and dairy products are commonly incorporated. However, while these additions improve taste and texture, they also influence phenolic bioaccessibility, potentially altering the absorption and functionality of cocoa's bioactive compounds. The interactions between phenolics and various food matrices play a critical role in determining their effectiveness, particularly in systems involving dairy proteins. **Shahidi & Pan (2021)** in their review stated that many factors can affect the absorption of phytochemical compounds during digestion due to complexity of the food components consumed. One example is the study by **(Qie *et al.*, 2022)**, which demonstrate that non-covalent interactions between milk proteins and coffee polyphenols can mitigate oxidative degradation of polyphenols during gastrointestinal digestion.

The binding of phenolic compounds to other food components is influenced by multiple factors, including molecular size, structure, polarity, and the specific configuration of bound or free phenolics. This interaction has been demonstrated in a study by **Kelemen *et al.* (2022)**, which examined phenolic content variations in a mixture of raspberry juice and brown rice protein. The findings revealed a decline in anthocyanin levels and total phenolics as the proportion of brown rice protein in the mixture increased. However, a review by **Günal-Köroğlu *et al.* (2023)** highlighted other studies reporting enhanced phenolic bioavailability due to the formation of protein-phenolic complexes. These contrasting results indicate that the impact of protein-phenolic interactions within a food matrix is not necessarily uniform across different food types. **Günal-Köroğlu *et al.* (2023)** further concluded that while numerous studies have modeled protein-phenolic interactions and assessed their effects in specific food matrices, the behavior of these interactions remains unpredictable in more complex systems.

Expanding on this topic, **Sari *et al.* (2025)** conducted an evaluation of phenolic bioaccessibility changes in pasteurized cocoa beverages formulated with varying milk matrices. Their findings indicated that, in general, the inclusion of milk enhanced phenolic bioaccessibility in pasteurized cocoa drinks. Further analysis was carried out in current study to examine variations in phenolic profile as individual compounds in cocoa beverages with added milk matrices, excluding pasteurization treatment. This allowed for the assessment of phenolic behavior resulting solely from milk addition. Understanding how phenolic compounds interact within different formulations is essential for optimizing the nutritional and functional properties of cocoa-based beverages. This study supports the development of scientifically informed functional beverages that maximize phenolic bioavailability and health potential.

MATERIAL AND METHODS

Chemicals and reagents

Cocoa powder used in this study were commercial products purchased from local market. Cocoa powder was non-alkalized and made from a blend of Trinitario and Forestero cocoa beans originating from Lampung, South Sulawesi and East Nusa Tenggara, Indonesia.

The powdered dairy products consist of full cream milk powder (F), skim milk powder (S), and whey protein powder (W). These products were commercially manufactured by Fonterra Cooperative Group (Auckland, New Zealand) and purchased online. The nutritional composition of each type of powdered dairy product is as follows: protein 24.23% (F), 32.94% (S), and 12.5% (W); fat 26.18% (F), 0.72% (S), and 0.70% (W); and carbohydrate 40.3% (F), 54.5% (S), and 76.1% (W).

Chemicals used for in vitro simulated digestion included pepsin enzyme (P7000), pancreatin enzyme (P7545), and bile salt (B8631) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), along with KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2 \cdot 6H_2O$, $(NH_4)_2CO_3$, HCl, sodium bicarbonate, and NaOH. Analytical-grade reagents such as Folin–Ciocalteu reagent (Merck), Na_2CO_3, gallic acid, disodium ethylenediaminetetraacetic acid (EDTA) salt, ethanol, $AlCl_3$, quercetin, acetonitrile and formic acid (UHPLC-analytical grade).

Preparation of cocoa beverages

Cocoa beverage samples were prepared following the method outlined by **Sari *et al.* (2025)** with slight modifications. Cocoa powder was mixed with full cream milk powder (CF), skim milk powder (CS), and whey protein powder (CW) in 1:1 (w/w) ratio. A total of 0.5 g of this mixture was dissolved in 50 mL of boiling distilled water (approximately 95 °C). A control sample (C) was prepared by dissolving only cocoa powder into the same volume of boiling water. All samples were cooled in room temperature, then freeze-dried using a freeze dryer (ALPHA 1-4 Ldplus, Martin Christ, Germany) for subsequent extraction.

In vitro gastrointestinal digestion

Cocoa beverages sample was prepared shortly prior to the simulated digestion. Gastric (SGF) and intestinal (SIF) juices were simulated refers to INFOGEST protocol as stated in **Minekus *et al.* (2014)**. Gastric mixture was prepared by adding 10 ml of gastric fluid stock solution to the 15 ml sample, along with HCl to adjust the pH to 3, followed by the addition of 7.5 µl CaCl2 and 2.4 ml pepsin (EC 3.4.23.1). The compositions of gastric fluid stock mixtures refers to **Minekus *et al.* (2014)**. To achieve a 50:50 (v/v) final volume ratio between sample and all gastric juices, 2.29 ml aquadest was added. The mixture was incubated at 37°C for 2 hours in shaking waterbath (LSB 045S, Daihan Labtech, Indonesia) and then soaked in ice bath to stop pepsin activity. Finally, 10 ml of gastric digestion was taken as a gastric phase fraction then centrifuged for 1 hour at 25°C and 3000 rpm (Eppendorf 5810 R). Supernatants were taken for characterization of dissolved phenolic compounds in the gastric phase.

The small intestine phase began by adding 11 ml SIF stock solution to the digestive mixture, adjusting the pH to 7 with NaOH (1 N), and adding 40 µl CaCl2 (0.3 M) and 5 ml pancreatin. The compositions of intestinal fluid stock mixtures refers to **Minekus *et al.* (2014)**. A total of 1.31 ml of aquadest was added to establish a 50:50 (v/v) final volume ratio between sample and all intestinal phase fluids. The mixture was incubated at 37°C for 2 hours, and then centrifuged for 1 hour at 25°C and 3000 rpm. Supernatants were taken for characterization of dissolved phenolic compounds in the intestinal phase. Aquadest was used as a negative control and standard solution such as gallic acid and quercetine was also subjected to this simulated digestion for considering the samples calculation.

Sample extraction

Method for extracting cocoa beverage samples before and after simulated digestion were based on the procedure described by **Sari *et al.* (2025)**. A 50% ethanol solution (v/v) was used as the solvent. For samples after digestion, the solvent was added in a 1:5 (v/v) ratio. For samples before digestion, the ratio was 1:20 (w/v). The samples were mixed for 30 minutes at 50°C using a magnetic stirrer (Wina Instruments 206, Indonesia), then centrifuged at 25°C and 3000 rpm for 15 minutes. The supernatant was stored at 4°C for 24 hours and centrifuged again under the same conditions. Finally, it was filtered using Whatman No. 41 filter paper.

Insoluble Phenolic Hydrolysis

Insoluble fraction of digested sample which had the lowest total phenolic bioaccessibility was hydrolyzed reffering to **Suwannachot *et al.* (2022)**. This solution is considered as an undigested fraction and has the potential to be carried to the colon so that the phenolic content was measured. Bound phenolics from insoluble fraction are hydrolyzed with 4M NaOH (containing 1% ascorbic acid and 10mM EDTA 1:2 v/v). Hydrolysis was carried out in a shaking waterbath for 4 hours at 100 rpm, 20ºC. Subsequently, the solution's pH is lowered to 2 by adding 6M HCl and then centrifuged for 5 minutes at 3000 rpm, 4°C. Finally, the phenolic fraction is extracted using the previously described extraction method.

Total phenolic content (TPC)

Analysis of undigested and digested samples was conducted following the method described by **Sari *et al.* (2025)**. To each sample extract, 1.5 ml of 10% Folin-Ciocalteu reagent was added, followed by 1.5 ml of distilled water and 2.5 ml of 20% Na_2CO_3 solution. The mixture was incubated in the dark for 30 minutes. Absorbance was measured at 750 nm using a UV-Vis spectrophotometer (Uv mini-1240 Shimadzu, Japan), with distilled water serving as a blank for absorbance measurements. The total phenolic content was determined using gallic acid standard curve and expressed as milligrams of gallic acid equivalent per gram of dry sample (mg GAE/g). Total phenolic content of insoluble fraction was also analyzed with this method.

Total flavonoid content (TFC)

Total flavonoid content was analyzed following the method outlined by **Sari *et al.* (2025)**. For both undigested and digested samples, 5 ml of 2% $AlCl_3$ solution was added and the mixture was left for 10 minutes in the dark. The absorbance was measured at 415 nm using a UV-Vis spectrophotometer (Uv mini-1240 Shimadzu, Japan), with distilled water used as a blank. Total flavonoid content was expressed in mg of quercetin equivalent per gram of sample (mg QE/g) based on the quercetin standard curve. Total flavonoid content of insoluble fraction was also analyzed with this method.

Phenolic profile analysis by UHPLC-HRMS

Phenolic profile analysis was conducted using UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS to identify the profile of phenolic compounds in samples which had highest and lowest total phenolic bioaccessibility index. Additionally, insoluble fraction of digested sample, which has the lowest total phenolic bioaccessibility index, was also analyzed. Analytical method followed the procedure outlined by **Sari *et al.* (2025) and Syarpin *et al.* (2023)**. Ethanol extract of sample was filtered through a 0.2µ PTFE membrane and injected into a UHPLC using a C18 column (100 x 2.1 mm, 1.5 m, ThermoScientific) at a flow rate of 0.2 ml/min. Mobile phase consisted of water + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B) with a gradient of 0-1 minutes (5% B), 1-25 minutes (5-95% B), 25-28 minutes (95% B), 28-30 minutes (5% B). Column temperature was set at 30ºC with injection volume of 2.0 µL. Analysis was carried out with molecular weights in interval 100-1500 m/z and negative ionization mode. Compound Discoverer 3.2, PubChem, Chemspider and mzCloud database was used to identify phenolic compounds from chromatogram.

Recovery and bioaccessibility index

Calculation of recovery index is intended to determine the amount of phenolic compounds in the sample that can be released after undergoing gastric digestion, while bioaccessibility index indicates its quantity released during the intestinal phase. Recovery (Eq. 1) and bioaccessibility (Eq. 2) index was calculated using following equation referred to **Sari *et al.* (2025)** :

$$RI\ (\%) = A/C \times 100$$

$$BI\ (\%) = B/C \times 100$$

where A is total phenolic or total flavonoid content of digested sample in gastric phase, B is total phenolic or total flavonoid content of digested sample in intestinal phase, and C is total phenolic or total flavonoid content of undigested sample

Statistical analysis

The experiment was conducted with two replicates for each treatment, and each replicate was analyzed in duplicate. Data were presented as the mean ± standard error and analyzed using analysis of variance (ANOVA) at a 95% confidence level. When significant differences were detected, Duncan's Multiple Range Test (DMRT) was applied for post-hoc comparison at the same confidence level. Statistical analyses were performed using IBM SPSS Statistics version 26 (SPSS 26.0, IBM Corporation, USA).

RESULTS AND DISCUSSION

Changes of TPC and TFC of cocoa beverage during simulated digestion

Table 1 presents total phenolic content of cocoa beverages subjected to simulated digestion phases. Statistical analysis showed that the different type of milk matrix significantly influenced phenolic levels across all phases ($p < 0.05$). According to Duncan's post hoc test, the cocoa beverage without milk addition (C) exhibited the highest mean phenolic content: 16.40 ± 0.8 mg GAE/g db (non-digested), 16.36 ± 0.09 (gastric), and 10.79 ± 0.34 (intestinal). These results suggest that milk components interact with cocoa phenolics, forming complexes that reduce measurable phenolic content throughout digestion.

Milk contains macronutrients such as proteins, fats, and lactose, which may interact with phenolic compounds, thereby forming derivatives and reducing their measurable concentration. Among the milk matrices tested, full cream milk (CFB) yielded significantly higher total phenolic values ($p < 0.05$) compared to other milk types: 7.70 ± 0.86 (non-digested), 8.86 ± 0.19 (gastric), and 5.57 ± 0.38 mg GAE/g db (intestinal). In contrast, the addition of whey protein (CW) to cocoa beverages resulted in the lowest values ($p < 0.05$): 4.94 ± 0.81, 7.48 ± 0.09, and 4.83 ± 0.87 mg GAE/g db, respectively. Total phenolic content of sample with skim milk addition (C) showed no significant difference when compared to CFB and CW.

Table 1 Total phenolic content, recovery index and bioaccessibility index of cocoa beverages without milk (C), with full cream milk (CF), skim milk (CS) and whey protein powder (CW) in each digestion phases

Sample	Total phenolic content (mg GAE/g db)			RI (%)	BI (%)
	Undigested	Gastric	Intestinal		
C	16,40 ± 0,8^{cB}	16,36 ± 0,09^{cC}	10,79 ± 0,34^{cA}	100,28 ± 5,50^{a}	66,29 ± 5,33^{a}
CF	7,70 ± 0,86^{bB}	8,86 ± 0,19^{bC}	5,57 ± 0,38^{bA}	118,54 ± 15,64^{a}	73,03 ± 3,15^{ab}
CS	4,78 ± 0,44^{abB}	8,69 ± 0,13^{abC}	6,15 ± 0,27^{abA}	184,44 ± 14,08^{a}	129,71 ± 5,99^{c}
CW	4,94 ± 0,81^{aB}	7,48 ± 0,09^{aC}	4,83 ± 0,87^{aA}	160,46 ± 27,98^{a}	97,13 ± 1,73^{b}
Gallic acid	15,99 ± 0,00^{c}	ns	9,29 ± 0,11^{c}	ns	58,09±0,67^{a}

Note: ns: not specified. Value is expressed in mean ± standard error (n = 2). Numbers followed by different lowercase letters in column or different capital letters in row showed significant differences based on Duncan's Multiple Range Test.

The increased phenolic content detected in the CFB sample is likely influenced by the naturally occurring phenolic compounds in full cream milk, including hydroxybenzoic acids, flavonoids, and anthocyanins originating from livestock feed **(Zeb, 2021)**. Studies have shown that fresh cow's milk contains significantly more phenolic compounds (420.34–490.72 mg GAE/100 mL) compared to whey powder (32.29–124.29 mg GAE/100 g), this variation is likely explained by the differing degrees of processing involved **(Sik *et al.*, 2023)**. Whey protein's lower phenolic measurements may also reflect its relatively low protein content, which influences the solubility of protein-phenolic complexes. When the protein-to-phenolic ratio is high, the resulting complexes tend to be more soluble, regardless of the specific molecular structure involved **(Shahidi & Dissanayaka, 2023)**. Conversely, lower protein content favors the formation of less soluble, precipitable complexes, which are less accessible to digestive enzymes and yield reduced measurable phenolic concentrations. Furthermore, the type of protein in the milk matrix affects its affinity to phenolic compounds. Casein, characterized by a high proportion of proline residues, has a loosely packed structure, making it more susceptible to enzymatic hydrolysis **(Ng-Kwai-Hang, 2011)**. Unlike casein-containing matrices such as full cream and skim milk, whey-based samples lack of casein, making the phenolic-protein complexes formed therein more stable and resistant to enzymatic breakdown during digestion. Compared to the findings of **Sari *et al.* (2025)**, the addition of whey to pasteurized cocoa beverages resulted in higher total phenolic content than other sample with milk matrix in the gastric phase. In contrast, in this study, the addition of whey to unpasteurized cocoa beverages produced the lowest phenolic content across than other sample in all digestive phases. These findings suggest that differences in thermal processing influence phenolic stability at specific stages of digestion.

The results in Table 1 also show that the gastric phase had the highest average total phenolic content and was significantly different, with values of 8.86 ± 0.19 mg GAE/g dw (CFB), 8.69 ± 0.13 mg GAE/g dw (C), and 7.48 ± 0.09 mg GAE/g dw (CW). In the C sample, the highest value was found in the undigested phase. Overall, the intestinal phase had the lowest phenolic content and was significantly different ($p < 0.05$), except in the C sample, where the lowest value occurred in the undigested phase. **Sari *et al.* (2025)** also reported that phenolic and flavonoid contents in pasteurized cocoa beverages were lowest in the intestinal phase. Several studies support that intestinal pH may oxidize phenolic compounds and promote complex formation with proteins, including digestive enzymes, thereby reducing measurable phenolic levels **(Qie *et al.*, 2021; Sari *et al.*, 2025; Tosif *et al.*, 2021)**.

Table 1 presents the recovery index (RI) and bioaccessibility index (BI) of total phenolics in cocoa beverages. These results showed that phenolic recovery index (RI) did not differ significantly across samples, while bioaccessibility index differed significantly ($p < 0.05$). The CS sample exhibited the highest BI (129.71 ± 5.99%), whereas the non-milk C sample had the lowest (66.29 ± 5.33%). Although in general the total phenolics of C were higher in each digestive phase, its intestinal retention was comparatively lower, indicating milk's role in stabilizing phenolics of cocoa beverages. This effect is attributed to casein's open structure and proline-rich regions, which facilitate non-covalent bonding with phenolics, protecting them during gastric digestion and enhancing their release in the intestinal phase **(Hamzalioglu *et al.*, 2023; Ng-Kwai-Hang, 2011)**. Study by **Sari *et al.* (2025)** presented contrasting results, with pasteurized cocoa beverages exhibiting bioaccessibility index ranging from 79.91% to 98.05% and showing no significant differences among the various milk matrices. In contrast, the non-pasteurized cocoa beverages in the present study demonstrated significantly different phenolic bioaccessibility values, ranging from 66.29% to 129.71%. Furthermore, **Sari *et al.* (2025)** reported that the inclusion of whey protein in pasteurized cocoa resulted in the highest and significantly different phenolic recovery index, a result that contrasts with the findings of the present study. These differences may stem from pasteurization-induced matrix disruption that facilitates gastric-phase phenolic release **(Lorenzo *et al.*, 2019)**, while non-pasteurized cocoa retains protein integrity, enabling more dynamic milk-phenolic interactions and contributing to the variation in phenolic bioaccessibility.Phenolic bioaccessibility index of gallic acid standard solution also shown in Table 1. This solution exhibited the lowest bioaccessibility value at 58.09 ± 0.67%, which was significantly different from CS and CW samples ($p < 0.05$). This indicates that isolated phenolics, represented by gallic acid, was less stable in gastrointestinal tract As the standard solution lacks a food matrix that facilitates additional phenolic release during digestion, its bioaccessibility is solely determined by the compound's intrinsic stability within the digestive system.

Table 2 Total flavonoid content, recovery index and bioaccessibility index of cocoa beverages without milk (C), with full cream milk (CF), skim milk (CS) and whey protein powder (CW) in each digestion phases

Sample	Total Flavonoid Content (mg QE/g db)			RI (%)	BI (%)
	Undigested	Gastric	Intestinal		
C	4,35 ± 0,45^{aC}	3,78 ± 0,02^{aB}	0,40 ± 0,01^{aA}	88,67 ± 9,53^{b}	9,40 ± 0,83^{b}
CF	7,33 ± 1,68^{aC}	1,64 ± 0,25^{aB}	0,22 ± 0,02^{aA}	26,80 ± 9,52^{a}	3,20 ± 0,46^{a}
CS	3,84 ± 0,73^{aC}	1,23 ± 0,18^{aB}	0,20 ± 0,00^{aA}	36,59 ± 11,77^{a}	5,60 ± 1,09^{ab}
CW	3,32 ± 0,17^{aC}	1,31 ± 0,10^{aB}	0,20 ± 0,01^{aA}	39,18 ± 0,99^{ab}	6,01 ± 0,56^{ab}
Quercetin	10,03 ± 0,00^{b}	ns	1,30 ± 0,03^{b}	ns	12,92 ± 0,31^{c}

Note: ns: not specified. Value is expressed in mean ± standard error (n = 2). Numbers followed by different lowercase letters in column or different capital letters in row showed significant differences based on Duncan's Multiple Range Test

Table 2 present total flavonoid content in cocoa beverage samples. The type of milk matrix showed no significant effect on total flavonoid levels, while each digestion phase differed significantly ($p < 0.05$). These findings indicate that digestive conditions, rather than milk composition, primarily influence flavonoid accessibility. All samples exhibited the highest flavonoid content in undigested phase: 4.35 ± 0.45 mg QE/g db (C), 7.33 ± 1.68 mg QE/g db (CFB), 3.84 ± 0.73 mg QE/g db (CB), and 3.32 ± 0.17 mg QE/g db (CW). Unlike total phenolics, which increased during gastric phase, flavonoid levels declined progressively from gastric to intestinal phases. This aligns with **Sari *et al.* (2025)**, who reported consistent reductions of flavonoid across digestion phases. The rise in total phenolic during gastric phase without a parallel increase in flavonoids suggests that non-flavonoid phenolics may dominate intestinal stability. **Gonzales *et al.* (2015)** noted that interactions between flavonoid and other macromolecule, particularly covalent bonding with carbohydrates, could restrict its bioaccessibility.

Quercetin standard solution was also subjected to simulated digestion. As a relatively stable and commonly used flavonoid reference in plant extract analyses **(Indiarto *et al.*, 2022, 2024)**, it exhibited a significantly higher bioaccessibility (12.92 ± 0.31%) than food-matrix samples. Unlike gallic acid, quercetin demonstrated greater digestive stability, highlighting the limitations of single-compound representation. Each phenolic compound, including flavonoids with varying structures and polymerization degrees, exhibits distinct digestive behavior. **van de Langerijt *et al.* (2023)** observed that polar phenolics tend to interact with milk proteins via hydrogen bonds, while non-polar ones form stronger hydrophobic interactions that are more resistant to release.

Phenolic profile of cocoa beverages before and after digestion

Identification of phenolic compounds by chromatography was carried out on samples that had the highest and lowest total phenolic bioaccessibility index. Table

3 presents the confirmed phenolic compounds found in the analyzed samples. Phenolic compounds contained in fresh cocoa beans are typically in the form of glycosides **(Żyżelewicz *et al.*, 2016)**. The absence of phenolic glycoside in this analysis may be attributed to the extensive processing of cocoa beans into powder and beverages, involving fermentation, drying, roasting, grinding, and brewing. Preparation for analysis also involves temperatures of 40-50ºC. Although the specific temperature range that is able to break the glycoside bonds of phenolic compound is unknown, intense heating and fermentation processes have been reported to break the glycosidic bonds between phenolic compound and sugars, converting them to produce aglycones, especially in cocoa powder **(Aaby & Amundsen, 2023; Leonard *et al.*, 2021; Rohn *et al.*, 2007)**.

Based on the results in Table 3, protocatechuic acid emerged as the phenolic compound with highest peak area in undigested sample. The presence of protocatechuic acid in cocoa powder has also been reported in previous studies, one of them was by Baranowska *et al.* **(Baranowska *et al.*, 2020)**. Protocatechuic acid is a phenolic compound that belongs to the group of phenolic acids and is one of the derivatives of water-soluble benzoic acid **(Zhang *et al.*, 2021)**. This compound associated with various biological activities such as anti-inflammatory, anti-diabetic, antiviral, antioxidant, and anticancer properties **(Cadena-Iñiguez *et al.*, 2024; Krzysztoforska *et al.*, 2019; Song *et al.*, 2020; Tanaka *et al.*, 2011)**.

Most of phenolic compounds identified in undigested C were also found in undigested CS sample, such as catechins, epicatechins, 2,5-dihydroxybenzaldehyde, N-caffeoyl-l-aspartic acid, N-coumaroyl-l-aspartic acid, 4-caffeoylshikimic acid, and 5-caffeoylshikimic acid. Meanwhile, complex compounds with $C_{37}H_{36}N_2O_{11}$ and $C_{14}H_{15}O_7P$ formula were only identified in undigested CS sample. Other compounds such as 4-hydroxybenzaldehyde, vanillin, 5,5'-dehydrodivanylate, and quinic acid were identified in undigested C sample but not in undigested CS sample. Interaction between protein in skim milk and phenolics in cocoa powder can lead to the formation of new complexes, reducing the number of free phenolic compounds and their antioxidant activity **(Gallo *et al.*, 2013)**. Both stable covalent and non-covalent bonds can be formed from the interaction between cocoa phenolic with whey protein, β-lactoglobulin and casein in skim milk **(Gallo *et al.*, 2013)**.

The presence of phenolic compounds identified in undigested C and CS samples has also been reported in several previous studies, regardless of the method of analysis used. Catechin and epicatechin are the most common flavonoid group found in cocoa beans **(Melo *et al.*, 2020)**. Simple phenolic compounds such as 2,5-dihydroxybenzaldehyde, 4-hydroxybenzaldehyde. Vanillin and 5,5'-dehydrodivanillate were also identified in cocoa bean samples by Barnaba *et al.* **(Barnaba *et al.*, 2017)**. Meanwhile, phenolic metabolite compounds such as N-caffeoyl-l-aspartic acid and N-coumaroyl-l-aspartic acid were identified in cocoa bean and cocoa powder samples in a study by Febrianto and Zhu and Greño *et al*. **(Febrianto & Zhu, 2021; Greño *et al.*, 2022)**. Caffeoylshikimic acid is an ester of caffeic acid and shikimic acid **(Angelino *et al.*, 2018)**. Caffeic acid is a group of phenolic acids that are also reported to be contained in cocoa products **(Flores, 2019)**. Similarly, quinic acid has been found in cocoa beans as chlorogenic acid (caffeoyl-quinic acid), an ester of caffeic acid and quinic acid, as reported in several studies **(Bertazzo *et al.*, 2013; Caprioli *et al.*, 2016; Ramos-Escudero *et al.*, 2021)**.

Table 3 Phenolics profile of cocoa beverages with skim milk and without milk

Compound	Formula	MW	MS1 [M-H]-	Peak area (%)				
				C		CS		HC
				Undigested	Intestinal	Undigested	Intestinal	
Protocatechuic acid	C7 H6 O4	154.03	153.02	2.74	nd	1.18	nd	0.55
2,5-dihydroxybenzaldehyde	C7 H6 O3	138.03	137.02	1.77	nd	1.17	nd	nd
Catechin	C15 H14 O6	290.08	289.07	1.70	nd	1.10	nd	0.30
N-caffeoyl-l-aspartic acid	C13 H13 N O7	295.07	294.06	1.16	nd	0.53	nd	nd
Epicatechin	C15 H14 O6	290.08	289.07	1.02	nd	0.91	nd	nd
N-coumaroyl-l-aspartic acid	C13 H13 N O6	279.07	278.07	0.84	nd	0.41	nd	nd
4-hydroxybenzaldehyde	C7 H6 O2	122.04	121.03	0.24	nd	nd	nd	nd
4-caffeoylshikimic acid	C16 H16 O8	336.08	335.08	0.24	nd	0.13	nd	nd
Vanillin	C8 H8 O3	152.05	151.04	0.23	nd	nd	nd	nd
5-caffeoylshikimic acid	C16 H16 O8	336.08	335.08	0.22	nd	0.17	nd	nd
5,5'-dehydrodivanillate	C16 H14 O8	334.07	333.06	0.20	nd	nd	nd	nd
Quinic acid	C7 H12 O6	192.06	191.06	0.19	nd	nd	nd	nd
Salicyloyl phytosphingosine	C25 H43 N O5	437.31	436.31	nd	0.17	nd	0.17	nd
3-Hydroxybenzoic acid	C7 H6 O3	138.03	137.02	nd	0.10	nd	0.10	1.68
4,7 dihydroxyisoflavone	C15 H10 O4	254.06	253.05	nd	0.10	nd	nd	nd
5,9-Dihydroxy-2-(2-hydroxy-2-propanyl)-1-(1-hydroxy-3,5,6-trimethoxy-10-methyl-9-oxo-9,10-dihydro-2-acridinyl)-10-methoxy-11-methyl-1,11-dihydrofuro[2,3-c]acridin-6(2H)-one	C37 H36 N2 O11	684.23	683.23	nd	nd	0.75	nd	nd
3-Hydroxy-2-[1-hydroxy-2-(4-hydroxyphenyl)ethyl] phenyl] dihydrogen phosphate	C14 H15 O7 P	326.06	325.05	nd	nd	0.12	nd	nd
2,3-Dihydroxybenzoic acid	C7 H6 O4	154.03	153.02	nd	nd	nd	nd	0.32
Eugenitin	C12 H12 O4	220.07	219.07	nd	nd	nd	nd	0.29
3,5-Dihydroxybenzoic acid	C7 H6 O4	154.03	153.02	nd	nd	nd	nd	0.18
Gingerdiol 3,5-diacetate	C19 H28 O6	352.19	351.18	nd	nd	nd	nd	0.17

Note: MW: molecular weight; nd: not detected, C: cocoa beverages without milk, CS: cocoa beverages with skim milk powder, HC: hydrolyzed insoluble fraction of C

The number of phenolic compounds identified in both samples in intestinal phase decreased significantly (Table 3). All phenolics identified in undigested phase were not identified in the sample after intestinal phase. This could be due to the breakdown of phenolic compounds in acidic stomach and alkaline intestine environment, as well as the presence of bile salts, which can cause the precipitation of phenolic compounds **(Ozkan *et al.*, 2023)**. In contrast, phenolic compounds identified in intestinal phase sample were new phenolic compounds that were not present in undigested phase, such as salicyloyl phytosphingosine and 3-

hydroxybenzoic acid, as well as 4,7 dihydroxyisoflavones that were only identified in C sample. Salicyloyl phytosphingosine compounds can be formed from the reaction between salicylic acid or other carboxylic acid derivatives with lipid such as phytosphingosine, as occurs in the formation of salicyloyl chitosan compounds reported by He *et al.* **(He *et al.*, 2011)**. The presence of salicylic acid in cocoa beans is rarely reported in studies, but Herrera-Rocha *et al.* **(Herrera-Rocha *et al.*, 2021)** identified the presence of salicylic acid in cocoa beans as a flavor component. Although salicylic acid was not detected in undigested sample, the release of this compound from food matrix may occur during digestion and then react with lipids to form new complexes. The same thing may occur to 3-Hydroxybenzoic acid and 4,7 dihydroxyisoflavones, which were not previously detected in undigested sample.

Cocoa beverages without milk addition (C) had the lowest phenolic bioaccessibility index compared to other samples, which was 66,29% (Table 1). Digested supernatants from this sample were analyzed as soluble fractions that are considered as biologically accessible. Meanwhile, the separated insoluble factions from the separation underwent hydrolysis in both acidic and alkaline conditions based on Suwannachot *et al.* **(Suwannachot *et al.*, 2022)**. The insoluble fraction contained complex phenolic compounds that covalently bound to the cell walls of other components in the food matrix, predominantly on macromolecules such as carbohydrates, proteins, pectins and others **(Suwannachot *et al.*, 2022)**. Colonic bacteria may release several phenolic compounds from this fraction to facilitate biological activities within digestive tract **(da Costa Pinaffi *et al.*, 2020)**. Total phenolic content measured in the insoluble fraction after hydrolysis was 2,128 mg GAE/g of cocoa powder, while flavonoid content was 1,068 mg QE/g of cocoa powder. Although phenolic content of this fraction remained lower compared to its soluble fractions (supernatant), the flavonoid content was higher (Figure 2).

Chromatographic analysis identified seven distinct phenolic compounds in this fraction, including protocatechuic acid, catechins, and 3-hydroxybenzoic acid, alongside four newly identified phenolic compounds which absent in other analyzed samples such as 2,3-dihydroxybenzoic acid, eugenitin, 3,5-dihydroxybenzoic acid, and gingerdiol 3,5-diacetate. The presence of new phenolic compounds in this fraction might be as a result of hydrolysis for 4 hours in alkaline conditions, allowing the release of phenolic compounds that were previously still in bonded form **(Suwannachot *et al.*, 2022)**. Isomers of hydroxybenzoic acid compounds were also detected in undigested sample but was not detected in intestinal phase sample. This indicate their binding during digestion, remaining undetected until intestinal phase then released after hydrolisis of its insoluble fractions. Eugenitin is a phenolic compound with antifungal activity, while Gingerdiol 3,5-diacetate has various health benefits such as antioxidant, anti-inflammatory, antimicrobial, and anticancer properties **(Mao *et al.*, 2019; Mittal *et al.*, 2014; Nishidono *et al.*, 2018; Vicidomini *et al.*, 2021)**. The presence of these compound in insoluble fraction sample potentially resulting from diverse reactions and interactions during hydrolysis process, gastric and intestinal phase digestion.

Results of this study confirm that hydrolysis of insoluble fractions in samples from gastrointestinal digestion can release some insoluble-bound phenolic compounds, indicating the potential re-metabolism of phenolic compounds in colon by microflora. This process facilitates the release of phenolic compounds into simpler forms with low molecular weights, enhancing their bioavailability and absorption in the colon **(Chen *et al.*, 2024)**.

CONCLUSION

Current study demonstrates that adding milk matrices to cocoa beverages significantly enhance the simulated gastrointestinal bioaccessibility of phenolics, despite an initial decline in total measured phenolics. Adding skim milk powder to cocoa beverages produced the highest phenolic bioaccessibility index among other formulations, suggesting suggesting it is the preferred matrix for maximizing phenolics accessibility. Chromatographic analysis indicated the presence of 10 confirmed phenolic compounds in undigested phase of CS, but only 2 were identified in its intestinal phase. This finding showed that insoluble fraction hydrolysis were able to release bound phenolic compounds that were previously unidentified in intestinal phase, implies a potential for phenolic release in the colon. However, as the findings were based on in vitro models, it should be cautiously interpreted due to inherent limitations in replicating the complexity of human gastrointestinal physiology. Future studies may implement dynamic and multi-compartment digestion systems or in vivo validation to confirm intestinal and colonic phenolic release and absorption. Colonic fermentation with human fecal inocula is also recommended to simulate microbial biotransformation of insoluble-bound phenolics and asses resulting bioactivities.

Acknowledgments: This work was supported by the Ministry of Finance, Republic of Indonesia in the form of LPDP scholarship 2023-2024.

REFERENCES

Aaby, K., & Amundsen, M. R. (2023). The stability of phenolic compounds and the colour of lingonberry juice with the addition of different sweeteners during thermal treatment and storage. *Heliyon*, *9*(5), e15959. https://doi.org/10.1016/j.heliyon.2023.e15959

Angelino, D., Tassotti, M., Brighenti, F., Del Rio, D., & Mena, P. (2018). Niacin, alkaloids and (poly)phenolic compounds in the most widespread Italian capsule-brewed coffees. *Scientific Reports*, *8*(1), 1–11. https://doi.org/10.1038/s41598-018-36291-6

Baranowska, M., Suliborska, K., Todorovic, V., Kusznierewicz, B., Chrzanowski, W., Sobajic, S., & Bartoszek, A. (2020). Interactions between bioactive components determine antioxidant, cytotoxic and nutrigenomic activity of cocoa powder extract. *Free Radical Biology and Medicine*, *154*(April), 48–61. https://doi.org/10.1016/j.freeradbiomed.2020.04.022

Barnaba, C., Nardin, T., Pierotti, A., Malacarne, M., & Larcher, R. (2017). Targeted and untargeted characterisation of free and glycosylated simple phenols in cocoa beans using high resolution-tandem mass spectrometry (Q-Orbitrap). *Journal of Chromatography A*, *1480*, 41–49. https://doi.org/10.1016/j.chroma.2016.12.022

Bertazzo, A., Comai, S., Mangiarini, F., & Chen, S. (2013). Composition of Cacao Beans. In *Chocolate in Health and Nutrition* (pp. 1–553). https://doi.org/10.1007/978-1-61779-803-0

Cadena-Iñiguez, J., Santiago-Osorio, E., Sánchez-Flores, N., Salazar-Aguilar, S., Soto-Hernández, R. M., Riviello-Flores, M. de la L., Macías-Zaragoza, V. M., & Aguiñiga-Sánchez, I. (2024). The Cancer-Protective Potential of Protocatechuic Acid: A Narrative Review. *Molecules*, *29*(7). https://doi.org/10.3390/molecules29071439

Caprioli, G., Fiorini, D., Maggi, F., Nicoletti, M., Ricciutelli, M., Toniolo, C., Prosper, B., Vittori, S., & Sagratini, G. (2016). Nutritional composition, bioactive compounds and volatile profile of cocoa beans from different regions of Cameroon. *International Journal of Food Sciences and Nutrition*, *67*(4), 422–430. https://doi.org/10.3109/09637486.2016.1170769

Chen, J., Chen, X., Zhang, Y., Feng, Z., Zhu, K., Xu, F., & Gu, C. (2024). Bioactivity and influence on colonic microbiota of polyphenols from noni (Morinda citrifolia L.) fruit under simulated gastrointestinal digestion. *Food Chemistry: X*, *21*(June 2023), 101076. https://doi.org/10.1016/j.fochx.2023.101076

da Costa Pinaffi, A. C., Sampaio, G. R., Soares, M. J., Shahidi, F., de Camargo, A. C., & Torres, E. A. F. S. (2020). Insoluble-bound polyphenols released from guarana powder: Inhibition of alpha-glucosidase and proanthocyanidin profile. *Molecules*, *25*(3). https://doi.org/10.3390/molecules25030679

Fayeulle, N., Preys, S., Roger, J. M., Boulanger, R., Hue, C., Cheynier, V., & Sommerer, N. (2020). Multiblock analysis to relate polyphenol targeted mass spectrometry and sensory properties of chocolates and cocoa beans. *Metabolites*, *10*(8), 1–13. https://doi.org/10.3390/metabo10080311

Febrianto, N. A., & Zhu, F. (2021). Composition of methylxanthines, polyphenols, key odorant volatiles and minerals in 22 cocoa beans obtained from different geographic origins. *Lwt*, *153*(August 2021), 112395. https://doi.org/10.1016/j.lwt.2021.112395

Flores, M. E. J. (2019). Cocoa flavanols: Natural agents with attenuating effects on metabolic syndrome risk factors. *Nutrients*, *11*(4). https://doi.org/10.3390/nu11040751

Gallo, M., Vinci, G., Graziani, G., De Simone, C., & Ferranti, P. (2013). The interaction of cocoa polyphenols with milk proteins studied by proteomic techniques. *Food Research International*, *54*(1), 406–415. https://doi.org/10.1016/j.foodres.2013.07.011

Gonzales, G. B., Smagghe, G., Grootaert, C., Zotti, M., Raes, K., & Camp, J. Van. (2015). Flavonoid interactions during digestion, absorption, distribution and metabolism: A sequential structure-activity/property relationship-based approach in the study of bioavailability and bioactivity. *Drug Metabolism Reviews*, *47*(2), 175–190. https://doi.org/10.3109/03602532.2014.1003649

Greño, M., Herrero, M., Cifuentes, A., Marina, M. L., & Castro-Puyana, M. (2022). Assessment of cocoa powder changes during the alkalization process using untargeted metabolomics. *Lwt*, *172*(August). https://doi.org/10.1016/j.lwt.2022.114207

Günal-Köroğlu, D., Lorenzo, J. M., & Capanoglu, E. (2023). Plant-Based Protein-Phenolic Interactions: Effect on different matrices and in vitro gastrointestinal digestion. *Food Research International*, *173*(July). https://doi.org/10.1016/j.foodres.2023.113269

Hamzalioglu, A., Tagliamonte, S., Gökmen, V., & Vitaglione, P. (2023). Casein-phenol interactions occur during digestion and affect bioactive peptide and phenol bioaccessibility. *Food and Function*, *14*(20), 9457–9469. https://doi.org/10.1039/d3fo02630b

He, G., Chen, X., Yin, Y., Zheng, H., Xiong, X., & Du, Y. (2011). Synthesis, characterization and antibacterial activity of salicyloyl chitosan. *Carbohydrate Polymers*, *83*(3), 1274–1278. https://doi.org/10.1016/j.carbpol.2010.09.034

Herrera-Rocha, F., Cala, M. P., Aguirre Mejía, J. L., Rodríguez-López, C. M., Chica, M. J., Olarte, H. H., Fernández-Niño, M., & Gonzalez Barrios, A. F. (2021). Dissecting fine-flavor cocoa bean fermentation through metabolomics analysis to break down the current metabolic paradigm. *Scientific Reports*, *11*(1), 1–18. https://doi.org/10.1038/s41598-021-01427-8

Indiarto, R., Herwanto, J. A., Filianty, F., Lembong, E., Subroto, E., & Muhammad, D. R. A. (2024). Total phenolic and flavonoid content, antioxidant activity and characteristics of a chocolate beverage incorporated with encapsulated

clove bud extract. *CYTA - Journal of Food*, *22*(1). https://doi.org/10.1080/19476337.2024.2329144
Indiarto, R., Rahimah, S., Subroto, E., Putri, N. A. G., & Pangawikan, A. D. (2022). Antioxidant activity and characteristics of a cocoa drink formulated with encapsulated green coffee extract. *International Journal of Food Properties*, *25*(1), 2477–2494. https://doi.org/10.1080/10942912.2022.2144883
Kelemen, V., Pichler, A., Ivić, I., Buljeta, I., Šimunović, J., & Kopjar, M. (2022). Brown Rice Proteins as Delivery System of Phenolic and Volatile Compounds of Raspberry Juice. *International Journal of Food Science and Technology*, *57*(4), 1866–1874. https://doi.org/10.1111/ijfs.15023
Krzysztoforska, K., Mirowska-Guzel, D., & Widy-Tyszkiewicz, E. (2019). Pharmacological effects of protocatechuic acid and its therapeutic potential in neurodegenerative diseases: Review on the basis of in vitro and in vivo studies in rodents and humans. *Nutritional Neuroscience*, *22*(2), 72–82. https://doi.org/10.1080/1028415X.2017.1354543
Lee, K. W., Kim, Y. J., Lee, H. J., & Lee, C. Y. (2003). Cocoa Has More Phenolic Phytochemicals and a Higher Antioxidant Capacity than Teas and Red Wine. *Journal of Agricultural and Food Chemistry*, *51*(25), 7292–7295. https://doi.org/10.1021/jf0344385
Leonard, W., Zhang, P., Ying, D., Adhikari, B., & Fang, Z. (2021). Fermentation transforms the phenolic profiles and bioactivities of plant-based foods. *Biotechnology Advances*, *49*(April), 107763. https://doi.org/10.1016/j.biotechadv.2021.107763
Lorenzo, J. M., Estévez, M., Barba, F. J., Thirumdas, R., Franco, D., & Munekata, P. E. S. (2019). Polyphenols: Bioaccessibility and bioavailability of bioactive components. In *Innovative Thermal and Non-Thermal Processing, Bioaccessibility and Bioavailability of Nutrients and Bioactive Compounds*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-814174-8.00011-1
Mao, Q. Q., Xu, X. Y., Cao, S. Y., Gan, R. Y., Corke, H., Beta, T., & Li, H. Bin. (2019). Bioactive compounds and bioactivities of ginger (zingiber officinale roscoe). *Foods*, *8*(6), 1–21. https://doi.org/10.3390/foods8060185
Melo, T. S., Pires, T. C., Engelmann, J. V. P., Monteiro, A. L. O., Maciel, L. F., & Bispo, E. da S. (2020). Evaluation of the content of bioactive compounds in cocoa beans during the fermentation process. *Journal of Food Science and Technology*, *58*(5), 1947–1957. https://doi.org/10.1007/s13197-020-04706-w
Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., MacIerzanka, A., MacKie, A., … Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food-an international consensus. *Food and Function*, *5*(6), 1113–1124. https://doi.org/10.1039/c3fo60702j
Mittal, M., Gupta, N., Parashar, P., Mehra, V., & Khatri, M. (2014). Phytochemical evaluation and pharmacological activity of syzygium aromaticum: A comprehensive review. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(8), 67–72.
Ng-Kwai-Hang, K. (2011). Milk Proteins: Heterogeneity, Fractionation, and Isolation. In *Encyclopedia of Dairy Sciences* (Second Ed., pp. 751–764). Elsevier Academic Press.
Nishidono, Y., Saifudin, A., Nishizawa, M., Fujita, T., Nakamoto, M., & Tanaka, K. (2018). Identification of the chemical constituents in Ginger (Zingiber officinale) responsible for thermogenesis. *Natural Product Communications*, *13*(7), 869–873. https://doi.org/10.1177/1934578x1801300722
Ozkan, G., Sakarya, F. B., Tas, D., Yurt, B., Ercisli, S., & Capanoglu, E. (2023). Effect of In Vitro Digestion on the Phenolic Content of Herbs Collected from Eastern Anatolia. *ACS Omega*, *8*(14), 12730–12738. https://doi.org/10.1021/acsomega.2c07881
Qie, X., Cheng, Y., Chen, Y., Zeng, M., Wang, Z., Qin, F., Chen, J., Li, W., & He, Z. (2022). In vitro phenolic bioaccessibility of coffee beverages with milk and soy subjected to thermal treatment and protein–phenolic interactions. *Food Chemistry*, *375*(March 2021), 131644. https://doi.org/10.1016/j.foodchem.2021.131644
Qie, X., Wu, Y., Chen, Y., Liu, C., Zeng, M., Qin, F., Wang, Z., Chen, J., & He, Z. (2021). Competitive interactions among tea catechins, proteins, and digestive enzymes modulate in vitro protein digestibility, catechin bioaccessibility, and antioxidant activity of milk tea beverage model systems. *Food Research International*, *140*(July 2020), 110050. https://doi.org/10.1016/j.foodres.2020.110050
Ramos-Escudero, F., Casimiro-Gonzales, S., Fernández-Prior, Á., Cancino Chávez, K., Gómez-Mendoza, J., Fuente-Carmelino, L. de la, & Muñoz, A. M. (2021). Colour, fatty acids, bioactive compounds, and total antioxidant capacity in commercial cocoa beans (Theobroma cacao L.). *Lwt*, *147*(January). https://doi.org/10.1016/j.lwt.2021.111629
Rohn, S., Buchner, N., Driemel, G., Rauser, M., & Kroh, L. W. (2007). Thermal degradation of onion quercetin glucosides under roasting conditions. *Journal of Agricultural and Food Chemistry*, *55*(4), 1568–1573. https://doi.org/10.1021/jf063221i
Sari, D. I. P., Prangdimurti, E., & Indrasti, D. (2025). Bioaksesibilitas Senyawa Fenolik Minuman Kakao Pasteurisasi dengan Penambahan Matriks Susu. *Jurnal Teknologi Dan Industri Pangan*, *36*(1), 28–39. https://doi.org/10.6066/jtip.2025.36.1.28
Shahidi, F., & Dissanayaka, C. (2023). Phenolic-protein interactions: insight from in-silico analyses – a review. *Food Production, Processing and Nutrition*, *5*(1). https://doi.org/10.1186/s43014-022-00121-0
Shahidi, F., & Pan, Y. (2021). Influence of food matrix and food processing on the chemical interaction and bioaccessibility of dietary phytochemicals: A review. *Critical Reviews in Food Science and Nutrition*, *62*(23), 6421–6445. https://doi.org/10.1080/10408398.2021.1901650
Sik, B., Buzás, H., Kapcsándi, V., Lakatos, E., Daróczi, F., & Székelyhidi, R. (2023). Antioxidant and polyphenol content of different milk and dairy products. *Journal of King Saud University - Science*, *35*(7), 102839. https://doi.org/10.1016/j.jksus.2023.102839
Song, J., He, Y., Luo, C., Feng, B., Ran, F., Xu, H., Ci, Z., Xu, R., Han, L., & Zhang, D. (2020). New progress in the pharmacology of protocatechuic acid: A compound ingested in daily foods and herbs frequently and heavily. *Pharmacological Research*, *161*(July), 105109. https://doi.org/10.1016/j.phrs.2020.105109
Suwannachot, J., Reginio, F. C., Hamauzu, Y., & Ogawa, Y. (2022). Assessment of free, esterified, and insoluble-bound phenolics of green and red perilla leaves and changes during simulated gastrointestinal digestion. *Food Chemistry Advances*, *1*(January), 100018. https://doi.org/10.1016/j.focha.2022.100018
Syarpin, Permatasari, S., & Pujianto, D. A. (2023). Analysis of phytochemical constituents and antioxidant activity from the fractions of Luvunga sarmentosa root extract using LCMS/MS. *Biodiversitas*, *24*(2), 733–740. https://doi.org/10.13057/biodiv/d240208
Tanaka, T., Tanaka, T., & Tanaka, M. (2011). Potential Cancer Chemopreventive Activity of Protocatechuic Acid. *Journal of Experimental and Clinical Medicine*, *3*(1), 27–33. https://doi.org/10.1016/j.jecm.2010.12.005
Tosif, M. M., Najda, A., Bains, A., Krishna, T. C., Chawla, P., Dyduch-Siemińska, M., Klepacka, J., & Kaushik, R. (2021). A comprehensive review on the interaction of milk protein concentrates with plant-based polyphenolics. *International Journal of Molecular Sciences*, *22*(24). https://doi.org/10.3390/ijms222413548
van de Langerijt, T. M., O'Callaghan, Y. C., Tzima, K., Lucey, A., O'Brien, N. M., O'Mahony, J. A., Rai, D. K., & Crowley, S. V. (2023). The influence of milk with different compositions on the bioavailability of blackberry polyphenols in model sports nutrition beverages. *International Journal of Dairy Technology*, *76*(4), 828–843. https://doi.org/10.1111/1471-0307.12987
Vicidomini, C., Roviello, V., & Roviello, G. N. (2021). Molecular basis of the therapeutical potential of clove (Syzygium aromaticum L.) and clues to its anti-COVID-19 utility. *Molecules*, *26*(7). https://doi.org/10.3390/molecules26071880
Zeb, A. (2021). Phenolic Antioxidants in Dairy Products. In *Phenolic Antioxidants in Foods: Chemistry, Biochemistry and Analysis* (pp. 281–296). Springer, Cham. https://doi.org/10.1007/978-3-030-74768-8_10
Zhang, S., Gai, Z., Gui, T., Chen, J., Chen, Q., & Li, Y. (2021). Antioxidant Effects of Protocatechuic Acid and Protocatechuic Aldehyde: Old Wine in a New Bottle. *Evidence-Based Complementary and Alternative Medicine*, *2021*. https://doi.org/10.1155/2021/6139308
Żyżelewicz, D., Krysiak, W., Oracz, J., Sosnowska, D., Budryn, G., & Nebesny, E. (2016). The influence of the roasting process conditions on the polyphenol content in cocoa beans, nibs and chocolates. *Food Research International*, *89*, 918–929. https://doi.org/10.1016/j.foodres.2016.03.026