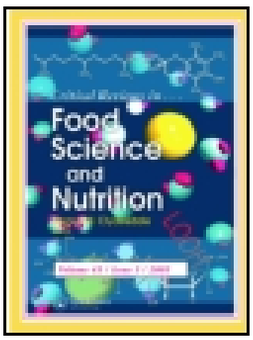

Critical Reviews in Food Science and Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

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To cite this article: Noor Ariefandie Febrianto, Sunan Wang & Fan Zhu (2021): Chemical and biological properties of cocoa beans affected by processing: a review, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2021.1928597

To link to this article: https://doi.org/10.1080/10408398.2021.1928597

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2021.1928597

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REVIEW

Chemical and biological properties of cocoa beans affected by processing: a review

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ABSTRACT

Cocoa (*Theobroma cacao* L.) is widely cultivated in tropical countries. The cocoa beans are a popular ingredient of confectionery. Cocoa beans contain various chemicals that contribute to their bioactivity and nutritional properties. There has been increasing interest in developing cocoa beans for "healthy" food products. Cocoa beans have special combination of nutrients such as lipids, carbohydrates, proteins and other compounds of biological activities. The bioactive phytochemicals include methylxanthines, polyphenols, biogenic amines, melanoidins, isoprostanoids and oxalates. These phytochemicals of cocoa are related to various *in vivo* and *in vitro* biological activities such as antioxidation, anti-cancer, anti-microbial, anti-inflammation, anti-diabetes, cardiovascular protection, physical improvement, anti-photoaging, anti-depression and blood glucose regulation. The potential of bioactive compounds in cocoa remains to be maximized for food and nutritional applications. The current processing technology promotes the degradation of beneficial bioactive compounds, while maximizing the flavors and its precursors. It is not optimized for the utilization of cocoa beans for "healthy" product formulations. Modifications of the current processing line and non-conventional processing are needed to better preserve and utilize the beneficial bioactive compounds in cocoa beans.

KEYWORDS

Theobroma cacao; cacao; post-harvest processing; fermentation; functional food; health effect

1. Introduction

Cocoa beans obtained from the cacao plant (*Theobroma cacao* L.) are a highly commercialized product in confectionery and food industries. Cocoa beans refer to the raw or dried seeds of *T. cacao*. Cocoa beans are usually sold in fermented and dried forms, while unfermented cocoa beans are also available in small amounts. Cocoa nibs/cotyledons are the most important part of the beans. Cocoa nibs are processed into various products, such as cocoa mass/liquor, cocoa powder and cocoa butter in grinding industries (Muñoz et al. 2020). These products can be used in the manufacturing of bakery and confectionery products. Beans from other species of *Theobroma* such as *T. bicolor* and *T. grandiflorum* have been used as the ingredient of a confectionery (alternative chocolate) and beverages in some niche market (Zarrillo et al. 2018).

Variation in chemical composition is related to quality diversity of cocoa beans available in the market. The diversity is influenced by genotype/variety, geographical conditions, cultivation methods, and postharvest practices (D'Souza et al. 2017; Elwers et al. 2009; Febrianto and Zhu 2019a; Kumari et al. 2018; Muñoz et al. 2020). Fermentation, as one of the most important operations in cocoa bean processing, is mostly done in traditional way using spontaneous fermentation and based on local practices. The lack of optimal conditions in post-harvest processing leads to to the variations in the quality of cocoa beans (Muñoz et al. 2020).

The majority of cocoa beans available in the market are processed by means of conventional method (Figure 1). In this report, conventional processing refers to the commonly applied methods by farmers or cocoa industries (Figure 1B), while non-conventional processing (Figure 1A,C) relates to alternative methods or has additional steps of operations added to the conventional processing. In conventional processing, freshly harvested cocoa beans are immediately subjected to spontaneous fermentation for 2–7 days (depending on the cocoa varieties, regions of growth and common practices) before drying (Urbańska et al. 2019; Muñoz et al. 2020). This type of processing is generally sufficient to produce cocoa beans for consumption purpose (Di Mattia et al. 2017; Muñoz et al. 2020). Non-conventional processing operations are carried out to either improve the sensory characteristics of cocoa beans, to deal with cocoa beans with specific properties (unfermented, high acidity, off-flavored, etc.) (Figure 1C) or to better preserve the beneficial bioactive compounds such as polyphenols in cocoa beans (Figure 1A). Increasing consumer awareness toward "healthy" products requires cocoa beans containing a considerable amount of bioactive compounds.

The consumption of cocoa based products rich in bioactive compounds can be related to decreased risk in

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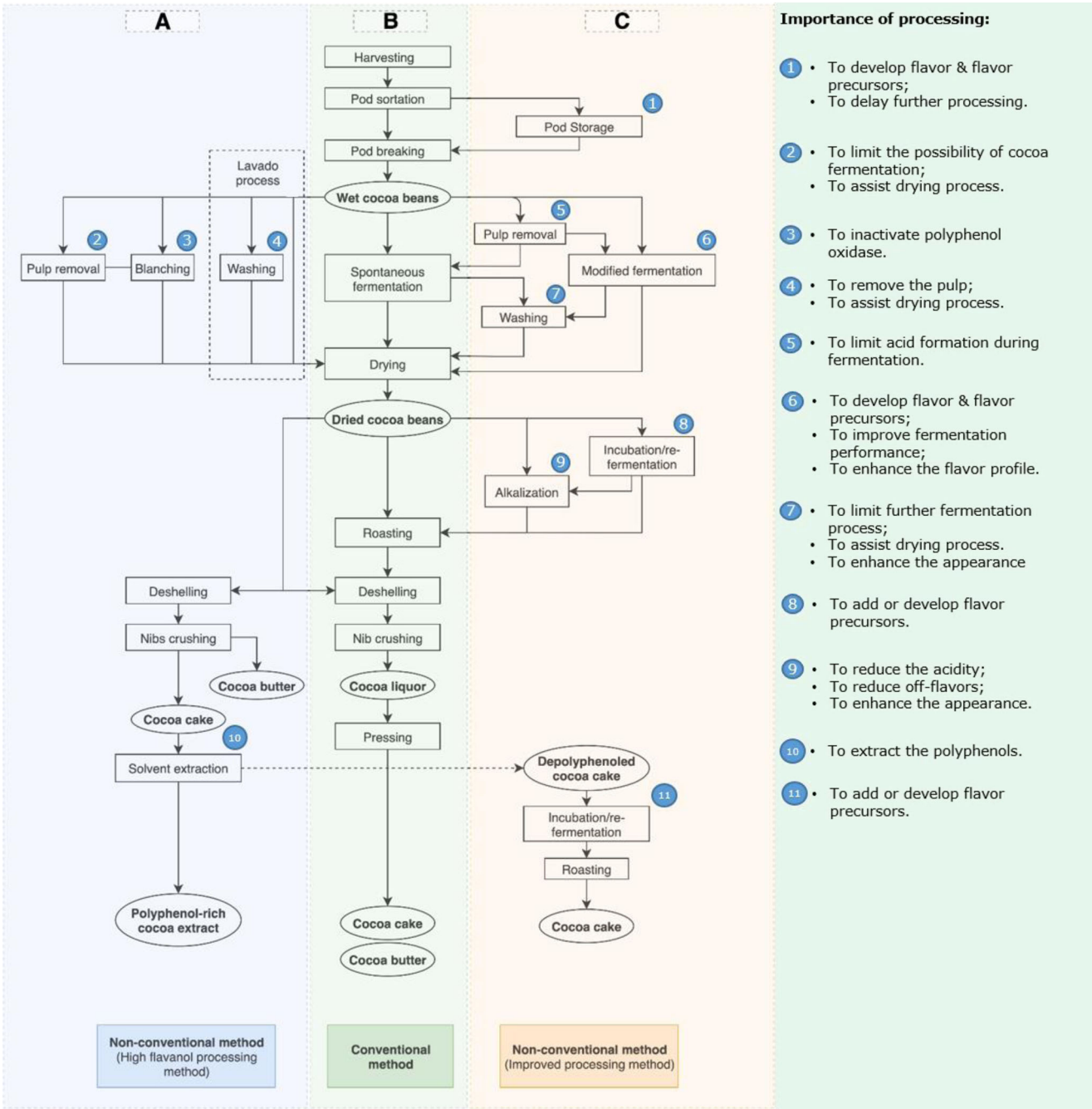

Figure 1. Primary post-harvest and grinding operations of whole cocoa beans. (A) Non-conventional processing method to produce cocoa products with high flavonoid content; (B) Conventional processing of whole cocoa beans; (C) Non-conventional processing method to improve the quality of cocoa beans and cocoa products. The circled number in the "Importance of processing" column explains the purpose of each processing step marked with the same circled number in the columns A, B, and C. Summarized from Ziegleder (2017); González-Barrio et al. (2020); Misnawi, Nazamid, and Jamilah (2002); Oliveira et al. (2011); Rovati (2016); Figueroa-Hernández et al. (2019); Kongor et al. (2016); Aprotosoaie, Luca, and Miron (2016); Muñoz et al. (2020); Urbańska et al. (2019).

coronary heart problems, hypertension, diabetes, and depression (Cordero-Herrera et al. 2015; Cordero-Herrera et al. 2015; Messaoudi et al. 2008; Ramos, Martín, and Goya 2017). Various *in vitro* and *in vivo* studies have been done to evaluate the health effects of different compounds in cocoa beans (Bauer et al. 2016; Hernández-Hernández et al. 2018; Ramos, Martín, and Goya 2017; Todorovic et al. 2017). The nutritional benefits of cocoa based products were reported to be mainly contributed by polyphenols in the beans. The health benefits of cocoa may also be contributed by compounds other than polyphenols such as Maillard reaction products and microbial metabolites (Baranowska et al. 2020; Di Mattia et al. 2017; Quiroz-Reyes and Fogliano 2018; Ryan et al. 2016).

Various reviews on cocoa beans have been published with main focuses on the factors affecting its flavor profile (Aprotosoaie, Luca, and Miron 2016; Kongor et al. 2016; Muñoz et al. 2020), effect of processing (Urbańska et al. 2019), biotechnology (Wickramasuriya and Dunwell 2018), valorization of by-products (Rojo-Poveda et al. 2020), traceability and health benefits of functional microbial metabolites (Mota-Gutierrez et al. 2019), and health effects (Katz,

Doughty, and Ali 2011). However, systematic knowledge of the detailed chemical composition and non-conventional processing methods for cocoa beans remains to be collected.

2. Scope and approach of the review

This review summarizes the detailed chemical composition and biological properties of cocoa beans. It is focused on how the chemical composition affects the choice of processing methods, as well as the impact of different post-harvest processing methods on the chemical profiles of cocoa beans. The choice of alternative non-conventional processing methods in relation to the properties of cocoa beans is discussed. This focused literature review provides a broad perspective in the body of the current knowledge of cocoa quality and processing. It also aims to identify the current research gaps in the cocoa-related research. The results from different studies are critically compared and analyzed. Each reference was narratively reviewed to better understand the variability of the chemical composition of cocoa, to better utilize the active ingredients by processing and to promote the further application of cocoa in food industries.

The literature search was done employing various online databases, including Google Scholar, Google Patents, Web of Science, PubMed®, and SCOPUS. The literature collection was done from April 2020 until May 2021. The keywords for searching contained general terms of "cocoa" OR "cacao" OR "cocoa beans" OR "cocoa extract" followed by (AND) the subject of interests related to chemical composition/components ("proximate," "lipid," "protein," "carbohydrate," "polyphenol," "alkaloid," "ash," "fiber," "flavor volatile" and "mineral") or country of origins ("Ivory Coast"/"Cote d'Ivoire," "Ghana," "Nigeria," "Indonesia," "Brazil," "Malaysia." "Peru," "Ecuador," "Venezuela," "Madagascar," "Togo," and "China"), or processing method ("post-harvest," "fermentation," "pod storage," "pulp conditioning," "roasting," "alkalization," "blanching," "washing," and "conching"), or health effects ("health effect," "in vitro," "in vivo," "antioxidant," "anti-microbial," "anti-inflammatory," "diabetic," "cancer," "inflammation," and "cardiovascular") OR consumer perception ("consumer perception," "hedonic," and, "liking"). In the case that aforementioned keywords did not provide satisfactory results, the derivative terms were used including "butter," "amino acid," "peptide," "dietary fiber," "starch," methylxanthine," "flavan-3-ol," "flavonol," "proanthocyanidin," "phytochemical," "procyanidin," "anthocyanin," "nibs," "cotyledon," "shell," "forced-fermentation," "artificial fermentation," "starter culture," "Maillard reaction," "unfermented," "lavado," "modified fermentation," "alternative process," "improved process," "bitterness," "astringency," "bitter," astringent," "chocolate," "dark chocolate," and "milk chocolate." The included publications were preferably in English and were published from 2000 to 2021. Papers of other languages with great significance related to the topic are also included. The publications before 2000 were considered if there were little studies related to the particular topic of interest. The data in the cited publications are presented in their original values. Inclusion criteria of content were of cocoa or cocoa-derived products studied for their chemical composition, post-harvest processing, and in vivo and in vitro health effects. Exclusion criteria were of evaluating the parts of cacao except for the beans such as leaves, bark, and roots.

3. Current processing technology of cocoa beans

3.1. Conventional processing

Cocoa beans are mostly processed using the conventional method on cacao farm, farmer's house, or co-operative facility (Muñoz et al. 2020; CAOBISCO/ECA/FCC 2015). The primary steps of conventional cocoa processing include harvesting, pod sortation, pod-breaking, spontaneous fermentation, and drying. Secondary processing steps of the grinding industries include roasting, deshelling, nib-crushing, and liquor pressing (Figure 1B) (Di Mattia et al. 2017). Spontaneous fermentation is usually done by piling up the wet cocoa beans in heaps, wooden boxes, wooden basket, or any convenience container for 2–7 days (Urbańska et al. 2019; Muñoz et al. 2020; Figueroa-Hernández et al. 2019). Fermentation is essential to build up the flavors and their precursors (Mota-Gutierrez et al., 2018; Kongor et al. 2016). The cocoa beans are then sun-dried or mechanically dried until the moisture content reaches 5–7% (Urbańska et al. 2019). In the industries, the roasting of cocoa beans is done using high temperature (>100 °C) to develop the desired flavor profile (Ziegleder 2017). The conventional processing of cocoa is efficient to produce good quality cocoa beans for confectionery purposes.

3.2. Non-conventional processing

Cocoa beans may be processed non-conventionally. Several alternatives can act as substitute or as addition to the conventional processing (Figure 1C). The process may include pulp conditioning (pod storage and pulp removal), modified fermentation, washing, incubation/re-fermentation of dried beans, and alkalization (Oliveira et al. 2011; Hinneh et al. 2018; Misnawi, Nazamid, and Jamilah 2002; Muñoz et al. 2020; Urbańska et al. 2019). Pulp conditioning could be done by pod storage and pulp removal methods (pressing, pre-drying, and mechanical de-pulping) (Muñoz et al. 2020). Pod storage is commonly practiced some countries such as Ghana. Other cocoa-producing countries may adopt another methods of pulp conditioning (Bangerter et al. 1991). In this paper, pulp conditioning is classified into non-conventional processing since it is considered an additional step in the processing.

Different fermentation methods can also be used. Starter culture or flavoring substances can be added to modify the fermentation process. Environmental conditions such as temperature and pH may also be modified to perform artificial/forced fermentation. The use of these methods may either increase the concentrations of flavor precursors produced (Santos et al. 2020), improve the fermentation performance (Figueroa-Hernández et al. 2019; John et al. 2020),

Table 1. Comparison of proximate composition of cocoa beans from different origins, genotypes and processing methods.

Samples and source locations	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Carbohydrate (%)	Fiber (%)	References
Unfermented dry beans							
Ivory Coast[b]	6.3	22.4	46.3	3.2	22.9	5.5	Kouadio et al. (2020)
Ghana[b]	4.2	21.6	55.2	3.5	15.5	n.d	Afoakwa et al. (2013)
Nigeria[b]	5.0	17.5	62.9	4.4	9.3[c]	5.9	Aremu, Agiang, and Ayatse (1995)
Indonesia[b]	8.1	15.4	34.9	5.1	n.d	n.d	Mulyawanti, Hidayat, and Risfaheri (2018)
Venezuela *Criollo* (different genotypes)	4.4–6.0	14.7–20.5	46.1–56.4	3.6–5.2	8.7–28.2	4.23–9.0[a]	Liendo, Padilla, and Quintana (1997)
Venezuela hybrid (*Forastero* × *Criollo*)	5.7–7.17	14.8–16.2	47.3–54.2	3.4–3.9	20.3–25.3	5.7–8.8[a]	Padilla et al. (2000)
Peru[b]	4.8	15.7	48.9	4.4	31.0	n.d	Salazar et al. (2020)
Fermented dry beans							
Ecuador[b]	5.9	12.8	43.5	4.0	33.8	19.4	Torres-Moreno et al. (2015)
Ivory Coast[b]	6.5	20.7	43.9	3.3	26.0	6.8	Kouadio et al. (2020)
Ghana[b]	5.1	12.8	41.9	3.6	36.6	11.3	Torres-Moreno et al. (2015)
Ghana Hybrid	5.3	14.0	33.0	1.2	51.9	n.d	Grassia et al. (2019)
Indonesia[b]	4.7	15.4	37.3	3.9	n.d	n.d	Mulyawanti, Hidayat, and Risfaheri (2018)
Brazil[d]	0.9–1.3	7.9–9.1	34.8–41.8	1.5–1.9	46.4–54.1	n.d	Melo et al. (2020)
Cameroon *Trinitario*	5.9	17.9	41.2	7.3	27.7	n.d	Djikeng et al. (2018)
Nigeria[b]	2.2–5.3	14.6–19.8	42.2–55.7	3.2–5.6	15.8–35.6	3.1–5.6	Aremu, Agiang, and Ayatse (1995)
Malaysia[b]	5.7	22.5	42.7	3.3	25.8	17.2	Agus, Mohamad, and Hussain (2018)
Malaysia[b]	5.3	12.3	41.2	5.1	26.8	9.8	Abdullahi et al. (2018)
Peru[b]	3.1	15.8	47.7	4.5	32.0	n.d	Salazar et al. (2020)
Peru *Criollo*	4.2	9.9	49.4	0.9	39.7	n.d	Grassia et al. (2019)
Ecuador *Criollo*	4.6	12.3	48.8	1.2	37.6	n.d	
Fermented dry beans with pod storage (Ghana mixed hybrids) (Values vary between different time of pod storage)	3.8–4.9	17.6 – 21.6	50.5–55.2	2.3–3.5	15.5–24.9	n.d	Afoakwa et al. (2013)

n.d: not determined.
[a]Calculated as crude fiber.
[b]Information of genotype is not available.
[c]As nitrogen-free extract (by difference).
[d]Various genotypes.

or enhance the flavor profiles of cocoa beans (Visintin et al. 2017; Dario and Eskes 2009). Furthermore, dried unfermented and partially-fermented beans can be incubated with added enzymes or chemicals to increase the number of flavor precursors, or re-fermented (by the addition of water or buffer) to reactivate the enzymes in cocoa beans (Hansen et al. 2004; Oliveira et al. 2011; Misnawi, Nazamid, and Jamilah 2002). This can improve the flavor quality of cocoa beans during roasting.

Non-conventional process can be carried out to produce cocoa beans with specific characteristics. For example, fresh cocoa beans can be directly dried to preserve the bioactive compounds (Figure 1A). Washing, pulp reduction, and blanching may also be done to assist the drying process and inactivation of polyphenol oxidase in cocoa beans. In this context, the practice of washing the cocoa beans followed by sun-drying is commonly known in Latin America as “lavado” processing (Chin et al. 2013; Ciaramelli et al. 2021). This process results in unfermented cocoa beans with high polyphenol content (Chin et al. 2013; González-Barrio et al. 2020). The cocoa beans could then be processed to produce "healthy" products. The polyphenols of the beans can then be extracted using solvents for further applications. The non-conventional processing methods can improve the quality of the cocoa beans and offer the possibility of their diverse applications.

4. Chemical composition of cocoa beans

4.1. Proximate composition

The contents of moisture, ash, lipid, protein and carbohydrate, and fiber of cocoa nibs ranged from 2.2–7.17%, 0.9–7.3%, 33–63%, 7.9–22.5% and 8.7–51.9%, and 3.1–19.4% (dry basis, DB), respectively (Table 1). The compositions depend on country of origin, genetics, growing and post-harvest practices of cocoa beans (Abdullahi et al. 2018; Afoakwa et al. 2013; Agus, Mohamad, and Hussain 2018; Djikeng et al. 2018; Grassia et al. 2019; Liendo, Padilla, and Quintana 1997; Salazar et al. 2020; Torres-Moreno et al. 2015). In the subsequent discussion, the term cocoa beans, unless specified, refer to the cocoa nibs/cotyledon part of the cocoa beans. The analysis carried out and cocoa-related studies were mainly done only on that particular part.

4.2. Lipids

Cocoa lipid (commonly recognized as cocoa butter) constitutes a major one of cocoa beans. Cocoa butter is rich in saturated fatty acids (FAs) (56.6–64.1%) (Table 2). Saturated FAs in cocoa are lauric acid, myristic acid, pentadecylic acid, palmitic acid, margaric acid, stearic acid, arachidic acid, and behenic acid. Unsaturated FAs account for ∼34.6–43.4% of cocoa butter. The monounsaturated FAs include pentadecenoic acid, palmitoleic acid, heptadecenoic acid, oleic acid, and gondoic acid. Essential FAs such as linoleic and linolenic acids are present in small amounts (2–4.5%) in cocoa butter (Grassia et al. 2019; Liendo, Padilla, and Quintana 1997; Torres-Moreno et al. 2015). Generally, palmitic, stearic and oleic acids are the major FAs in cocoa butter (Grassia et al. 2019; Liendo, Padilla, and Quintana 1997; Torres-Moreno et al. 2015).

These FAs are in the form of triacylglycerols (TAG) in cocoa butter. Major TAGs of cocoa are 1-palmitoyl-2-oleoyl-3-stearin (POS) (39–49%), 2-oleoyl distearin (SOS)

Table 2. Comparison of fatty acid composition of cocoa butter from different origins, genotypes and processing methods.

	C12:0	C14:0	C15:0	C15:1	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	Total saturated	Total unsaturated
Unfermented dry beans																	
Dominican Republic[a]	–	–	–	–	27.2	–	–	–	36.0	33.6	3.13	–	–	–	–	–	–
Madagascar[a]	–	–	–	–	27.4	–	–	–	36.2	33.9	2.49	–	–	–	–	–	–
Ecuador[a]	–	–	–	–	28.3	–	–	–	35.7	33.5	2.52	–	–	–	–	–	–
Venezuela *Criollo* (different genotype)	–	–	–	–	20.3-30.1	–	–	–	33.8-38.8	35.0-40.8	0-3.2	0-1.3	–	–	–	56.5-63.9	36.2-43.4
Fermented dry beans																	
Ivory Coast[a]	–	–	–	–	25.8	–	–	–	36.9	32.9	2.80	0.20	1.20	–	0.20	64.1	35.9
Ghana[a]	–	0.09	0.03	0.01	25.0	0.20	0.32	0.03	36.4	34.3	2.02	0.18	1.24	0.04	0.14	63.2	36.7
Ghana Hybrid	0.10	0.02	0.03	–	26.8	0.21	0.20	0.01	36.5	32.1	1.16	0.92	0.06	0.21	0.22	63.9	34.6
Indonesia[a]	–	–	–	–	24.1	–	–	–	37.3	34.3	2.70	0.20	1.20	–	0.20	62.8	37.2
Nigeria[a]	–	–	–	–	26.5	–	–	–	37.1	33.1	2.25	–	1.03	–	–	–	–
Brazil[a]	–	–	–	–	25.1	–	–	–	33.3	36.5	3.50	0.20	1.20	–	0.20	59.8	40.2
Ecuador[a]	0.02	0.06	0.03	0.01	27.6	0.32	0.27	0.02	33.8	34.7	2.43	0.14	1.09	0.05	0.15	62.3	37.7
Togo[a]	–	0.09	–	–	26.2	0.21	0.18	–	35.8	33.7	2.80	–	0.94	–	–	–	–
China[a]	–	–	–	–	24.3	–	–	–	35.8	33.3	3.80	1.8[b]	–	–	–	–	
Malaysia[a]	–	–	–	–	23.6	–	–	–	35.5	33.4	4.20	1.9[b]	–	–	–	–	
Peru *Criollo*	–	0.06	0.04	–	28.2	0.25	0.36	0.06	30.8	35.3	3.43	0.16	0.96	0.06	0.16	60.6	39.2
Ecuador *Criollo*	–	0.08	–	–	26.3	0.27	0.26	0.50	35.1	33.6	2.87	0.16	1.00	0.05	0.16	62.9	37.5

[a]Information of genotype is not available.
[b]Data were presented as linolenic acid + arachidic acid. Summarized from Grassia et al. (2019); Liendo, Padilla, and Quintana (1997); Lipp and Anklam (1998); Torres-Moreno et al. (2015); Foubert et al. (2006); Żyżelewicz et al. (2014); Marty and Marangoni (2009); Servent et al. (2018).

(21–43%) and 2-oleoyl dipalmitin (POP) (6.9–17%) (Sirbu et al. 2018). The variation in TAG composition was affected by origin of cocoa beans and post-harvest practice (Grassia et al. 2019; Sirbu et al. 2018). Cocoa butter is valued due to its unique melting point and crystallization properties. The FA and TAG composition of cocoa butter related to melting properties of chocolate in mouth (Servent et al. 2018).

4.2.1. Effect of conventional processing on lipids

4.2.1.1. Effect of fermentation. Fermentation affects the composition and extractability of cocoa butter. Fermentation did not change the free FA content in cocoa butter (Servent et al. 2018). Higher total lipid content was found in fermented beans, which may be contributed by the changes in lipid extractability (Sirbu et al. 2018). Extraction yields of cocoa butter were higher by 2–10% in fermented cocoa beans (144 h) than those of unfermented beans (Servent et al. 2018). Fermentation changed the TAG diversity and composition (Sirbu et al. 2018). Unfermented cocoa beans contained 117 TAG compounds, more than that of fermented beans (70 TAG compounds). Unfermented cocoa beans contained polar TAG, which were absent in fermented cocoa beans (Sirbu et al. 2018). Polar TAG appointed as 2-cacaoyl-1,3-diyl dipalmitate containing "cacaoic acid" was only found in the unfermented cocoa beans. The compound could be a marker of the quality of cocoa beans (Sirbu et al. 2018).

4.2.1.2. Effect of roasting. Roasting promotes the change in characteristics of cocoa butter. Oracz and Nebesny (2019) found roasting temperature-dependently reduced the level of lipids. Żyżelewicz et al. (2014) found that roasting reduced the peroxide value of cocoa butter, which was caused by either inhibition of fat oxidation or peroxide conversion toward secondary oxidation products. Roasting at 150 °C for 25 min (air flow rate (v) = 1 m/s and relative air humidity (RH) = 0.3%) induced the formation of *trans*-oleic acid. Oven roasting decreased the amounts of palmitic, stearic, arachidic, palmitoleic, oleic, linoleic and linolenic acids in cocoa beans, while superheated steam roasting changed the FA composition to a small extent (Zzaman, Bhat, and Yang 2017). The roasting reduced the contents of solid TAGs such as POP, POS and SOS in cocoa beans, reducing the overall solid fat content of cocoa butter (Zzaman, Bhat, and Yang 2017).

4.2.2. Effect of non-conventional processing on lipids

4.2.2.1. Pulp conditioning. Pod storage can affect the lipid composition of cocoa butter. Fermented cocoa beans obtained from the pods stored for 10 days had more free FAs and higher peroxide values than those of the non-stored pods and the pods stored for 3 and 7 days (Afoakwa et al. 2014). Prolonged pod storage may lead to seed germination. The activity of lipase during the germination process may lead to cocoa butter hydrolysis, increasing the free FA content (Guzmán-Ortiz et al. 2019).

4.2.2.2. Alkalization. Addition of alkaline reagent led to the hydrolysis and saponification of cocoa butter (García, Esteve, and Baviera 2020). The alkalization also reduced the content of cocoa butter. Alkalization should be done in low-fat material rather than in high-fat material to preserve the quantity and quality of cocoa butter (García, Esteve, and Baviera 2020).

4.3. Carbohydrates

4.3.1. Monosaccharides, disaccharides and oligosaccharides

Cocoa beans contain monosaccharides (fructose, glucose), disaccharides (sucrose, and melibiose), oligosaccharides (raffinose, 1-kestose, stachyose and verbascose), and sugar alcohols (myo-inositol, scyllo-inositol, mannitol, and galactinol) (Megías-Pérez et al. 2018; Redgwell, Trovato, and Curti 2003). Analysis of unfermented cocoa beans from different

origins showed great variability in the contents of sucrose, raffinose and stachyose due to genetic and environmental factors (Megías-Pérez et al. 2018). The sugar content of dried cocoa beans was also affected by the post-harvest storage and processing (fermentation). Unfermented cocoa beans contained more sugars (0.9–4.9%, fat free dry matter (ff DM) compared to fermented cocoa beans (0.2–0.4% ff DM) (Megías-Pérez et al. 2018).

4.3.2. Starch

Cocoa beans had 4.5–7% of starch (WB) and fat-free cocoa mass contained around 18.6% of starch (ff DM) (Schmiederand and Keeney 1980). In cocoa beans, starch granules are found along with lipid vacuoles and protein bodies. Granule size of cocoa starch varied from 2–12.5 µm (mean 4.6 µm). Cocoa starch was consisted of 31–36% of amylose. The amylose content contributed to higher viscosity of cocoa starch compared to corn starch. Gelatinization temperature of cocoa starch was 61 °C and the complete loss of birefringence of the granules was at 68 °C (Schmiederand and Keeney 1980). The crystallinity and molecular structure of cocoa starch should be better studied. Understanding the functional and biological properties of cocoa starch may be important for the formulations of "healthy" cocoa products.

4.3.3. Dietary fiber

Dried cocoa beans contained 11.3 to 19.4% of dietary fiber (WB) (Torres-Moreno et al. 2015). Valiente et al. (1994) found 17.8% (DB) of total dietary fiber (DF) in cocoa beans, with 14.3% insoluble DF (IDF) (DB) and 3.5% soluble DF (SDF) (DB). SDF was consisted of neutral sugars and uronic acid. The IDF contained lignins (Valiente et al. 1994). Cocoa bean shell (CBS, husk or hull) is a potential source of DF. The shell contained 60.5% TDF (DB), with IDF and SDF being 83.3% and 16.7%, respectively (Lecumberri et al. 2007). The IDF was composed of Klason lignin (32.4% DB), neutral sugars (14.5% DB) and uronic acid (3.48% DB) (Lecumberri et al. 2007). Indeed, cocoa beans can be a good source of dietary fiber. Further studies of under-utilized CBS are needed to better utilize it as a functional food ingredient.

4.3.4. Effect of conventional processing on carbohydrates

4.3.4.1. Effect of fermentation. Fermentation greatly affected carbohydrate profiles of cocoa beans (Megías-Pérez et al. 2018). The changes in the carbohydrate profile are affected by the activities of enzymes such as glycosidase and invertase (Muñoz et al. 2020). Sucrose content was greatly reduced during 4 days of fermentation as observed in cocoa beans obtained from Brazil, Cameroon, Ecuador, Ivory Coast and Malaysia (Megías-Pérez et al. 2018). Fermentation of up to 144–168 h increased the concentrations of several sugars and sugar alcohols such as fructose, glucose, mannitol, and melibiose in the cocoa beans. On the other hand, the concentrations of myo-inositol, galactinol, 1-kestose, raffinose, and stachyose were decreased (Megías-Pérez et al. 2018). The composition of sugars affects the flavor formation in cocoa beans during subsequent roasting process (Ziegleder 2017).

4.3.4.2. Effect of roasting. Roasting reduced the levels of glucose and fructose in cocoa beans, mainly due to their interactions with amino acids. The amount of reducing sugars was positively associated with flavor development during cocoa beans roasting (Aprotosoaie, Luca, and Miron 2016; Kongor et al. 2016). The contents of sucrose, raffinose, stachyose and verbascose were little affected or increased during roasting. There was no reaction between non-reducing sugars and amino acids during the roasting (Redgwell, Trovato, and Curti 2003). An increased concentration of CWM was found in roasted beans, mainly contributed by the increased amount of "Klason lignin" during roasting (Redgwell, Trovato, and Curti 2003). The term "Klason lignin" in the study did not refer to real lignin in cocoa beans but to Maillard reaction products (MRP) formed during roasting. Like Klason lignin, MRP are insoluble after sulfuric acid treatment, thus interfering with the calculation of "Klason lignin." Pectic and hemicellulosic polymers in cocoa beans were not affected by roasting (Redgwell, Trovato, and Curti 2003).

4.3.5. Effect of non-conventional processing on carbohydrates

4.3.5.1. Pulp conditioning. Longer pod storage (>2 days) increased the contents of some sugars such as glucose and fructose in cocoa beans (Afoakwa et al. 2013). This could be contributed by invertase from the fermentation-like activity during prolonged storage of cocoa beans (Hinneh et al. 2018). Physical de-pulping reduced the amount of pulp in cocoa beans, resulting in lower total carbohydrate content (Bangerter et al. 1991).

4.3.5.2. Modified fermentation. Fermentation of Scavina cocoa beans fermented with *Torulaspora delbrueckii* and *Candida parapsilosis* led to higher reducing sugar content (Santos et al. 2020). The pectinolytic activity of the inoculum facilitated the release of the reducing sugars from the beans. Those conditions helped to activate the cotyledon enzymes such as invertase (De Vuyst and Weckx 2016). The invertase hydrolyzed sucrose into fructose and glucose.

4.3.5.3. Incubation and re-fermentation. Reducing sugars can be added to cocoa beans to increase the amounts of flavor precursors (Hansen et al. 2004). Re-fermentation of dried beans re-activated invertase which converted sucrose to fructose and glucose (Misnawi, Nazamid, and Jamilah 2002). The modification of carbohydrate composition in cocoa beans was mainly intended to improve the flavor quality of cocoa beans.

4.3.5.4. Alkalization. Alkalization of cocoa beans using Na_2CO_3 at pH 8.0 reduced sucrose, while increasing the concentration of glucose in unroasted cocoa beans (Taş and Gökmen 2016). On the other hand, Li et al. (2012) found

that alkalization using NaOH solution (1–3%) significantly reduced the amounts of fructose and glucose. This difference could be attributed to the conditions of alkalization with different pH which led to different extents of degradation of the sugars (De Bruijn, Kieboom, and van Bekkum 2010). When the alkalization reduced the amount of reducing sugars in the cocoa beans, limited formation of cocoa flavor via Maillard reaction during roasting was obtained (García, Esteve, and Baviera 2020).

4.4. Proteins

Cocoa protein is mainly consisted of albumin and vicilin-like storage globulin, while kinase superfamily proteins were also found (Kratzer et al. 2009; Kumari et al. 2016). The amounts of essential amino acids were ~43 and 40% of protein in unfermented and fermented cocoa beans (calculated without histamine), respectively (Adeyeye et al. 2010). Cocoa beans contained relatively high amounts of lysine, aspartic acid, glutamic acid, arginine, and leucine (>50 mg/g of crude protein). Methionine and cysteine were the limiting amino acids in cocoa beans (<10 mg/g of crude protein) (Adeyeye et al. 2010).

Protein composition of cocoa beans is largely affected by defense and stress mechanisms, photosynthesis and hormone metabolisms during the growth of cacao tree (Scollo et al. 2020). Cocoa beans from the crop cultivated in soil with pH of 5–7 contained more protein than those cultivated in low pH (<4.5) soil (Kumari et al. 2018). During post-harvest processing, protein in cocoa beans is mostly degraded into peptides and amino acids which play important role in flavor development of the beans.

Protein in cocoa beans is also present as biogenic amines (BA) including phenylethylamine, tyramine, tryptamine, serotonin, histamine, putrescine, dopamine, spermidine, spermine and ethanolamine (Oracz and Nebesny 2014). The concentration of BAs in unroasted cocoa beans ranged from 2.66 to 11.4 mg/kg ff DM, depending on climate conditions in growing location. Several pathways of BA formation were proposed, including microbial metabolisms mainly during fermentation, amination/transamination of ketones and aldehydes, lipid peroxidation and high heat treatment such as roasting (Brito et al. 2017).

4.4.1. Effect of conventional processing on protein

4.4.1.1. Effect of fermentation. Protein profiles of unfermented and fermented cocoa beans are different. Fermentation reduced the total protein content of cocoa beans by 36–80% (Kumari et al. 2016). Kumari et al. (2018) and D'Souza et al. (2018) found that peptide diversity of cocoa beans increased during fermentation. Acidification of cocoa beans in the early stage of fermentation induced the release of free amino acids in the beans (Niemenak et al. 2020). Significance increase in the numbers of albumin, basic chitinase, lipoxygenase, peroxidase, 2S sulphur-rich seed storage proteins, and vicilin peptides were found in fermented cocoa beans. This could be used as an indicator for fermentation level of cocoa beans. Free peptides were mostly generated during 24–120 h of fermentation. They were not found in unfermented beans and beans obtained from later stages of fermentation (>120 h). After 120 h of fermentation, peptides were degraded into di/tri-peptides and free amino acids (Kumari et al. 2016). The presence of the peptides is important in cocoa beans. A previous study showed that cocoa-specific flavor could only be generated during roasting of amino acids and sugar mixtures in the presence of peptides (Scalone et al. 2019; Voigt, Textoris-Taube, and Wöstemeyer 2018).

Peptides in cocoa beans during fermentation were mostly composed of 2–5 amino acids (D'Souza et al. 2018). Underfermented cocoa beans contained less than 100 peptides and fairly fermented cocoa beans contained 100–300 peptides. The beans with the number of peptides exceeding 300 were well fermented (D'Souza et al. 2018). Fermentation also induced the change in biogenic amines. An elevated concentration of total BAs was found during 7 days fermentation (from 12.8 up to 39.6 mg/kg WB), mainly contributed by spermidine (38–55% of total BA) (Brito et al. 2017). BAs are important plant development regulators, and their content is usually increased in response to abiotic stress such as high-temperature. High-temperature during fermentation may help to release the BAs in cocoa beans (Chen et al. 2018).

4.4.1.2. Effect of roasting. Roasting significantly affects the composition of various chemical compounds in cocoa beans. Roasting induced conversion of reducing sugars and proteins of cocoa beans (Maillard reaction) into the formation of flavor compounds and melanoidins (Kongor et al. 2016; Muñoz et al. 2020). Roasting reduced the protein content in cocoa beans. The decrease of protein content up to 27.1% of the initial content (unroasted cocoa beans) was reported (Oracz and Nebesny 2019). Higher temperature roasting (≥135 °C) led to larger reduction of protein content. Roasting increased the concentration of biogenic amines in roasted cocoa beans (4.42 to 33.5 mg/kg ff DM), due to limited oxygen access (Oracz and Nebesny 2014). Different roasting temperature (110–150 °C) increased the amounts of BAs in cocoa beans from different origins to various extents (from 2.66–11.4 to 8.03–33.5 mg/kg ff DM). High temperature roasting conditions (≥135 °C) with increased air humidity (5%) resulted in the formation of more BAs (Oracz and Nebesny 2014).

4.4.2. Effect of non-conventional processing on protein

4.4.2.1. Pulp conditioning. Pod storage affects the composition of free amino acids in cocoa beans. Cocoa beans obtained from the pods stored for 7 days contained more free amino acids than the beans from those stored for 3 days (Hinneh et al. 2018). The degradation of proteins in cocoa beans during pod storage related to the cellular degradation and proteolysis in cocoa beans (Hinneh et al. 2018).

4.4.2.2. Modified fermentation. The use of inoculum in cocoa beans fermentation could increase the formation of amino acids. Higher free amino acid content was measured in cocoa beans fermented with *T. delbrueckii* compared to

control (without inoculum) (Santos et al. 2020). Proteases from the inoculum may diffuse into the cocoa beans, assisting amino acid formation through protein degradation (Santos et al. 2020). The use of inoculum with high proteolytic activity is recommended to assist cocoa fermentation. Under specific fermentation conditions, the peptide formation was modified in cocoa beans. Cocoa beans fermented at the temperature of 46–48 °C in an acidic environment had the highest peptide diversity and quantity compared to those obtained at lower temperature and neutral pH conditions (John et al. 2020). The modification of the fermentation conditions can significantly affect the enzyme activity and the fermentation outcomes.

4.4.2.3. Incubation and re-fermentation. Amino acids can be added to cocoa liquor to produce more flavor precursors. The addition of proline and rhamnose to cocoa liquor increased caramel and biscuit flavors in chocolate (Hansen et al. 2004). Incubation of cocoa liquor under acidic condition with/without the addition of protease enzymes could produce cocoa hydrolysate. This cocoa hydrolysate can generate chocolate flavor independently or mixed with a chocolate product to assist the flavor formation (Hansen et al. 2004). However, the chemical composition of the hydrolysate has not been studied in detail.

4.4.2.4. Alkalization. Alkalization reduced the amount of free amino acids in cocoa beans. Heavy alkalization led up to complete degradation of the amino acids (Li et al. 2012). Great reduction (>90%) of alanine, tyrosine, valine, phenylalanine, leucine, tyramine, arginine, cysteine, and lysine was obtained after heavy alkalization (3% NaOH). Hydrophobic amino acids were more affected by the alkalization than the hydrophilic ones.

4.5. Polyphenols

Polyphenol composition in cocoa beans has been intensively studied. Polyphenols constitute ~15% ff DM of cocoa beans (Urbańska et al. 2019). It is mostly composed of proanthocyanindins (PAs), flavan-3-ols and anthocyanins.

4.5.1. Flavonoids

4.5.1.1. Flavan-3-ols and derivatives. Flavan-3-ols and derivatives are the most abundant cocoa polyphenols (Kelm et al. 2006; Kongor et al. 2016; Ryan et al. 2016; Urbańska et al. 2019). Epicatechin is the major flavan-3-ol monomer in cocoa beans and the main backbone of cocoa proanthocyanidins (PAs) (Kelm et al. 2006) (Figure 2). PAs are responsible for bitter and astringent taste of cocoa. Epicatechin concentration in cocoa beans is high in unfermented cocoa beans (6–52 g/kg ff DM) (Elwers et al. 2009; Febrianto and Zhu 2019a; Hernández-Hernández et al. 2018). Epicatechin content is affected by genetics (Febrianto and Zhu 2019a; Hernández-Hernández et al. 2018), origin (D'Souza et al. 2017), fertilization (Elwers et al. 2009), and stress response during harvesting (D'Souza et al. 2017). Catechin is generally present in a lower concentration than epicatechin in cocoa beans (Elwers et al. 2009; Febrianto and Zhu 2019b; Hernández-Hernández et al. 2018).

PAs in cocoa beans are mostly composed by epicatechin. PA B-type dimer, PA A-type dimer, PA B-type trimer, PA B-type tetramer, and their glycosides were the common PAs in cocoa (D'Souza et al. 2017; Febrianto and Zhu 2019a; Kelm et al. 2006; Robbins et al. 2013; Rodríguez-Carrasco et al. 2018; Ryan et al. 2016). Cocoa beans are rich in non-extractable proanthocyanidin (NEPA). The content of NEPA in unfermented Indonesia cocoa beans (26 genotypes) varied from 11.1 to 27.4 g/kg DB (Febrianto and Zhu 2019a).

4.5.1.2. Anthocyanins. Anthocyanins are responsible for color of cocoa beans. Total anthocyanin content of cocoa beans could reach 4% of total polyphenols (Muñoz et al. 2020). Deep purple color of *Forastero* cocoa beans is associated with high concentration of anthocyanins. Cyanidin-3-galactoside and cyanidin 3-arabinoside were the major anthocyanins in cocoa beans (Figure 2) (Aprotosoaie, Luca, and Miron 2016; Febrianto and Zhu 2019a; De Taeye et al. 2016). During post-harvest treatment, anthocyanin content decreased due to enzymatic hydrolysis and polyphenol oxidase (PPO) catalyzed oxidation (Muñoz et al. 2020). Anthocyanins were absent in white/ivory *Criollo* cocoa beans (Kongor et al. 2016).

4.5.1.3. Flavonols. Flavanols in cocoa beans are mostly in the form of flavonol glycosides. Quercetin-based flavonols such as quercetin-3-O-hexoside (hyperoside and isoquercitrin) and quercetin-3-O-arabinoside were the most reported flavonol glycosides in cocoa beans (D'Souza et al. 2017). Other flavonols such as kaempferol-3-O-hexoside (with glucose and neohesperoside moieties) and kaempferol-3-O-rutinoside were also detected in cocoa (D'Souza et al. 2017; Rodríguez-Carrasco et al. 2018).

4.5.1.4. Flavones. Apigenin, luteoin, apigenin hexoside, luteoin hexoside, amentoflavone, and biapigenin were detected in cocoa (D'Souza et al. 2017; Rodríguez-Carrasco et al. 2018). Chocolate products derived from *Forastero*, *Trinitario* and *Criollo* beans contained flavones in a range of 46 to 110 mg/kg WB (around 9.4% of total polyphenols) (Rodríguez-Carrasco et al. 2018).

4.5.1.5. Flavanones. Naringenin based flavanones were detected in cocoa beans and cocoa-products. Flavanone glycosides with rhamnosidoglucose and glucose moieties were found in unfermented and fermented cocoa beans and chocolate (D'Souza et al. 2017; Rodríguez-Carrasco et al. 2018). Chocolate products derived from *Trinitario* cocoa beans contained considerably more flavanones (>50 mg/kg WB) than those of *Forastero* and *Criollo* (Rodríguez-Carrasco et al. 2018)

4.5.2. Phenolic acids and derivatives

Phenolic acids in cocoa were hydroxybenzoic, hydroxycinnamic acids and amino acid conjugated derivatives (N-phenylpropenoyl-L-amino acids, NPA) (D'Souza et al. 2017; Febrianto and Zhu 2019a; Rodríguez-Carrasco et al. 2018).

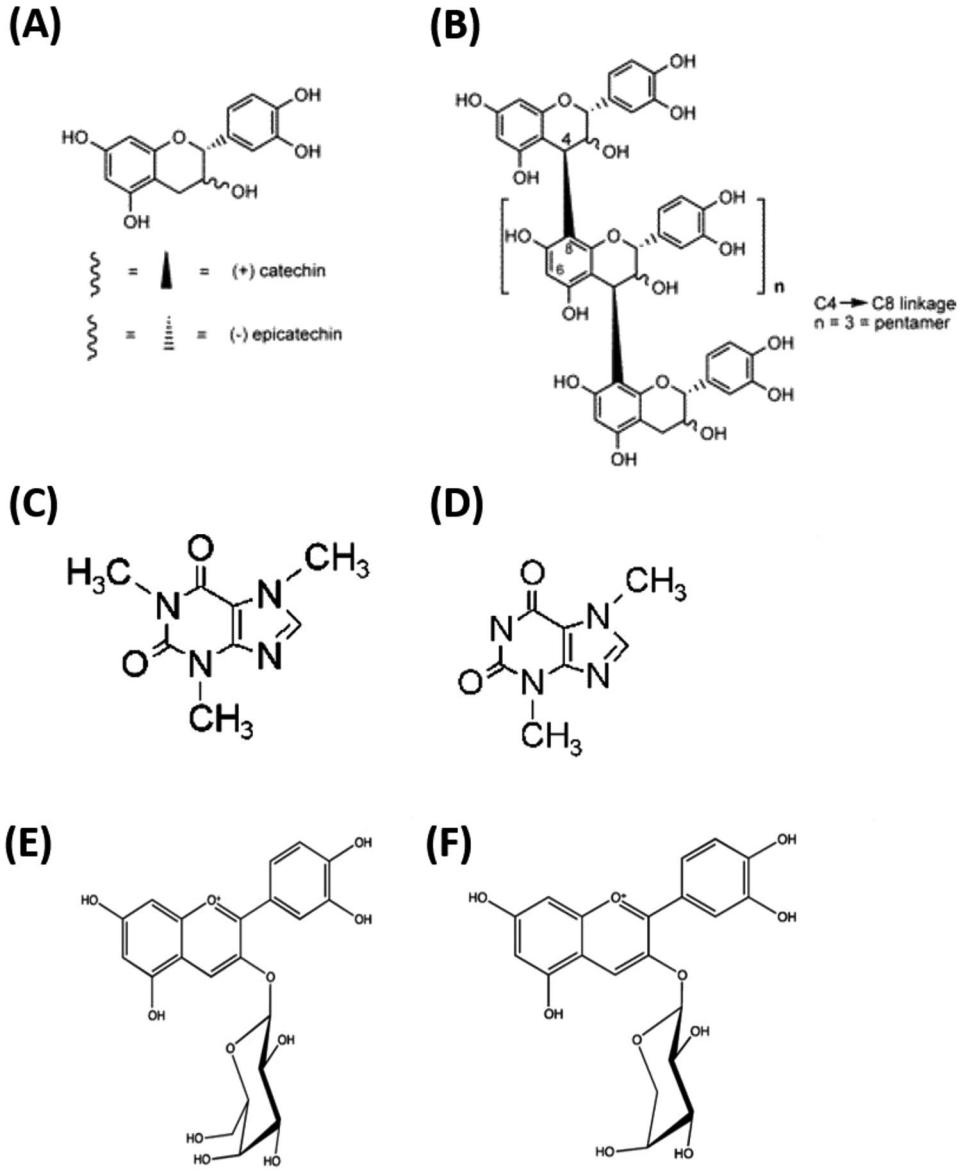

Figure 2. Some representative polyphenols in cocoa: (A) Chemical structures of (–)-epicatechin and (+) catechin, the major flavan-3-ol monomers in cocoa beans; (B) Chemical structure of B-type proanthocyanidin (PA), mainly composed of (–)-epicatechin; (C) Chemical structure of caffeine; (D) Chemical structure of theobromine; (E) Chemical structure of cyanidin-3-galactoside; (F) Chemical structure of cyanidin-3-arabinoside. (Caligiani et al. 2010; De Taeye et al. 2016; Kelm et al. 2006) (Reprinted with permission from American Chemical Society).

Hydroxybenzoic and hydroxycinnamic acids in cocoa beans included gallic, protocathechuic, chlorogenic, ferulic, caffeic and coumaric acids. Caffeoyl, feruloyl, and *p*-coumaryl-based amino acid conjugated derivatives were the frequently detected NPA in cocoa beans (D'Souza et al. 2017).

Total phenolic acid contents of different cocoa genotypes from Indonesia ranged from 2.03–6.19 g chlorogenic acid equiv/kg DB, with caffeoyl aspartic acid being predominant followed by *p*-coumaryl aspartic acid, clovamide and *p*-coumaryl tyrosine (Febrianto and Zhu 2019a). Phenolic acids were prone to bond with high molecular weight fraction of melanoidins in roasted cocoa beans, which could be separated by means of acid and alkaline hydrolysis (Oracz and Nebesny 2019).

4.5.3. Effect of conventional processing on polyphenols

4.5.3.1. Effect of fermentation. Concentrations of flavan-3-ols, anthocyanins, phenolic acids and flavones were generally reduced as the fermentation degree increased (Albertini et al. 2015; Elwers et al. 2009; Febrianto and Zhu 2020; Urbańska et al. 2019). The reduction of anthocyanin and epicatechin contents during fermentation was more drastic than that of flavonols and phenolic acid NPA (Febrianto and Zhu 2020). The reduction of polyphenols was mostly associated with the activity of PPOs during fermentation (Muñoz et al. 2020). Epicatechin concentration in cocoa beans was drastically decreased during fermentation (Elwers et al. 2009; Febrianto and Zhu 2020). The oxidation of free PAs resulted in the formation of quinones which are reactive toward proteins. PAs could also bind with insoluble CWM (Zhu 2018). Due to these mechanisms, free PAs in cocoa beans are largely converted into NEPA.

4.5.3.2. Effect of roasting. The dynamics of bioactive compounds during roasting was intensively studied. Roasting

significantly reduced the polyphenol content and changed its composition (Urbańska et al. 2019; Żyżelewicz et al. 2016). Roasting reduced the amounts of total flavan-3-ols and NPAs in cocoa beans (temperature-relative humidity dependent) (Oracz and Nebesny 2019). Żyżelewicz et al. (2016) found increased catechin content during 20 min of roasting at 135 and 150 °C (5% of relative humidity, velocity = 0.5 and 1 m/s) by 250% and 375%, respectively. Such increases may be induced by the epimerization of flavan-3-ol monomers and PAs. Polymerization of low-molecular-weight compounds may occur, increasing the level of PAs with high degree of polymerization (Urbańska et al. 2019).

4.5.4. Effect of non-conventional processing on polyphenols

4.5.4.1. Blanching. Blanching of wet cocoa beans can be done right after harvesting to preserve the polyphenols in cocoa beans. Blanching is carried out by dipping wet cocoa beans in hot water (95 °C) for 5 min before drying. The increase of the temperature in the inner part of the cocoa beans helped to inactivate the PPOs, preventing the degradation of the polyphenols. (González-Barrio et al. 2020). The unroasted cocoa powder obtained from this process contained significantly more flavanols (44.6 g/kg WB) than that obtained using the conventional processing method (3.5 g/kg WB) (González-Barrio et al. 2020).

4.5.4.2. "Lavado" processing. Lavado process can produce cocoa beans with high polyphenol content. For example, the cocoa beans produced contained more polyphenols (up to 3 times higher) and more flavan-3-ols (up to 5 times higher) than fully fermented cocoa beans (Albertini et al. 2015; Febrianto and Zhu 2020). The extract from the "lavado" processed cocoa beans had more catechin, epicatechin than naturally processed (fermented) cocoa beans (Ciaramelli et al. 2021). The proanthocyanidin content of "lavado" processed beans was 3.7 times higher than that of fermented beans (Chin et al. 2013).

4.6. Alkaloids

Theobromine and caffeine were the major methylxanthines in cocoa beans (Figure 2) (Martínez-Pinilla, Oñatibia-Astibia, and Franco 2015; Urbańska et al. 2019). Theobromine and caffeine are responsible for psychoactive potential of cocoa and partially contribute to bitter taste (Stark, Bareuther, and Hofmann 2006). The concentration of theobromine was higher than that of caffeine (2.85–3.95% and 0.3–0.6% ff DM, respectively) (Aprotosoaie, Luca, and Miron 2016). Among different varieties of cocoa, *Forastero* cocoa beans contained the highest amount of theobromine, followed by *Trinitario* and *Criollo*. On contrary, caffeine concentration was the highest in *Criollo* cocoa beans, followed by *Trinitario* and *Forastero* (Aprotosoaie, Luca, and Miron 2016).

4.6.1. Effect of conventional processing on alkaloids

4.6.1.1. Effect of fermentation. Fermentation reduced theobromine and caffeine contents of cocoa beans (Febrianto and Zhu 2020; Hernández-Hernández et al. 2018; Júnior et al. 2020). The reduction of theobromine during fermentation was caused by its diffusion to husk of cocoa beans, resulting in elevated concentration of theobromine in the husk samples (Hernández-Hernández et al. 2018). Theobromine and caffeine could also migrate into cocoa butter (Aprotosoaie, Luca, and Miron 2016). The ratio of theobromine and caffeine concentration was relatively stable during fermentation, which can be used as indicator to categorize cocoa beans regardless of the post-harvest process applied (Febrianto and Zhu 2020).

4.6.1.2. Effect of roasting. Roasting reduced the alkaloid content in cocoa beans. During roasting, theobromine and caffeine could bond with diketopiperazines, resulting in lower concentration of free alkaloids (Aprotosoaie, Luca, and Miron 2016).

4.6.2. Effect of non-conventional processing on alkaloids

4.6.2.1. Pulp conditioning. Reduced amount of theobromine and increased amount of caffeine were found in cocoa beans that were previously treated with pulp conditioning (spreading/pre-drying, pressing, and pod storage) (Nazaruddin et al. 2006). Caffeine may be released from the cells due to physical disruption, leading to better solvent extraction and higher caffeine content measured (Pham et al. 2019). Fermentation-like activity during pod storage may induce bio-detheobromination by microorganisms (Mat et al. 2016; Oduro-Mensah et al. 2018; Wang et al. 2005).

4.6.2.2. Modified fermentation. Different types of starter culture led to different alkaloid composition in Malaysian cocoa beans. Ghanaian cocoa beans (obtained from hybrids of *Forastero*) fermented using the starter culture containing *Saccharomyces cerevisiae* H5S5K23, *Limosilactobacillus fermentum* 222 and *Acetobacter pasteurianus* 386B had more caffeine and theobromine than the cocoa beans obtained from spontaneous fermentation (Lefeber et al. 2012). The use of *Candida* sp. for the fermentation resulted in the absence of theobromine at the end of fermentation (Mat et al. 2016). The mechanism of alkaloid degradation is still unclear. It was reported that some microorganisms from the genus of *Aspergillus*, *Rhizopus* and *Mucor* exhibited the ability to degrade theobromine (bio-detheobromination) and to facilitate caffeine formation (Mat et al. 2016; Oduro-Mensah et al. 2018; Wang et al. 2005).

4.6.2.3. Alkalization. Alkalization significantly reduced the content of methylxanthines in cocoa beans. The reduction was contributed by the formation of salts or the interactions of the methylxanthines with the alkali agent (García, Esteve, and Baviera 2020).

4.7. Vitamins and minerals

Cocoa is a source of vitamin D_2. Ergosterol, a precursor of vitamin D_2 was produced mainly during drying of cocoa beans by means of fungal activities. Analysis of various cocoa products from Germany showed that raw cocoa beans contained 2 µg/kg (DB) of vitamin D_2, whereas dark chocolate had 39.5 µg/kg (DB) (Kühn et al. 2018). Great diversity of vitamin D_2 contents may be due to the effect of processing and formulations of the products. Cocoa butter contained vitamin E mainly in the form of γ-tocopherol, while α, β, and δ-tocopherols were also present in small quantities (Oracz, Nebesny, and Żyżelewicz 2014).

Cocoa beans are rich in potassium, magnesium, phosphorus and calcium. Depending on pod storage conditions, potassium was the predominant mineral in Ghanaian cocoa beans (2,071–2,558 mg/100 g DB), followed by magnesium (262–364 mg/100 g DB), phosphorus and calcium (196–382 and 140–171 mg/100 g DB, respectively). Sodium, zinc, copper, and iron were also found in low concentration (<20 mg/100 g DB) (Afoakwa et al. 2013).

4.7.1. Effect of conventional processing on vitamins and minerals

4.7.1.1. Effect of fermentation. Fermentation of fresh cocoa beans reduced the concentrations of Fe, Cu, Na, and P, and increased that of Mg, Zn, Ca, and K (Afoakwa et al. 2013). Fermentation may help the release of minerals from cellular matrices of the cocoa beans through enzymatic actions. Their utilization by microorganisms may also contribute to the changes of the mineral composition (Gadd 2010).

4.7.1.2. Effect of roasting. Roasting of cocoa beans under low temperature (110 °C) (air humidity of 5%) increased the concentrations of δ- and γ-tocopherols in cocoa beans. High temperature roasting (150 °C) led to degradation of tocopherols, while the use of humid air for roasting helped preserve tocopherols (Oracz, Nebesny, and Żyżelewicz 2014).

4.7.2. Effect of non-conventional processing on minerals and vitamins

4.7.2.1. Pulp conditioning. Pod storage reduced the concentrations of Fe, Zn, and Na, while increasing that of Cu, Mg, Ca, P, and K. Longer duration of pod storage led to further changes in the mineral composition, which was contributed to the activities of microorganisms (Afoakwa et al. 2013).

4.7.2.2. Alkalization. The use of sodium based alkali agents increased the Na content of alkalized cocoa powder. No significant difference was found in other mineral contents between natural and alkalized cocoa powders (Adeyeye 2016).

4.8. Volatiles

Volatile compounds contribute to aroma and flavor of cocoa products (Aprotosoaie, Luca, and Miron 2016; Frauendorfer and Schieberle 2019; Ziegleder 2017). Cocoa volatiles are mostly composed of carboxylic acids, alcohols, aldehydes, esters, ketones, ethers, hydrocarbons and pyrazines. Volatiles in cocoa beans are present naturally and are developed during the processing of cocoa beans. Volatiles can be generated through microbial and biochemical activities during fermentation and from Maillard reactions during drying and roasting (Stark, Bareuther, and Hofmann 2006; Kadow 2020; Febrianto and Zhu 2020; Frauendorfer and Schieberle 2019; Muñoz et al. 2020). The pulps of cocoa beans were rich in alcohols, esters, ketones and terpenes volatiles (Hegmann et al. 2020). Carboxylic acids, alcohols, esters and ketones were dominant in raw fermented cocoa beans, while unfermented cocoa beans contained mostly of alcohol volatiles (Aprotosoaie, Luca, and Miron 2016; Febrianto and Zhu 2020). Total volatile content of cocoa mass was in range of ~5–200 mg/kg DB (calculated as 1-octen-3-ol equivalent), depending on the fermentation duration (Febrianto and Zhu 2020). Another study showed that total volatile content was more than 330 mg/kg WB in roasted cocoa beans (Frauendorfer and Schieberle 2019). The difference could be due to several factors such as the genetic variation of the samples and the use of different standards for quantification (e.g., octen-1-ol or others) in different studies.

Among numerous identified volatiles in cocoa beans, at least 30 compounds significantly contribute to the aroma of cocoa powder (Frauendorfer and Schieberle 2019). Acetic acid was the most abundant volatile in roasted cocoa beans with the highest odor activity value (OAV, concentration of odorant divided by its threshold value) of more than 2660. Other volatiles with high OAV included 3-methylbutanal (>2000), 3-methylbutanoic acid (>440), phenylacetaldehyde (>245) and ethyl-2-methylbutanoate (>135) (Frauendorfer and Schieberle 2019). The presence of *n*-methylpropanoic acid and *n*-methylbutanoic acid was related to over-fermentation of cocoa beans, contributing to hammy, sweat and rancid aroma (Ziegleder 2017). Aldehydes and pyrazines contributed to unique chocolate aroma. They were mostly developed during roasting process (Frauendorfer and Schieberle 2019).

4.8.1. Effect of conventional processing on volatiles

4.8.1.1. Effect of fermentation. Microbial actions during fermentation may led to enhanced flavor characteristics of cocoa beans. The concentrations of important volatiles such as some aldehydes, ketones, carboxylic acids and pyrazines increased during fermentation (Cevallos-Cevallos et al. 2018; Febrianto and Zhu 2020; Rodriguez-Campos et al. 2012). Acetic acid is a major volatile produced by acetic acid bacteria (Aprotosoaie, Luca, and Miron 2016). Prolonged fermentation increased the diversity of volatiles in roasted samples (Febrianto and Zhu 2020). On the other hand, over-fermentation of cocoa beans resulted in detrimental effect on cocoa beans due to the formation of off-flavor volatiles (Ziegleder 2017).

4.8.1.2. Effect of roasting. Roasting induced the formation of flavor compounds in cocoa beans mainly through Maillard

reactions (Frauendorfer and Schieberle 2019; Ziegleder 2017). The diversity of volatiles in roasted fermented cocoa beans was similar or larger compared to the unroasted fermented samples (Febrianto and Zhu 2020). The amount of total key volatiles of roasted cocoa beans was generally lower than unroasted samples, mainly due to large reduction of acetic acid during roasting (Febrianto and Zhu 2020; Frauendorfer and Schieberle 2019). The increase in concentrations of aldehydes and pyrazines was the most prominent, contributing to roasty, nutty, malty, honey-like and caramel-like aroma (Febrianto and Zhu 2020; Frauendorfer and Schieberle 2019). However, the reduction of such compounds during roasting could occur due to limited availability of precursors in cocoa beans (Ziegleder 2017). Roasting process can be done using whole beans, nibs and cocoa mass/liquor. Off flavors such as volatile acids can be better evaporated using the latter (Ziegleder 2017).

4.8.2. Effect of non-conventional processing on volatiles

4.8.2.1. Pulp conditioning. Storage of cocoa pods resulted in pulp volume reduction and discoloration of pods due to microbial activities (Hinneh et al. 2018). Pod storage for 7 days had similar effects on "fermentation" by developing the "sweating" (leaching of cocoa pulp) phenomenon and strong alcoholic odor. After fermentation and roasting, the obtained cocoa beans had more ketones and pyrazines than those from 3 days of storage and the beans without storage (Hinneh et al. 2018).

4.8.2.2. Modified fermentation. The use of inoculum to assist fermentation may improve the flavor profiles of cocoa beans. The mechanism was related to the increased amounts of flavor precursors (as mentioned in sections 3.3.5.2 and 3.4.2.2) and also to the metabolites produced by yeast and microbial fermentation. Visintin et al. (2017) reported that the addition of *S. cerevisiae* and *T. debrueckii* resulted in higher contents of 2-acetylpyrrole, phenethyl acetate, and 2-phenyl ethanol. The use of natural flavoring agents such as fruit juice and plant parts during the fermentation to enrich the flavor of cocoa beans has also been reported (Dario and Eskes 2009).

4.8.2.3. Incubation and re-fermentation. The addition of flavor precursors to cocoa beans/mass during incubation facilitates flavor development during roasting. The addition of proline, rhamnose, and fructose increased the formation of 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 2-acetyl-1-pyrroline in cocoa beans during roasting, resulting in stronger caramel, biscuit, and cookie flavors in the cocoa mass (Hansen et al. 2004). Re-fermentation of dried cocoa beans can reactivate some enzymes in under-fermented cocoa beans (Misnawi, Nazamid, and Jamilah 2002). These enzymes facilitate the formation of flavors precursors in dried cocoa beans required for flavor formation during roasting.

4.8.2.4. Alkalization. Alkalization affects flavor composition of cocoa beans through several mechanisms. The addition of NaOH solution (1–3%) significantly reduced the amount of acetic acid formed, reducing the intensity of the acidic off-flavor. Alkalization also reduces the amounts of flavor precursors such as amino acids and reducing sugars, reducing the degrees of Maillard reactions during roasting. This resulted in less cocoa flavor compounds in the alkalized cocoa beans. Alkali agent addition also gave alkaline flavor in the alkalized cocoa powder (García, Esteve, and Baviera 2020). Alasti et al. (2020) found that alkalization helped to balance the flavor profile of cocoa powder by facilitating the formation of 2,3,5-trymethylpyrazine during roasting.

4.9. Melanoidins

Melanoidins are high molecular weight compounds produced from Maillard reactions during cocoa bean roasting. The formation of melanoidins during roasting is affected by several factors. Quiroz-Reyes and Fogliano (2018) found elevated concentration of melanoidins in the samples with a high amount of sugars and a low catechin content. Oracz and Zyzelewicz (2019) showed that various phenolic compounds such as catechin, epicatechin, protocatchualdehyde, ellagic acid and *N*-phenylpropenoyl-L-amino acid were present in bonded form with melanoidins. These phenolic compounds could be released under acidic condition, increasing the nutritional value of cocoa beans.

4.9.1. Effect of conventional processing on melanoidins

4.9.1.1. Effect of fermentation. Fermentation is mainly responsible for generating reducing sugars and amino acids required for Maillard reactions and production of melanoidins (Kongor et al. 2016).

4.9.1.2. Effect of roasting. Higher roasting temperature and lower humidity environment promoted formation of melanoidins in cocoa beans (Oracz and Nebesny 2019; Sacchetti et al. 2016). For example, almost 2-fold difference in melanoidin content was found in cocoa beans roasted at high temperature (~16 µmol/g DB, 145 °C) than at low temperature (~9 µmol/g DB, 125 °C) (Sacchetti et al. 2016).

4.9.2. Effect of non-conventional processing on melanoidins

Formation of melanoidins during roasting may be reflected by the changes of its precursors affected by non-conventional processing (as discussed in Sections 3.3.5.2 and 3.4.2.2). More studies regarding the formation of melanoidins affected by non-conventional processing are still required.

4.10. Isoprostanoids (phytoprostanes and phytofurans)

Phytoprostanes (PhytoPs) and phytofurans (PhytoFs) in low concentrations (221–1589 and 1.18–13.1 ng/g WB, respectively) were detected in dry cocoa beans. They were formed

from lipid peroxidation of α-linoleic acid (León-Perez et al. 2019). PhytoPs are biologically active, mainly accumulated in plant tissues due to oxidative stress. PhytoPs have immunomodulatory effect and can affect the development of brain and central nervous system (Collado-González et al. 2015). However, further study on the effect of post-harvest processing on PhytoPs and PhytoFs is required.

4.11. Undesirable compounds

4.11.1. Oxalates

Raw cocoa beans varying in maturity stages contained 6.0–6.7 mg/g DB of oxalates. Most of them were soluble oxalates (5.19–6.03 mg/g DB) (Nguyễn, Lê, and Savage 2018). Chocolate products contained a notable amount of oxalates (∼7.29 mg/g DB) with ∼64% of soluble and ∼36% of insoluble oxalates (Schroder, Vanhanen, and Savage 2011).

4.11.2. Polycyclic aromatic hydrocarbon

Benzo[*a*]pyrene, benzo[*a*]antrhacen, chrysene, benzo[*b*]fluoranthene are polycyclic aromatic hydrocarbons (PAHs) in cocoa beans and related products. The PAHs concentration in cocoa and chocolate products from the market of Asia, Africa and America (year of 1999–2102) (5.88–10.1 µg/kg fat) was lower than the limit set by European Union (EU No 835/2011) (30 µg/kg fat) (Raters and Matissek 2014).

4.11.3. Acrylamide

Acrylamide is a Maillard reaction product in cocoa formed during roasting. Acrylamide contents of cocoa and its products in Germany were low (<490 µg/kg WB) (Raters and Matissek 2018). Semi-finished cocoa product such as cocoa mass, cocoa nibs and cocoa powder were found to contain more acrylamide (190 µg/kg WB) than chocolate and finished cocoa-containing products (50 µg/kg WB), mainly due to the addition of other ingredients such as milk and sugars (Raters and Matissek 2018).

4.11.4. Effect of processing on undesirable compounds

4.11.4.1. Effect of fermentation. Fermentation reduced the content of oxalate in cocoa beans, whereas drying had little effect on that (Nguyễn, Lê, and Savage 2018). The use of pectinase during fermentation assisted the release of soluble oxalate from cocoa beans (Nguyễn, Lê, and Savage 2018). It was proposed that lactic acid bacteria and *Bacillus subtilis* are responsible for the degradation and the production of oxalate content in cocoa beans during fermentation, respectively (Nguyễn, Lê, and Savage 2018).

4.11.4.2. Effect of roasting. Higher temperature and prolonged time of roasting lead to the formation of acrylamide. Żyżelewicz et al. (2017) found that whole bean roasting resulted in higher level of acrylamide in cocoa beans compared to that of nibs roasting. This could be caused by longer time required in whole bean roasting. Roasting could increase and decrease the concentration of PAHs. Cocoa beans roasted in high temperature (150 °C) contained higher concentration of PAHs compared to that of roasted in lower temperature (135 °C) (Żyżelewicz et al. 2017).

4.11.4.3. Strategies to reduce the undesirable compounds. Several strategies may be used to reduce the formation of undesirable compounds in cocoa beans. High hygiene standards on cocoa handling by farmers and industries should be maintained to prevent PAHs contamination. Drying of the cocoa beans by burning fossil fuel and woods may be replaced by sun-drying (Misnawi 2012). Low-temperature roasting may lower the formation of PAHs and acrylamide (FoodDrinkEurope 2019). Addition of calcium salt and ultrasonication of cocoa powder could reduce the oxalate content (Huynh, Nguyen, and Nguyen 2020). Calcium could bind soluble oxalate to form insoluble oxalate complexes. Ultrasonication induces sonolytic degradation of the oxalates. However, the application of such techniques should be better studied since they may also affect other components in cocoa powder.

4.12. Comparative studies on chemical composition of cocoa beans of different genotypes, origins, and degrees of fermentation

Comparative studies of chemical composition of different cocoa beans were done (D'Souza et al. 2017; Elwers et al. 2009; Kumari et al. 2018; Lebot et al. 2020; Sirbu et al. 2018). Among different cocoa varieties, *Forastero* cocoa beans contained more polyphenols such as flavan-3-ols and anthocyanins than *Trinitario* and *Criollo*. The latter contained more alkaloids with much less anthocyanins (D'Souza et al. 2017; Elwers et al. 2009). Different cocoa varieties (*Criollo, Forastero, Amelonado, Trinitario* and hybrids) cultivated in Vanuatu had high caffeine contents (0.1–1.3% DB). West African bulk cocoa liquor had more volatiles than fine Ecuadorian cocoa liquor (Tuenter et al. 2020). Cocoa beans from Brazil, Ecuador, Indonesia and Malaysia contained relatively less lipids than those of Tanzania and Ivory Coast (Sirbu et al. 2018). Ecuadorian cocoa butter had more POP and less POS and SOS than that from Venezuela and Malaysia. Indonesian, Tanzanian and Brazilian cocoa butter were characterized by higher proportions of minor TAGs such as PLS (1-palmitoyl-2-linoleoyl-3-stearoyl-glycerol), POO (1-palmitoyl-2,3-dioleoyl-glycerol), SLS (1,3-distearoyl-2-linoleoyl-glycerol) and SOO (1-stearoyl-2,3-dioleoyl-glycerol) (Sirbu et al. 2018). Fermented cocoa beans contained more peptides, reducing sugars, and volatiles, and less polyphenols and oxalates (D'Souza et al. 2018; Febrianto and Zhu 2020; Muñoz et al. 2020; Nguyễn, Lê, and Savage 2018). Great variations of polyphenol composition between cocoa varieties (*Forastero, Trinitario* and *Criollo*) indicated great intra-group diversity (Elwers et al. 2009; Febrianto and Zhu 2020). Indeed, there is high variability in the characteristics of the cocoa beans. The variability reflects specific characteristics of different cocoa beans, representing unique sensory experiences from its-derived products. This variability can support emerging taste-specific products such as those of

single-origin, single-variety/genotype, and specially processed products. However, the variability in the quality due to numerous factors (origin, processing methods, genotypes, and environment) may pose a problem for the industries seeking uniformity. In this context, ensuring the supply of cocoa beans with consistent quality may be the most critical.

Different experimental procedures used for the analysis contribute to the variation of chemical composition reported above. For example, the use of different AOAC methods (965.13, 948.22, and 989.05) for the determination of cocoa butter content was reported in different studies (Abdullahi et al. 2018; Afoakwa et al. 2013; Agus, Mohamad, and Hussain 2018, 2018; Djikeng et al. 2018; Grassia et al. 2019; Liendo, Padilla, and Quintana 1997; Salazar et al. 2020; Torres-Moreno et al. 2015). This contributed to different results of cocoa butter content. Comparison of those different methods using the same samples and apparatus (Soxtec) was reported. Servent et al. (2018) found that the cocoa butter content measured by AOAC 965.13 (with acid hydrolysis) was higher than that of AOAC 989.05 (with weak base hydrolysis) and AOAC 948.22 (without any hydrolysis). Acid hydrolysis before fat extraction (AOAC 965.13) releases lipids, leading to a higher estimation of the extracted fat. In industrial applications, AOAC 948.22 may be preferred since it accurately measures the amount of extractable fat of cocoa beans. For different studies on other components such as bioactives and flavors, the use of different solvents, standards, and instrumental conditions contributed greatly to the variations of data (Hinneh et al. 2018; Frauendorfer and Schieberle 2019). These sources of data variations could be minimized by using the same experimental procedures and conditions. On the other hand, moisture and fat contents should be included to ease the conversion between various calculation bases such as WB, DB, and ff DM. Overall, great variations of the chemical composition of cocoa beans are greatly affected by genotypes, origins, environmental conditions, processing and analytical methods used for their determination. Further studies could be focused on the variations of chemical compounds based on the more specific group classification to better explain the relation between cocoa genetic and their chemical composition. Analytical method should be standardized to provide unbiased comparison and results.

5. Effect of chemical composition of cocoa beans on the choice of post-harvest processing methods and conditions

There is significant variation of cocoa beans quality in the market. Common classifications of quality include “fine cocoa” and “bulk cocoa” (based on genotype), and “unfermented,” “partially-fermented/under-fermented” and “fully-fermented” (based of post-harvest processing) (Kongor et al. 2016; Misnawi, Nazamid, and Jamilah 2002). The industrial processing of cocoa beans is based on these classifications of quality. Primary post-harvest practices of cocoa beans include pod-breaking, bean extraction, fermentation, and drying (Figure 1B) (Aprotosoaie, Luca, and Miron 2016; Figueroa-Hernández et al. 2019; Kongor et al. 2016; Muñoz et al. 2020; Urbańska et al. 2019). Secondary processes of cocoa beans are mostly done by industries, where the processing may be varied from one to another. The secondary processes of cocoa beans include roasting, deshelling, grinding, and the separation of cocoa butter and cocoa mass (Aprotosoaie, Luca, and Miron 2016; Figueroa-Hernández et al. 2019; Kongor et al. 2016; Muñoz et al. 2020; Urbańska et al. 2019).

We argue here that the choice of processing should be made based on chemical composition of cocoa beans for maximum quality. Currently, different types and conditions of processing (e.g., different fermentation time, and different roasting temperature) have been done on the 3 major varieties of cocoa (*Criollo*, *Forastero* and *Trinitario*) to maximize their sensory quality. However, there are great variations of chemical composition between these cocoa varieties and even among different genotypes of the same cocoa group (Elwers et al. 2009; Febrianto and Zhu 2019b). Using the same general method of processing may not be suitable to include all the cocoa beans of different genetics and varieties. Thus, understanding of the interactions between cocoa genotypes/varieties, chemical composition and processing methods should be better developed.

There are some processing steps that can be used to improve the quality of cocoa beans and/or to preserve the bioactive compounds in cocoa beans. Table 3 summarizes the examples of non-conventional processing methods that can be applied differently based on the characteristics of cocoa beans, while the pathways of processing are presented in Figure 1A,C. Fresh Malaysian cocoa beans contained a high amount of pulp. Fermentation of such cocoa beans increased the acid production. This can result in cocoa beans with high acidity (Bangerter et al. 1991). The amount of pulp should be reduced prior to fermentation to limit acid formation during fermentation. The pulp removal can be done by mechanical de-pulping, pre-drying and pressing of cocoa beans prior to fermentation (Bangerter et al. 1991) Dried up cocoa beans with a low amount of pulp may hinder the fermentation process resulting in partially-fermented cocoa beans. In this case, addition of pulp from other sources (freshly harvested cocoa beans) or artificial pulp is necessary to ensure optimum fermentation (Lee et al. 2019).

Modified fermentation can be applied in cocoa beans instead of the commonly used spontaneous fermentation. Cocoa beans were mixed with starter culture, enzyme, and/or other substances such as fruit, fruit juice or aromatic plant-parts (Dario and Eskes 2009). The addition of starter culture containing yeast and/or lactic acid bacteria and/or acetic acid bacteria may increase the fermentation performance by improving sensory quality of cocoa beans which were more uniform (Figueroa-Hernández et al. 2019). Another alternative such as controlled incubation system for fermentation may open new possibility to tune the quality of cocoa beans (John et al. 2020). The controlled incubation system had temperature-controlled environment and special media (solution of ethanol, acetic acid and lactic acid) to

Table 3. Examples of non-conventional processing operations on different characteristics of cocoa raw materials.

Raw material	Pulp conditioning	Blanching	Fermentation	Washing	Dried bean post-processing	Alkalization	Roasting	Results	References
Fresh cocoa beans	Mechanical de-pulping	95 °C for 5 min	No	No	No	No	Heat treatment at 121 °C for 1 min on cocoa powder	Cocoa powder obtained from the treatment contained 12-fold more of flavan-3-ol monomers, 15-fold more of proanthocyanidin dimers and showed 4-times higher antioxidant activity (ORAC) compared to conventionally processed powder	González-Barrio et al. (2020)
Fresh cocoa beans with a high amount of pulp	No	No	5 days fermentation	No	No	No	Yes	Dried cocoa beans with high acidity (pH of nibs < 5)	Bangerter et al. (1991)
Fresh cocoa beans with a high amount of pulp	Pod storage 5–11 days	No	5 days fermentation	No	No	No	Yes	Chocolate prepared from the beans had improved flavor properties albeit being inconsistent	Bangerter et al. (1991)
Fresh cocoa beans with a high amount of pulp	Mechanical de-pulping (10–30% of pulp removal)	No	3 days fermentation	No	No	No	Yes	Dried cocoa beans with low acidity (pH > 5). Chocolate prepared from the beans had low acidity, less off-flavors and enhanced flavor	Bangerter et al. (1991)
Fresh cocoa beans (*Amelonado* × *Forastero* hybrid)	No	No	Modified fermentation with addition of fruit/plant material (juice/pulp)	No	No	No	Yes	Nibs of dried cocoa beans and chocolate prepared from the beans had fruity flavor resembling the fruit used during fermentation	Dario and Eskes (2009)
Fresh *Forastero* cocoa beans (German hybrid)	No	No	Fermentation with the addition of either yeast extract-based supplement or starter culture containing *S. cerevisiae* and *A. pasteurianus*. Fermentation was done at 30 and 45 °C for 7 days	No	No	No	120 °C for 30 min	Cocoa liquor produced from treated beans had unique characteristics. The addition of starter culture in fermentation media led to the formation of cocoa liquor with strong acidity and fruity flavor compared to that added with yeast extract-based supplement	John et al., (2019)
Fresh *Forastero* cocoa beans (German hybrid)	Pulp removal by washing using high pressure running water for 15 mins	No	Fermentation in solutions containing various concentrations of ethanol, acetic acid, and lactic acid. Fermentation was carried out at temperatures ranged from 25 to 60 °C for 2 days	No	No	No	No	Manipulation of fermentation conditions regulated the peptide and flavanol profiles after fermentation. Temperature, acetic acid concentration and incubation time were the most prominent factors affecting fermentation. Fermentation at $45 - 50$ °C could optimize the peptide diversity and quantity in cocoa beans	John et al. (2020)
Fresh cocoa beans	No	No	Fermentation using various yeasts. Fermentation was carried out in wooden boxes (45 kg) for 6 days	No	No	No	No	Cocoa beans obtained from the treatment contained lower acidity than the control. Cocoa beans fermented using *Rhodotorula mucilaginosa, Torulaspora delbrueckii, Candida parapsilosis,* and *Pichia galeiformis* culture contained more reducing sugars and free amino acids than that of control. Starter cultures containing *Candida parapsilosis, Torulaspora delbrueckii,* and *Pichia kluyveri* had potential to be used for flavor improvement	Santos et al. (2020)
Cocoa hybrid from Ghana, Ivory	No	No	Fermentation using starter culture of *L. fermentum* 222 and *A. pasteurianus*	No	No	No	140 °C for 30 min for chocolate production	The use of starter culture in fermentation yielded more consistent quality of cocoa beans compared to that of spontaneous	Lefeber et al. (2012)

(*continued*)

Table 3. Continued.

Raw material	Pulp conditioning	Blanching	Fermentation	Washing	Dried bean post-processing	Alkalization	Roasting	Results	References
Coast and Malaysia			386B with/without *S. cerevisiae* H5S5K23. Fermentation was done in heaps (Ghana and Ivory Coast) and wooden box (Malaysia) for 5 to 6 days					fermentation. With the use of starter culture, similar sensory characteristics of chocolates were obtained regardless of the origins and the fermentation methods (heaps or box) of the cocoa beans. The chocolate obtained from some of the fermentation with starter culture showed enhanced sensory properties than the control (spontaneous fermentation)	
Defatted cocoa powder of dried unfermented and under-fermented cocoa beans (hybrid variety).	No	No	no	No	Incubation in acetate buffer, pH 5.5 at 45 °C for 16 h	No	No	Incubated cocoa powder showed improved fermentation index (from < 0.9 to > 1.2) to a similar level with cocoa powder from fermented cocoa beans (1.58). Incubated cocoa powder also showed increased amounts of free amino acids and sugars to a similar level with cocoa powder from fermented cocoa beans	Misnawi, Nazamid, and Jamilah (2002)
Dried unfermented and under-fermented cocoa beans (hybrid variety)	No	No	Unfermented = 0 days fermentation; Under-fermented = 2 days fermentation	No	Incubation in distilled water for 16 h at 45 °C. Incubation was carried out with or without polyphenol oxidase or tyrosine enzyme enrichment	No	No	Incubated cocoa beans had increased fermentation index from 0.6 to 0.8 (unfermented), and from 0.9 to 1 (under-fermented) compared to 1.6 in full-fermented beans. Cut test score was also improved by at least 30% in under-fermented beans and 50% in unfermented beans	Misnawi, Jamilah, and Nazamid (2003)
Under-fermented cocoa nibs	No	No	Yes	No	Incubation with various proteases and carboxypeptidases at 50 °C for 8 h	No	120 °C for 12 min	Significant improvements in sensory properties were obtained. The use of enzymes from microbe and vegetal-origin resulted in a cocoa liquor with the highest acceptance	Oliveira et al. (2011)
Cocoa liquor	No	No	Yes	No	Incubation with the addition of proline and rhamnose	No	Yes	Incorporation of cocoa liquor obtained from treatment in chocolate resulted in the increase of caramel and biscuit flavor attributes	Hansen et al. (2004)
Dried unfermented cocoa	No	No	No	No	Incubation with artificial cocoa pulp medium. The incubation was carried out in controlled temperature (25–48 °C) for 6 days	No	No	The use of artificial cocoa pulp as medium could mimic on-farm fermentation conditions. The cocoa beans obtained had increased levels of ethanol, acetic acid, lactic acid and volatiles, and decreased level of polyphenols	Lee et al. (2019)
Commercial cocoa powder	n.i	n.i	n.i	n.i	Solvent extraction using aqueous ethanol with different concentrations and ratios (50–80% w/v, 5–20 weight to volume ratios). The extraction was done at temperature between 35–40 °C for at least 2 h. The extract was then purified by filtration/centrifugation, vacuum evaporation, liquid-liquid extraction and column chromatography	n.i	n.i	The purified extracts had more flavan-3-ols than unpurified extract (23.7% to 3.4%, respectively) and more polyphenols (90.1% gallic acid equivalent to 31%, respectively)	Rovati (2016)

Nibs of Cameroon *Forastero* cocoa beans	n.i	n.i	Yes	n.i	n.i	Alkalization using NaOH, K_2CO_3, and NH_4HCO_3 at various concentrations	130 °C for 20 min	Changes in color of powdered cocoa nibs were observed. The color varied from reddish brown to blackish brown in alkalized samples compared to light brown in non-alkalized powder. Alkalized cocoa powder had more balanced aromatic profile shown by a lower ratio of 2,3,5,6-tetramethylpyrazine to 2,3,5-trimethylpyrazine (0.8–1.1) than non-alkalized powder (1.2)	Alasti et al. (2020)

n.i: no information regarding this process in the study.

facilitate the fermentation of the cocoa beans (John et al. 2020).

Unfermented or partially-fermented cocoa beans do not have enough flavor precursors to generate flavor volatiles during roasting for sensory quality (Aprotosoaie, Luca, and Miron 2016; Kongor et al. 2016). Additional processing of the dried cocoa beans can be used to generate flavor precursors prior to roasting process. The additional processing steps include one or the combination of: (1), enzymatic treatment using protease and/or invertase to generate flavor precursors in cocoa beans (US5888562A 1999); (2), re-fermentation through incubation to re-activate the remaining key enzymes in cocoa, which would assist the development of flavor precursors in cocoa beans (Misnawi, Nazamid, and Jamilah 2002); and (3), the infusion of flavor precursors from external source such as rhamnose and proline (Hansen et al. 2004).

Cocoa beans of different varieties require different roasting conditions. *Criollo* cocoa beans are roasted in gentler conditions. This is mainly to preserve the aromatic volatiles in the beans. On the other hand, *Forastero* cocoa beans are roasted under more intense conditions mainly to yield higher chocolate-related aroma by means of optimizing Maillard reactions (Kongor et al. 2016). The characteristics of *Trinitario* cocoa beans could be similar to either that of *Criollo* or *Forastero* or between that of *Criollo* and *Forastero* (Elwers et al. 2009; Febrianto and Zhu 2019b). *Criollo*-like *Trinitario* cocoa beans may be processed using the *Criollo*-based processing method, while *Forastero*-like *Trinitario* can be processed using the *Forastero*-based processing method. For the *Trinitario* cocoa beans similar to neither *Criollo* nor *Forastero*, special conditions of processing method should be developed based on those of *Forastero* and *Criollo* processing.

Unsuitable processing conditions of post-harvest practices used in different regions may produce cocoa beans with a high amount of off-flavors. High amounts of acetic and lactic acids in cocoa beans lead to the excessive sour taste of cocoa beans (CAOBISCO/ECA/FCC 2015). High amounts of volatiles responsible for musty and moldy flavor could be found in over-fermented and moldy cocoa beans (CAOBISCO/ECA/FCC 2015). Alkalization of cocoa beans is required to reduce the amounts of acetic acid and other acids as well as moldy notes (García, Esteve, and Baviera 2020). Chocolate/liquid cocoa mass produced with these cocoa beans may also require conching process at high temperature to efficiently reduce acetic acid level in the products (Ziegleder 2017).

The preservation of polyphenols can be achieved through bean blanching and without fermentation and roasting steps (González-Barrio et al. 2020). Cocoa beans of *Forastero* and *Forastero*-like *Trinitario* are more suitable to be processed using this method than those of *Criollo*. However, cocoa beans with a high content of polyphenols are unpalatable. Methods to optimize both of health promoting potential and flavor of cocoa beans should be used. Isolation of polyphenols via solvent extraction may be conducted to obtain polyphenol-rich cocoa extracts for subsequent usage (Rovati 2016). Incubation or re-fermentation of cocoa cake residue using enzymes and/or flavor precursors may be then used before roasting to improve its palatability.

6. Biological activities of cocoa beans

6.1. In vitro studies

In vitro bioactivities of cocoa and cocoa-related products have been analyzed using chemistry and cell based assays (Table 4).

6.1.1. Antioxidation

Antioxidant activity (AA) of cocoa-related samples was commonly measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), total reactive antioxidant potential (TRAP), and oxygen radical absorbance capacity (ORAC) assays. AA of cocoa beans is contributed mainly by polyphenols (Pedan, Fischer, and Rohn 2016) and Maillard reaction products (Ioannone et al. 2015). Pedan, Fischer, and Rohn (2016) found that methylxanthines (such as theobromine and caffeine) had no reactions with DPPH radicals. Theobromine and caffeine showed AA in calf thymus DNA (*in-vitro*) by inhibiting oxidative DNA breakage and the generation of hydroxyl radicals (Azam et al. 2003). Thus, the contribution of alkaloids to the AA was dependent on the types of assays. Maillard reaction products may contribute to the AA by their high antioxidant activities (Ioannone et al. 2015). Melanoidin bound phenolics were naturally-occurring antioxidant compounds in chocolate (Oracz and Nebesny 2019). Baranowska et al. (2020) showed that AA of cocoa ethanolic extract was due to synergistic effects of catechin, epicatechin, gallocatechin, procyanidin B1, procyanidin trimer, quercetin, and protocatechuic acids.

6.1.2. Anti-cancer

Cocoa bioactive compounds exhibited anti-proliferation against various cancer cells. Cocoa extract increased the apoptosis rate of human lung carcinoma cell line (A549 cells). The addition of cocoa extracts (100 µg/mL) reduced the viability of A549 cells by up to 40–50% in 48 h (Bauer et al. 2016). Several mechanisms of anti-cancer properties of cocoa have been discussed. Epicatechin increased the production of intracellular reactive oxygen species (ROS) and upregulated pro-apoptotic protein expression, resulting in the decrease of the viability of MDA-MB-231 and MCF-7 breast cancer cells. Epicatechin also upregulated death receptor (DR4/DR5) in MDA-MB-231 cells (Pereyra-Vergara et al. 2020).

6.1.3. Anti-microbial

Cocoa extract showed anti-microbial activity toward various Gram negative and Gram positive bacteria. Gram negative bacteria was more sensitive to phenolic content of cocoa extract than Gram positive bacteria (Todorovic et al. 2017). Lyophilized extract of cocoa beans inhibited the growth of *Helicobacter pyroli* with a minimum inhibitory concentration (MIC) of 80–90 mg/mL (Lawal, Olorunnipa, and Adeniyi 2014). Pectin from cocoa pod husk also possessed moderate anti-microbial activities against *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Shigella* spp., *Staphylococcus aureus, Bacillus subtilis*, *Enterococcus* spp., and *Listeria* spp. (Adi-Dako et al. 2016). Cocoa powder could be used as prebiotics to promote the growth of beneficial lactic acid bacteria, while inhibiting the growth of foodborne pathogens. The enrichment with 3% of cocoa powder in bacteria-specific media (MRS and lactose broth) time-dependently increased the number of *Lactobacillus casei, Lacticaseibacillus rhamnosus* and *L. plantarum* from 0.27–1 log CFU/mL, and inhibited the growth of *E. coli, S. typhimurium,* and *L. monocytogenes* (Peng et al. 2015). Cocoa extracts acted as anti-microbial agents through several mechanisms. Polyphenols can suppress the microbial growth by disrupting amino acid production and reducing cell membrane fluidity (Todorovic et al. 2017). Flavonoids and phenolic acids can reduce the flagellum and motility of microbial, limiting their attachment and invasion toward human intestinal cells (Peng et al. 2015). Methylxanthines inhibited the excision repair mechanisms in the microbes, mainly due to their abilities to bind single-stranded DNA (Azam et al. 2003).

6.1.4. Anti-inflammatory

Cocoa extracts were anti-inflammatory via various physiological mechanisms. Methylxanthines acted as phosphodiesterase inhibitors which downregulated the secretion of pro-inflammatory cytokines (Martínez-Pinilla, Oñatibia-Astibia, and Franco 2015). Proanthocyanidin B_2-rich water extract of cocoa powder reduced inflammatory cytokines (such as IL-5, IL-1β, IL-6, IL-8 and human IL-15) without interfering with cells viability (Kramer et al. 2019). PAs of cocoa inhibited inflammatory reactions by modulating cellular maintenance, and transcriptional activity and regulating the expression of V-ATPase-involved genes of dendritic cells (Midttun et al. 2018).

6.2. In vivo studies

In vivo biological activities of cocoa and cocoa-related products were obtained using both animal and clinical models (Table 5). These studies indicated versatile uses of cocoa as ingredient in making functional foods.

6.2.1. Antioxidation

The addition of the extract of raw and roasted cocoa beans in rat diet significantly increased the AA of water-soluble and lipid-soluble fractions of rat blood serum (Żyżelewicz et al. 2020). Analysis on myocardial tissue samples of ischemia-reperfusion (I/R) induced rats showed that the supplementation of cocoa extract lowered the levels of malondialdehyde, ROS, and nitrotyrosine in myocardial tissue. This indicated that oxidative stress and nitrosative stress was lowered (Ahmed et al. 2020). Cocoa prevented oxidative injury through several mechanisms. Flavonoids could interfere with inducible nitric-oxide synthase activity of macrophages, preventing overproduction of nitric oxides and

Table 4. *In vitro* studies of health effects of cocoa-related products.

Bioactivity	Sample information	Experimental	Major findings	Proposed key bioactive compounds	References
Antioxidation	50% acetone/water extract of unroasted cocoa beans	AA was analyzed by means of online NP-HPLC-DPPH, and DPPH assay. Correlations between PA, methylxanthines and AA were measured	High AA was measured for PA, while methylxanthines didn't show any AA. PA hump in the end of chromatogram showed the highest AA	Oligomeric (DP 2–10) and polymeric proanthocyanidin	Pedan, Fischer, and Rohn (2016)
Antioxidation	Extract of cocoa beans from 25 genotypes	AA was analyzed by DPPH assay, correlation between AA and TPC was measured	EC_{50} (DPPH) values were in the range of 4.83–9.69 mg of dry extract/mL. AA was significantly correlated ($R = -0.86$) with TPC	Theobromine, epicatechin and catechin	Hernández-Hernández et al. (2018)
Antioxidation	Four chocolate formulations were analyzed (raw-unroasted beans 70% and 85% and traditional-roasted beans 70% and 80%). Soluble fraction was prepared by acetone extraction and insoluble-bound fraction was obtained by extraction with diethyl ether and ethyl acetate after alkaline and acid hydrolysis	Methylxanthines, alkaloids and phenolic compounds were determined by using UHPLC. TPC was determined. AA was analyzed by DPPH, FRAP, ORAC and ABTS	Soluble fraction of raw samples showed higher AA compared to that of traditional sample with the same formulation. On contrary, insoluble-bound fraction of traditional samples showed higher AA compared to that of raw samples (measured by ABTS and FRAP). AA of soluble fraction was generally higher than that of insoluble-bound fraction	Epicatechin, catechin, protocatechuic acid	Mudenuti et al. (2018)
Antioxidation	Acetone/water/acetic acid extract of fermented cocoa beans obtained from different roasting temperature and duration	TPC (GAE) and AA (FRAP and TRAP) were analyzed. Flavanols and PA content were determined by means of RP and NP-HPLC analysis, respectively	Antioxidant activity of the roasted cocoa beans' extract was higher (~220 μmol TE/g for samples roasted at 125 °C for 60 mins) than that of unroasted (measured by TRAP)	Maillard reaction products	Ioannone et al. (2015)
Antioxidation	Clovamide and methanolic extract of raw and roasted cocoa beans, winnowed nibs and winnowed side products	Composition of methanolic extracts were analyzed using HPLC-DAD-ESI-MS^2. AA was measured using Hydrodynamic voltammetry at a rotating ring-disk electrode system	Raw cocoa beans had the highest AA, followed by roasted beans, winnowed nibs and winnowed side products. There was significant correlation between AA and clovamide content in the cocoa extract	Clovamide	Ye et al. (2021)
Antioxidation and anti-cancer	Cocoa extract of slate beans and fermented cocoa beans. Cocoa extract was prepared by means of extraction using methanol, methanol 50% and methanol 50%:acetone 70% (1:1).	AA was analyzed by DPPH, ABTS/TEAC, and FRAP. Cytotoxicity of the extract was determined in-vitro using human lung carcinoma cell line (A549)	Cocoa extract prepared using methanol 50%:acetone 70% (1:1) showed higher AA than other cocoa extracts. The extract of unroasted well fermented (UWF) cocoa beans showed the highest AA (DPPH and ABTS), while the extract of unroasted-slate cocoa beans showed highest FRAP value. UWF extract with concentration of 100 μg reduced the viability of A549 cells around 40% in 48 h after exposure. Treatment of A549 cells with the extracts of UWF and slate beans (10 mg/mL) resulted in significant increase of apoptosis rate	Unspecified	Bauer et al. (2016)
Antioxidation and anti-diabetic	Cocoa extract of natural *Forastero* cocoa powder (CPE) and epicatechin (EC). Cocoa extract was prepared by washing 1 g of cocoa powder with acidic methanol 50% and acetone 70%	Oxidative stress-induced human HepG2 cells were treated with 10 μM EC or 1 μg/mL CPE for 24 h. ROS production, GSH, protein oxidation, GPx, GR, CAT, GST, glucose uptake analysis and western blot assay were carried out	CPE and EC upregulated GSH level and normalized the level of ROS, protein carbonyl content, GPx, GR, CAT, and GST, indicating their protective effect toward oxidative stress in oxidative-stress-induced human HepG2 cells. CPE increased the activity of GPx and GR cells in normal HepG2 cells further stimulated the activation of Nrf2, indicating the responses of cell protection. CPE also modulated MAPKs, improved cellular redox status and insulin resistance of high-glucose-induced HepG2 cells	Epicatechin	Cordero-Herrera et al. (2014)
Antioxidation and anti-microbial	Acetone/water/acetic acid extracts of natural and alkalized cocoa powder	AA was analyzed by DPPH, ABTS TEAC, RACI and GAS methods. Anti-microbial activity was tested against *Staphylococcus aureus, S. epidermidis, Bacillus subtilis, Erscherichia coli, Klebsiella*	AA significantly correlated with total phenolic, total flavanol, proanthocyanidins and flavan-3-ols contents. AA of natural cocoa extracts were higher than that of alkalized. Natural cocoa extracts had higher anti-microbial activity toward Gram positive bacteria	Polyphenols and unspecified alkalization products	Todorovic et al. (2017)

(*continued*)

Table 4. Continued.

Bioactivity	Sample information	Experimental	Major findings	Proposed key bioactive compounds	References
		pneumoniae, Pseudomonas aeruginosa, Salmonella abony, and *Candida albicans*	than toward Gram negative bacteria. Cocoa extract from alkalized samples showed enhanced anti-microbial activity toward Gram negative bacteria than that of natural cocoa samples		
Anti-microbial	Pectin was isolated from cocoa pod husk (CPH) by means of hot aqueous and hot aqueous citric acid (4% w/v) extraction	Anti-microbial and anti-fungal activity of CPH pectin was measured in agar diffusion method against amoksiklav, ciprofloxacin and nystatin as positive control	CPH pectin (dose dependent, 1.25–10 mg/mL) showed moderate activity toward *S. aureus, P. aeruginosa, B. subtilis, E. coli, S. typhi* and *Shigella* spp. It also showed slight and weak activity toward *Enterococcus, A. niger* and *Listeria* spp	Pectin	Adi-Dako et al. (2016)
Anti-microbial	Cocoa methanolic extract	Cocoa methanolic extract was tested against 41 strains of *H. pyroli*. Agar diffusion method was used; determination of MIC and time-kill assay were carried out	The MIC of cocoa extract was in range of 80–90 mg/mL. A dose of 100 mg/mL of cocoa extract activities against *H. pyroli*. The use of cocoa extract at 2–4 times of MIC gave a similar activity to that of 40 μg/mL of ofloxacin (positive control) in *H. pyroli* strain BAA009 and BAA026	Unspecified	Lawal, Olorunnipa, and Adeniyi (2014)
Anti-microbial	MRS broth, LB broth, milk or skim milk enriched with defatted cocoa powder-enriched (3% w/v)	Viability assay was performed on *Lactobacillus casei, L. rhamnosus, L. plantarum, L. acidophilus, Bacillus subtilis, Enterococcus faecalis, Streptococcus thermophilus, Escherichia coli, Salmonella typhimurium*, and *Listeria monocytogenes*. Invasion assay was used using human intestinal INT407 cells	Cocoa powder supplementation increased the viability of *Lactobacillus, B. subtilis, E. faecalis*, and *S. thermophilus* and inhibited the growth, adhesive activities and invasive ability of *E. coli, S. Typhimurium*, and *L. monocytogenes*	Flavonoids and phenolic acids	Peng et al. (2015)
Anti-inflammatory	Water extract of natural cocoa powder	Cocoa extract was tested against Caco-2 cells line. Its effect on cell viability, efficacy toward TG2 inhibition and inflammatory cytokines was analyzed	Lower activity of TG2 level was found in IFN-γ and p-31-43-treated Caco-2 cells. Cocoa extract addition significantly reduced the levels of IL-1β, IL-6, IL-15 and IL-8 in IFN-γ and gliadin treated Caco-2 cells. No significant difference on cell viability was observed with the addition of cocoa extract at different concentration	PA B2	Kramer et al. (2019)
Anti-inflammatory	Purified PAs fraction (DP ~5.6) of cocoa beans' acetone/water extract	Cocoa extract was tested against monocytes isolated from Buffy coats. Microarray, gene pathway analysis, qPCR, and ELISA analyses were carried out	PA showed anti-inflammatory effects by regulating the expression of genes responsible for V-ATPase and second-messenger activity, resulting in the inhibition of pro-inflammatory cytokine secretion	PA	Midttun et al. (2018)

AA, antioxidant activity; DPPH, 2,2-diphenyl-1-picrylhydrazyl; NP-HPLC-DPPH, normal phase-high performance liquid chromatography-2,2-diphenyl-1-picrylhydrazyl; PA, proanthocyanidin; DP, degree of polymerization; TPC, total phenolic content; EC_{50}, half maximal effective concentration; UPLC, ultra-high performance liquid chromatography; FRAP, ferric reducing antioxidant power; ORAC, oxygen radical absorbance capacity; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); TEAC, Trolox equivalent antioxidant capacity; TRAP, total reactive antioxidant potential; HPLC-DAD-ESI-MS^2, high-performance liquid chromatography-diode array detector-electrospray ionization-tandem mass spectrometry; RACI, relative antioxidant capacity index; GAS, global antioxidant score; GSH, glutathione; GPx, glutathione peroxidase; GR, glutathione reductase; CAT, catalase; GST, glutathione-S-transferase; ROS, reactive oxygen species; MIC, minimum inhibitory concentration; MAPKs, mitogen-activated protein kinases; TG2, transglutaminase 2; IFN-γ, interferon gamma; IL, interleukin; ELISA, enzyme-linked immunosorbent assay.

Table 5. *In vivo* studies of health effects of cocoa-related products.

Bioactivity	Sample type	Experimental	Major findings	Proposed key bioactive compounds	References
Antioxidation	Bread supplemented with water extract of raw and roasted *Forastero* cocoa beans (contained total phenolics of 0.141 and 0.035 mg/g dry weight, respectively)	Male Wistar laboratory rats (n = 24) were fed with dried and crumbed cocoa extract-supplemented bread for 4 weeks. The feces were collected during the trial. At the termination of study (*post-mortem*), intestinal contents, blood and tissue samples were collected. Volatile fatty acid profile, glycolytic enzymes, blood glucose level, cholesterol, uric acid, reduced and oxidized glutathione, TBARS assay, antioxidant activity analyses were carried out	Higher ratio of reduced to oxidized glutathione was found in the livers of cocoa-treated rats, indicating increased antioxidant activity	Unspecified	Żyżelewicz et al. (2018)
Antioxidation, anti-inflammatory, anti-cancer, anti-necrotic	Cocoa extract (CE) and epicatechin (EC)	Female BALB/cN mice (2–3 months, 20–25 g of weight) were intoxicated with carbon-tetrachloride (CCl_4, 1 mL/kg body weight, twice a week). Post-treatment was done by supplementing 34.5 mg/kg cocoa extract and 2.51 mg/kg epicatechin, twice a week for 2 weeks. The blood was collected from orbital sinus. After 2 weeks, the mice were sacrificed and the liver was collected and stored. Blood biochemistry, liver damage, ELISA, liver tissue fractionation, SDS-PAGE, western blot and antioxidative enzyme assessment were carried out	CE and EC supplementation could suppress the elevation of AST, ALT, ALP, and TAG due to CCl_4 intoxication indication protection effect toward hepatotoxicity. CE and EC supplementation also normalized blood glucose and lactate level. CE and EC reduced the levels of TNF-α, and IFN-γ in CCl_4 intoxicated mice, indicating anti-inflammatory activity. Inhibition of Hsp90 was also found, indicating inhibition of oncogenesis. CE supplementation improved antioxidant activity by amelioration of CAT activity, Mt-SOD activity modulation and total protein thiols increase	Unspecified (extract contained high amount (>10 mg/g DM) of EGC, EC and PA B2)	Giacometti et al. (2016)
Anti-diabetic	AIN-93G containing 10% of *Forastero* cocoa powder	Male Zucker diabetic fatty rats (n = 16, 9 weeks old) were supplemented with diet contained 10% of cocoa. Blood samples were collected after 10 weeks and kidneys were collected for histological analysis. Biochemical analysis on blood and urine were carried out. Other analyses included glucose tolerance test, Western blot, and histological analyses	Supplementation of cocoa alleviated hyperglycemia and hyperinsulinanemia in diabetic rats. Cocoa supplementation also helped to restore renal functionality	Unspecified	Álvarez-Cilleros et al. (2019)
Anti-diabetic	AIN-93G containing 10% of *Forastero* cocoa powder	Male Zucker diabetic fatty rats (n = 16, 9 weeks old) were supplemented with diet contained 10% of cocoa. After 20 weeks of supplementation, the rats were sacrificed. Blood samples, colon, and fecal samples were collected and analyzed. The analyses included histologic, and immune-histological analysis, terminal deoxynucleotidyl tTransferase dUTP nick end labeling (TUNEL) assay, DNA analysis, fatty acids and lactate analysis	Faster renewal rate of colonic epithelium was found in cocoa-supplemented diabetic rats. Cocoa supplementation also inhibited the expression of pro-inflammatory cytokines. Cocoa diet inhibited the growth of *Eschericia*, *Tepidibacter*, *Lactobacillus*, and *Enterococcus*, preventing the change of intestinal microbial composition induced by diabetes	Unspecified	Álvarez-Cilleros et al. (2020)
Anti-inflammatory, oxidative stress regulation and	CocoaVia® methanolic extract (contained 250 mg procyanidins/g cocoa extract)	Methanolic extract in water with different doses (5, 10, 15, 20 and 25 mg/kg body weight) were administered to 25	Elevated activity of p-Erk1/2 and p-Akt was measured, indicating higher cell survival during Ischemia-	Flavan-3-ol monomers and PA	Ahmed et al. (2020)

(*continued*)

Table 5. Continued.

Bioactivity	Sample type	Experimental	Major findings	Proposed key bioactive compounds	References
cardiovascular protection		Sprague Dawley male rats via oral gavage. Polyphenol levels in blood sample analysis was carried out. Induced ischemia/ reperfusion injury and tissue collection were carried out on 10 rats. The myocardial tissue and heart section were then subjected to immunohistochemistry staining analysis, oxidative stress measurement and TUNEL assay	reperfusion injury. Analysis on the cocoa-treated heart tissues showed reduced apoptosis level. Supplementation of cocoa extract was associated with enhanced phosphorylation of Erk1.2 and Akt, resulting in cardio protection activity		
Anti-cancer	AIN-93G diet containing 5% and 10% cocoa	Female BALB/c mice (n = 50, 6–8 weeks old, 25–30 g) were induced to have colitis-associated cancer by azoxymethane and dextran sodium sulfate. The mice were then fed with cocoa supplemented diet for 62 days. The mice were then sacrificed, and the colon were collected. Disease activity index was measured weekly. The colon was analyzed for malondialdehyde, enzymatic and non-enzymatic antioxidant level. Other analysis included immune-histochemical, Western blot, and analysis	The supplementation of 5% cocoa in diet help to inhibit tumor growth in colon, while 10% cocoa in diet reduced branching and budding in crypts. Cocoa diet alleviated the activity of enzymatic and non-enzymatic antioxidants. Cell-protection mechanism was also activated by cytoprotective enzymes in cocoa treated mice	Unspecified	Pandurangan et al. (2015)
Physical improvement	Flavanol-rich cocoa beverage (contained total flavanols of 528 mg) and non-flavanol beverage (0 g flavanol content)	Double-blind, placebo-controlled, crossover and randomized trial was carried out involving 7 African American and 7 Caucasian American. Flavanol-rich cocoa beverage (total flavanols 528 mg per serving) and non-flavanol containing beverages were consumed. The effects on cutaneous vasodilator response to local heating were assessed	Improved function on cutaneous microvascular of young African American was found. The consumption of flavanol-rich beverages (total flavanols 528 mg per serving) was related to the increase of nitric oxide bioavailability	Flavan-3-ol monomers and PA	Kim and Brothers (2020)
Physical improvement	Fat reduced cocoa powder and maltodextrin as control.	Randomized, parallel-group placebo-controlled trial was carried out involving 44 male endurance cross-country athletes (18-50 years of age). Dietary habits, anthropometry, body composition, maximal oxygen uptake, ventilator threshold, maximal aerobic speed, myostatin, follistatin and leptin analyses were carried out	The supplementation enhanced body composition of cocoa supplemented-subjects (5 g cocoa powder/day, contained 425 mg of flavanols) due to decreased body fats levels. The improvement was partially contributed by the changes in follistatin/myostatin ratio and the decrease in leptin levels	PA B2	García-Merino et al. (2020)
Therapeutic effect in people with PAD	Flavanol-rich cocoa beverages (contained 15 g of cocoa and 75 mg of epicatechin)	Double-blind, pilot randomized clinical trial was carried out involving 40 participants (20 treated and 20 control) with the age of >60 years and currently diagnosed with peripheral artery disease. Flavanols-rich cocoa beverages were consumed by 3 packets daily for 6 months. Walk test, physical activity test and muscle tissues analysis were carried out during the clinical trial	Cocoa beverages-treated participants improved calf muscle perfusion and its COX enzyme activity, capillary density and abundance of central nuclei	Unspecified	McDermott et al. (2020)
Enzymes modulation,	Water extract of raw and roasted *Forastero*	Cocoa extracts and blank samples were delivered in daily diet	Cocoa extract supplementation (2% of	Melanoidins	Żyżelewicz et al. (2020)

(*continued*)

Table 5. Continued.

Bioactivity	Sample type	Experimental	Major findings	Proposed key bioactive compounds	References
antioxidation and lipid peroxidation inhibition	cocoa beans (contained total phenolics of 32.48 and 26.62 mg/g dry weight)	(2%) to 28 male 6 week old Wistar laboratory rats. Body composition (body lean and fat tissue) analysis were carried out in 0 and final hour of experiment. Small intestine, cecum, colon, and other tissue samples (liver, kidneys and heart) were collected at the termination of study. The analysis carried out included gastrointestinal enzymes activity, volatile fatty acids profile, glycolytic enzymes activity, blood's glucose, cholesterol, uric acid, antioxidant activity, reduced and oxidized glutathione and TBARS assay	diet) increased the activity of β-glucosidase and α-galactosidase. Blood antioxidant effect in cocoa extract treated sample was increased. The concentration of TBARS in heart was decreased		
Anti-photoaging	Cocoa powder (CP) (containing 71.5 mg/g flavanol content)	Female albino hairless mice (6 week old) were treated with cocoa powder diet in various doses (39.1 and 156.3 mg/kg of cocoa powder) and positive control (625 mg/kg pycogenol). The supplementation was done through oral and administered for 8 weeks. The mice were irradiated with UV lamp with emission spectrum between 275–380 nm. Irradiation dose was set at 50 mJ/cm^2 and increased into 200 mJ/cm^2 (maintained after reached 200 mJ/cm^2) for 8 weeks. RNA analysis was done in frozen skin tissue and wrinkle formation was recorded and assessed. Reverse transriptase PCR, western blotting, immunohistochemistry and luciferase reporter gene assay were also carried out	CP-administered mice (156.3 mg/kg) showed significant decreases of wrinkle formation. Mice with CP-supplementation also showed higher recovery of collagen levels and skin esthetic level comparable to positive control. CP-supplementation also regulate cathepsin G and serpin b6c, indicating its potential as antiphotoaging agents	Unspecified	Kim et al. (2016)
Circadian rhythm modulation	Milk chocolate	Adult Wistar rats were exposed in different conditions of 6-h phase advance to mimic jet-lag condition. Milk chocolate (5 g) was administered daily at different time (onset of previous night or start of new night). On the other hand, slow rotating wheel was used to simulate shift-work condition. Chocolate (5 g) was administered at different time (dinner and breakfast). General activity, core temperature, metabolic and hormonal rhythm were measured. Immuno-histochemical analysis, SCN c-Fos quantification and cosinor analysis were carried out	Consumption of chocolate during breakfast restored the c-Fos expression in the dorsal suprachiasmatic nucleus of jet lagged rats and restored daily peaks of glucose, triglyceride, and core temperature in shift-worked rats The consumption of chocolate during breakfast helped prevent circadian disruption due to shift work and jet-lag conditions in rat model	Fat and sugar	Escobar et al. (2020)

PA, proanthocyanidin; PAD, peripheral artery disease; COX, cytochrome c oxidase; TBARS, thiobarbituric acid reactive substances; ELISA, enzyme-linked immunosorbent assay; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TAG, triacylglycerol; TNF-α, tumor necrosis factor alpha; IFN-γ, interferon gamma; CAT, catalase activity; Mt-SOD, superoxide dismutase in mitochondria fraction; DM, dry matter; EGC, epigallocatechin.

superoxide anions (Nijveldt et al. 2001). Flavonoids may also prevent oxidative injuries through the inhibition of xanthine oxidase activity; reduce the number of immobilized leukocytes responsible for production of oxidants and inflammatory mediators; and interact with enzyme systems such as peroxidase, proteolytic enzymes, and lipoxygenase (Nijveldt et al. 2001).

6.2.2. Anti-diabetic

Cocoa supplementation alleviated hepatic insulin resistance and oxidative stress in Zucker diabetic fatty rats. Cocoa supplementation (100 g/kg) exhibited preventive and inhibitive activities toward renal diseases in type-2 diabetic patients by modulating related pathways and restoring the renal functionality (Álvarez-Cilleros et al. 2019). The supplementation of cocoa polyphenols in beverage decreased the spikes of blood glucose levels of healthy panelists (Campbell, Foegeding, and Harris 2016). On the other hand, the addition of 10% cocoa powder into diet regulated the gut microbiota composition in male Zucker diabetic fatty rats. Cocoa supplementation improved the abundance of *Blautia* and *Flavobacterium,* while inhibiting the growth of *Parabacteirodes* and *Sutterella* in diabetic animals with increased levels of ZO-1 and reduced levels of glycemia, cytokines and CD45 (Álvarez-Cilleros et al. 2020)

Various mechanisms of anti-diabetic activity of the cocoa bioactive were reported. Flavan-3-ols and PAs modulated the insulin secretion through the preservation of insulin secretion cells, regulation of insulin signaling and enhancement of insulin receptors (Ramos, Martín, and Goya 2017). PAs also showed insulinomimetic effect in some cells such as skeletal muscle and adipose tissues. Epicatechin was able to improve liver performance by reducing the accumulation of lipid droplets, non-esterified FAs, triglycerides and cholesterol (Cordero-Herrera et al. 2015). Further, flavan-3-ols and PAs acted as antioxidants and anti-inflammatory agents to prevent oxidative stress and inflammation occurred due to the disease (Ramos, Martín, and Goya 2017).

6.2.3. Anti-inflammatory

The supplementation of cocoa extract decreased the level of pro-inflammatory enzyme in CCl_4 intoxicated mice (Giacometti et al. 2016). Cocoa extract (34.5 mg/kg) showed higher activity in suppressing the levels of IFN-γ than pure (-)-epicatechin (2.24 mg/kg) (Giacometti et al. 2016). Ahmed et al. (2020) found the reduction in levels of inflammatory mediators such as IL-6 and NF-κB in the heart of cocoa extract-treated Sprague Dawley male rat (dose of 15 mg/kg, contained 250 mg of flavan-3-ols/g). Various compounds may contribute to the anti-inflammatory activity of cocoa extracts. Epicatechin decreased the amount of pro-inflammatory cytokines and modulated the level of serum TNF-α (Giacometti et al. 2016). Combination of quercetin and flavan-3-ols were able to modulate cytokines by inhibiting the transcription factors of NF-κB and AP-1, and reducing the MAPK activity (Serafini, Peluso, and Raguzzini 2010).

6.2.4. Cardiovascular protection

An improvement in microvascular function was found in young African Americans, which mainly related to the increased availability of nitric oxide after cocoa consumption (Kim and Brothers 2020). The consumption of cocoa polyphenols (600 and 1200 mg/kg/day) in neo-natal Sprague-Dawley rats had no toxicity, while inhibiting LV dysfunction induced by pressure overload (Sari et al. 2020). The consumption of PAs induced the relaxation of endothelium by the activation of NO synthase. Epicatechin and PAs contributed to the improvement of brachial artery by means of flow-mediated vasodilatation (Corti et al. 2009). Epicatechin metabolites inhibited NADPH oxidase, resulting in the prevention of NO inactivation by free radicals. Epicatechin also modulated NO bioavailability and reduced the level of endothelin-1 in plasma, preventing vasoconstriction of endothelium (Corti et al. 2009). Theobromine increased the level of high-density lipoprotein (HDL) and decreased that of low-density lipoproteins (LDL) in plasma, which may be contributed by its inhibitory ability against adenosine receptors (Martínez-Pinilla, Oñatibia-Astibia, and Franco 2015).

6.2.5. Anti-cancer

Potential of cocoa as chemo-preventive agent in colorectal carcinogenesis was mainly contributed by its ability to induce the activities of enzymatic and non-enzymatic antioxidant, while suppressing the iNOS, COX-2 and Nrf2 in BALB/c mice. These changes indicated reduced inflammation and activation of cell-protections by cytoprotective genes (Pandurangan et al. 2015). Inhibition of Hsp90 expression was also found in CCl_4-induced mice with the supplementation of cocoa extract and epicatechin. This indicated inflammatory prevention and carcinogenesis prevention (Giacometti et al. 2016). Theobromine also regulated the activities of PDE4 (phosphodiesterase 4), Akt (protein kinase B), and NF-κB during the malignant glioblastoma proliferation (Martínez-Pinilla, Oñatibia-Astibia, and Franco 2015).

6.2.6. Physical improvement

Long term consumption of cocoa (>10 weeks) was reported to increase the follistatin level and decrease the leptin level, while improving the physique in terms of body fat reduction in male cross-country athletes (García-Merino et al. 2020). This improvement may be contributed by ability of polyphenols, specifically oligomeric PAs, to modulate α-glycosidase and limiting the intestinal absorption (García-Merino et al. 2020; Ryan et al. 2016). Further, methylxanthines showed an ability to stimulate lipolysis by inhibiting A1 receptors and PDE4, while increasing the levels of catecholamines. Methylxanthines also inhibited the adipogenesis by disturbing the Akt and ERK axis and activating AMPK (5′AMP-activated protein kinase). This led to the weight loss in obese subjects (Carrageta et al. 2018).

6.2.7. Anti-photoaging

Supplementation of cocoa powder in diet exhibited anti-photoaging effect by inhibiting the adverse effect of ultraviolet-B (UVB) irradiation such as wrinkle formation, while promoting the recovery of collagen in mice skin (Kim et al. 2016). Epicatechin and PAs in cocoa beans were metabolized into 5-(3′, 4′-dihydroxyphenyl)-γ-valerolactone (DHPV). DHPV was able to inhibit AP-1 activity and suppressed the UVB-induced MMP-1 protein expression and gene transcription. DHPV also downregulated the level of cathepsin G and upregulated serpin b6c, reducing the wrinkle formation in the skin (Kim et al. 2016).

6.2.8. Anti-depressant

Supplementation of cocoa polyphenolic extract may act as anti-depressant. This was indicated by reduced duration of immobility of Wistar-Unilever rats during forced swimming test (Messaoudi et al. 2008). Anti-depressant effect of cocoa was related with the ability of flavan-3-ols and PAs to block the monoamine uptake in the brain under stress conditions (Messaoudi et al. 2008). Anti-depressant effect of chocolate may be also related to phenylethylamine, a neuromodulator for mood regulation. Further, the palatability of cocoa products also contributed to mood improvement of the consumers after eating the products (Jackson et al. 2019). Vitamin D_2 was distributed in the hypothalamus and may affect the levels of neuropeptide and gene expression related to depression (Shi, Wang, and Xu 2017). Methylxanthines, caffeine in particular, could help to release dopamine in prefrontal cortex, providing anti-depressant drug-like effect (Szopa et al. 2016).

6.2.9. Circadian rhythm modulation

The consumption of chocolate during breakfast helped prevent circadian disruption due to shift work and jet-lag conditions in rat model (Escobar et al. 2020). The effect may be related to the high palatability of chocolate contributed by high fat and high sugar composition. High palatability of food activated brain reward system, accelerating the re-entrainment after shift work or jet-lag conditions (Escobar et al. 2020).

6.2.10. Adverse effects

The consumption of cocoa and chocolate was associated with the over-inflammation and worsening of acne (Netea et al. 2013). The consumption of chocolate may be related to the induction of mononuclear cells in the blood to release IL-1β and TNFα, altered the cytokines profiles, and over-inflammation upon stimulation of acne bacteria. While the responsible bioactive compounds are still to be identified, it may be related with the additional compounds such as fat and sugars (Netea et al. 2013).

7. Process to optimize the biological activities of cocoa beans and their derived products

The biological activities of cocoa are contributed by various chemical components. In most of the studies discussed in this paper, polyphenols are the point of interest and responsible for many of the biological activities. Unfortunately, the current processing methods are focused on the palatability of cocoa by optimizing the development of flavor profiles and Maillard reaction products. The polyphenols are largely degraded during the processing of cocoa beans. The applications of non-conventional processing operations may better preserve the polyphenols, improving polyphenol-based biological activities of cocoa products (González-Barrio et al. 2020). However, cocoa beans obtained from the non-conventional methods are less palatable. The combination of processes or food ingredients/products may be necessary to maximize the health potential of cocoa. Cocoa products obtained from conventional processing may be combined with those from non-conventional processing to obtain "healthy" formulations with good palatability.

In terms of maximizing the health benefits and palatability of cocoa products, several alternative processes deserve to be more studied. Isolation of polyphenol-rich cocoa extracts may ease their utilizations for food and pharmaceutical applications. In the form of extracts, polyphenols are a versatile product. It can be used as a supplement for food products to enhance their health benefits. Furthermore, more studies on the methods of modified fermentation may be carried out to explore the possibility of obtaining good palatability cocoa with a considerable amount of bioactive compounds remained. A study on forced fermentation revealed the possibility of tuning the outcomes of fermentation (John et al. 2020). In this case, the conditions of forced fermentation may be directed to produce flavor precursors while preserving the bioactive compounds.

Incorporation/combination of polyphenols with other bioactive chemical compounds may improve the biological activities for targeted applications. Apart from polyphenols, the biological activities of the processed cocoa products may also be contributed by heat generated compounds such as Maillard reaction products (Di Mattia et al. 2017), methylxanthines (Carrageta et al. 2018; Martínez-Pinilla, Oñatibia-Astibia, and Franco 2015), vitamins, fibers, fats and microbial metabolites (Ryan et al. 2016). Cocoa powder and extracts contained various compounds which may have synergistic or antagonistic effects on biological activities. More comparative studies should be carried out to evaluate the most prominent components and the effect of processing on the biological activities of cocoa beans.

8. Roles of polyphenols in human health and consumer perception of polyphenol rich food products

Polyphenols in the cocoa products are responsible for various health claims. The claimed health effects included antioxidation, anti-cancer, anti-microbial, anti-inflammatory and anti-diabetic capacities, cardiovascular protection, physical improvement, and anti-photoaging (Tables 4 and 5). Similar effects were reported for the polyphenols extracted from other natural products such as grapes (Rasines-Perea and Teissedre 2017), berries (Olas 2018; Lavefve, Howard, and

Carbonero 2020), and tea (Khan and Mukhtar 2018). Polyphenols, in general, regardless of the sources, have been reported to possess the aforementioned health effects (Sajadimajd et al. 2020; Bendokas et al. 2020; Ullah et al. 2020; Drewnowski and Gomez-Carneros 2000).

Polyphenols can contribute to undesirable bitterness and astringency of food products (Drewnowski and Gomez-Carneros 2000). Excessive levels of bitterness and astringency of cocoa bean products are related to the presence of polyphenols in cocoa mass (Febrianto and Zhu 2020). High bitterness and astringency negatively affect consumer perception of cocoa-derived confectioneries (Del Prete and Samoggia 2020) and also other products such as green tea and black tea (Liou et al. 2020). Compared to several bitter-tasting beverages, extra dark chocolate (99% of cocoa) was less preferred by consumers than green tea, black tea, and coffee (liking scores of 2.29, 4.59, 5.98, and 6.11, respectively) (Donadini, Fumi, and Lambri 2012). It was mainly caused by the higher levels of bitterness and astringency of the dark chocolate. The different consumer preferences could be due to the variations in the polyphenol composition. Different phenolic compounds have different threshold values for sensory perception (Stark, Bareuther, and Hofmann 2006). Cocoa is rich in epicatechin and oligomeric proanthocyanidins. Green tea is rich in epigallocatechin-3-gallate, epigallocatechin, epicatechin-3-gallate, and epicatechin (Ravindranath et al. 2006). Black tea is rich in theaflavins and proanthocyanidin dimers (B1 and B2) (Luximon-Ramma et al. 2005; Deka et al. 2021), and coffee is rich in caffeoyl-quinic acid and feroyl-quinic acid derivatives (Gigl et al. 2021). Even though chocolate is generally considered as an unhealthy product due to high contents of saturated fat, the chocolate with higher cocoa content or low fat cocoa products such as cocoa powder may be considered as healthier options by customers (Katz, Doughty, and Ali 2011; Sepúlveda et al. 2021).

Most consumers prefer polyphenol-containing products with a balanced level of bitterness and sweetness. Sugars can effectively reduce the bitterness and astringency of polyphenol-enriched products, thus increasing their sensory acceptability (Jaeger et al. 2009). However, excess sugar consumption has negative health effects (Lustig, Schmidt, and Brindis 2012). Strategies to reduce the bitterness and astringency have been reported, including cyclodextrin- or polymer-based encapsulations (Fang and Bhandari 2010) and emulsion-based delivery systems (Coupland and Hayes 2014). Applications of taste-masking techniques for cocoa polyphenol-based products are expected to increase the sensory quality. In addition, recent technological developments promote natural product-based drug discovery (Atanasov et al. 2021). Focusing on pharmaceutical applications, the role of cocoa polyphenols in health outcomes and development of cocoa polyphenol-based drugs remain to be studied.

9. Conclusions and research outlook

Special combination of nutrients and bioactive compounds in cocoa beans relate to various biological activities. The activities include antioxidation, anti-diabetic property, anti-inflammatory, cardiovascular protection, anti-microbial capacity, anti-cancer, physical improvement, anti-photoaging, anti-depressant, and circadian rhythm modulation. These effects are contributed by various components, including polyphenols, methylxanthines, Maillard reaction products, and vitamins in the cocoa beans. Processing cocoa beans significantly alters the composition of cocoa beans. Current conventional processing is not suitable to optimize the health potentials of cocoa. The fermentation and roasting processes induce most of the chemical changes in the beans. The most noticeable changes are the degradation of carbohydrates and proteins to form flavor precursors (amino acids and reducing sugars), production of flavors, and reduction of bioactive compounds such as polyphenols. On the other hand, non-conventional processing such as "lavado" operation better preserves bioactive compounds and can produce more "healthy" products, albeit being less palatable. A combination of processes may be needed to obtain products with balanced characteristics. Overall, the cocoa bean processing methods should be carefully selected by taking the chemical variations into consideration to maximize the flavor and nutritional effects of the products.

To maximize desirable nutrients and bioactive derived from cocoa beans, the following aspects deserve attention: 1) Development of bioactive-rich cultivation; 2) Effect of genotype-processing interactions on chemical and biological properties; 3) Comparative studies of chemical composition based on the newest genotype classification; 4) Valorization of underutilized resources (leaves, bark, pod husk, and shell); 5) Process optimization for minimizing undesirable physiochemical characteristics and sensory attributes, and compounds; 6) Process modification focusing on the preservation of bioactive compounds; 7) Process modification to obtain balanced characteristics of biological functions and palatability; 8) Synergistic and antagonistic effect of various bioactive compounds in cocoa; 9) Increasing the bioavailability of cocoa's bioactive compounds; 10) Novel product development and formulation.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

N.A.F. is financially supported by a New Zealand Aid under the term of NZ ASEAN Scholarship.

References

Abdullahi, G., R. Muhamad, O. Dzolkhifli, and U. R. Sinniah. 2018. Analysis of quality retentions in cocoa beans exposed to solar heat treatment in cardboard solar heater box. *Cogent Food & Agriculture* 4 (1):1483061. doi: 10.1080/23311932.2018.1483061.

Adeyeye, E. I. 2016. Proximate, mineral and antinutrient compositions of natural cocoa cake, cocoa liquor and alkalized cocoa powders sourced in Nigeria. *Journal of Advanced Pharmaceutical Science and Technology* 1:12–28.

Adeyeye, E. I., R. O. Akinyeye, I. Ogunlade, O. Olaofe, and J. O. Boluwade. 2010. Effect of farm and industrial processing on the amino acid profile of cocoa beans. *Food Chemistry* 118 (2):357–63. doi: 10.1016/j.foodchem.2009.04.127.

Adi-Dako, O., K. Ofori-Kwakye, S. Frimpong Manso, M. E. L. Boakye-Gyasi, C. Sasu, and M. Pobee. 2016. Physicochemical and antimicrobial properties of cocoa pod husk pectin intended as a versatile pharmaceutical excipient and nutraceutical. *Journal of Pharmaceutics* 2016:1–12. doi: 10.1155/2016/7608693.

Afoakwa, E. O., E. Ofosu-Ansah, J. F. Takrama, A. S. Budu, and H. Mensah-Brown. 2014. Changes in chemical quality of cocoa butter during roasting of pulp preconditioned and fermented cocoa (*Theobroma cacao*) beans. *International Food Research Journal* 21: 2221–7.

Afoakwa, E. O., J. Quao, J. Takrama, A. S. Budu, and F. K. Saalia. 2013. Chemical composition and physical quality characteristics of Ghanaian cocoa beans as affected by pulp pre-conditioning and fermentation. *Journal of Food Science and Technology* 50 (6):1097–105. doi: 10.1007/s13197-011-0446-5.

Agus, B. A. P., N. N. Mohamad, and N. Hussain. 2018. Composition of unfermented, unroasted, roasted cocoa beans and cocoa shells from Peninsular Malaysia. *Journal of Food Measurement and Characterization* 12 (4):2581–9. doi: 10.1007/s11694-018-9875-4.

Ahmed, S., N. Ahmed, A. Rungatscher, D. Linardi, B. Kulsoom, G. Innamorati, S. A. Meo, M. A. Gebrie, R. Mani, F. Merigo, et al. 2020. Cocoa flavonoids reduce inflammation and oxidative stress in a myocardial ischemia-reperfusion experimental model. *Antioxidants* 9 (2):167. doi: 10.3390/antiox9020167.

Alasti, F. M., N. Asefi, R. Maleki, and S. S. SeiiedlouHeris. 2020. The influence of three different types and dosage of alkaline on the inherent properties in cocoa powder. *Journal of Food Science and Technology* 57 (7):2561–71. doi: 10.1007/s13197-020-04293-w.

Albertini, B., A. Schoubben, D. Guarnaccia, F. Pinelli, M. D. Vecchia, M. Ricci, G. C. D. Renzo, and P. Blasi. 2015. Effect of fermentation and drying on cocoa polyphenols. *Journal of Agricultural and Food Chemistry* 63 (45):9948–53. doi: 10.1021/acs.jafc.5b01062.

Álvarez-Cilleros, D., M. E. López-Oliva, M. A. Martín, and S. Ramos. 2019. Cocoa ameliorates renal injury in Zucker diabetic fatty rats by preventing oxidative stress, apoptosis and inactivation of autophagy. *Food & Function* 10 (12):7926–39. doi: 10.1039/C9FO01806A.

Álvarez-Cilleros, D., S. Ramos, M. E. López-Oliva, F. Escrivá, C. Álvarez, E. Fernández-Millán, and M. Á. Martín. 2020. Cocoa diet modulates gut microbiota composition and improves intestinal health in Zucker diabetic rats. *Food Research International* 132: 109058. doi: 10.1016/j.foodres.2020.109058.

Aprotosoaie, A. C., S. V. Luca, and A. Miron. 2016. Flavor chemistry of cocoa and cocoa products – An overview. *Comprehensive Reviews in Food Science and Food Safety* 15 (1):73–91. doi: 10.1111/1541-4337.12180.

Aremu, C. Y., M. A. Agiang, and J. O. I. Ayatse. 1995. Nutrient and antinutrient profiles of raw and fermented cocoa beans. *Plant Foods for Human Nutrition* 48 (3):217–23. doi: 10.1007/BF01088443.

Atanasov, A. G., S. B. Zotchev, V. M. Dirsch, and C. T. Supuran. 2021. Natural products in drug discovery: Advances and opportunities. *Nature Reviews Drug Discovery* 20 (3):200–16. doi: 10.1038/s41573-020-00114-z.

Azam, S., N. Hadi, N. U. Khan, and S. M. Hadi. 2003. Antioxidant and prooxidant properties of caffeine, theobromine and xanthine. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research* 9 (9):BR325–330.

Bangerter, U., B. H. Beh, A. B. Callis, and I. J. Pilkington. 1991. Improved cocoa fermentation. EP0442421A2.

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

Bauer, D., J. P. de Abreu, H. S. S. Oliveira, A. Goes-Neto, M. G. B. Koblitz, and A. J. Teodoro. 2016. Antioxidant activity and cytotoxicity effect of cocoa beans subjected to different processing conditions in human lung carcinoma cells. *Oxidative Medicine and Cellular Longevity* 2016:7428515. doi: 10.1155/2016/7428515.

Bendokas, V., K. Skemiene, S. Trumbeckaite, V. Stanys, S. Passamonti, V. Borutaite, and J. Liobikas. 2020. Anthocyanins: From plant pigments to health benefits at mitochondrial level. *Critical Reviews in Food Science and Nutrition* 60 (19):3352–65. doi: 10.1080/10408398.2019.1687421.

Brito, B. D. d. C., R. C. Chisté, R. da Silva Pena, M. B. A. Gloria, and A. S. Lopes. 2017. Bioactive amines and phenolic compounds in cocoa beans are affected by fermentation. *Food Chemistry* 228: 484–90.

Caligiani, A., D. Acquotti, Cirlini, M., and G. Palla. 2010. 1H NMR study of fermented cocoa (*Theobroma cacao* L.) beans. *Journal of Agriculture and Food Chemistry* 58:12105–11.

Campbell, C. L., E. A. Foegeding, and G. K. Harris. 2016. Cocoa and whey protein differentially affect markers of lipid and glucose metabolism and satiety. *Journal of Medicinal Food* 19 (3):219–27. doi: 10.1089/jmf.2015.0044.

CAOBISCO/ECA/FCC. 2015. *Cocoa beans: Chocolate and cocoa industry quality requirements*. Brussels: CAOBISCO.

Carrageta, D. F., T. R. Dias, M. G. Alves, P. F. Oliveira, M. P. Monteiro, and B. M. Silva. 2018. Anti-obesity potential of natural methylxanthines. *Journal of Functional Foods* 43:84–94. doi: 10.1016/j.jff.2018.02.001.

Cevallos-Cevallos, J. M., L. Gysel, M. G. Maridueña-Zavala, and M. J. Molina-Miranda. 2018. Time-related changes in volatile compounds during fermentation of bulk and fine-flavor cocoa *(Theobroma cacao)* beans. *Journal of Food Quality* 2018:1758381.

Chen, D., Q. Shao, L. Yin, A. Younis, and B. Zheng. 2018. Polyamine function in plants: Metabolism, regulation on development, and roles in abiotic stress responses. *Frontiers in Plant Science* 9:1945. doi: 10.3389/fpls.2018.01945.

Chin, E., K. B. Miller, M. J. Payne, W. J. Hurst, and D. A. Stuart. 2013. Comparison of antioxidant activity and flavanol content of cacao beans processed by modern and traditional Mesoamerican methods. *Heritage Science* 1 (1):9. doi: 10.1186/2050-7445-1-9.

Ciaramelli, C., A. Palmioli, A. De Luigi, L. Colombo, G. Sala, M. Salmona, and C. Airoldi. 2021. NMR-based Lavado cocoa chemical characterization and comparison with fermented cocoa varieties: Insights on cocoa's anti-amyloidogenic activity. *Food Chemistry* 341: 128249. doi: 10.1016/j.foodchem.2020.128249.

Collado-González, J., T. Durand, F. Ferreres, S. Medina, A. Torrecillas, and Á. Gil-Izquierdo. 2015. Phytoprostanes. *Lipid Technology* 27 (6): 127–30. doi: 10.1002/lite.201500020.

Cordero-Herrera, I., M. Á. Martín, E. Fernández-Millán, C. Álvarez, L. Goya, and S. Ramos. 2015. Cocoa and cocoa flavanol epicatechin improve hepatic lipid metabolism in *in vivo* and *in vitro* models. Role of PKCζ. *Journal of Functional Foods* 17:761–73. doi: 10.1016/j.jff.2015.06.033.

Cordero-Herrera, I., M. Á. Martín, L. Goya, and S. Ramos. 2014. Cocoa flavonoids attenuate high glucose-induced insulin signalling blockade and modulate glucose uptake and production in human HepG2 cells. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association* 64:10–9. doi: 10.1016/j.fct.2013.11.014.

Corti, R., A. J. Flammer, N. K. Hollenberg, and T. F. Lüscher. 2009. Cocoa and cardiovascular health. *Circulation* 119 (10):1433–41. doi: 10.1161/CIRCULATIONAHA.108.827022.

Coupland, J. N., and J. E. Hayes. 2014. Physical approaches to masking bitter taste: Lessons from food and pharmaceuticals. *Pharmaceutical Research* 31 (11):2921–39. doi: 10.1007/s11095-014-1480-6.

D'Souza, R. N., A. Grimbs, S. Grimbs, B. Behrends, M. Corno, M. S. Ullrich, and N. Kuhnert. 2018. Degradation of cocoa proteins into oligopeptides during spontaneous fermentation of cocoa beans. *Food Research International* 109:506–16. doi: 10.1016/j.foodres.2018.04.068.

D'Souza, R. N., S. Grimbs, B. Behrends, H. Bernaert, M. S. Ullrich, and N. Kuhnert. 2017. Origin-based polyphenolic fingerprinting of *Theobroma cacao* in unfermented and fermented beans. *Food Research International* 99:550–9. doi: 10.1016/j.foodres.2017.06.007.

Dario, A., and A. Eskes. 2009. Process for the fermentation of cocoa beans to modify their aromatic profile. WO2009/103137A2.

De Bruijn, J. M., A. P. G. Kieboom, and H. van Bekkum. 1987. Alkaline degradation of monosaccharides V: Kinetics of the alkaline isomerization and degradation of monosaccharides. *Recueil Des Travaux Chimiques Des Pays-Bas* 106 (2):35–43. doi: 10.1002/recl.19871060201.

De Taeye, C., V. J. Eyamo Evina, G. Caullet, N. Niemenak, and S. Collin. 2016. Fate of anthocyanins through cocoa fermentation. Emergence of new polyphenolic dimers. *Journal of Agricultural and Food Chemistry* 64 (46):8876–85. doi: 10.1021/acs.jafc.6b03892.

De Vuyst, L. and S. Weckx. 2016. The cocoa bean fermentation process: From ecosystem analysis to starter culture development. *Journal of Applied Microbiology* 121:5–17.

Deka, H., P. P. Sarmah, A. Devi, P. Tamuly, and T. Karak. 2021. Changes in major catechins, caffeine, and antioxidant activity during CTC processing of black tea from North East India. *RSC Advances* 11 (19):11457–67. doi: 10.1039/D0RA09529J.

Del Prete, M., and A. Samoggia. 2020. Chocolate consumption and purchasing behaviour review: Research issues and insights for future research. *Sustainability* 12 (14):5586. doi: 10.3390/su12145586.

Di Mattia, C. D., G. Sacchetti, D. Mastrocola, and M. Serafini. 2017. From cocoa to chocolate: The impact of processing on *in vitro* antioxidant activity and the effects of chocolate on antioxidant markers *in vivo*. *Frontiers in Immunology* 8:1207. doi: 10.3389/fimmu.2017.01207.

Djikeng, F. T., W. T. Teyomnou, N. Tenyang, B. Tiencheu, A. T. Morfor, B. A. H. Touko, S. N. Houketchang, G. T. Boungo, M. S. L. Karuna, F. C. Ngoufack, et al. 2018. Effect of traditional and oven roasting on the physicochemical properties of fermented cocoa beans. *Heliyon* 4 (2):e00533. doi: 10.1016/j.heliyon.2018.e00533.

Donadini, G., M. D. Fumi, and M. Lambri. 2012. The hedonic response to chocolate and beverage pairing: A preliminary study. *Food Research International* 48 (2):703–11. doi: 10.1016/j.foodres.2012.06.009.

Drewnowski, A., and C. Gomez-Carneros. 2000. Bitter taste, phytonutrients, and the consumer: A review. *The American Journal of Clinical Nutrition* 72 (6):1424–35. doi: 10.1093/ajcn/72.6.1424.

Elwers, S., A. Zambrano, C. Rohsius, and R. Lieberei. 2009. Differences between the content of phenolic compounds in *Criollo*, *Forastero* and *Trinitario* cocoa seed (*Theobroma cacao* L.). *European Food Research and Technology* 229 (6):937–48. doi: 10.1007/s00217-009-1132-y.

Escobar, C., E. Espitia-Bautista, M. A. Guzmán-Ruiz, N. N. Guerrero-Vargas, M. Á. Hernández-Navarrete, M. Ángeles-Castellanos, B. Morales-Pérez, and R. M. Buijs. 2020. Chocolate for breakfast prevents circadian desynchrony in experimental models of jet-lag and shift-work. *Scientific Reports* 10 (1):6243. doi: 10.1038/s41598-020-63227-w.

Fang, Z., and B. Bhandari. 2010. Encapsulation of polyphenols – A review. *Trends in Food Science & Technology* 21 (10):510–23. doi: 10.1016/j.tifs.2010.08.003.

Febrianto, N. A., and F. Zhu. 2019a. Diversity in composition of bioactive compounds among 26 cocoa genotypes. *Journal of Agricultural and Food Chemistry* 67 (34):9501–9. doi: 10.1021/acs.jafc.9b03448.

Febrianto, N. A., and F. Zhu. 2019b. Intravariety diversity of bioactive compounds in *Trinitario* cocoa beans with different degrees of fermentation. *Journal of Agricultural and Food Chemistry* 67 (11):3150–8. doi: 10.1021/acs.jafc.8b06418.

Febrianto, N. A., and F. Zhu. 2020. Changes in the composition of methylxanthines, polyphenols, and volatiles and sensory profiles of cocoa beans from the Sul 1 genotype affected by fermentation. *Journal of Agricultural and Food Chemistry* 68 (32):8658–75. doi: 10.1021/acs.jafc.0c02909.

Figueroa-Hernández, C., J. Mota-Gutierrez, I. Ferrocino, Z. J. Hernández-Estrada, O. González-Ríos, L. Cocolin, and M. L. Suárez-Quiroz. 2019. The challenges and perspectives of the selection of starter cultures for fermented cocoa beans. *International Journal of Food Microbiology* 301:41–50. doi: 10.1016/j.ijfoodmicro.2019.05.002.

FoodDrinkEurope. 2019. *Acrylamide toolbox 2019*. Brussels: FoodDrinkEurope.

Foubert, I., P. Vanrolleghem, O. Thas, and K. Dewettinck. 2006. Influence of chemical composition on the isothermal cocoa butter crystallization. *Journal of Food Science* 69 (9):E478–487. doi: 10.1111/j.1365-2621.2004.tb09933.x.

Frauendorfer, F., and P. Schieberle. 2019. Key aroma compounds in fermented *Forastero* cocoa beans and changes induced by roasting. *European Food Research and Technology* 245 (9):1907–15. doi: 10.1007/s00217-019-03292-2.

Gadd, G. M. 2010. Metals, minerals and microbes: Geomicrobiology and bioremediation. *Microbiology (Reading, England)* 156 (Pt 3):609–43. [Database] doi: 10.1099/mic.0.037143-0.

García, D. V., É. P. Esteve, and J. M. B. Baviera. 2020. Changes in cocoa properties induced by the alkalization process: A review. *Comprehensive Reviews in Food Science and Food Safety* 19:2200–21.

García-Merino, J. A., D. Moreno-Pérez, B. de Lucas, M. G. Montalvo-Lominchar, E. Muñoz, L. Sánchez, F. Naclerio, K. M. Herrera-Rocha, M. R. Moreno-Jiménez, N. E. Rocha-Guzmán, et al. 2020. Chronic flavanol-rich cocoa powder supplementation reduces body fat mass in endurance athletes by modifying the follistatin/myostatin ratio and leptin levels. *Food & Function* 11 (4):3441–50. doi: 10.1039/D0FO00246A.

Giacometti, J., D. Muhvić, A. Pavletić, and L. udarić. 2016. Cocoa polyphenols exhibit antioxidant, anti-inflammatory, anticancerogenic, and anti-necrotic activity in carbon tetrachloride-intoxicated mice. *Journal of Functional Foods* 23:177–87. doi: 10.1016/j.jff.2016.02.036.

Gigl, M., O. Frank, J. Barz, A. Gabler, C. Hegmanns, and T. Hofmann. 2021. Identification and quantitation of reaction products from quinic acid, quinic acid lactone, and chlorogenic acid with Strecker aldehydes in roasted coffee. *Journal of Agricultural and Food Chemistry* 69 (3):1027–38. doi: 10.1021/acs.jafc.0c06887.

González-Barrio, R., V. Nuñez-Gomez, E. Cienfuegos-Jovellanos, F. J. García-Alonso, and M. J. Periago-Castón. 2020. Improvement of the flavanol profile and the antioxidant capacity of chocolate using a phenolic rich cocoa powder. *Foods* 9 (2):189. doi: 10.3390/foods9020189.

Grassia, M., G. Salvatori, M. Roberti, D. Planeta, and L. Cinquanta. 2019. Polyphenols, methylxanthines, fatty acids and minerals in cocoa beans and cocoa products. *Journal of Food Measurement and Characterization* 13 (3):1721–8. doi: 10.1007/s11694-019-00089-5.

Guzmán-Ortiz, F. A., J. Castro-Rosas, C. A. Gómez-Aldapa, R. Mora-Escobedo, A. Rojas-León, M. L. Rodríguez-Marín, R. N. Falfán-Cortés, and A. D. Román-Gutiérrez. 2019. Enzyme activity during germination of different cereals: A review. *Food Reviews International* 35 (3):177–200. doi: 10.1080/87559129.2018.1514623.

Hansen, C. E., A. Klueppel, E. Raetz. 1995. Enzymatic treatments of cocoa. US5888562A.

Hansen, C. E., C. Budwig, S. Kochhar, M. A. Jullerat, J.-C. Spandone, P. Nicolas, R. Redgwell, E. Armstrong, and D. Sievert. 2004. Chocolate flavor manipulation. US 2004/0191403 A1.

Hegmann, E., W. Niether, C. Rohsius, W. Phillips, and R. Lieberei. 2020. Besides variety, also season and ripening stage have a major influence on fruit pulp aroma of cacao (*Theobroma cacao* L.). *Journal of Applied Botany and Food Quality* 93:266–75.

Hernández-Hernández, C., I. Viera-Alcaide, A. M. Morales-Sillero, J. Fernández-Bolaños, and G. Rodríguez-Gutiérrez. 2018. Bioactive compounds in Mexican genotypes of cocoa cotyledon and husk. *Food Chemistry* 240:831–9. doi: 10.1016/j.foodchem.2017.08.018.

Hinneh, M., E. Semanhyia, D. Van de Walle, A. De Winne, D. A. Tzompa-Sosa, G. L. L. Scalone, B. De Meulenaer, K. Messens, J. Van Durme, E. O. Afoakwa, et al. 2018. Assessing the influence of pod storage on sugar and free amino acid profiles and the implications on some Maillard reaction related flavor volatiles in *Forastero* cocoa beans. *Food Research International* 111:607–20. doi: 10.1016/j.foodres.2018.05.064.

Huynh, N. K., D. H. M. Nguyen, and H. V. H. Nguyen. 2020. Reduction of soluble oxalate in cocoa powder by the addition of calcium and ultrasonication. *Journal of Food Composition and Analysis* 93:103593. doi: 10.1016/j.jfca.2020.103593.

Ioannone, F., C. D. Di Mattia, M. De Gregorio, M. Sergi, M. Serafini, and G. Sacchetti. 2015. Flavanols, proanthocyanidins and antioxidant activity changes during cocoa (*Theobroma cacao* L.) roasting as affected by temperature and time of processing. *Food Chemistry* 174: 256–62. doi: 10.1016/j.foodchem.2014.11.019.

Jackson, S. E., L. Smith, J. Firth, I. Grabovac, P. Soysal, A. Koyanagi, L. Hu, B. Stubbs, J. Demurtas, N. Veronese, et al. 2019. Is there a relationship between chocolate consumption and symptoms of depression? A cross-sectional survey of 13,626 US adults. *Depression and Anxiety* 36 (10):987–95. doi: 10.1002/da.22950.

Jaeger, S. R., L. G. Axten, M. W. Wohlers, and D. Sun-Waterhouse. 2009. Polyphenol-rich beverages: Insights from sensory and consumer science. *Journal of the Science of Food and Agriculture* 89 (14):2356–63. doi: 10.1002/jsfa.3721.

John, W. A., N. L. Böttcher, M. Aßkamp, A. Bergounhou, N. Kumari, P.-W. Ho, R. N. D'Souza, E. Nevoigt, and M. S. Ullrich. 2019. Forcing fermentation: Profiling proteins, peptides and polyphenols in lab-scale cocoa bean fermentation. *Food Chemistry* 278:786–94. doi:10.1016/j.foodchem.2018.11.108.

John, W. A., N. L. Böttcher, B. Behrends, M. Corno, R. N. D'souza, N. Kuhnert, and M. S. Ullrich. 2020. Experimentally modelling cocoa bean fermentation reveals key factors and their influences. *Food Chemistry* 302:125335. doi: 10.1016/j.foodchem.2019.125335.

Júnior, P. C. G., V. B. dos Santos, A. S. Lopes, J. P. I. de Souza, J. R. S. Pina, G. C. A. Chagas Júnior, and P. S. B. Marinho. 2020. Determination of theobromine and caffeine in fermented and unfermented Amazonian cocoa (*Theobroma cacao* L.) beans using square wave voltammetry after chromatographic separation. *Food Control* 108:106887. doi: 10.1016/j.foodcont.2019.106887.

Kadow, D. 2020. The biochemistry of cocoa flavor – A holistic analysis of its development along the processing chain. *Journal of Applied Botany and Food Quality* 93:300–12.

Katz, D. L., K. Doughty, and A. Ali. 2011. Cocoa and chocolate in human health and disease. *Antioxidants & Redox Signaling* 15 (10): 2779–811. doi: 10.1089/ars.2010.3697.

Kelm, M. A., J. C. Johnson, R. J. Robbins, J. F. Hammerstone, and H. H. Schmitz. 2006. High-performance liquid chromatography separation and purification of cacao (*Theobroma cacao* L.) procyanidins according to degree of polymerization using a diol stationary phase. *Journal of Agricultural and Food Chemistry* 54 (5):1571–6. doi: 10.1021/jf0525941.

Khan, N., and H. Mukhtar. 2018. Tea polyphenols in promotion of human health. *Nutrients* 11 (1):39. doi: 10.3390/nu11010039.

Kim, J.-E., D. Song, J. Kim, J. Choi, J. R. Kim, H.-S. Yoon, J.-S. Bae, M. Han, S. Lee, J. S. Hong, et al. 2016. Oral supplementation with cocoa extract reduces UVB-induced wrinkles in hairless mouse skin. *Journal of Investigative Dermatology* 136 (5):1012–21. doi: 10.1016/j.jid.2015.11.032.

Kim, K., and R. M. Brothers. 2020. Acute consumption of flavanol-rich cocoa beverage improves attenuated cutaneous microvascular function in healthy young African Americans. *Microvascular Research* 128:103931. doi: 10.1016/j.mvr.2019.103931.

Kongor, J. E., M. Hinneh, D. Van de Walle, E. O. Afoakwa, P. Boeckx, and K. Dewettinck. 2016. Factors influencing quality variation in cocoa (*Theobroma cacao*) bean flavor profile – A review. *Food Research International* 82:44–52. doi: 10.1016/j.foodres.2016.01.012.

Kouadio, P. A., B. K. Attioua, F. A. Kabran, B. A. Kassi, R. A. Yao, H. K. Yeboue, M. N. Kasse, and E. K. Amoikon. 2020. Comparative physico-chemical study of fermented and unfermented cocoa beans from Côte d'Ivoire. *International Journal of Biosciences* 16:355–62.

Kramer, K., M. Yeboah-Awudzi, N. Magazine, J. M. King, Z. Xu, and J. N. Losso. 2019. Procyanidin B2 rich cocoa extracts inhibit inflammation in Caco-2 cell model of in vitro celiac disease by down-regulating interferon-gamma- or gliadin peptide 31-43-induced transglutaminase-2 and interleukin-15. *Journal of Functional Foods* 57:112–20. doi: 10.1016/j.jff.2019.03.039.

Kratzer, U., R. Frank, H. Kalbacher, B. Biehl, J. Wöstemeyer, and J. Voigt. 2009. Subunit structure of the vicilin-like globular storage protein of cocoa seeds and the origin of cocoa- and chocolate-specific aroma precursors. *Food Chemistry* 113 (4):903–13. doi: 10.1016/j.foodchem.2008.08.017.

Kühn, J., A. Schröter, B. M. Hartmann, and G. I. Stangl. 2018. Cocoa and chocolate are sources of vitamin D_2. *Food Chemistry* 269: 318–20. doi: 10.1016/j.foodchem.2018.06.098.

Kumari, N., A. Grimbs, R. N. D'Souza, S. K. Verma, M. Corno, N. Kuhnert, and M. S. Ullrich. 2018. Origin and varietal based proteomic and peptidomic fingerprinting of *Theobroma cacao* in non-fermented and fermented cocoa beans. *Food Research International (Ottawa, Ont.)* 111:137–47. doi: 10.1016/j.foodres.2018.05.010.

Kumari, N., K. J. Kofi, S. Grimbs, R. N. D'Souza, N. Kuhnert, G. Vrancken, and M. S. Ullrich. 2016. Biochemical fate of vicilin storage protein during fermentation and drying of cocoa beans. *Food Research International (Ottawa, Ont.)* 90:53–65. doi: 10.1016/j.foodres.2016.10.033.

Lavefve, L., L. R. Howard, and F. Carbonero. 2020. Berry polyphenols metabolism and impact on human gut microbiota and health. *Food & Function* 11 (1):45–65. doi: 10.1039/c9fo01634a.

Lawal, T. O., T. A. Olorunnipa, and B. A. Adeniyi. 2014. Susceptibility testing and bactericidal activities of *Theobroma cacao* Linn. (cocoa) on *Helicobacter pylori* in an *in vitro* study. *Journal of Herbal Medicine* 4 (4):201–7. doi: 10.1016/j.hermed.2014.09.004.

Lebot, V., M. Melteras, A. Pilecki, and J.-P. Labouisse. 2020. Chemometric evaluation of cocoa (*Theobroma cacao* L.) and coffee (*Coffea* spp.) germplasm using HPTLC. *Genetic Resources and Crop Evolution* 67 (4):895–911. doi: 10.1007/s10722-020-00888-6.

Lecumberri, E., R. Mateos, M. Izquierdo-Pulido, P. Rupérez, L. Goya, and L. Bravo. 2007. Dietary fibre composition, antioxidant capacity and physico-chemical properties of a fibre-rich product from cocoa (*Theobroma cacao* L.). *Food Chemistry* 104 (3):948–54. doi: 10.1016/j.foodchem.2006.12.054.

Lee, A. H., A. P. Neilson, S. F. O'Keefe, J. A. Ogejo, H. Huang, M. Ponder, H. S. S. Chu, Q. Jin, G. Pilot, and A. C. Stewart. 2019. A laboratory-scale model cocoa fermentation using dried, unfermented beans and artificial pulp can simulate the microbial and chemical changes of on-farm cocoa fermentation. *European Food Research and Technology* 245 (2):511–9. doi: 10.1007/s00217-018-3171-8.

Lefeber, T., Z. Papalexandratou, W. Gobert, N. Camu, and V. L. De. 2012. On-farm implementation of a starter culture for improved cocoa bean fermentation and its influence on the flavor of chocolates produced thereof. *Food Microbiology* 30 (2):379–92. doi: 10.1016/j.fm.2011.12.021.

León-Perez, D., S. Medina, J. Londoño-Londoño, M. Cano-Lamadrid, Á. Carbonell-Barrachina, T. Durand, A. Guy, C. Oger, J. M. Galano, F. Ferreres, et al. 2019. Comparative study of different cocoa (*Theobroma cacao* L.) clones in terms of their phytoprostanes and phytofurans contents. *Food Chemistry* 280:231–9. doi: 10.1016/j.foodchem.2018.12.072.

Li, Y., Y. Feng, S. Zhu, C. Luo, J. Ma, and F. Zhong. 2012. The effect of alkalization on the bioactive and flavor related components in commercial cocoa powder. *Journal of Food Composition and Analysis* 25 (1):17–23. doi: 10.1016/j.jfca.2011.04.010.

Liendo, R., F. C. Padilla, and A. Quintana. 1997. Characterization of cocoa butter extracted from *Criollo* cultivars of *Theobroma cacao* L. *Food Research International* 30 (9):727–31. doi: 10.1016/S0963-9969(98)00025-8.

Liou, B.-K., Y.-M. Jaw, G. C.-C. Chuang, N. N. J. Yau, Z.-Y. Zhuang, and L.-F. Wang. 2020. Important sensory, association, and postprandial perception attributes influencing young Taiwanese consumers' acceptance for Taiwanese specialty teas. *Foods* 9 (1):100. doi: 10.3390/foods9010100.

Lipp, M., and E. Anklam. 1998. Review of cocoa butter and alternative fats for use in chocolate – Part A. Compositional data. *Food Chemistry* 62 (1):73–97. doi: 10.1016/S0308-8146(97)00160-X.

Lustig, R. H., L. A. Schmidt, and C. D. Brindis. 2012. The toxic truth about sugar. *Nature* 482 (7383):27–9. [Database] doi: 10.1038/482027a.

Luximon-Ramma, A., T. Bahorun, A. Crozier, V. Zbarsky, K. P. Datla, D. T. Dexter, and O. I. Aruoma. 2005. Characterization of the antioxidant functions of flavonoids and proanthocyanidins in Mauritian

black teas. *Food Research International* 38 (4):357–67. doi: 10.1016/j.foodres.2004.10.005.

Martínez-Pinilla, E., A. Oñatibia-Astibia, and R. Franco. 2015. The relevance of theobromine for the beneficial effects of cocoa consumption. *Frontiers in Pharmacology* 6:30. doi: 10.3389/fphar.2015.00030.

Marty, S., and A. G. Marangoni. 2009. Effects of cocoa butter origin, tempering procedure, and structure on oil migration kinetics. *Crystal Growth & Design* 9 (10):4415–23. doi: 10.1021/cg9004505.

Mat, S. A., I. S. M. Daud, M. H. M. Rojie, N. Hussain, and Y. Rukayadi. 2016. Effects of *Candida* sp. and *Blastobotrys* sp. starter on fermentation of cocoa (*Theobroma cacao* L.) beans and its antibacterial activity. *Journal of Pure and Applied Microbiology* 10 (4):2501–10. doi: 10.22207/JPAM.10.4.04.

McDermott, M. M., M. H. Criqui, K. Domanchuk, L. Ferrucci, J. M. Guralnik, M. R. Kibbe, K. Kosmac, C. M. Kramer, C. Leeuwenburgh, L. Li, et al. 2020. Cocoa to improve walking performance in older people with peripheral artery disease: The COCOA-PAD pilot randomized clinical trial. *Circulation Research* 126 (5):589–99. doi: 10.1161/CIRCRESAHA.119.315600.

Megías-Pérez, R., S. Grimbs, R. N. D'Souza, H. Bernaert, and N. Kuhnert. 2018. Profiling, quantification and classification of cocoa beans based on chemometric analysis of carbohydrates using hydrophilic interaction liquid chromatography coupled to mass spectrometry. *Food Chemistry* 258:284–94. doi: 10.1016/j.foodchem.2018.03.026.

Melo, C. W. B. D., M. D. J. Bandeira, L. F. Maciel, E. d. S. Bispo, C. O. D. Souza, and S. E. Soares. 2020. Chemical composition and fatty acids profile of chocolates produced with different cocoa. *Food Science and Technology* 40 (2):326–33. doi: 10.1590/fst.43018.

Messaoudi, M., J.-F. Bisson, A. Nejdi, P. Rozan, and H. Javelot. 2008. Antidepressant-like effects of a cocoa polyphenolic extract in Wistar-Unilever rats. *Nutritional Neuroscience* 11 (6):269–76. doi: 10.1179/147683008X344165.

Midttun, H. L. E., A. Ramsay, I. Mueller-Harvey, and A. R. Williams. 2018. Cocoa procyanidins modulate transcriptional pathways linked to inflammation and metabolism in human dendritic cells. *Food & Function* 9 (5):2883–90. doi: 10.1039/c8fo00387d.

Misnawi, J. S., B. Jamilah, and S. Nazamid. 2003. Effects of incubation and polyphenol oxidase enrichment on colour, fermentation index, procyanidins and astringency of unfermented and partly fermented cocoa beans. *International Journal of Food Science and Technology* 38 (3):285–95. doi: 10.1046/j.1365-2621.2003.00674.x.

Misnawi, J. S., S. Nazamid, and B. Jamilah. 2002. Activation of remaining key enzymes in dried under-fermented cocoa beans and its effect on aroma precursor formation. *Food Chemistry* 78:407–17.

Misnawi. 2012. Effect of cocoa bean drying methods on polycyclic aromatic hydrocarbons contamination in cocoa butter. *International Food Research Journal* 19:1589–94.

Mota-Gutierrez, J., C. Botta, I. Ferrocino, M. Giordano, M. Bertolino, P. Dolci, M. Cannoni, and L. Cocolin. 2018. Dynamics and Biodiversity of Bacterial and Yeast Communities during Fermentation of Cocoa Beans. *Applied and Environmental Microbiology* 84 (19). doi:10.1128/AEM.01164-18.

Mota-Gutierrez, J., L. Barbosa-Pereira, I. Ferrocino, and L. Cocolin. 2019. Traceability of functional volatile compounds generated on inoculated cocoa fermentation and its potential health benefits. *Nutrients* 11:884.

Mudenuti, N. V., de R, A. C. de Camargo, F. Shahidi, T. B. Madeira, E. Y. Hirooka, and M. V. E. Grossmann. 2018. Soluble and insoluble-bound fractions of phenolics and alkaloids and their antioxidant activities in raw and traditional chocolate: A comparative study. *Journal of Functional Foods* 50:164–71. doi: 10.1016/j.jff.2018.10.003.

Mulyawanti, I., T. Hidayat, and N. F. N. Risfaheri. 2018. Pengaruh jenis fermentor terhadap mutu biji kakao kering non fermentasi [The influences of fermentor type on quality of dried unfermented cocoa bean]. *Jurnal Penelitian Pascapanen Pertanian* 15 (2):91–8. In Indonesian. doi: 10.21082/jpasca.v15n2.2018.91-98.

Muñoz, M. S., J. R. Cortina, F. E. Vaillant, and S. E. Parra. 2020. An overview of the physical and biochemical transformation of cocoa seeds to beans and to chocolate: Flavor formation. *Critical Reviews in Food Science and Nutrition* 60 (10):1593–613. doi: 10.1080/10408398.2019.1581726.

Nazaruddin, R., L. K. Seng, O. Hassan, and M. Said. 2006. Effect of pulp preconditioning on the content of polyphenols in cocoa beans (*Theobroma cacao*) during fermentation. *Industrial Crops and Products* 24 (1):87–94. doi: 10.1016/j.indcrop.2006.03.013.

Netea, S. A., S. A. Janssen, M. Jaeger, T. Jansen, L. Jacobs, G. Miller-Tomaszewska, T. S. Plantinga, M. G. Netea, and L. A. B. Joosten. 2013. Chocolate consumption modulates cytokine production in healthy individuals. *Cytokine* 62 (1):40–3. doi: 10.1016/j.cyto.2013.02.003.

Nguyễn, H. V. H., H. M. Lê, and G. P. Savage. 2018. Effects of maturity at harvesting and primary processing of cocoa beans on oxalate contents of cocoa powder. *Journal of Food Composition and Analysis* 67:86–90. doi: 10.1016/j.jfca.2018.01.007.

Niemenak, N., V. Jos, A. S. D. Mouafi, A. G. T. Ngouambe, C. Bernhardt, R. Lieberei, and B. Bisping. 2020. Assessment of the profile of free amino acids and reducing sugars of cacao beans from local Cameroonian *Trinitario* (SNK varieties) and *Forastero* (TIKO varieties) using fermentation-like incubation. *Journal of Applied Botany and Food Quality* 93:321–9.

Nijveldt, R. J., van Nood, E. D. E. C. van Hoorn, P. G. Boelens, K. van Norren, and P. A. M. van Leeuwen. 2001. Flavonoids: A review of probable mechanisms of action and potential applications. *The American Journal of Clinical Nutrition* 74 (4):418–25. doi: 10.1093/ajcn/74.4.418.

Oduro-Mensah, D., A. Ocloo, S. T. Lowor, C. Mingle, L. K. N. A. Okine, and N. A. Adamafio. 2018. Bio-detheobromination of cocoa pod husks: Reduction of ochratoxin A content without change in nutrient profile. *Microbial Cell Factories* 17 (1):79. doi: 10.1186/s12934-018-0931-x.

Olas, B. 2018. Berry phenolic antioxidants – Implications for human health? *Frontiers in Pharmacology* 9:78. doi: 10.3389/fphar.2018.00078.

Oliveira, H. S. S., M. E. O. Mamede, A. Góes-Neto, and M. G. B. Koblitz. 2011. Chocolate flavor in poor-quality cocoa almonds by enzymatic treatment. *Journal of Food Science* 76 (5):C755–759. doi: 10.1111/j.1750-3841.2011.02168.x.

Oracz, J., and D. Zyzelewicz. 2019. *In vitro* antioxidant activity and FTIR characterization of high-molecular weight melanoidin fractions from different types of cocoa beans. *Antioxidants* 8:560. doi: 10.3390/antiox8110560.

Oracz, J., and E. Nebesny. 2014. Influence of roasting conditions on the biogenic amine content in cocoa beans of different *Theobroma cacao* cultivars. *Food Research International* 55:1–10. doi: 10.1016/j.foodres.2013.10.032.

Oracz, J., and E. Nebesny. 2019. Effect of roasting parameters on the physicochemical characteristics of high-molecular-weight Maillard reaction products isolated from cocoa beans of different *Theobroma cacao* L. groups. *European Food Research and Technology* 245 (1):111–28. doi: 10.1007/s00217-018-3144-y.

Oracz, J., E. Nebesny, and D. Żyżelewicz. 2014. Effect of roasting conditions on the fat, tocopherol, and phytosterol content and antioxidant capacity of the lipid fraction from cocoa beans of different *Theobroma cacao* L. cultivars. *European Journal of Lipid Science and Technology* 116 (8):1002–14. doi: 10.1002/ejlt.201300474.

Padilla, F. C., R. Liendo, and A. Quintana. 2000. Characterization of cocoa butter extracted from hybrid cultivars of Theobroma cacao L. *Archivos Latinoamericanos De Nutricion* 50 (2):200–5. PMC: 11048595

Pandurangan, A. K., Z. Saadatdoust, N. M. Esa, H. Hamzah, and A. Ismail. 2015. Dietary cocoa protects against colitis-associated cancer by activating the Nrf2/Keap1 pathway. *BioFactors (Oxford, England)* 41 (1):1–14. doi: 10.1002/biof.1195.

Pedan, V., N. Fischer, and S. Rohn. 2016. An online NP-HPLC-DPPH method for the determination of the antioxidant activity of condensed polyphenols in cocoa. *Food Research International* 89:890–900. doi: 10.1016/j.foodres.2015.10.030.

Peng, M., U. Aryal, B. Cooper, and D. Biswas. 2015. Metabolites produced during the growth of probiotics in cocoa supplementation and the limited role of cocoa in host-enteric bacterial pathogen interactions. *Food Control* 53:124–33. doi: 10.1016/j.foodcont.2015.01.014.

Pereyra-Vergara, F., Olivares-Corichi, I. M. A. G. Perez-Ruiz, J. P. Luna-Arias, and J. R. García-Sánchez. 2020. Apoptosis induced by (−)-epicatechin in human breast cancer cells is mediated by reactive oxygen species. *Molecules* 25 (5):1020. doi: 10.3390/molecules25051020.

Pham, V. T. T., T. Ismail, M. Mishyna, K. S. Appiah, Y. Oikawa, and Y. Fujii. 2019. Caffeine: The allelochemical responsible for the plant growth inhibitory activity of Vietnamese tea (*Camellia sinensis* L. Kuntze). *Agronomy* 9 (7):396. doi: 10.3390/agronomy9070396.

Quiroz-Reyes, C. N., and V. Fogliano. 2018. Design cocoa processing towards healthy cocoa products: The role of phenolics and melanoidins. *Journal of Functional Foods* 45:480–90. doi: 10.1016/j.jff.2018.04.031.

Ramos, S., M. A. Martín, and L. Goya. 2017. Effects of cocoa antioxidants in type 2 diabetes mellitus. *Antioxidants* 6 (4):84. doi: 10.3390/antiox6040084.

Rasines-Perea, Z., and P.-L. Teissedre. 2017. Grape polyphenols' effects in human cardiovascular diseases and diabetes. *Molecules* 22 (1):68. doi: 10.3390/molecules22010068.

Raters, M., and R. Matissek. 2014. Quantitation of polycyclic aromatic hydrocarbons (PAH4) in cocoa and chocolate samples by an HPLC-FD method. *Journal of Agricultural and Food Chemistry* 62 (44): 10666–71. doi: 10.1021/jf5028729.

Raters, M., and R. Matissek. 2018. Acrylamide in cocoa: A survey of acrylamide levels in cocoa and cocoa products sourced from the German market. *European Food Research and Technology* 244 (8): 1381–8. doi: 10.1007/s00217-018-3051-2.

Ravindranath, M. H., T. S. Saravanan, C. C. Monteclaro, N. Presser, X. Ye, S. R. Selvan, and S. Brosman. 2006. Epicatechins purified from green tea (*Camellia sinensis*) differentially suppress growth of gender-dependent human cancer cell lines. *Evidence-Based Complementary and Alternative Medicine: eCAM* 3 (2):237–47. doi: 10.1093/ecam/nel003.

Redgwell, R. J., V. Trovato, and D. Curti. 2003. Cocoa bean carbohydrates: Roasting-induced changes and polymer interactions. *Food Chemistry* 80 (4):511–6. doi: 10.1016/S0308-8146(02)00320-5.

Robbins, R. J., J. Leonczak, J. Li, J. C. Johnson, T. Collins, C. Kwik-Uribe, H. H. Schmitz, J. Austad, S. Bhandari, T. Cifuentes, et al. 2013. Flavanol and procyanidin content (by degree of polymerization 1-10) of chocolate, cocoa liquors, cocoa powders, and cocoa extracts: First action 2012.24. *Journal of AOAC International* 96 (4): 705–11., doi: 10.5740/jaoacint.13-109.

Rodriguez-Campos, J., H. B. Escalona-Buendía, S. M. Contreras-Ramos, I. Orozco-Avila, E. Jaramillo-Flores, and E. Lugo-Cervantes. 2012. Effect of fermentation time and drying temperature on volatile compounds in cocoa. *Food Chemistry* 132 (1):277–88. doi: 10.1016/j.foodchem.2011.10.078.

Rodríguez-Carrasco, Y., A. Gaspari, G. Graziani, A. Santini, and A. Ritieni. 2018. Fast analysis of polyphenols and alkaloids in cocoa-based products by ultra-high performance liquid chromatography and orbitrap high resolution mass spectrometry (UHPLC-Q-Orbitrap-MS/MS). *Food Research International* 111:229–36. doi: 10.1016/j.foodres.2018.05.032.

Rojo-Poveda, O., L. Barbosa-Pereira, G. Zeppa, and C. Stévigny. 2020. Cocoa bean shell – A by-product with nutritional properties and biofunctional potential. *Nutrients* 12 (4):1123. doi: 10.3390/nu12041123.

Rovati, L. C. 2016. Purified cocoa beans extracts, production and use thereof for the treatment of central and peripheric human diseases. WO 2016/020854.

Ryan, C., L. Ye, S. O'Keefe, and A. P. Neilson. 2016. Unfermented and fermented cocoa extracts as inhibitors of pancreatic α-amylase, α-glucosidase, and pancreatic lipase. *The FASEB Journal* 30: 691.30.

Sacchetti, G., F. Ioannone, M. De Gregorio, C. D. Mattia, M. Serafini, and D. Mastrocola. 2016. Non enzymatic browning during cocoa roasting as affected by processing time and temperature. *Journal of Food Engineering* 169:44–52. doi: 10.1016/j.jfoodeng.2015.08.018.

Sajadimajd, S., R. Bahramsoltani, A. Iranpanah, J. Kumar Patra, G. Das, S. Gouda, R. Rahimi, E. Rezaeiamiri, H. Cao, F. Giampieri, et al. 2020. Advances on natural polyphenols as anticancer agents for skin cancer. *Pharmacological Research* 151:104584.

Salazar, E., R. Valenzuela, M. Aguilar, N. Aranda, A. Sotelo, G. C. Chire-Fajardo, and M. Ureña. 2020. Physicochemical properties and microbial group behavior of postharvest Peruvian cocoa bean. *Enfoque Ute* 11 (4):48–56. doi: 10.29019/enfoqueute.v11n4.602.

Santos, D. S., R. P. Rezende, T. F. Dos Santos, E. d. L. S. Marques, A. C. R. Ferreira, A. B. d. C. e Silva, C. C. Romano, D. W. da Cruz Santos, J. C. T. Dias, and J. D. T. Bisneto. 2020. Fermentation in fine cocoa type Scavina: Change in standard quality as the effect of use of starters yeast in fermentation. *Food Chemistry* 328:127110. doi: 10.1016/j.foodchem.2020.127110.

Sari, N., Y. Katanasaka, H. Honda, Y. Miyazaki, Y. Sunagawa, M. Funamoto, K. Shimizu, S. Shimizu, H. Wada, K. Hasegawa, et al. 2020. Cacao bean polyphenols inhibit cardiac hypertrophy and systolic dysfunction in pressure overload-induced heart failure model mice. *Planta Medica* 86 (17):1304–12. doi: 10.1055/a-1191-7970.

Scalone, G. L. L., K. Textoris-Taube, B. D. Meulenaer, N. D. Kimpe, J. Wöstemeyer, and J. Voigt. 2019. Cocoa-specific flavor components and their peptide precursors. *Food Research International (Ottawa, Ont.)* 123:503–15. doi: 10.1016/j.foodres.2019.05.019.

Schmiederand, R. L., and G. Keeney. 1980. Characterization and quantification of starch in cocoa beans and chocolate products. *Journal of Food Science* 45 (3):555–7. doi: 10.1111/j.1365-2621.1980.tb04099.x.

Schroder, T., L. Vanhanen, and G. P. Savage. 2011. Oxalate content in commercially produced cocoa and dark chocolate. *Journal of Food Composition and Analysis* 24 (7):916–22. doi: 10.1016/j.jfca.2011.03.008.

Scollo, E., D. C. A. Neville, M. J. Oruna-Concha, M. Trotin, and R. Cramer. 2020. UHPLC–MS/MS analysis of cocoa bean proteomes from four different genotypes. *Food Chemistry* 303:125244. doi: 10.1016/j.foodchem.2019.125244.

Sepúlveda, W. S., M. T. Maza, P. Uldemolins, E. G. Cantos-Zambrano, and I. Ureta. 2021. Linking dark chocolate product attributes, consumer preferences, and consumer utility: Impact of quality labels, cocoa content, chocolate origin, and price. *Journal of International Food & Agribusiness Marketing*. doi: 10.1080/08974438.2021.1908924.

Serafini, M., I. Peluso, and A. Raguzzini. 2010. Flavonoids as anti-inflammatory agents. *The Proceedings of the Nutrition Society* 69: 273–8.

Servent, A., R. Boulanger, F. Davrieux, M.-N. Pinot, E. Tardan, N. Forestier-Chiron, and C. Hue. 2018. Assessment of cocoa (*Theobroma cacao* L.) butter content and composition throughout fermentations. *Food Research International (Ottawa, Ont.)* 107: 675–82. doi: 10.1016/j.foodres.2018.02.070.

Shi, H., B. Wang, and X. Xu. 2017. Antidepressant effect of vitamin D: A literature review. *Neuropsychiatry* 7:337–41.

Sirbu, D., A. Grimbs, M. Corno, M. S. Ullrich, and N. Kuhnert. 2018. Variation of triacylglycerol profiles in unfermented and dried fermented cocoa beans of different origins. *Food Research International (Ottawa, Ont.)* 111:361–70. doi: 10.1016/j.foodres.2018.05.025.

Stark, T., S. Bareuther, and T. Hofmann. 2006. Molecular definition of the taste of roasted cocoa nibs (*Theobroma cacao*) by means of quantitative studies and sensory experiments. *Journal of Agricultural and Food Chemistry* 54 (15):5530–9. doi: 10.1021/jf0608726.

Szopa, A., E. Poleszak, E. Wyska, A. Serefko, S. Wośko, A. Wlaź, M. Pieróg, A. Wróbel, and P. Wlaź. 2016. Caffeine enhances the antidepressant-like activity of common antidepressant drugs in the forced swim test in mice. *Naunyn-Schmiedeberg's Archives of Pharmacology* 389 (2):211–21. doi: 10.1007/s00210-015-1189-z.

Taş, N. G., and V. Gökmen. 2016. Effect of alkalization on the Maillard reaction products formed in cocoa during roasting. *Food Research International* 89:930–6. doi: 10.1016/j.foodres.2015.12.021.

Todorovic, V., M. Milenkovic, B. Vidovic, Z. Todorovic, and S. Sobajic. 2017. Correlation between antimicrobial, antioxidant activity, and

polyphenols of alkalized/nonalkalized cocoa powders. *Journal of Food Science* 82 (4):1020–7. doi: 10.1111/1750-3841.13672.

Torres-Moreno, M., E. Torrescasana, J. Salas-Salvadó, and C. Blanch. 2015. Nutritional composition and fatty acids profile in cocoa beans and chocolates with different geographical origin and processing conditions. *Food Chemistry* 166:125–32. doi: 10.1016/j.foodchem.2014.05.141.

Tuenter, E., C. Delbaere, A. De Winne, S. Bijttebier, D. Custers, K. Foubert, J. V. Durme, K. Messens, K. Dewettinck, and L. Pieters. 2020. Non-volatile and volatile composition of West African bulk and Ecuadorian fine-flavor cocoa liquor and chocolate. *Food Research International (Ottawa, Ont.)* 130:108943. doi: 10.1016/j.foodres.2019.108943.

Ullah, H., A. De Filippis, C. Santarcangelo, and M. Daglia. 2020. Epigenetic regulation by polyphenols in diabetes and related complications. *Mediterranean Journal of Nutrition and Metabolism* 13 (4):289–310. doi: 10.3233/MNM-200489.

Urbańska, B., Derewiaka, D. A. Lenart, and J. Kowalska. 2019. Changes in the composition and content of polyphenols in chocolate resulting from pre-treatment method of cocoa beans and technological process. *European Food Research and Technology* 245 (10):2101–12. doi: 10.1007/s00217-019-03333-w.

Valiente, C., R. M. Esteban, E. Mollá, and F. J. López-Andréu. 1994. Roasting effects on dietary fiber composition of cocoa beans. *Journal of Food Science* 59 (1):123–4. doi: 10.1111/j.1365-2621.1994.tb06914.x.

Visintin, S., L. Ramos, N. Batista, P. Dolci, F. Schwan, and L. Cocolin. 2017. Impact of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* starter cultures on cocoa beans fermentation. *International Journal of Food Microbiology* 257:31–40. doi: 10.1016/j.ijfoodmicro.2017.06.004.

Voigt, J., K. Textoris-Taube, and J. Wöstemeyer. 2018. pH-Dependency of the proteolytic formation of cocoa- and nutty-specific aroma precursors. *Food Chemistry* 255:209–15. doi: 10.1016/j.foodchem.2018.02.045.

Wang, X., S. Hu, X. Wan, and C. Pan. 2005. Effect of microbial fermentation on caffeine content of tea leaves. *Journal of Agricultural and Food Chemistry* 53 (18):7238–42. doi: 10.1021/jf050495h.

Wickramasuriya, A. M., and J. M. Dunwell. 2018. Cacao biotechnology: Current status and future prospects. *Plant Biotechnology Journal* 16 (1):4–17. doi: 10.1111/pbi.12848.

Ye, N., S. Belli, F. Caruso, G. Roy, and M. Rossi. 2021. Antioxidant studies by hydrodynamic voltammetry and DFT, quantitative analyses by HPLC-DAD of clovamide. *Food Chemistry* 341 (Pt 2):128260. doi: 10.1016/j.foodchem.2020.128260.

Zarrillo, S., N. Gaikwad, C. Lanaud, T. Powis, C. Viot, I. Lesur, O. Fouet, X. Argout, E. Guichoux, F. Salin, et al. 2018. The use and domestication of *Theobroma cacao* during the mid-Holocene in the upper Amazon. *Nature Ecology & Evolution* 2 (12):1879–88. doi: 10.1038/s41559-018-0697-x.

Zhu, F. 2018. Interactions between cell wall polysaccharides and polyphenols. *Critical Reviews in Food Science and Nutrition* 58 (11):1808–31. doi: 10.1080/10408398.2017.1287659.

Ziegleder, G. 2017. Flavour development in cocoa and chocolate. In *Beckett's industrial chocolate manufacture and use*, ed. S. T. Beckett, M. S. Fowler, and G. R. Ziegler, 5th ed., 185–215. Chichester: John Wiley and Sons Ltd.

Żyżelewicz, D., M. Bojczuk, G. Budryn, Z. Zduńczyk, J. Juśkiewicz, A. Jurgoński, and J. Oracz. 2018. Influence of diet based on bread supplemented with raw and roasted cocoa bean extracts on physiological indices of laboratory rats. *Food Research International (Ottawa, Ont.)* 112:209–16. doi: 10.1016/j.foodres.2018.06.039.

Żyżelewicz, D., G. Budryn, W. Krysiak, J. Oracz, E. Nebesny, and M. Bojczuk. 2014. Influence of roasting conditions on fatty acid composition and oxidative changes of cocoa butter extracted from cocoa bean of *Forastero* variety cultivated in Togo. *Food Research International* 63:328–43. doi: 10.1016/j.foodres.2014.04.053.

Żyżelewicz, D., W. Krysiak, J. Oracz, D. Sosnowska, G. Budryn, and E. Nebesny. 2016. The influence of the roasting process conditions on the polyphenol content in cocoa beans, nibs and chocolates. *Food Research International* 89:918–29. doi: 10.1016/j.foodres.2016.03.026.

Żyżelewicz, D., J. Oracz, M. Bojczuk, G. Budryn, A. Jurgoński, J. Juśkiewicz, and Z. Zduńczyk. 2020. Effects of raw and roasted cocoa bean extracts supplementation on intestinal enzyme activity, biochemical parameters, and antioxidant status in rats fed a high-fat diet. *Nutrients* 12 (4):889. doi: 10.3390/nu12040889.

Żyżelewicz, D., J. Oracz, W. Krysiak, G. Budryn, and E. Nebesny. 2017. Effects of various roasting conditions on acrylamide, acrolein, and polycyclic aromatic hydrocarbons content in cocoa bean and the derived chocolates. *Drying Technology* 35 (3):363–74. doi: 10.1080/07373937.2016.1175470.

Zzaman, W., R. Bhat, and T. A. Yang. 2017. Impact of convectional and superheated-steam roasting on the physycochemical and microstructural properties of cocoa butter extracted from cocoa beans. *Journal of Food Processing and Preservation* 41 (4):e13005. doi: 10.1111/jfpp.13005.