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Review

Bioactives in Cocoa: Novel Findings, Health Benefits, and Extraction Techniques

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Abstract: *Theobroma cacao* L. seeds, commonly known as cocoa beans, are the foundation for cocoa and chocolate production. Following harvest, these beans undergo a multi-step processing chain including fermentation, drying, roasting, and grinding. This process yields cocoa solids, cocoa butter, and cocoa powder—all fundamental ingredients in the food and beverage industry. Beyond its sensory appeal (flavor, aroma, and texture), cocoa has garnered significant interest for its potential health benefits attributed to a rich profile of bioactive compounds. Cocoa is a well-documented source of polyphenolics, specifically flavanols, alongside methylxanthines, phytosterols, and dietary fibers. These constituents have been associated with a diverse range of bioactivities, including antioxidant, anti-carcinogenic, anti-diabetic, anti-inflammatory, anti-obesity, and anti-allergenic properties, potentially contributing to overall health maintenance. Efficient extraction techniques are crucial for maximizing the recovery of these valuable bioactive components from cocoa plant material. Modern methods are continuously being explored to optimize this process. This review focuses on the established health benefits associated with the bioactive compounds present in cocoa. Additionally, it will explore and discuss contemporary approaches for the extraction of these bioactive compounds from this plant source.

Keywords: cocoa; health benefits; polyphenolic and methylxanthine compounds; extraction

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1. Introduction

Theobroma cacao L. trees, native to tropical regions of Central and South America, are cultivated for their seeds, known as cocoa beans. These beans thrive within a narrow equatorial band, often referred to as the "cocoa belt" [1]. The beans are encased within pods containing a sweet, mucilaginous pulp [2]. Following harvest, the beans undergo fermentation, drying, roasting, and subsequent processing into various cocoa products like cocoa powder and cocoa butter. Cocoa powder originates from grinding roasted cocoa beans, while cocoa butter is the extracted fat component. Both hold significant value in the food industry, with cocoa powder finding applications in baking, confectionery, and beverages, while cocoa butter is a key ingredient in chocolate production and cosmetics.

Cocoa boasts a long history of human consumption, with evidence tracing its use back to the Maya civilization in Central America (400 AD) as a ceremonial and potentially pleasurable food source. Notably, the Maya referred to their hot water cocoa beverage as the "Food of the Gods" [3]. As shown in Table 1, from a compositional standpoint, cocoa is a rich source of fiber (40–26%), lipids (24–10%), proteins (20–15%), carbohydrates (15%), and micronutrients (<2%) encompassing minerals (P, Ca, K, Na, Mg, Zn, and Cu) and vitamins (A, B, E) [4]. Additionally, cocoa is recognized for its abundance of polyphenols, particularly flavanols, which have been linked to a variety of health benefits [5]. These

bioactive components in cocoa have been associated with antioxidant, anti-carcinogenic, anti-diabetic, anti-inflammatory, anti-obesity, and anti-allergenic properties, potentially contributing to overall health. Several studies suggest that cocoa consumption may offer health advantages, including a reduced risk of chronic diseases like cardiovascular ailments, metabolic disorders, and cancer [5–7]. Furthermore, research indicates positive effects on the nervous system, visual function, and skin [8–10].

Table 1. The nutritional content of cocoa in percentage per 100 g [11].

Macronutrients per 100 g	Percentage
Protein	15–20%
Carbohydrates	~15%
Lipids	10–25%
Fiber	25–40%
Micronutrients per 100 g	**Per 100 g**
Vitamin A (Retinol)	<0.2 mg
Vitamin E (Tocopherol)	2.5 mg
Vitamin B1 (Thiamine)	0.3 mg
Vitamin B1 (Riboflavin)	0.4 mg
Vitamin B3 (Niacin)	0.7 mg
Sodium (Na)	0.03 g
Potassium (K)	4.3 g
Calcium (Ca)	151 mg
Phosphorus (P)	700 mg
Iron (Fe)	26 mg
Magnesium (mg)	555 mg
Copper (Cu)	5 mg

2. Bioactives in Cocoa

Bioactive compounds are naturally occurring molecules in plants and foods that demonstrate health benefits when consumed at appropriate levels. These compounds can influence physiological functions beyond basic nutrition and may contribute to chronic disease risk reduction or overall well-being through antioxidant and anti-inflammatory properties [12]. Ongoing research continues to identify novel bioactive molecules and their potential health impacts, fueling interest in functional foods, nutraceuticals, and dietary supplements designed to deliver concentrated forms of these compounds.

Unprocessed cocoa beans are a rich source of polyphenols (12–18% dry weight), encompassing major groups like flavanols, anthocyanidins, and proanthocyanidins [13–15]. Naturally occurring polyphenols have been extensively studied for their health benefits, including combating free radicals detrimental to human health and food systems [16]. These compounds are linked to various functionalities in functional foods, including cardiovascular protection, anti-tumor activity, anti-inflammatory properties, and benefits for neurodegeneration, bacterial control, and dental health [17]. Cocoa products are also abundant in methylxanthines such as caffeine, theobromine, and theophylline. While these compounds offer health benefits, they can negatively impact taste by introducing astringency and bitterness. Additionally, they can influence the stability and digestibility of products high in these components [17–19]. Consequently, subsequent treatments like fermentation, drying, and roasting are crucial for achieving the unique sensory characteristics of cocoa products (Figure 1).

The synthesis of bioactive compounds during cocoa post-harvest processing is influenced by factors such as reducing sugars, peptides, and amino acids [19]. Additionally, genotype, bean maturity, geographical origin, and processing methods (fermentation and drying) play a role [20]. Yeasts and lactic acid bacteria initiate fermentation by degrading pulp sugars primarily into ethanol. This exothermic process elevates cocoa mass temperature and oxygen tension, leading to acetic acid production from ethanol [21,22]. Fermentation reduces pH and increases titratable acidity, triggering embryo death and

cell wall rupture through the action of ethanol and acetic acid. Furthermore, fermentation decreases bitterness and astringency by reducing phenolic content due to phenol oxidase activity [23,24]. However, inadequate fermentation processes can be detrimental, leading to decreased phenolic content and antioxidant activity in cocoa beans [18,25].

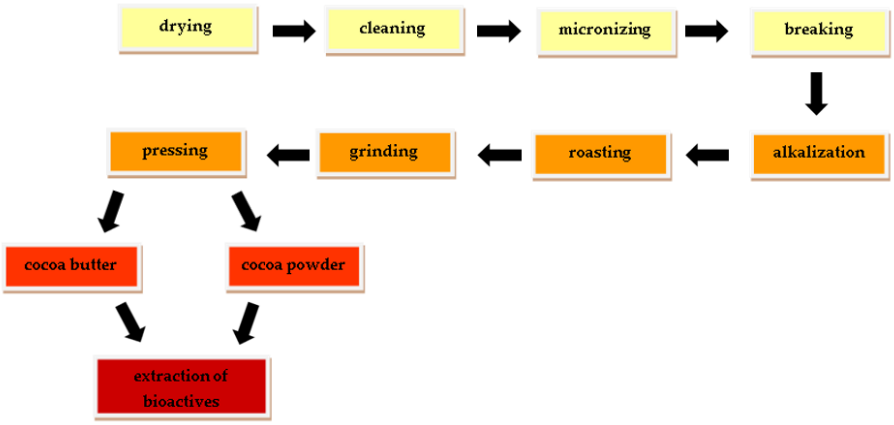


Figure 1. Processing of cocoa beans.

2.1. Polyphenols

Cocoa polyphenols as phytocompounds or phytochemicals, are widespread across the plant kingdom, serving as a defense mechanism against herbivores, pathogens, and ultraviolet radiation. Cocoa stands out for its abundance of polyphenolic compounds, particularly flavanols (also known as flavan-3-ols)—a specific subclass of flavonoids [26] (Figure 2).

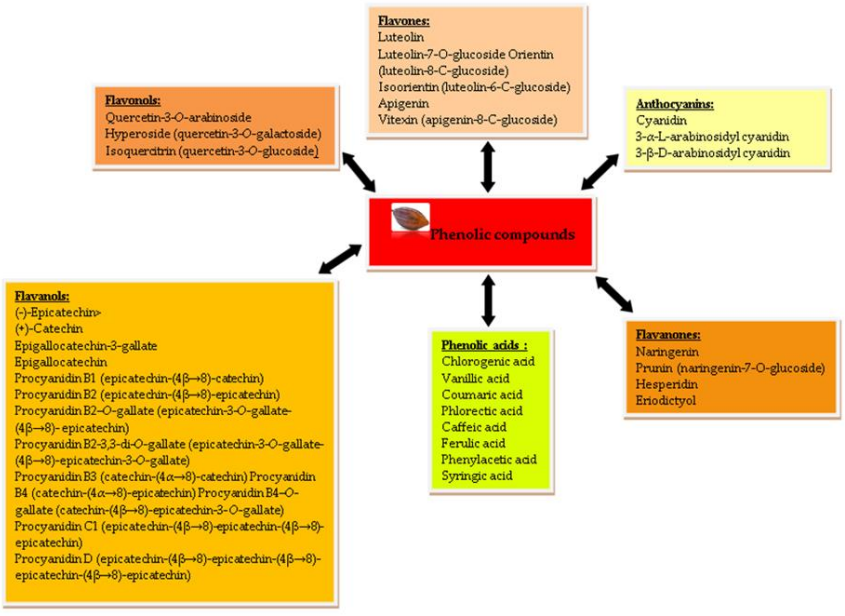


Figure 2. Main groups of cocoa polyphenols.

As mentioned, cocoa polyphenols, classified as secondary metabolites, contribute to the characteristic bitterness and astringency of cocoa beans and chocolate [27]. These diverse compounds encompass over 8000 identified phenolic structures [28]. The core flavonoid structure features 15 carbon atoms with two aromatic rings (A and B) linked by a three-carbon bridge (C). Variations in hydroxylation patterns and the chromane ring (C) classify flavonoids into subgroups like anthocyanins, flavan-3-ols (flavanols), flavones, flavanones, and flavonols [29].

Unfermented, dried cocoa beans contain roughly 13.5% phenolics. These include monomeric flavanols like (−)-epicatechin and (+)-catechin, along with procyanidins (dimeric, particularly B2 and B1) and oligomeric/polymeric forms [30]. While present in lower quantities, cocoa also boasts other polyphenols such as flavones (luteolin and apigenin), flavanones (naringenin), flavonols (quercetin, isoquercitrin, and hyperoside), phenolic acids, and anthocyanins [10,31]. Procyanidins are the primary contributors to cocoa's antioxidant activity [32]. However, high flavanol content, particularly, is responsible for the characteristic bitterness, significantly influencing chocolate's organoleptic properties and palatability [33].

Cocoa polyphenol content exhibits significant variability. Several factors influence this, including cocoa subspecies, geographical origin, cultivation practices (soil type, altitude, and sun exposure), and bean maturity [34,35]. During development, polyphenols gradually accumulate in storage cells, leading to increased levels with maturity [36]. For instance, catechin content can rise from 6.39 ± 0.02 g/100 g dry matter in stage 1 (purple-red pods) to 8.04 ± 0.24 g/100 g dry matter in stage 4 (orange pods) [37]. Manufacturing and processing significantly impact the final quality and quantity of polyphenols retained in cocoa products [8]. Defatted cocoa seeds experience a substantial decrease in total soluble polyphenols (from 20% to 6%) after drying [38]. Fermentation can be even more impactful, leading to a 70% reduction in overall phenolic content and a 90% decrease in (−)-epicatechin content [39]. Acidic conditions, heat, and enzymatic activity are believed to be the primary factors contributing to the decline in polyphenols and methylxanthines during processing.

Upon ingestion, the human body recognizes polyphenols as foreign compounds (xenobiotics). Their absorption is primarily influenced by structural complexity rather than concentration. Generally, polyphenols exhibit low bioaccessibility and bioavailability, with most remaining unabsorbed in their natural state [40]. Several factors govern the fate of ingested polyphenols: (i) individual variability (genetic differences in metabolic enzymes, efflux pumps, and transporters can influence individual polyphenol metabolism); (ii) gut microbiota interactions (bidirectional interactions with gut bacteria play a role, potentially leading to synergistic or antagonistic effects with other dietary components); and (iii) overall metabolism (these mechanisms modulate the rate of absorption, distribution, metabolism, and excretion of polyphenols [41]). For optimal absorption, polyphenols require extensive modification through hydrolysis, conjugation, and microbial degradation into secondary metabolites with enhanced bioactivity and bioavailability [42].

The metabolic journey of cocoa polyphenols commences in the oral cavity. Here, flavonoid glucosides are converted into aglycones, further transformed into absorbable bioactive compounds by the oral epithelium. Reaching the stomach, oligomeric polyphenols are broken down into their monomeric units [43]. Small intestine absorption is the primary route for cocoa polyphenol metabolites. Monomers rapidly reach the liver, where phase II enzymes conjugate them into sulfates, glucuronides, and methylated metabolites. Additionally, enterohepatic recirculation may occur through the excretion of some flavanols via bile. Oligomeric procyanidins demonstrate poor gastrointestinal absorption and are primarily metabolized by gut microbiota alongside monomeric flavanols within the large intestine. Estimates suggest only 5–10% of polyphenols are absorbed in the small intestine, with the remaining 90–95% reaching the colon [39]. Here, colonic microbial fermentation transforms them into secondary metabolites with various physiological consequences influencing gut ecology and human health [44,45]. This microbial biotransformation appears to be the most efficient pathway for generating small, bioavailable secondary metabolites capable of entering circulation, reaching target organs, and exerting their bioactivities [41].

A study by Llerena et al. [46] investigated the bioactive compound profile and antioxidant activity in cocoa residues (mucilage and bean shells) of two varieties: Nacional x Trinitario (Fine Aroma) and CCN-51. The Nacional × Trinitario mucilage exhibited the highest concentrations of procyanidin B1, B2, C1, epicatechin, and catechin, with procyanidin B2 (3.52 mg/100 mL) and catechin (3.54 mg/100 mL) being the most abundant. When comparing epicatechin and catechin content between mucilage and bean shells, the bean shells displayed

higher levels, particularly epicatechin. Interestingly, no significant differences were observed in total polyphenol concentration between the two mucilage samples. However, significant differences ($p < 0.05$) were found for epicatechin and catechin content in the bean shells, highlighting the influence of variety on these specific compounds. Consistent with previous research by Martinez et al. [47] and Okiyama et al. [48], flavan-3-ols (catechin, epicatechin, and procyanidins) were the dominant phenolic compounds identified, contributing to the bitter taste observed in cocoa derivatives and residues. Notably, unlike cocoa mucilage, cocoa bean shells possess a distinct phenolic profile compared to cocoa beans themselves [46]. Febrianto and Zhu's 2022 study [49] examined the chemical composition (methylxanthines, polyphenols, key odorant volatiles, and minerals) of 22 cocoa samples from various Indonesian regions. Significant variations were observed in flavan-3-ol monomer composition between under-fermented and fermented samples, with the exception of those from Sulawesi. Partially fermented beans displayed lower flavan-3-ol content compared to unfermented ones, likely due to degradation during fermentation. Conversely, under-fermented samples exhibited higher phenolic acid content compared to fermented beans [50]. The authors suggest that factors like genetics, growing environment, and plant defense mechanisms influence phenolic acid profiles.

2.2. Methylxanthin

Theobromine (3,7-dimethyxanthine) and caffeine (1,3,7-trimethylxanthine) are the predominant purine alkaloids in cocoa seeds, with theophylline (1,3-dimethylxanthine) present in trace amounts [51] (Figure 3). These secondary metabolites contribute to the astringency and bitterness characteristic of cocoa and its derivatives. Similar to polyphenol content, cocoa methylxanthine content exhibits variation based on the genetic background, geographic origin, and maturity stage of the beans [5]. Studies have reported a significant increase (up to 74.2%) in theobromine and caffeine content between immature and mature cocoa beans [5]. Theobromine, the primary alkaloid responsible for the mild stimulant effects of cocoa, possesses high bioavailability and diverse biological activities. Recent research has explored the potential of theobromine to elevate serum HDL cholesterol levels through dietary intervention with cocoa products [52]. Additionally, theobromine demonstrates stimulatory effects on heart muscle, promotes the relaxation of bronchial smooth muscle in the lungs, and may play a role in intracellular signaling pathways [53].

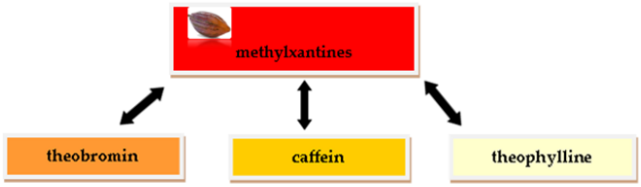


Figure 3. Cocoa methylxantines.

Studies indicate that cocoa mucilage and bean shells harbor higher concentrations of methylxanthines, particularly theobromine. This observation held true for both Nacional and Nacional × Trinitario cocoa varieties. Theobromine content peaked at 2.66 mg/100 mL in the mucilage of Nacional × Trinitario beans, with significant differences observed between theobromine and caffeine levels [46]. Theobromine and caffeine content varies depending on the fermentation process, with under-fermented beans generally exhibiting higher levels of both alkaloids compared to fermented beans [50]. Fermentation triggers bio-detheobromination, leading to a decrease in theobromine content. Among fermented samples, those from Sulawesi displayed the highest theobromine concentration, while Balinese beans had the lowest. Interestingly, Sulawesi beans also had the highest caffeine content, with Mamuju-3 samples reaching levels (3.07 g/kg dry weight) comparable to under-fermented beans and fermented Jember-Edel beans (3.33 g/kg dry weight). This suggests a potential role for caffeine overexpression as a plant defense mechanism against pathogens [50], with further studies showing that pathogen attack can induce an eight-fold increase in caffeine content in cacao stems [54]. Environmental

factors like water availability, seasonal variations, temperature fluctuations, and light intensity significantly impact the methylxanthine profile of plants, including cocoa. The observed variability in methylxanthine content likely stems from a combination of geographical and environmental factors at the origins of the bean samples, along with inherent genetic differences in the cocoa plants themselves [55].

2.3. Phytosterols

Phytosterols, plant-based steroids found in various plant parts (roots, stems, leaves, fruits, and grains), are crucial components of plant cell membranes [56]. Daily human intake of phytosterols ranges from 100 to 400 mg, primarily from vegetable oils, grains, nuts, and vegetables [56–59]. Current research suggests diverse physiological functions associated with phytosterols, including antioxidant activity, anti-inflammatory and antipyretic effects, and potential hormonal influence [60–62]. Notably, their most significant function lies in reducing cholesterol absorption and the concentration of low-density lipoprotein cholesterol (LDL-C) [60,61]. Due to their structural similarity to cholesterol, phytosterols compete for intestinal absorption, thereby lowering plasma LDL-C levels. Additional potential benefits include improved insulin sensitivity, enhanced lipid metabolism, and reduced risk of cancer, Alzheimer's disease, and atherosclerosis-related cardiovascular diseases (CVDs) [62–66]. These positive health impacts have driven the widespread use of phytosterols in functional foods. Cocoa beans represent a good source of phytosterols (200–300 mg/100 g fat), existing in both free and esterified forms. Beta-sitosterol and stigmasterol are the most prevalent phytosterols in cocoa beans, accounting for 59% and 22% of total sterols, respectively. Other phytosterols like campesterol, cycloartenol, 24-methylene cycloartenol, delta-5 avenasterol, and sitostanol are present in considerably lower quantities [67]. A study by Zarabadipour et al. investigated the sterol profiles of two cocoa powder brands (Delphi and Bensef). Beta-sitosterol, stigmasterol, and campesterol were identified as the major sterol components in both samples, with no significant compositional differences observed. Variations in the sterol content of cocoa powders could potentially stem from differences in the plant source or processing and storage conditions [68].

2.4. Dietary Fibers

The concept of dietary fiber has evolved from its simple definition in the 1970s as "plant cell remnants resistant to human digestion". Today, the Codex Alimentarius Commission provides a more comprehensive definition, classifying dietary fibers as carbohydrate polymers with ten or more monomeric units that resist hydrolysis by human small intestine enzymes. These fibers can be categorized into three groups: naturally occurring, extracted, and synthetic [69].

Observational studies reveal a strong association between high dietary fiber intake and a reduced risk of chronic diseases like cardiovascular disease, stroke, type 2 diabetes, colorectal cancer, and diverticular disease [70–73]. Notably, meta-analyses of prospective cohort studies suggest a 15–16% decrease in all-cause mortality among individuals with high fiber consumption compared to those with lower intake [72,73]. These studies indicate an adequate daily intake of 25–29 g, with potential benefits exceeding 30 g per day [72].

Dietary fiber from cocoa bean husk has shown potential against various health conditions. Studies have investigated the production of soluble, insoluble, and total dietary fiber from cocoa bean shells, analyzing their hypoglycemic and cholesterol-lowering effects. Notably, soluble fiber exhibited the highest glucose adsorption capacity, α-amylase inhibition activity, and cholesterol/sodium cholate binding capacity [74]. According to a study by Braojos et al., cocoa shell flour and cocoa shell extract significantly reduce the accumulation of fat, triglycerides, and cholesterol in HepG2 cells. The authors therefore suggest that cocoa shells can be used as safe ingredients that have the ability to regulate lipid metabolism [75]. The high dietary fiber content of cocoa bean husk makes it a valuable ingredient in food formulations. While chocolate is generally considered low in nutrients and high in calories, incorporating fiber can enhance its nutritional profile. However, this

addition may decrease the polyphenol content [76]. Barišić et al. successfully added cocoa shell (as a fiber source) to dark and milk chocolate, achieving increased dietary fiber content without significantly impacting polyphenols. The resulting chocolate quality was comparable to commercially available products [77]. Rojo-Poveda et al. utilized cocoa bean shells to create high-fiber functional biscuits exhibiting α-glucosidase inhibitory activity [78]. Studies have also shown that incorporating cocoa hulls into cakes increases dietary fiber content, phenolic content, and antioxidant activity [79]. Research has explored the influence of cocoa husk dietary fiber on the physicochemical and sensory properties of emulsion-type pork sausages at varying cocoa powder concentrations (0.25–2%). The findings indicate that cocoa powder enhances emulsion stability, improves flavor acceptability and overall product acceptance, and significantly inhibits lipid peroxidation during refrigerated storage [80].

3. Health Benefits of Bioactives from Cocoa

Cocoa consumption has been linked to potential benefits in preventing chronic diseases. These protective effects are attributed to various bioactive compounds within cocoa, particularly its rich phenolic content. Cocoa phenolics possess potent antioxidant properties, enabling them to neutralize free radicals in the body. Uncontrolled free radicals contribute to oxidative stress, which is associated with cellular damage and the development of chronic diseases and aging processes [81]. By mitigating oxidative stress, cocoa may offer protection against these conditions.

Certain cocoa compounds are being investigated for their ability to regulate the cell cycle, a crucial process for cell growth, division, and death. Additionally, cocoa may possess anti-cancer properties through its ability to induce apoptosis (programmed cell death) in cancer cells, thus hindering tumor development [82]. Studies also suggest that cocoa flavonoids may inhibit angiogenesis, the formation of new blood vessels necessary for tumor growth and spread. By limiting the blood supply to tumors, these flavonoids may impede their growth [83].

Flavonoids, such as catechins and epicatechins, have been extensively studied for their anti-inflammatory effects. Inflammation is a natural immune response triggered by harmful stimuli. Acute inflammation is typically short-lived and serves a protective purpose. Chronic inflammation, however, persists for extended periods and is associated with various diseases. Bioactive compounds in cocoa are believed to modulate the body's inflammatory response and reduce excessive inflammation. Certain cocoa flavonoids, including catechins and procyanidins, may possess antimicrobial properties. These flavonoids have demonstrated inhibitory effects against various bacteria and viruses, potentially contributing to cocoa's overall antimicrobial activity [11]. Studies also suggest some level of antimicrobial activity in theobromine, particularly against certain oral bacteria associated with dental caries and periodontal disease [17]. However, theobromine's antimicrobial activity is generally considered weaker compared to conventional medicinal antimicrobials [11].

3.1. Effects on Cardiovascular Diseases

Numerous studies in recent decades have explored the potential link between cocoa products and reduced CVD prevalence. Ren et al. investigated dose-dependent relationships between chocolate consumption and CVD incidence. Their findings suggest a weekly intake of 45 g of chocolate may be optimal for reducing CVD risk, with higher intakes potentially negated by adverse effects associated with high sugar content [84]. Similarly, Jafarnejad et al. demonstrated the positive impact of cocoa and its derivatives on reducing pulse wave velocity (PWV) and augmentation index (AIx) in both short- and long-term studies [85].

Oxidative stress and chronic inflammation are implicated in various cardiovascular conditions including atherosclerosis, hypertension, and myocardial infarction. Cocoa bioactives may offer protection against these conditions due to their antioxidant and anti-inflammatory properties. Cocoa flavanols have been shown to improve endothelial

function, crucial for maintaining healthy blood vessels. Endothelial dysfunction is an early marker in the development of CVD. Flavanols promote the production of nitric oxide, a molecule that relaxes blood vessels and improves blood flow, potentially reducing the risk of hypertension and atherosclerosis. A review by Sun et al. compiled data on improved endothelial function observed after sustained cocoa flavanol intake (over 2 weeks). Notably, they reported optimal effects on fibromuscular dysplasia with specific flavanol intakes: total flavanols (710 mg), (−)-epicatechin (95 mg), and (+)-catechin (25 mg) [86]. Studies suggest that even a modest reduction in systolic blood pressure (5 mmHg) can significantly decrease CVD risk over time (20% over 5 years) [87]. The flavanol-mediated improvement in endothelial function and enhanced nitric oxide availability are believed to contribute to blood pressure reduction. While the evidence regarding improvements in circulating lipid profile (cholesterol, triglycerides, HDL, and LDL) with cocoa consumption remains inconclusive, cocoa bioactives have been shown to inhibit platelet aggregation and reduce blood clot formation. By preventing excessive clotting, cocoa flavanols may help reduce the risk of thrombotic events.

Despite evidence supporting the cardiovascular benefits of cocoa bioactives, further research, including large-scale clinical trials, is needed to fully understand the mechanisms of action and establish optimal cocoa intake levels and consumption patterns for maximizing cardiovascular health benefits. It is important to remember that the potential benefits of cocoa consumption should be considered within the context of an overall healthy diet and lifestyle.

3.2. Effects on Cognitive Functions

Growing evidence suggests a link between cardiovascular changes, oxidative stress, and neuroinflammation in the development of cognitive decline [88]. Cognitive processes encompass mental activities involved in acquiring, processing, storing, and retrieving information. Studies like Lamport et al. demonstrate improvements in verbal episodic memory in healthy young adults following dark chocolate consumption (70% cocoa) [89]. Notably, the exact flavanol content was unknown but estimated to be around 80–90 mg, suggesting potential benefits even with lower flavanol intake compared to other studies.

Two main mechanisms are proposed for the potential cognitive benefits of cocoa: (i) direct interaction (flavanoids may directly interact with signaling pathways promoting neuronal function and brain connectivity [90]) and (ii) improved blood flow (cocoa may improve cerebral blood flow, potentially influencing memory processing [91]). A third emerging mechanism involves the gut–brain axis, where gut microbiota may influence the bioavailability of cocoa's polyphenols [92].

It has been shown that flavanols found in cocoa are associated with greater cerebral blood volume and that the neuroprotective effect of bioactives in cocoa is a result of having antioxidant and anti-inflammatory properties that potentially reduce the risk of cognitive decline and neurodegenerative diseases like Alzheimer's disease. Furthermore, cocoa contains theobromine and phenylethylamine, which can have mood-enhancing effects. Improvements in mood can indirectly benefit cognitive function by reducing stress and anxiety, while caffeine content in cocoa can increase alertness and improve focus, leading to enhanced cognitive performance in tasks that require sustained attention.

Although studies suggest that habitual consumption of flavonoid-rich foods increases levels of neurotrophic nerve growth factor in plasma, enhances cognitive function and performance, and thereby reduces the risk of cognitive decline and supports cognitive health both in young and older adults, the effects may vary depending on factors such as individual differences in metabolism and overall diet and lifestyle.

3.3. Effects on Gut and Gut Microbiota

Due to its abundance of fibers, cocoa has a positive effect on bowel movements. Sarrià et al. confirmed that fact in their two-stage, randomized, crossover, single-blind intervention in which volunteers (healthy adults aged 18–55) received two servings of

fiber-enriched cocoa (2.26 and 6.60 g/day of non-starch polysaccharides) for four weeks. Besides the more frequent daily bowel movements, the shorter time intervals between bowel movements and less feelings of constipation were also noted [93]. Furthermore, Fox et al. found no effects of ingesting 100 g of dark (72% cocoa, 250 mg flavanols) or white (0% cocoa) chocolate for 5 days on upper gastrointestinal function, but it was found that dark chocolate intake tends to slow colonic transit and increase stool consistency. The following was explained by the fact that at high levels, cocoa methylxanthines can accelerate colonic transit, whereas the low content found in dark chocolate may not trigger this response [94]. A potential mechanism leading to slower colonic transit is the inhibition of chloride channels induced by cocoa flavanols and reducing the water transport across the colonic epithelium [5].

Intestinal mucosa acts as a permeable barrier and regulates the intestinal immune system facing dietary pathogens or microbiota [95]. Oxysterol, derived from dietary cholesterol, is one of these pathogens, causing dysfunction and epithelium permeability and damaging intestinal mucosa by inducing inflammation and reactive oxygen species (ROS) overproduction [5]. The use of cocoa bean shell (CBS) was shown to upregulate nuclear erythroid 2 p45-related factor 2 (Nrf2) expression, which is a crucial transcription factor protecting cell response against redox stressors [96]. The use of CBS can also prevent the decrease of tight junction protein levels, which are involved in mucosa permeability [95]. Kramer et al. showed that procyanidin B2 from cocoa reduces the levels of the enzyme tissue transglutaminase-2 (TG2), the leading marker in diagnosing celiac disease (CD), and also reduces the proinflammatory cytokines IL-15, IL-1, IL-6, and IL-8 [97].

The gut microbiota refers to the community of microorganisms, including bacteria, viruses, fungi, and other microbes, that reside in the digestive tract of animals, including humans. The total number of intestinal bacteria exceeds ten times the number of eukaryotic cells in the body, and due to their important metabolic activity, the intestinal microbiota is often referred to as a "virtual" and "essential" organ that modulates the host's health phenotype with its secondary genome. This complex ecosystem plays a crucial role in various aspects of human health, including digestion, metabolism, immune function, and even mood regulation. Healthy gut microbiota is characterized by a diverse range of microbial species, meaning that greater diversity is generally associated with better health outcomes as well as that the balance of different microbial species in the gut is essential for maintaining health. Disruptions in this balance, known as dysbiosis, are often defined as a qualitative and quantitative change in microbiota, its metabolic activity, and local distribution. Dysbiosis has been linked to various health issues, including inflammatory bowel diseases (IBDs), obesity, diabetes, and autoimmune disorders. Diet is one of the most significant factors influencing gut microbiota composition. A diet rich in fiber, fruits, vegetables, and fermented foods supports a diverse and healthy gut microbiota. On the other hand, a diet high in processed foods, sugars, and saturated fats can negatively impact gut microbial diversity.

Recent studies suggest that unmodified dietary polyphenols from cocoa may influence gut microbiota through prebiotic effects and selective targeting of pathogenic bacteria [98,99]. Specific species, including *Escherichia coli*, *Bifidobacterium* spp., *Lactobacillus* spp., *Bacteroides* spp., and *Eubacterium* spp., seem to be primarily responsible for metabolizing cocoa polyphenols [100]. This gut microbial transformation converts these polyphenols into smaller, more bioavailable compounds compared to the original aglycones produced in the upper digestive tract [41]. Polyphenol supplementation appears to promote the growth of beneficial bacteria like Lactobacillus and Bifidobacterium while potentially reducing the abundance of pathogenic Clostridium species, such as *Clostridium perfringens* [101].

Theobromine also appears to influence gut microbiota. Martín-Peláez et al. conducted a two-week animal study where cocoa or theobromine alone led to decreased *Escherichia coli* levels compared to a control diet, suggesting a role in reducing Gram-negative bacteria [102]. Interestingly, theobromine alone (not whole cocoa) decreased *Bifidobacterium* spp., *Streptococcus* spp., *Clostridium histolyticum*, and *Clostridium perfringens*. These findings suggest potential

interactions between cocoa components, with polyphenols and fibers possibly counteracting or enhancing theobromine's effects [103].

In humans, cocoa polyphenols may promote Bifidobacteria growth by creating a gut environment favorable for these bacteria. These effects seem to be beneficial in both healthy and unhealthy guts, potentially improving overall microbiota composition [99]. Consuming cocoa flavanol-rich foods is associated with increased Lactobacilli and Bifidobacteria in humans, potentially influencing immunological tolerance. This suggests cocoa flavanols might act as prebiotics, affecting gut microbiota and potentially modulating the immune system. Notably, changes in fecal Bifidobacteria and Lactobacilli levels have been linked to decreased C-reactive protein (CRP) concentrations, a marker of inflammation [39]. Wiese et al. conducted a clinical trial investigating the prebiotic potential of dark chocolate (Trinitario cocoa beans) with or without added lycopene in healthy individuals with moderate obesity. Their results suggest that dark chocolate consumption led to decreased Bacteroidetes and increased Lactobacillus levels. Additionally, they observed reductions in liver-associated blood markers of oxidative damage and inflammation. The study also reported dose-dependent changes in gut microbiota profiles, along with blood, liver metabolism, skeletal muscle, and skin parameters [104].

3.4. Effects on Diabetes

Diabetes mellitus (diabetes) is a chronic metabolic disorder characterized by sustained hyperglycemia, with two different types known in medicine: type 1 (T1D) and type 2 (T2D). Several large observational studies suggest that moderate consumption of cocoa and cocoa products may be associated with a reduced risk of T2D [105–108]. Maskarinec et al. conducted a large, multi-ethnic cohort study (MEC) and found that individuals with higher intakes of chocolate products and cocoa flavanols had a lower risk of developing T2D, even after accounting for sugar intake, diet quality, and other dietary factors [109]. Clinical trials investigating the effects of cocoa products in diabetic patients are limited. However, some studies suggest potential benefits for reducing cardiovascular disease (CVD) risk in this population. Jafarirad et al. demonstrated that daily supplementation with high-cocoa dark chocolate (84%) for 8 weeks decreased inflammatory markers (hs-CRP, TNF-α, and IL-6) in diabetic patients. Interestingly, they also observed reductions in fasting blood glucose, HbA1c, LDL-C, and triglycerides [110]. Conflicting results exist regarding the impact of cocoa on glycemic control. Dicks et al. found no changes in blood sugar, insulin, lipids, or blood pressure in patients with T2D and hypertension following regular intake of a low-dose flavanol-rich cocoa powder for 12 days. This lack of effect may be due to existing medications targeting similar pathways as cocoa flavanols [111]. Limited research has explored the postprandial (after-meal) effects of cocoa in diabetic patients. Rynarzewski et al. observed no significant effects on postprandial glucose, lipids, or blood pressure in well-controlled diabetic patients consuming a flavanol-rich cocoa powder with a meal [112]. However, Davis et al. reported that a polyphenol-rich cocoa drink consumed with a high-fat meal may improve postprandial dyslipidemia (abnormal fat levels) and inflammation in adults with T2D [113].

Overall, these studies suggest that short-term cocoa consumption by diabetic patients may offer some benefits for glycemic control and, more consistently, for factors associated with CVD risk. Potential mechanisms likely involve the established positive effects of cocoa flavanols on vascular function and their ability to influence key proteins involved in insulin signaling, inflammation, oxidative stress, and gut microbiota [114–116].

3.5. Effects on Obesity

Obesity is a chronic condition characterized by excessive body fat accumulation. It is typically measured by body mass index (BMI), with a score of 30 or higher indicating obesity. Obesity is associated with numerous health risks, such as type 2 diabetes, cardiovascular diseases, dyslipidemia, certain cancers, sleep apnea, osteoarthritis, fatty liver disease, and reproductive problems.

The link between cocoa bioactives and obesity prevention remains inconclusive. However, research suggests that altered gut microbiota (dysbiosis) found in obese individuals might be a target for improving health. Studies show positive effects of cocoa consumption on cardiovascular markers (blood pressure and flow-mediated dilation) in obese adults. Additionally, obesity is linked to mental health issues like anxiety and depression. Ibero-Baraibar et al. conducted a trial where overweight/obese individuals consumed a calorie-restricted diet with or without a daily cocoa extract dose (1.4 g, 645 mg polyphenols) for 4 weeks [116]. Cocoa consumption led to increased plasma homovanillic acid levels, which was correlated with reduced depressive symptoms. Preclinical and cellular studies suggest potential anti-obesity mechanisms associated with cocoa, including lipid metabolism modulation, reduced adipogenesis, attenuated inflammation and oxidative stress [117,118], and microbiota reshaping [119].

4. Extraction of Bioactive Compounds from Cocoa

The growing demand for bioactive compounds in various industries, including pharmaceuticals, food, and chemicals, has intensified the search for eco-friendly extraction techniques. Conventional methods often rely on harsh chemicals or high temperatures, raising environmental concerns. Novel extraction approaches, referred to as "green technologies", offer promising alternatives. These techniques utilize various principles to achieve efficient extraction while minimizing environmental impact. Some examples include the following:

- Ultrasound-assisted extraction (UAE): utilizes ultrasound waves to disrupt plant cell walls, enhancing solvent penetration and bioactive compound release;
- Enzyme-assisted extraction (EAE): employs enzymes to selectively break down plant cell walls, facilitating the release of target compounds;
- Microwave-assisted extraction (MAE): harnesses microwave energy to rapidly heat solvents, promoting faster extraction and potentially improving yield;
- Pulsed electric field-assisted extraction (PEF): applies short, high-voltage electric pulses to create temporary pores in plant cell membranes, allowing for improved solvent access and extraction efficiency;
- Supercritical fluid extraction (SFE): utilizes fluids above their critical point (high temperature and pressure) to act as both solvent and solute carrier, offering high selectivity and purity for extracted compounds;
- Liquid extraction under pressure (PLE): employs heated pressurized solvents to enhance extraction efficiency, often utilizing pressurized static or dynamic extraction processes;
- Ohmic heater-assisted extraction (OHAE): applies an electric current to directly heat the extraction solution, offering rapid and efficient heating for improved extraction yields.

While these green technologies offer significant advantages, achieving optimal extraction efficiency still requires careful optimization. Key variables influencing extraction efficiency include particle size, solvent selection, temperature, extraction time, and sample-to-solvent ratio [120]. Optimizing these parameters forms the foundation for establishing efficient and environmentally friendly protocols for extracting bioactive compounds from plant materials.

4.1. Ultrasound-Assisted Extraction (UAE)

Ultrasound-assisted extraction (UAE) is a widely used novel technique for extracting phytochemicals [121]. It offers several advantages, including reduced time and energy requirements, low temperatures, and preservation of extract quality. UAE utilizes high-intensity sound waves to extract bioactive compounds from plant material. The ultrasound waves disrupt plant tissue through physical forces generated during acoustic cavitation. This disruption facilitates the release of extractable components into the solvent in a significantly shorter time by enhancing mass transfer [122]. Optimum extraction with UAE requires precise control of the frequency, power, duty cycle, temperature, time, solvent type, and liquid-to-solid ratio.

As described by Kumar et al. [123], ultrasound-assisted extraction (UAE) is considered an environmentally friendly technique due to its high productivity with minimal solvent and time consumption. Additionally, it is suitable for extracting thermally sensitive molecules. The frequency used in UAE for extracting bioactive compounds typically ranges between 20 and 120 kHz. Low-frequency, high-intensity ultrasound generates strong shear and mechanical forces, which are desirable for the extraction process. Conversely, high-frequency, low-power density ultrasound produces a large number of reactive radicals. The power delivered during UAE can be expressed as either amplitude percentage (ranging from 0 to 100%, where 100% represents the equipment's rated power) or power density (W/mL), calculated as the power dissipated per unit volume of the extraction medium. Duty cycle, expressed as a percentage, represents the ratio of pulse duration to the cycle time of the ultrasonic wave. Pulse duration refers to the time the ultrasonic transducer remains "on", while the pulse interval is the duration between pulses. This interval is also sometimes referred to as cycle time. Increasing the temperature can enhance the UAE yield due to its dual effect on both the solute and the solvent. A temperature rise increases the desorption properties and solubility of the solute in the solvent. Conversely, it decreases the solvent's viscosity, leading to increased diffusivity of the solvent within the tissue matrix. Similar to temperature, increasing sonication time initially leads to a rise in yield. However, further extension of sonication time can result in a decrease in yield, similar to the effect of increasing power and temperature. The initial increase in sonication time enhances the cavitation effect of the ultrasound, promoting swelling, hydration, fragmentation, and pore formation within the plant tissue matrix, facilitating the extraction of the target solutes. Regarding solvents, various options can be employed for UAE depending on the target compounds. These include acidified water, ethanol, other alcohols, acetone, and water. Optimization of the UAE process should encompass the selection of the appropriate solvent, particle size, temperature, time, and solvent-to-solid ratio, as in traditional methods. However, it should also include optimization of the specific ultrasound parameters (power and frequency) for optimal results [124,125].

There are several novel studies dealing with the optimization of the UAE process for cocoa bioactives extraction [76,126,127]. Jafari et al. used UAE to extract bioactives from cocoa shells, which they encapsulated in maltodextrin. They optimized the UAE process with regard to temperature (45–65 °C), extraction time (30–60 min), and ethanol concentration (60–100%) [126]. Yusof et al. optimized the UAE extraction process in regard to ethanol concentration (70–90%), temperature (45–65 °C), and irradiation time (30–60 min). They found that the highest total flavonoid yield obtained was 7.47 mg RE/g dw at 80% ethanol, 55 °C, and 45 min process duration [127].

4.2. Enzyme-Assisted Extraction (EAE)

Enzyme-assisted extraction (EAE) is a method that utilizes enzymes to extract bioactive compounds from natural sources. Enzymes act as biological catalysts, accelerating chemical reactions without being consumed themselves. In EAE, specific enzymes are chosen to break down cell walls, disrupt cellular structures, and release the target bioactive compounds from the source material. The selection of enzymes is based on their ability to hydrolyze specific chemical bonds present in the cell walls or storage structures of the source material. Commonly used enzymes include cellulases, pectinases, proteases, lipases, and carbohydrases, depending on the composition of the source material. Enzymes can be derived from bacteria, fungi, yeasts, archaea, animal organs, or plant extracts. EAE offers several advantages: (i) selective extraction: enzymes can be chosen to target specific components of the cell wall, enabling the selective extraction of desired bioactive compounds; (ii) mild extraction conditions: enzymes function under mild temperature and pH conditions, minimizing the degradation of heat-sensitive compounds; (iii) enhanced extraction efficiency: enzymes facilitate the breakdown of cell walls, increasing the accessibility of bioactive compounds and improving extraction yields; and (iv) environmentally friendly:

EAE typically uses water or buffer solutions as extraction solvents, generating less waste compared to chemical extraction methods.

EAE finds wide application in various industries, including food processing, pharmaceuticals, cosmetics, and natural product research, for the extraction of bioactive compounds from a diverse range of natural sources. Optimizing the choice of enzymes and extraction conditions allows for achieving maximum extraction efficiency and desired product characteristics [128].

In cocoa bioactives extraction, enzymes are often used in combination with other extraction methods to enhance the extraction process. E.g., Huynh et al. explored the potential of EAE in combination with UAE for extraction of alkaloid and phenolic compounds from cocoa bean shells. Viscozyme L beta-glucanase from *Aspergillus aculeatus* was used as an extraction enzyme, and it was concluded that incubation with 1.5% Viscozyme L at 50 °C for 40 to 60 min resulted in the highest amounts of theobromine and catechin in the extract [129].

4.3. Microwave-Assisted Extraction (MAE)

Microwave-assisted extraction (MAE) is a rapid and efficient technique for extracting bioactive compounds from natural sources such as plants, herbs, and agricultural products. In MAE, microwave energy is directly applied to the sample mixture, accelerating the extraction process by generating heat within the sample matrix. The frequency range of microwaves typically falls between 300 MHz and 300 GHz. The magnetic and electric fields in microwaves are perpendicular to each other. The electric field is responsible for heating by inducing ionic conduction and dipole rotation within the sample [130]. Depending on their dielectric properties, different components in the sample absorb microwave radiation to varying degrees. This absorption promotes cell rupture, allowing the solvent to penetrate more effectively through the plant matrix. For optimal MAE extraction, several factors need to be considered, including the nature and volume of the solvent, extraction time, microwave power, operating temperature, and the properties of the starting material [131].

In the study of Mellinas et al., microwave-assisted extraction of cocoa bean shell (CBS), a main by-product obtained from the cocoa industry, was optimized using response surface methodology. Effects of pH, time, temperature and solid–liquid ratio on the extraction yield, total uronic acid content, total phenolic content (TPC), and antioxidant capacity were evaluated. The optimal MAE conditions were t = 5 min, pH = 12, T = 97 °C, and S/L ratio = 0.04 g/mL. pH had the biggest influence on the CBS extraction, showing that alkaline MAE extracts (pH 12) were the most enriched in polysaccharides, proteins, and polyphenols with good antioxidant, foaming, and emulsifying properties [132].

4.4. Pulsed Electric Field-Assisted Extraction (PEF)

Pulsed electric field (PEF)-assisted extraction is an emerging technique that involves the application of short, high-voltage electrical pulses to the sample matrix, leading to the disruption of cell membranes and the release of intracellular compounds. PEF represents a "green" approach for enhancing the mass transfer and recovery of valuable bioactive compounds [133]. The advantages of PEF include (i) enhanced extraction efficiency: PEF disrupts cell membranes more efficiently than traditional extraction methods, leading to higher extraction yields; (ii) selectivity: PEF can be optimized to target specific compounds or components of the sample matrix, allowing for selective extraction of bioactive compounds; (iii) reduced processing time: PEF can significantly reduce extraction times compared to conventional methods, making it a rapid and efficient extraction technique; (iv) minimal use of solvents: PEF requires less solvent compared to conventional extraction methods, resulting in reduced solvent consumption and environmental impact; (v) preservation of bioactivity: PEF operates at relatively low temperatures, minimizing thermal degradation of heat-sensitive compounds and preserving the bioactivity of extracted compounds. On the other hand, the primary disadvantage of PEF applications is that the efficacy of the treatment can be affected by PEF device parameters and external factors (e.g., conductivity, pH, and

solution concentration) [134]. So far, it has applications in industries such as food processing, pharmaceuticals, biotechnology, and environmental science.

Barbosa-Pereira et al. evaluated the potential advantages of the combined use of PEF pretreatment with solid–liquid extraction to enhance the yield of bioactive compounds from two food by-products, cocoa bean shell (CBS) and coffee silver skin (CS). The parameters of the PEF pretreatment (electric field intensity, time of treatment, and the number of cycles), as well as the parameters of the solid–liquid extraction (ethanol concentration and extraction time), were optimized using the response surface methodology statistical approach. They compared the optimized methodology with conventional extraction and found that PEF-assisted extraction had higher (approximately 20%) recovery yields of polyphenols and methylxanthines than conventional extraction [135]. Carpentieri et al. also demonstrated that the application of PEF pretreatment of moderate intensity (3–5 kV/cm) and relatively low energy input (15–40 kJ/kg) before solid–liquid extraction (SLE) with green solvents (ethanol–water mixture and propylene glycol) can represent an approach to intensify the extractability of theobromine and caffeine from cocoa bean shells [136].

4.5. Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) is an ecologically friendly way to extract bioactives from sustainable sources, including herbs, spices, and plants. This method uses supercritical fluids, which enable the extraction of bioactive molecules [137,138]. A supercritical fluid (SCF) is any substance maintained above its critical pressure and temperature, where it has a mix of properties between liquid and gas. They are considered to be environmentally safe, and substances such as water, carbon dioxide, ammonia, ethane, fluoroform, nitrous oxide, propane, and xenon can be used as supercritical fluids [139,140]. Among them, carbon dioxide is the one that is used the most frequently because it is inexpensive, ecofriendly, and has a GRAS label [141]. Furthermore, CO_2 is gaseous at normal temperature and pressure, making extract recovery relatively straightforward and enabling the production of extracts without the need for a solvent. Temperature, pressure, supercritical CO_2 flow, the presence of a modifier, and extraction time are the main variables that influence SC-CO_2 extraction [142,143]. As highlighted by Melloul et al. [144], SFE with CO_2 has opened doors to extracting a wide variety of bioactive compounds from plant sources. These compounds have found applications in various sectors, including food, pharmaceuticals, and cosmetics. SFE offers several advantages over traditional extraction methods, such as (i) selectivity: supercritical fluids can be tuned to selectively extract specific compounds while leaving undesirable components behind, allowing for high-purity extracts; (ii) mild operating conditions: the extraction process can be conducted at relatively low temperatures, preserving the integrity of heat-sensitive compounds; (iii) being environmentally friendly: supercritical fluid extraction typically utilizes non-toxic and environmentally benign solvents, reducing environmental impact compared to conventional solvent extraction methods; and (iv) scalability: the process can be easily scaled up from laboratory-scale to industrial-scale operations, making it suitable for large-scale production.

Pico Hernández et al. determined the influence of main extraction parameters using supercritical CO_2 and ethanol for obtaining polyphenols and carotenoids from cocoa husk. According to their findings, extraction times higher than 120 min and particle sizes under 1.105 mm did not present significant effects on total polyphenol content (TPC). According to their result, TPC rises up to 53.84% with increases in pressure and mass concentration of ethanol, while temperature generates a reduction in TPC near 48.28% [145].

4.6. Pressurized Liquid Extraction (PLE)

Pressurized liquid extraction (PLE), also known as accelerated solvent extraction (ASE) or pressurized fluid extraction (PFE), emerged in the mid-1990s. This technique aims to reduce extraction time and solvent usage compared to traditional methods. PLE operates by utilizing a liquid solvent at elevated temperatures and pressures but always below the critical point to maintain the solvent in its liquid state [146,147]. These conditions alter the

solvent's physical and chemical properties, allowing for deeper penetration into the sample matrix. This enables increased solubility of target compounds (analytes), reduced solvent surface tension and viscosity, and enhanced mass transfer rate. These combined effects then lead to a faster extraction process with high yields and minimal solvent consumption [146]. The typical PLE process involves dispersing the sample with an inert material, placing the mixture in a pressurized vessel, pumping the solvent and heating the vessel (typically 75–200 °C), raising the pressure to around 100 atm, extracting the solvent, potentially repeating cycles for improved analyte recovery, and using compressed gas to purge the extract from the vessel [148].

Compared to microwave-assisted extraction (MAE), PLE offers a pre-filtered extract, eliminating the need for separate solid residue removal. Additionally, PLE allows for potential in-cell purification, enhancing selectivity. However, PLE generally requires more labor-intensive cell preparation and comes with a higher instrument cost [146–148].

The advantages of PLE are (i) high extraction efficiency—PLE allows rapid and efficient extraction of bioactive compounds due to the combined effects of high pressure, elevated temperature, and solvent penetration; (ii) reduced solvent consumption: PLE typically requires smaller volumes of solvent compared to traditional extraction methods, resulting in reduced costs and environmental impact; (iii) automation and reproducibility: PLE instruments can be automated, allowing for precise control over extraction parameters and ensuring reproducible results; and (iv) versatility: PLE can be applied to a wide range of sample types, including solid, semi-solid, and viscous samples, making it suitable for various applications in fields such as pharmaceuticals, food, environmental analysis, and natural product research.

In cocoa bioactives extraction research, PLE is mentioned in several studies and often compared to the conventional extraction methods. E.g., Pagliari et al., developed and optimized a pressurized hot water extraction process for the recovery of theobromine and caffeine from cocoa by-products. In comparison to the results obtained using ultrasound-assisted liquid extraction, under optimized conditions (ethanol 15%, temperature 90 °C, 5 cycles, and static time 6 min), the extraction efficiency increased by 156% for theobromine and 160% for caffeine [149].

5. Future Research Trends

This review explores recent research findings highlighting the bioactives of cocoa, their extraction techniques, and potential health benefits. Cocoa and cocoa products are well-established sources of phytochemicals with recognized nutritional and therapeutic value. Accumulating scientific evidence suggests that cocoa components with antioxidant and anti-inflammatory properties contribute positively to human health. Despite existing evidence on the health benefits of cocoa consumption, further research is needed to further quantify their biological activity and explore their health potential and their bioavailability. Furthermore, the development of environmentally friendly extraction techniques is a must-have in a world battling environmental and climate changes. By addressing these research gaps, scientists can develop a more comprehensive understanding of cocoa's potential health benefits and pave the way for future applications.

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