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The Effect of Processing Operations on the Polyphenol Content of Cocoa Beans: A Review

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Cocoa beans undergo processing operations to develop the aroma, and flavour precursors of chocolate and other cocoa products, in line with commercial preferences. However, processing has a negative effect on the polyphenol content of cocoa beans, reducing their health benefits, particularly their antioxidant properties. Therefore, the selection of suitable methods or conditions to minimise polyphenol degradation during cocoa bean processing is crucial. This review primarily focuses on understanding the adverse effects of drying, fermentation, roasting, and alkalisation on cocoa polyphenols. Additionally, this paper discusses the influence of extraction parameters on cocoa polyphenols, including method, solvent, time, and temperature. A comprehensive review of the relationship between processing operations and the polyphenol content of cocoa beans provides valuable insight into the mechanisms underlying these changes and outlines the necessary conditions for optimising processing techniques to preserve or enhance the polyphenol content, thereby improving the nutritional and health benefits of cocoa-based products. Past studies have reported the negative influences of processing operations on cocoa bean polyphenols; however, degradation is favoured due to the astringency and bitterness of cocoa polyphenols, which are not preferred in cocoa products. Therefore, more data on processing operations that focus on minimising polyphenol degradation, are still needed for a better understanding of the effect of each processing step on the polyphenol content of cocoa beans.

Keywords: Cocoa beans; polyphenol content; processing operations

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Recently [2–4], research on cocoa beans (*Theobroma cacao L.*) has increasingly focused on polyphenol compounds due to their known antioxidant properties. Cocoa contains a higher polyphenol content than red wine or tea [5], comprising 12-18 % of the dry-weight of cocoa beans [6]. Among the monomers of flavanols, (-)-epicatechin, followed by (+)-catechin, are the most abundant, as well as their oligomers, procyanidins [7]. Flavanol's structural properties, such as hydroxylation of the basic flavan ring system, oligomer chain length, and stereochemical features, serve as the molecular basis for its hydrogen-donating and metal-chelating antioxidant properties [8]. The anti-oxidant properties of cocoa beans have been associated with many bioactive properties including the inhibition of low-density lipoprotein (LDL) oxidation, suppression of platelet activation, and improvement in its plasma antioxidant, anti-inflammatory, antidiabetic, and anti-obesity activity [9–16].

Aside from health benefits, cocoa beans are globally utilised as raw materials in the confectionery and food industries for the production of cocoa liquor, cocoa powder, and cocoa butter [17–19]. Moreover, the health benefits of cocoa have prompted manufacturers to use it as a primary ingredient in the pharmaceutical and cosmetic industries [20] as previously reported [21–23]. Before cocoa beans are processed into the final product, they must undergo postharvest processing including pod opening, bean removal from the pod, bean fermentation, and drying [19]. Fermentation is the most critical step in the processing of cocoa beans, where it develops the flavour quality attributes of commercial cocoa beans [19]. Subsequently, the beans are dried to reduce the moisture content and prevent mould infestation during storage [24]. Fermentation and drying of cocoa beans are usually performed by farmers before sale or trade. The fermented and dried beans are then roasted, cracked and ground to give a powdery mass. The production of chocolate liquor and cocoa powder involves a roasting process [19]. Roasting removes undesirable volatile compounds and enhance aroma and flavour. The cocoa nibs, cocoa liquor and cocoa powder can be further modified by treatment with an alkaline solution, which is known as the alkalisation or Dutching process [7].

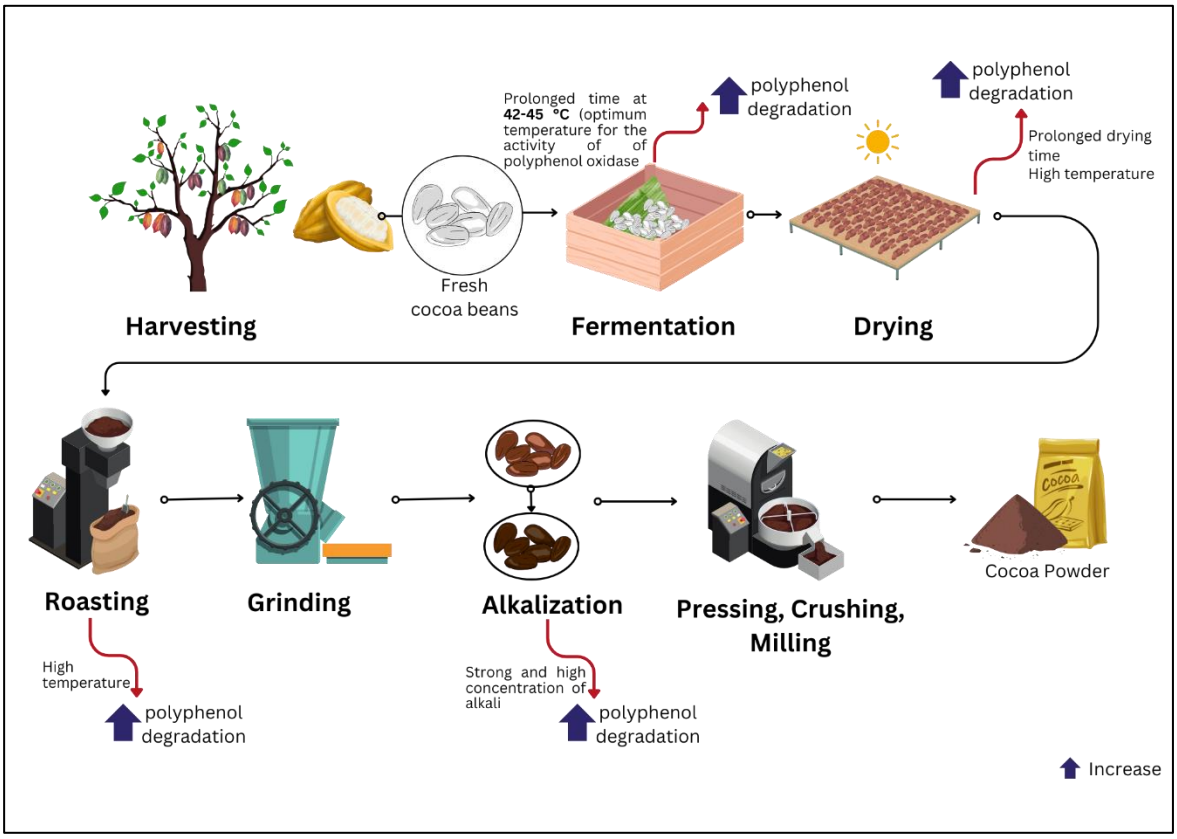

Figure 1. Processing of cocoa beans to semi-finished products with an indication of factors that increase polyphenol degradation during processing.

The reduction of polyphenol content in cocoa beans during processing has been reported in previous studies on the fermentation, drying, roasting, and alkalisation process [25–32]. Besides that, the extraction process is also crucial for the extraction of polyphenol compounds from cocoa beans. Extraction parameters, including the extraction type, solid-to-solvent ratio, extraction solvent, time, and temperature, can affect the efficiency of polyphenol extraction. This review focuses on the current knowledge of cocoa bean processing operations, providing a brief description of the commonly used fermentation, drying, roasting, alkalisation and extraction processes, and their influence on the polyphenol content of cocoa.

Fermentation

1. Biochemical Reactions Involved in Fermentation

Fermentation is the first step in cocoa bean processing. It is a compulsory process to prevent the germination of cocoa beans and to develop favourable flavour and aroma precursors. At the onset of fermentation, the presence of pulp decreases oxygen diffusion within the seed mass, creating anaerobic conditions [33]. The low oxygen availability, initial fermentation temperature of 25-35 °C, with a low pH of 3.6 due to the high concentration of citric acid present in the cocoa pulp, initiate yeast growth [34]. Spontaneous fermentation involves a diverse and abundant yeast population with *Saccharomyces cerevisiae* commonly detected during cocoa fermentation, probably due to its rapid growth, pectinolytic activity, and ethanol tolerance [35,36]**.** Yeast metabolises on glucose, fructose and sucrose in the cocoa pulp, producing ethanol and carbon dioxide [37]. The primary conversion of sugars to pyruvate, which results in the production of ATP and reduced NADH cofactors, marks the beginning of yeast metabolism [34]. Pyruvate undergoes a two-way anaerobic conversion to ethanol. Pyruvate decarboxylase (PDC), which also releases carbon dioxide, first converts pyruvate to acetaldehyde. Alcohol dehydrogenase (ADH) then converts acetaldehyde to ethanol. This type of oxidoreductase can catalyse the reversible interconversion of alcohols and their corresponding aldehydes or ketones [38].

Yeast and lactic acid bacteria (LAB) grow simultaneously for approximately 24-36 hours during the anaerobic phase of cocoa fermentation process [39]. The yeast population dominates the first 24 hours of fermentation and is subsequently reduced by the succession of the LAB population, primarily due to

pectin degradation [34]. LAB metabolises fructose and performs citric acid conversion. Fructose is converted to pyruvate either through glycolysis or the phosphoketolase pathway. Acetic acid and oxaloacetic acid are produced by citric acid conversion. Both compounds are then further converted to pyruvate, ultimately producing either lactic acid, acetic acid or pyruvate. Metabolites such as butanedione and 3-hydroxybutanone, which have a buttery flavour, are produced during this process [39].

Pectinolytic yeasts eventually deteriorate the seed pulp after the first 48 hours, draining the liquid trapped in the parenchymatous pulp. This increases aeration and encourages the growth of acetic acid bacteria (AAB) [18, 33]. The conversion from an anaerobic phase to an aerobic phase is influenced by air exposure during cocoa sweating and regular mixing of the cocoa beans during fermentation [39]. Members of the Acetobacter genus are common because of their ability to grow in an ethanol environment [36]. The most frequent AAB species present is *Acetobacter pasteurianus* [36, 40]. AABs are responsible for the simultaneous oxidation of ethanol and the conversion of lactic acid to acetic acid and acetoin [40, 41]. The primary sources of energy and carbon supply for AAB are ethanol produced by yeast and lactate produced by LAB [39]. The oxidation of ethanol into acetic acid is catalysed by two sequential membrane bound catalytic reactions: oxidation of ethanol into acetaldehyde catalysed by pyrroloquinoline quinone (PQQ)-dependent alcohol dehydrogenase, followed by the oxidation of acetaldehyde to acetic acid catalysed by aldehyde dehydrogenase [41]. Simultaneous with the oxidation of ethanol, lactic acid is oxidised into acetoin, and partly into acetic acid due to the low pyruvate decarboxylase activity of AAB [39]. Lactic acid oxidation begins with the conversion of lactic acid into pyruvate by lactate dehydrogenase. Pyruvate is further decarboxylated into acetaldehyde by pyruvate decarboxylase. Acetaldehyde is then oxidised into acetic acid by acetyl dehydrogenase [41]. The oxidation of lactic acid into acetoin is possible via two pathways. One of these pathways is identical to the formation of acetoin in yeast, where pyruvate is decarboxylated by a pyruvate decarboxylase, whereby bound active acetaldehyde undergoes carboligation with another acetaldehyde molecule to produce acetoin. An alternative pathway involves decarboxylation of α-acetolactate, which is formed from two molecules of pyruvate [41]. Acetic acid is the main product during fermentation, which can be overoxidised to carbon dioxide and water. An increase in temperature during aerobic reactions and a decrease in pH due to the penetration of acetic acid and ethanol into the cotyledons lead to embryo death [42, 43].

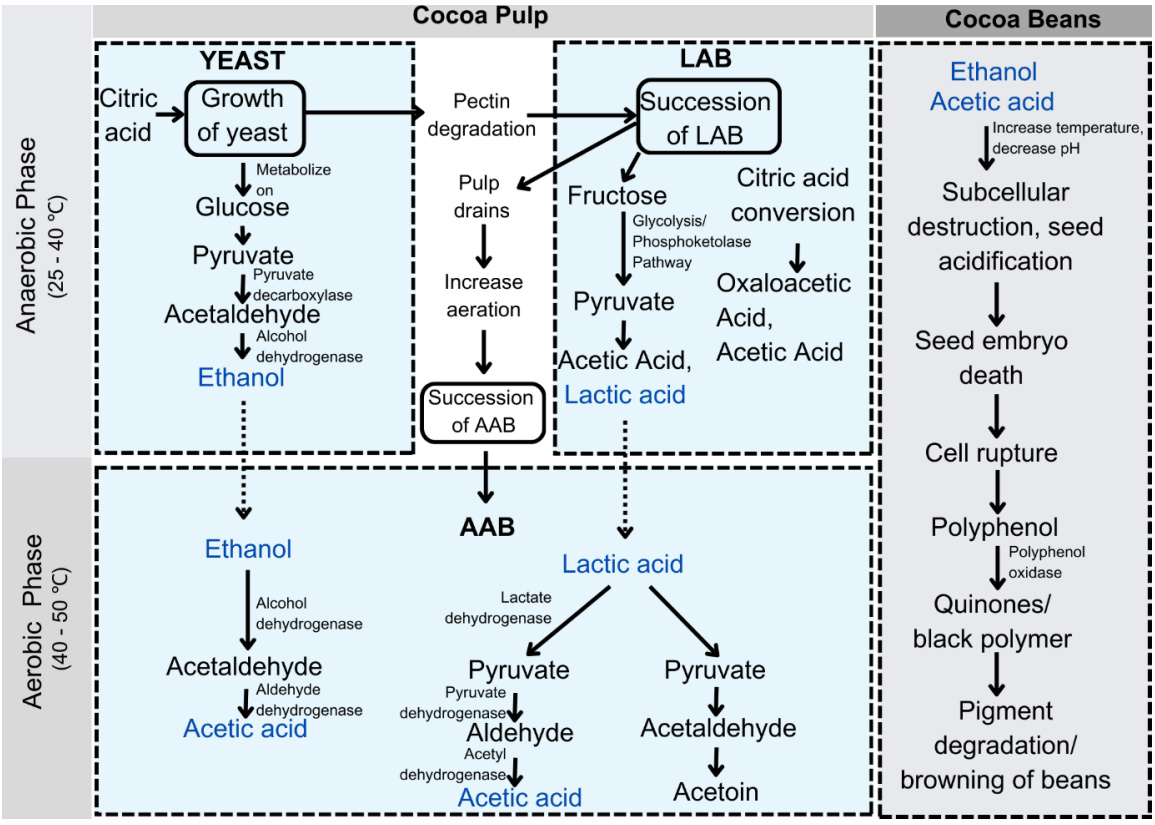

Figure 2. Biochemical transformation of cocoa beans during spontaneous fermentation, where LAB stands for lactic acid bacteria and AAB stands for acetic acid bacteria.

2. Influence of the Fermentation Process on Polyphenol Compounds in Cocoa Beans

During fermentation, cell rupture occurs in addition to the biochemical changes. The cellular components of cocoa cotyledons are released as a result of this process, creating ideal conditions for the development of taste precursors and pigment degradation [43]. During the anaerobic phase, polyphenol oxidase produces quinones from the oxidation of epicatechin, which further forms brown to black polymers [39,44]. The oxidation reaction significantly reduces the content of phenolic compounds responsible for the bitterness and astringency of the beans [36]. The optimal temperature for polyphenol oxidase activity ranges from 42 to 45 °C, and fermentation lasting longer within this temperature range results in a greater loss of phenolic content [18]. Glycosidase α-arabinosidase and β-galactosidase, which are optimally active under acidic conditions (pH 4.0-4.5), extract sugar moieties from anthocyanins (arabinose and galactose from cyanidin-3-α-L-arabinoside and cyanidin-3-β-D-galactoside, respectively) and convert them into anthocyanidins [39]. Anthocyanin degradation and polyphenol oxidation result in the browning of typically violet cotyledons [33].

2.1. Spontaneous Fermentation

Biochemical reactions during fermentation could lead to changes in the polyphenol content. Several studies have reported a reduction in phenolic content during fermentation [25,44,45]. A lower total phenolic content was observed in fermented cocoa beans (43 mg GAE/g) than in unfermented cocoa beans (115 mg GAE/g). The total phenolic content is expressed as gallic acid equivalent (GAE). The reduction in polyphenols was due to the diffusion of polyphenol compounds from the storage cells and oxidation by polyphenol oxidase and peroxidase to form high molecular-weight insoluble tannins [44]. Another study on the optimisation of the fermentation process of cocoa beans reported a low total phenolic content of 12.17 mg GAE/g after an optimal fermentation process, and a higher total phenolic content of 20.41 mg GAE/g in poorly fermented cocoa beans [25].

The total phenolic content of cocoa beans showed a reduction over the fermentation duration but at variable rates [45]. A previous study reported that the polyphenol content decreased by 20 % after 100 hours of fermentation [42]. The total phenolic content was reduced to 39.22 % from the initial phenolic content of 395.15 mg ECE/g after 144 hours of fermentation [46]. Meanwhile, a lower degradation rate of 31 % was reported after 168 hours of fermentation [47]. Additional data from past studies on the degradation rate of polyphenol content during fermentation are presented in **Table 1**. It was suggested that 48 hours is an adequate fermentation time to maintain high concentrations of bioactive compounds and antioxidant activity [46]. However, conventional fermentation practice is usually conducted for a longer time to achieve a better flavour profile and thus better commercial value.

Several studies have also investigated the reduction of monomeric phenol content in cocoa beans during fermentation [46,48]. Large reductions in catechin, procyanidin and epicatechin content, by 40-65 %, 38-67 % and 51-78 %, respectively, were reported after 120 hours of fermentation [18]. The rapid degradation of epicatechin compared with catechin has also been reported in other studies [46, 48, 49]. The high degradation rate of epicatechin is influenced by the reactivity and selectivity of peroxidase towards epicatechin during fermentation [18]. Reduced epicatechin content is favourable in the industry as it contributes to lower astringency in cocoa end-products [18]. Flavan-3-ol also showed degradation during fermentation [50]. A study reported a 63.81 % reduction rate in total flavonoid content after 144 hours of fermentation [46].

Polyphenols, namely anthocyanins, catechins and proanthocyanidins, are responsible for the pigmentation of cocoa beans. Anthocyanins produce red to purple pigments in unfermented cocoa beans [42], and this colour turns brown after fermentation [44]. Anthocyanins showed a 79 % reduction rate after 168 hours of fermentation [47]. This decrease in anthocyanin content is associated with conversion to anthocyanidins and complex tannins [51]. Anthocyanins are hydrolysed to anthocyanidins and sugars such as arabinose and galactose. Consequently, these sugars polymerise with catechins to form complex tannins [52]. Polyphenol oxidase is partly responsible for the colour changes in cocoa beans as this enzyme converts o-dihydroxyphenols to o-benzoquinones, which results in browning, affecting both the flavour and colour of the product [52].

2.2. Inoculated Fermentation

Inoculated fermentation involves the addition of a starter culture to initiate the fermentation of cocoa beans instead of spontaneous fermentation. A proper starter culture comprises at least one strain of yeast, lactic acid bacteria and acetic acid bacteria. Proper selection of lactic acid bacteria is vital to avoid the overproduction of lactic acid during fermentation, which could negatively impact the quality of cocoa beans. Some yeast species are valued because of their high capacity to produce ethanol and hydrolyse pectin and proteins. The production of ethanol and hydrolysis of pectin and proteins facilitate the release of sugars and nitrogenous compounds, which are relevant precursors for a high-quality flavour profile for chocolate [53]. Yeast cultures have been used in cocoa fermentation on an industrial scale [43]. It has been reported that yeasts from the *Saccharomyces, Pichia,* and *Hanseniaspora* genera are preferred as starter cultures in the inoculated fermentation of cocoa beans because of their ability to produce aromatic

compounds with high growth and pectinolytic activity [43]. Specifically, *Saccharomyces cerevisiae* and *Pichia kudriavzevii* produce esters, alcohols and aldehydes that impart desirable aromas in chocolate, such as 'fruity', 'floral,' and 'sweet'. These species can adapt to diverse environmental conditions and can inhibit the growth of putrefactive microorganisms. In addition, they reduce fermentation time by facilitating the drainage of pulp and increasing aeration, which allows rapid succession of lactic and acetic acid bacteria [18].

The flavour profile obtained from inoculated fermentation has a more positive impact than spontaneously fermented chocolate [33, 43, 53, 54]. The chocolate produced with inoculated cocoa beans was described as having 'fruity', 'acid', 'yogurt' and 'balsamic' flavours. In contrast, spontaneously fermented chocolates were described as 'sweet', 'cocoa' and 'caramel' flavours [43]. Yeasts are responsible for producing volatile compounds that are essential for developing fruity flavours and aromas in cocoa products. The volatile compounds produced are higher alcohols (such as 3-methylbutanol and 2-phenylethanol) and esters (such as ethyl acetate and 2-phenylethyl acetate), which contribute to the floral and fruity notes of cocoa beans [34]. Moreover, yeast is also responsible for pectinolytic hydrolysis during the cocoa fermentation process [55]. The pectinolytic activity of yeast strains, specifically *Kluyveromyces marxianus,* improved mass aeration and increased liquid drainage during the first 24 hours of fermentation, which are fundamental to developing bean quality [56]. Therefore, it may be concluded that inoculated fermentation results in a more desirable flavour profile for cocoa products than spontaneous fermentation.

Inoculated fermentation of cocoa beans has been studied mainly to improve the flavour and sensorial profile of the product. Since polyphenol compounds give an astringent and bitter taste which is least favourable, several studies have been reported related to the influence of inoculated fermentation on polyphenol compounds. However, some studies have reported a positive impact on polyphenol compounds by inoculated fermentation [18,26,57]. *Pichia kudriavzevii* is a potential starter culture that resulted in significantly higher values for total phenolic content, total flavonoid content and antioxidant activity, compared with the spontaneous fermentation of cocoa beans at various fermentation periods [57]. Another study reported a higher phenolic content obtained with *Saccharomyces cerevisiae* inoculated fermentation compared to spontaneous fermentation at 24-168 hours [18]. The degradation of phenolic content over time with *S. cerevisiae, P. kudriavzevii,* and spontaneous fermentation is shown in **Figure 1**. The combination of S. *cerevisiae* and *P. kudriavzevii* as starter cultures has promising potential in cocoa fermentation as it shortens the fermentation period from 7 to 6 days while giving the highest levels of epicatechin and catechin, compared with spontaneous fermentation [18]. The fermentation of cocoa beans conducted using 10 % of mixed strains consisting of *S. cerevisiae*, *Lactobacillus plantarum*, and *Acetobacter aceti* shortened the fermentation time to three days while producing a total phenolic content almost similar to that of natural fermentation, which was 34 and 39 mg ECE/g, respectively. However, a decreasing trend was observed in the total phenolic content as the concentration of inoculum increased from 10 % to 60 %, hence the use of low inoculum percentage in cocoa fermentation [26]. Several past studies related to the influence of inoculated fermentation on the polyphenol content of cocoa beans are compiled in **Table 1**.

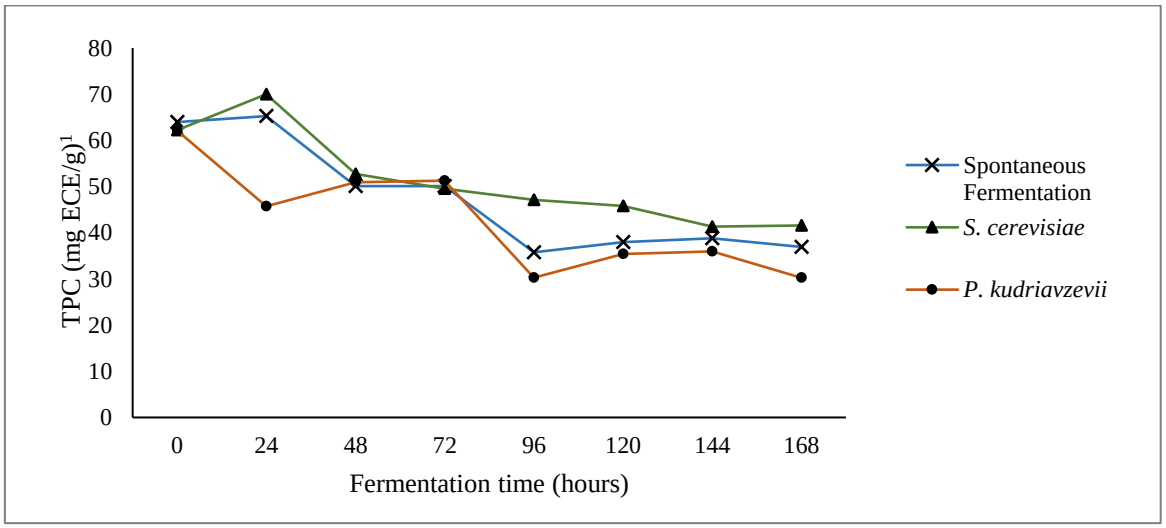

Figure 3. Degradation of phenolic content over time for *S. cerevisiae* and *P. kudriavzevii,* and spontaneous fermentation derived from [18]. [1] Equivalent to miligram epicatechin per gram of sample.

Table 1. Effect of fermentation process on the polyphenol content of cocoa beans during fermentation. **TPC** stands for total phenolic content. **GAE**, **ECE** and **CE** stand for gallic acid equivalent, epicatechin equivalent and catechin equivalent, respectively. DW indicates dry weight of sample.

Fermentation technique	Parameters	Outcomes	References
Spontaneous	Capacity: 50 kg Duration: 7 days Facility: wooden box Mixing: every 24 h	A gradual decrease in procyanidin yield was observed, from 13.8 % on the initial day to 6.2 % by the final day of fermentation.	[58]
Spontaneous	150 kg, 6 days	Total phenolic content of 76 mg CE/g DW	[59]
Spontaneous	5 days	At an average fermentation temperature of 39.89 ℃, the total phenolic content, catechin, epicatechin and procyanidin values decreased by 7 %, 65 %, 78 % and 53 %, respectively.	[18]
Spontaneous	200 kg, 4 days, wooden box, once at 48 hours	A 58 % reduction in (-)-epicatechin content was observed following a five-day fermentation period, resulting in a concentration of 9.37 mg/g.	[48]
Spontaneous and inoculated	100 kg, 7 days, wooden box, every 24 h from day 3 to 7, Starter culture: *Saccharomyces cerevisiae*	Significant reduction in total phenolic content was observed in cocoa beans that undergone inoculated fermentation compared to those undergone spontaneous fermentation.	[60]
Spontaneous and inoculated	7 days, wooden box, pectinase	Inoculated fermentation using pectinase resulted in a 43.9 % decrease in proanthocyanidin content, whereas spontaneous fermentation led to a 24.0 % reduction.	[61]
Inoculated	50 kg, 7 days, wooden box, *S. cerevisiae, P. kudriavzevii*	Fermented beans using *S. cerevisiae* exhibited a slightly higher total phenolic content (41.55 mg ECE/g) compared to those undergone spontaneous fermentation (36.95 mg ECE/g) and *P. kudriavzevii* (30.25 mg ECE/g).	[62]
Inoculated	36 kg, 4 days, plastic vessels, *Saccharomyces ceriviciae, Hanseniaspora opuntiae*	The cocoa isolate *H. opuntiae* strain was outcompeted by a stronger yeast strain, leading to under-fermented cocoa beans, which was however reflected by higher levels of polyphenols.	[55]
Inoculated	20 kg, 7 days, wooden box, isolated pure culture (yeast + LAB + AAB)	The identified optimal fermentation conditions (pH 5.32, 7 days, fermentation index: 1.05) that aimed at reducing astringency resulted in the lowest polyphenol content (12.99 mg GAE/g).	[25]
Inoculated	500 ml cocoa pulp media in 15 ml yeast starter culture, 5 days, 16 different species of yeast	The reduction of total phenolic content in beans subjected to inoculated fermentation was not significant. The least reduction occurred in the one treated with *C. quarcitrusa* while the highest was in the sample treated with *C. ethanolica*.	[57]

Drying

Drying is a compulsory process in the postharvest practice of cocoa bean processing. Drying process is stopped after the cocoa beans reach a moisture content of 7-8 % [17,63,64]. High moisture content favours mould growth, while excessive drying results in brittle beans [65]. The oxidative reaction during fermentation continues during drying, reducing the astringency, bitterness and acidity of cocoa beans [63]. The oxidative degradation of polyphenols involves the transformation of these compounds, first into quinones and then to melanin, by the enzymatic activity of polyphenol oxidase. However, high temperature drying (>60 ℃) stops enzymatic activity and leads to thermal degradation and the onset of the Maillard process, which is known as nonenzymatic browning [66]. The enzymatic oxidation of polyphenols is associated with the development of the brown colour of chocolate [63]. The reaction rates of both the polyphenol oxidation reaction and diffusion of polyphenols in cocoa beans increase with relative humidity and temperature [67]. The drying methods used for cocoa

beans include the sun, oven, microwave and freeze-drying. [27, 28, 68–71]. Additional data from past studies on the degradation rate of polyphenols during the drying process are presented in **Table 2**.

3. Influence of Drying Process on the Polyphenol Content of Cocoa Beans

3.1. Sun Drying

Sun drying is widely used in cocoa bean processing due to its simple setup, cost-effectiveness and renewable energy source. This drying method is usually conducted by spreading the beans on wooden or stainless-steel platform, or concrete floor [65, 72, 73]. Drying cocoa beans under direct sunlight must be closely monitored in case of rain. Furthermore, turning procedures are required every hour to ensure drying uniformity [72]. Due to weather dependency, the drying of cocoa beans usually takes up to 22 days during rainy or wet conditions, and up to 7 days during the dry season [27]. In addition, beans dried at ground level are easily contaminated by vermin and biotic contaminants [74]. In brief, solar drying is time-consuming, weather-dependent, and very susceptible to contamination.

There is a loss of polyphenol compounds in cocoa beans that are subjected to a longer drying period and exposure to intense sunlight. A longer drying period allows a longer time for the polyphenol oxidase to catalyses the hydroxylation and oxidation reactions of polyphenol to form melanin and water [66]. Additionally, a longer drying period triggers the nonenzymatic Maillard reaction between reducing sugars and amino acids. The condensation of amino acid groups and reducing sugars produces Schiff bases (glucosyl and fructose amines) and water [66]. Exposure to intense sunlight elevates the temperature of the cocoa beans and causes thermal degradation [69]. A drying duration of 4 days at ambient temperatures resulted in a lower total phenolic content and (+)-catechin content in sun-dried cocoa beans, compared to those dried using the oven and freeze dryer [75]. It was also reported that five days of sun drying gave a total phenolic content of 787.05-1249.71 mg GAE/g, slightly higher than that of oven-dried beans which ranged from 672.95 to 1229.93 mg GAE/g [68].

Besides long drying periods and intense sunlight, different sun drying methods also influence the degradation rate of phenolic content. For example, cocoa beans dried using wooden platforms under direct sunlight showed the lowest degradation rates of catechin and epicatechin content of 1.24 % and 7.5 % respectively. On the other hand, cocoa beans dried using a steel platform under a plastic roof with UV protection showed high degradation rates of 91.43 % and 50.0 % for catechin and epicatechin, respectively [65].

3.2. Oven Drying

An oven is used as a drying device by combining heat, low humidity, and air flow [76]. The moisture removal capacity of oven drying depends on the temperature and moisture content. When hot air in the oven come into contact with the material to be dried, heat is transferred to the material surface and leads to vaporization [77]. The different pressures between the surface and inner part of the material lead to moisture removal through vapour [77]. Oven-dried cocoa beans are subjected to a tempering process to redistribute the internal moisture to the outer bean layer after the drying cycle. This process is usually done by leaving the beans at room temperature overnight after 8 hours of oven drying [72].

Temperature and exposure time in the oven play critical roles in polyphenol degradation [28,70]. It has been reported that the degradation rates of total polyphenols, (+)-catechin, and (-)-epicatechin content in samples dried at 60, 70 and 80 ℃ were not significantly different. The duration of drying at 70 °C and 80 °C was shorter, while the samples which were dried at 60 °C were subjected to heating for a longer period [75]. Therefore, the factors of exposure time and temperature could explain the insignificant differences. Oven drying at temperatures of up to 65 °C resulted in a 25.26 % reduction in total phenolic content compared to freeze-dried cocoa beans, at 94.4 mg GAE/g and 126.3 mg GAE/g, respectively [28]. Cocoa beans subjected to oven drying at 60 ℃ for 24 hours resulted in a higher total phenolic content (42.1 mg GAE/g) than sun drying for 96 hours (15.0 mg GAE/g). Meanwhile, two days of oven drying at 55 ℃ showed a polyphenol content of 1249.71 mg GAE/g, which was 9.50 % lower than that of freeze-dried cocoa beans, which was 1380.86 mg GAE/g [68].

3.3. Freeze Drying

The process of freeze-drying is called lyophilization or molecular drying. Sublimation is the basic concept involved in the elimination of water by freeze-drying, where water transitions from solid to gas state, bypassing the liquid state [78]. This process is carried out under negative temperature conditions and a significantly reduced pressure of 1-50 Pa. First, the material to be dried is subjected to freezing at -40 ℃ to -50 ℃ for a short period to avoid the formation of large ice crystals. Then, the material is exposed to a combination of high vacuum and low temperatures in the freeze dryer. Freeze drying for up to 2 days allows for the removal of up to 95 % of water in the material. Finally, the material is re-dried through the desorption stage at 40 – 50 ℃. Through desorption, the strongly bound water molecules that remain in the dried material are eliminated, which results in only 1-2 % of water remaining in the freeze-dried material [79].

The freeze-drying method may minimise polyphenol degradation in cocoa beans, resulting in products with a higher phenolic content compared to sun and oven-drying methods [28, 68, 75]. The high

polyphenol recovery is due to the sub-zero temperatures induced by the vacuum conditions of the freeze dryer which inactivates enzyme activity and prevents the browning process [28]. Three days of freeze drying resulted in a total phenolic content ranging from 888.55 to 1380.86 mg GAE/g, and a total flavonoid content of 4536 mg QE/g, which was reported to be higher compared to sun drying and oven drying [68]. Further, a slightly higher epicatechin content was observed in freeze-dried cocoa beans (12.8 mg/g), compared to the sun-dried variety (12.4 mg/g) [80].

3.4. Microwave Drying

Microwave drying is based on a distinct volumetric heating mode facilitated by electromagnetic radiation at 915 or 2,450 MHz [81]. Microwave energy (300 MHz-300 GHz) is produced by magnetrons under the combined force of perpendicular electric and magnetic fields. Polar materials orient and reorient themselves according to the direction of the fast-alternating electric field created by microwaves. The fast shift in the electromagnetic field at 2450 MHz produces molecular reorientation, generates friction and heat [27]. Microwaves can inactivate polyphenol oxidase, which minimises cocoa phenolic content degradation. The increase in microwave energy up to 1.08 kJ/g causes the inactivation of polyphenol oxidase. The increase in microwave energy simultaneously increases the temperature of cocoa beans from 49.0 ℃ to 76.4 ℃ and decreases moisture content, which inactivates polyphenol oxidase [71]. Although poly-phenol oxidase is relatively stable from 50-70 ℃, its optimal activity occurs at 45 ℃ [82]. Cocoa beans dried using a microwave for 5 seconds showed a lower polyphenol degradation rate of 13.59 %, compared to sun drying for 72 hours (39.44 %) [66]. Therefore, the microwave drying method is a possible alternative to improve polyphenol recovery in cocoa beans.

Previous studies have reported that intermittent microwave drying could reduce the rate of polyphenol degradation rate of cocoa beans [66]. Generally, this method involves short exposures of 5-30 seconds at temperatures of 50-100 ℃ to prevent cocoa beans from being burned. The duration of exposure time during microwave drying does influence the degradation of polyphenol content in cocoa beans. The polyphenol degradation rate was 13.59 %, 31.74 % and 42.61 % at 5, 15 and 30 seconds, respectively. The 5 second exposure time allowed heating at 50 °C, which was lower than the 15 and 30 second exposure times, in which temperatures reached 70 °C and 100 °C, respectively. The observed variation in phenolic degradation rates may thus be explained by temperature differences.

Table 2. Effect of different drying methods on the polyphenol content of cocoa beans. TPC and TFC stand for total phenolic content and total flavonoid content, respectively. GAE and QE stand for gallic acid equivalent and quercetin equivalent, respectively. DW stands for dry weight of sample.

Drying technique	Parameter	Outcomes	References
Open sun drying and solar drying	Facility: bamboo mats Capacity: 1 kg Duration: 96 – 120 hours	The total phenolic content of cocoa beans, following a seven-day fermentation period, was greater when subjected to drying using a solar biomass hybrid dryer (711.44 mg GAE/g DW) in contrast to those dried using the sun drying method (362.69 mg GAE/g DW)	[82]
Open sun drying and greenhouse drying	3 kg, 27 hours Temperature: 55 °C	A modified greenhouse dryer resulted in the highest total phenolic content (85.03 mg GAE/ 100g DW) and total flavonoid content (54.11 mg QE/ 100 g DW), which were slightly higher compared to those obtained through open sun drying (82.76 mg GAE/ g DW and 51.69 mg QE/g DW, respectively).	[83]
Sun drying	Wooden tray, 3 days	Total phenolic content, anthocyanins, catechin, and epicatechin values were 85.75 mg GAE/g DW, 0.76 mg/g, 1.82 mg/g and 1.41 mg/g, respectively.	[84]
Oven drying	30, 40, 50, 60, and 70 ℃ (until moisture content reached <10%).	Procyanidin content decreased by 15-25 % with the rise in drying temperature. Polyphenol stability was optimised at 40 ℃ for 40 hours, which resulted in higher epicatechin and procyanidin levels compared to freeze drying for 68 hours.	[85]
Oven and sun	Oven drying:	The retention of polyphenols for oven and sun	[69]

drying	60, 70 and 80 °C, 300 g, meshed tray Sun drying: 12 days (9 hours daily)	drying ranged from 23.2 to 8.3 % and 23.2 to 11.4 %, respectively, when compared to the initial fresh sample. Oven drying showed lower polyphenol retention, due to the prolonged activation period of polyphenol oxidase during sun drying.	
Sun drying **Oven drying** **Freeze drying**	Sun drying: 5 days Oven drying: 55 °C, 2 days Freeze drying: -80 °C, 3 days	Freeze dried beans had the highest polyphenol content, followed by oven- and sun-dried beans.	[68]
Oven and freeze drying	Oven drying: 50 °C, 70 °C Freeze drying: -84 °C	Drying samples at 50 °C for 20 hours led to a total phenolic content that was 10 % lower than that achieved by drying at 70 °C for 3 hours. The difference in total phenolic content obtained with the freeze drying and oven drying methods at 70 °C was not significant.	[86]
Microwave drying	20-100 g, 60-180 seconds interval, 49.0–76.4 ℃	The highest polyphenol content (101.97 mg GAE/g) was observed in 100 g cocoa beans dried by microwave for 180 s at 600 W (microwave power, E = 1.08 KJ/g).	[71]
Microwave drying	Cocoa beans submerged in water at 95 ℃ for 5 minutes followed by thermal shocking with water at 25 ℃.	The reduced (-)-epicatechin content obtained in comparison to freeze dried beans was a result of the high temperature used. Conventional polyphenol oxidase inhibition at a temperature of 95 ℃ was not suitable to preserve the (-)-epicatechin content. (-)-Epicatechin = 17.456 mg/g and TPC = 202.1441 mg GAE/g.	[85]
Microwave drying	40, 50, 60, and 70 ℃, 23 – 41 hours until water content reached <10% by weight.	Procyanidins decreased in concentration between 15-25 % as the drying temperature was increased from 40 to 70 ℃. It led to 12.6 %, 19.5 % and 21.5 % degradation in the content of dimers, trimers and tetramers, respectively. Catechin content increased with drying temperature as a result of the dimer degradation products.	[85]

Roasting

The primary aim of roasting is to achieve several key characteristics in cocoa beans. These include developing the characteristic brown colour, enhancing the aroma and flavour, ensuring an acceptable brittleness, and the minimizing the presence of undesirable volatile compounds [50,87,88]. Unroasted cocoa beans are undesirable for their distinctive bitter, acidic, astringent and nutty flavour which are due to the high concentrations of volatile acids. [19]. Generally, the moisture content of cocoa beans is reduced to 2 % during roasting [88, 89]. Convection roasting is the most commonly used method in roasting cocoa beans, in which raw beans are subjected to a forced flow of hot air. Thermal processing during roasting involves high temperatures between 130 and 150 ℃ for 15 to 45 minutes [87]. The elevated temperature during roasting promotes lipid oxidation and nonenzymatic browning, which results in the reduced nutritional value of cocoa beans and polyphenol loss [90].

Past studies have reported that the degradation of polyphenol compounds during roasting is influenced by the temperature and duration of thermal processing [32, 87, 91]. Thermal treatments at higher temperatures of 120 and 150 °C generally resulted in gradual decrease in the total phenolic content in the Nacional and Trinitario cultivars [31]. The total phenolic content of cocoa beans roasted at 110 °C remained the same or slightly decreased, but beans roasted at 150 °C exhibited a loss of 2.5–15.0 % compared to unroasted beans [31]. The total flavonoid and phenolic content during roasting gradually decreased with time and temperature [91]. The highest degradation rates in both total phenolic content and total flavonoid content were observed at a roasting temperature of 250 °C for 50 minutes, while the lowest rates were observed at 150 °C for 10 minutes. Polyphenol compounds undergo structural changes during roasting, particularly the epimerization of both monomers and polymers. A rapid loss of (-)-epicatechin and (+)-catechin compounds was observed at high roasting temperatures as a result of heat-related epimerization from (-)-epicatechin [92].

Different roasting techniques can influence the reduction of polyphenols in cocoa beans [90]. Traditional roasting at 5 to 10 minutes significantly reduced the polyphenol content of cocoa beans, while oven roasting showed otherwise [90]. Oven roasting was conducted using an electric air-dried oven at 180 ℃, while traditional roasting was conducted using a cooking pot that could reach a temperature range of 200-220 ℃. Low molecular weight phenolic compounds are easily volatilised at high temperatures, hence the reduction in polyphenol content has been attributed to the higher processing temperatures used in traditional roasting [90]. Additionally, roasting cocoa beans using a superheated steam roasting method that involved a steam oven decreased the total phenolic content from 2.88 % to 24.57 %, and flavonoid content from 3.10 % to 35.31 %, respectively [91].

Alkalisation

Alkalisation, also known as dutching, is the washing of cocoa powder with an alkaline solution that neutralizes cocoa acidity to a pH of 7 [29]. Alkali treatment primarily aims to enhance the solubility of cocoa particles in aqueous media, produce the desired darker colour, and reduce astringency and bitterness [93–95]. Alkalisation is a further step of the Maillard reaction. It induces interactions between polyphenols and some Maillard products [93]. Polyphenols such as catechin, anthocyanins and procyanidins may transform into quinones during the alkalisation process, which can tolerate polymerization or form heavy insoluble brown compounds through protein linking [96]. Alkalisation has been reported to significantly reduce the polyphenol content of cocoa powder [29, 30].

During alkalisation, some factors, including the type and concentration of the alkali solution, influence the polyphenol degradation of cocoa beans [93,97]. It is known that a lower polyphenol content is associated with a less astringent taste and darker colour of cocoa powder [97]. Alkalised cocoa powder was found to have lower catechin and epicatechin content compared to untreated cocoa powder, with a reduction of 36 mg/100 g to 27.6 mg/100 g for catechin, and from 56.2 mg/100 g to 48.2 mg/100 g for epicatechin, respectively [29]. The reduction of monomeric polyphenols was also reported, with a 77 % decrease in epicatechin content and a 67 % decrease in catechin content observed after NaOH alkalisation at 0.02 MPa for 20 minutes [93]. Potassium carbonate (K_2CO_3), and sodium hydroxide (NaOH) are generally used for cocoa powder alkalisation [98]. The total polyphenol content of cocoa powders changed significantly with different alkali solutions. Alkalisation with a 1 % K_2CO_3 solution produced a lower polyphenol content (14.34 mg/g) than that with a 1 % NaOH solution (15.85 mg/g) [97].

The content of total polyphenols decreased with increased alkalisation [93, 97]. For example, a light degree of alkalisation (1 % NaOH solution for 20 minutes at 0.02 MPa) showed a 27.94 % decrease in polyphenols, while a heavy degree of alkalisation (3 % NaOH solution for 30 minutes at 0.1 MPa) resulted in a 39.16 % decrease, when compared to the non-alkalised cocoa powder [93]. Similarly, alkalisation with 3 % K_2CO_3 gave a lower total phenolic content of 10.49 mg/g compared to 1 % K_2CO_3, which gave 14.34 mg/g [97].

Extraction

4. Influence of Extraction Methods on the Polyphenol Content of Cocoa Beans

Different extraction methods have varying influence on the extraction of phenolic compounds from plants. Choosing a suitable extraction method is crucial to prevent the loss of bioactive compounds. A suitable method could improve extraction efficiency and reduce time and energy consumption. Additionally, it aids in environmental conservation by reducing solvent waste and hazardous substances [99]. Generally, the extraction of polyphenols from cocoa beans can be divided into conventional and non-conventional methods. Conventional methods are based on solid-liquid extraction with various solvents. The conventional extraction method requires large volumes of solvents as well as manual procedures which are labour intensive and primarily dependent on the investigator [100]. Despite these drawbacks, conventional methods are still employed because research has been done to enhance their conditions and expand their industrial-scale applicability [101]. Maceration is the common conventional method used to extract polyphenols from cocoa beans [99, 102]. Other methods, including infusion and decoction, have also been used, although no comparison studies have been done [84]. The limitation of conventional extraction methods prompted the development of environmentally friendly and efficient extraction techniques. Non-conventional extraction methods offer several advantages in terms of time, solvent consumption, yields, and reproducibility. However, production costs have to be assessed to use them on an industrial scale [101]. Therefore, non-conventional extraction methods such as ultrasonic-assisted extraction [68,103,104] and pressurized liquid extraction [105] have been reported for the extraction of polyphenols in cocoa beans. Additional data from past studies on the degradation rates of polyphenol content during extraction processes is presented in **Table 3**.

4.1. Maceration

Maceration is widely used for the extraction of phenolic compounds in cocoa beans. The process involves soaking ground samples in a suitable solvent in a closed system, and then agitating them continuously or occasionally at room temperature [100]. The duration of maceration extraction ranges from hours to days [101]. In the extraction of cocoa

beans, the duration can vary from 25 minutes (5 minutes of vortex agitation followed by 20 minutes of centrifugation) to 30 hours [102]. A separation process, such as filtration, decantation or purification, is used after the extraction phase [100]. The filtered liquid is then evaporated and concentrated using a rotary evaporator, and stored until further analysis. The speed of agitation and duration are the two critical parameters in this method. The speed of the magnetic stirrer induces vortex formation and, if set at different speeds, could lead to turbulence. Consequently, the mass transfer rate may increase [106]. Although the setup of the maceration technique is simple and inexpensive, this method requires a long extraction time, massive solvent consumption, and low extraction efficiency [107].

Studies on the comparison of maceration and ultrasonic-assisted extraction methods for the extraction of polyphenols in cocoa beans have been reported. Maceration of cocoa beans for 30 hours resulted in a lower yield of polyphenols (3.40%) than the ultrasonic-assisted extraction technique (8.37%) that was carried out for 75 minutes [102]. Additionally, as compared to ultrasonic-assisted extraction techniques utilizing methanol, ethanol, and acetone (with yields of 11.42 %, 10.91 %, and 2.76 % respectively), maceration of polyphenols in cocoa beans using the same extracting solvents exhibited significantly lower extraction yields (3.35 %, 4.92 %, and 1.92 %, respectively). According to another study, the total phenolic content of cocoa beans after 2 hours of maceration was lower (91.06 mg g^{-1}) than it was after 30 minutes of sonication (135.92 mg g^{-1}) [99]. These findings suggest that maceration is useable, but not an efficient method for extracting polyphenols in cocoa beans compared to the ultrasonic-assisted extraction method.

4.2. Ultrasonic-Assisted Extraction

Ultrasonic-assisted extraction (UAE) involves the application of high-intensity ultrasonic waves to samples [106]. Ultrasound creates solvent cavities, described as micro-sized bubbles [100]. The explosion of cavitation bubbles leads to cell wall rupture, which accelerates the diffusion of solutes into the solvent [107]. Extraction of polyphenols generally requires ultrasound with a frequency range of 20-2000 kHz [100, 101]. At lower sonication frequencies, like 20 kHz, the physical effect of cavitation phenomena, such as liquid circulating current and turbulence, dominates and controls the extraction process and duration. A smaller sample size is preferred for this method to increase the number of cells directly exposed to ultrasonically-induced cavitation [101]. Swelling rate, disruption, and particle size post-treatment are factors that influence extraction efficiency [106]. This method is widely used in the extraction of polyphenolic compound in plants, as it requires low solvent consumption, short extraction times, low temperatures, and a simple setup for both small and large-scale settings [106].

Several studies have compared the efficiency of ultrasound-assisted and maceration extraction methods in the extraction of polyphenols in cocoa beans [99, 102]. A past study reported that a cocoa bean extract obtained by UAE had a higher total phenolic content (135.92 mg g^{-1}) compared to one obtained by maceration (91.06 mg g^{-1}) [99]. The anti-oxidant activity was evaluated using 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, and the result was expressed as the half-maximal effective concentration (EC_{50}). EC_{50} is the amount of antioxidant necessary to decrease the initial concentration of DPPH radicals by half. The lower the EC_{50} value, the greater the activity of the extract as a DPPH radical scavenger [108]. The EC_{50} of a cocoa extract obtained by UAE (0.0124 mg ml^{-1}) was lower than that of a cocoa extract obtained by maceration (0.0164 mg ml^{-1}). High-pressure liquid chromatography (HPLC) analysis showed a positive correlation between total phenolic content and antioxidant activity, where the (-)-epicatechin content in the cocoa bean extract obtained with UAE (144 μg mg^{-1}) was higher compared to the extract obtained through maceration (132.88 μg mg^{-1}). Therefore, a higher total polyphenol content of cocoa beans was obtained with ultrasound compared to maceration [99].

UAE may be used in continuous or pulsed sonication modes. Pulsed UAE is reported to be more effective than continuous extraction for sensitive components like polyphenols in cocoa [109,110]. In contrast to continuous sonication, pulsed sonication has a period of inactivity that suppresses the constant generation of cavitation microbubbles during pulsation, and favours restoration of the cavitation zone [109]. During the active period, maximum wave power is reached and this decreases to a fixed constant value during the period of inactivity. The peaks of power, intensity and successive starts and shutdowns cause a state of shock in the solution which may initiate the disintegration of cavitation bubbles, hasten degradation of the plant matrix, and hence improve the release of the extract [110]. In addition, the inactivation period creates residual nuclei that initiate the formation of microbubbles in the next active period, which facilitates the homogenous distribution of these microbubbles throughout the system during the active period and significantly increases its yield [109]. Pulsed sonication also has a high energy conversion efficiency which may decrease the probability of thermal degradation of samples [111, 112]. The yield of epicatechin increased by 282 % when pulsed sonication ($1s_{(on)}/1s_{(off)}$) was used, compared to continuous sonication [109]. Pulsed sonication ($4s_{(on)}/2s_{(off)}$) also gave a better ascorbic acid yield compared to continuous sonication [110].

4.3. Pressurized Liquid Extraction

In pressurized liquid extraction, a high pressure

is applied to keep the solvent in a liquid state at a temperature above its boiling point [107]. This technique generally operates within a pressure range of 3.3-20.3 MPa and an elevated temperature of 40-200 ℃ [100]. High temperatures accelerate the disruption of solute-matrix interactions, resulting in analyte desorption from the matrix. This process also decreases solvent viscosity and surface tension. Low solvent viscosity and surface tension, combined with high pressure, may improve sample matrix penetration and extraction efficiency [105]. High pressures and temperatures accelerate diffusion rates and shorten extraction times [105,107]. The use of water as an extracting solvent reduces organic solvent waste. Furthermore, the high purity of the crude extract negates the need for a purification process. The disadvantages of pressurized liquid extraction including low analyte selectivity, potential interference during the extraction process, and the need for expensive advanced instrumentation. Additionally, the extract may become highly diluted, especially when using multiple cycles, [100]. Pressurized Hot Water Extraction (PHWE) is a technique that uses water as the solvent and applies the principle of pressurized liquid extraction. In this process, the dielectric constant of water decreases with increasing temperature. This trend implies that the polarizability of water can be adjusted by changing the temperature. Water's dielectric constant at 250 °C is similar to methanol's under ambient conditions. Therefore, water at high temperatures is a green alternative that could potentially replace conventionally-used organic solvents [105]. PHWE is more efficient than UAE for the extraction of polyphenols from cocoa beans [105]. The total phenolic content obtained with PHWE (218.1 mg/100 g sample) was higher than that obtained with UAE (146.2 g/100 g sample). The liquid water properties at elevated temperatures explained the high phenolic content extracted by PHWE. An increase in temperature triggers an increase in the solvation power of a liquid. Liquid water at elevated temperatures and pressures has a low polarity and density, enabling faster mass transfer and improved sample wetting due to higher diffusivity and lower viscosity and surface tension [105].

5. Influence of Extraction Conditions on the Polyphenol Content of Cocoa Beans

5.1. Extraction Solvent

Solvent selection is an important factor for extraction efficiency, as the recovery of phenolic content is affected by the solvent polarity [113]. Phenolic compounds are generally highly soluble in organic solvents, thus giving higher yields [106]. According to the law of similarity and intermiscibility, a solvent with a polarity value near the polarity of the solute is likely to perform better, and vice versa [107]. The solubility of polyphenols increases with the increasing polarity of the solvent. Methanol is the most polar, followed by ethanol, ethyl acetate, n-butanol, and water, in descending order [114]. The most common solvents used as extractants in the extraction of polyphenols are methanol and ethanol, often with different proportions of water. Although methanol is generally found to be more efficient in the extraction of polyphenols, ethanol is preferred in the food industry due to its lower toxicity [115]. The use of pure ethanol as an extraction solvent leads to the ineffectiveness of polyphenol extraction due to the presence of hydroxyl groups. As a solvent, water is also ineffective in extracting polyphenols due to the improved activity of polyphenol oxidases, which lead to a reduction in phenolic content. These enzymes are inactive in alcoholic media; therefore, water-ethanol is a very suitable extraction solvent. A proportionate mixture of water-ethanol solvent is sustainable, economical, and less toxic for industrial use [114]. Based on past studies, 70 % acetone [28,69,99,116] and 70 % ethanol [68, 117,118] are the most common extraction solvents used for the extraction of polyphenols from cocoa beans. The highest total phenolic content obtained was 1380.86 mg GAE/g extracted with 70 % ethanol using the UAE method [68]. In contrast, a comparison study of five different solvents, 100 % methanol, 70 % methanol, 60 % isopropanol, 80 % ethanol, and acetone/water/acetic acid (70:29.5:0.5 v/v), showed significant differences in their extraction efficiencies, with acetone/water/acetic acid (70:29.5:0.5 v/v) being statistically superior [103].

5.2. Extraction Time

Studies conducted on the extraction of phenolics from cocoa beans at different extraction times (30-90 minutes) showed that the highest TPC value in cocoa beans (40.33 mg GAE/g) was obtained at 74.5 minutes, after which the response variable started to decelerate [103]. It has been noted that an increase in extraction time results in a progressive increase in the polyphenol content of cocoa beans until the inflection time is reached [103, 107, 115]. In the UAE method, the inflection point for the extraction time is explained by the cavitation phenomenon, which occurs for prolonged sonication times, and this phenomenon leads to degradation of the polyphenol content in the sample [115]. The application of ultrasound in extraction enhances mass transfer phenomena and reduces the necessary extraction time to obtain maximum recovery of phenolic content. However, ultrasound may produce some chemical effects due to the production of free radicals within cavitation bubbles. Sonication of water results in the formation of highly reactive hydroxyl radicals, which may combine to form hydrogen peroxide and induce oxidation of phenolic content as the extraction time is increased. It has been suggested that extraction using sonication at

20 kHz for 30 minutes may obtain maximum yields of flavonoids and phenolic acid [101].

Extraction time alone does not determine the overall efficiency of extraction. Temperature, time, solid-solvent ratio and their interactions with each other significantly affect the phenolic content of cocoa beans [103]. An increase in temperature accelerates the diffusion coefficient of phenolic compounds, which leads to an increase in the mass transfer of phenolic compounds from the plant matrix to the solvent; therefore, the process may be accomplished in a shorter extraction time. Further, the smaller particle size of the raw material enhances the quantitative recovery of phenolic content in a shorter time due to mass transfer phenomena [101].

5.3. Extraction Temperature

An increase in extraction temperature has a significant influence on the total phenolic content [115]. A higher temperature reduces the surface tension and viscosity of the solvent [119]. This results in better interaction and improved analyte contact with the solvent, which reduces barrier properties and improves the solute diffusion process to the solvent [106]. In addition, heating may soften vegetable tissues and weaken the cell walls [103], which results in higher permeability of the cell walls, higher solubility of phenolic compounds, and increases in heat and mass transfer through the plant matrix [101]. Despite these advantages, many phenolic compounds are easily hydrolysed and oxidised at high temperatures, especially over an extended period. Generally, the reduction of total phenolic content was observed with extraction temperatures above 60 ℃ [120]. However, the sensitivity of a sample to temperature-induced polyphenol degradation depends on the type of polyphenol compounds present in the plant extracts, their physiochemical and biochemical characteristics, and solvent-sample interactions [119]. An optimization study was conducted using UAE of polyphenols in cocoa beans. An extraction time of 30 minutes and a temperature of 20-40 ℃ were used, and the optimum phenolic content was obtained at 39.3 ℃ [103].

5.4. Solid-Solvent Ratio

The solid-solvent ratio influences the yield of phenolic content. An increase in the solute-solvent ratio positively affects extraction yield, as explained by the mass transfer principle. A higher solvent-to-solid ratio accelerates the mass transfer phenomenon due to the greater differences in concentration between the solid matrix and the bulk phase of the solvent, which leads to a faster extraction rate [101]. The extraction of phenolics from cocoa beans was conducted at different solvent-solid ratios, starting from 10, 15 and 20 g/ml using response surface methodology and the maximum TPC of cocoa beans was achieved at a solid-solvent ratio of 22.8 g/ml [103]**.** However, a higher extraction rate does not necessarily extract more phenolic compounds, due to the coextraction of non-desirable compounds [101]. An increase in the solvent ratio can affect the whole extraction process and cause solvent wastage due to the formation of polyphenol oxidase through the reaction of polyphenol with hydrophilic compounds. This leads to difficulty in isolating polyphenolic compounds, due to improved enzyme activity [106].

Table 3: Effect of different extraction parameters on the polyphenol content of cocoa beans. TPC and TFC stand for total phenolic content and total flavonoid content, respectively. GAE, CE and RE stands for gallic acid equivalent and quercetin equivalent, and rutin equivalent respectively.

Extraction method	Parameter	Outcome	References
Ultrasound	Solid to solvent ratio = 1:20 (w/v) Solvent = Methanol/Water (50:50 v/v) Sonication time = 30 minutes Frequency = 25 – 45 kHz	A significant increase of 5% was recorded in the total phenolic content extraction rate at 25 kHz compared to extraction at 45 kHz (4% decrease) in 30 minutes.	[121]
Ultrasound	1:10 (w/v), Ethanol/Water (80:20 v/v), 30 minutes, 40 kHz, 25 ℃	Total phenolic, flavanol and flavonoid content values were 19.85 – 33.39 mg GAE/g, 9.99 – 22.30 mg CE/g and 13.78 – 35.93 mg RE/g, respectively.	[122]
Ultrasound	1:22.8 (w/v), Acetone/Water/Acetic acid (70:29.5:0.5 v/v/v), 74.5 minutes, 39.3 ℃	The optimal total phenolic content was 40.33 mg GAE/ g after optimization of solvent type, temperature, time and solute/ solvent ratio.	[103]
Ultrasound	1: 5 (w/v) Ethanol/Water (80:20 v/v), 10 minutes, 20 ℃	The crude extract obtained through sonication was further fractionated using a separating funnel with different solvents. The ethyl acetate fraction resulted in	[104]

		the highest, while the aqueous fraction showed the lowest total phenolic and flavonoid content values.	
Maceration and ultrasound	**Maceration:** 1:5 (w/v), 24 hours **Ultrasound**: 1:5 (w/v), 15 minutes (additional 1 hour of mixing)	The ultrasound extraction method was more effective in the extraction of polyphenols compared to maceration. Ethanol and methanol were helpful solvents for polyphenol extraction.	[102]
Maceration and ultrasound	Maceration: 1:20 (w/v), methanol: water (1:1 v/v), 2 hours, room temperature. Ultrasound: 1:20 (w/v), methanol: water (1:1 v/v), 30 minutes, 25 kHz	Higher polyphenol content values were obtained using ultrasonic radiation (135.92 mg/g) compared to maceration (91.06 mg/g).	[99]
Maceration	Acetone/water (70:30 v/v) and water, 24 hours, room temperature	Acetone/water (70:30 v/v) extracted more polyphenols compared to water, with 236.28 and 135.45 mg GAE/g, respectively.	[116]
Maceration	1:100 (w/v), methanol/water (70:30 v/v), 45 minutes (constant stirring)	The total phenolic content, (+)-catechin and (-)-epicatechin content of cocoa beans from different regions ranged from 33.55-71.66 mg GAE/g, 4.20 - 8.93 mg/g and 3.45-13.16 mg/g, respectively.	[123]
Maceration	1:10 (w/v), ethanol/water (70:30 v/v), 60 minutes, 60 ℃	Total phenolic content of 103-202 mg GAE/g	[117]
Infusion	1:20 (w/v), boiling water, 1 hour	Average total phenolic content ranged from 8.12 – 30.10 mg/10 g.	[84]
Decoction	1:10 (w/v), boiling water	The catechin and epicatechin content of unfermented and fermented cocoa beans were 4.56 - 15.14 mg/g and 26.22 – 52.32 mg/g, respectively.	[124]

CONCLUSION

Cocoa beans are an excellent choice for antioxidant intake as their polyphenol content surpasses the polyphenol levels found in green tea and red wine. Cocoa beans are the raw material for producing cocoa-based products in the food, confectionery, cosmetics and pharmaceutical industries. The production of final cocoa products requires technological processing, including fermentation, drying, alkalisation and roasting to develop favourable flavours and textures. Technological processing negatively impacts the polyphenol compounds, mostly due to extended processing periods and elevated temperatures. Both spontaneous and inoculated fermentation for extended periods lead to greater degradation of polyphenol compounds due to prolonged enzymatic activity that oxidises polyphenol compounds to complex tannins. Meanwhile, drying using a freeze dryer is favourable for the preservation of polyphenol compounds as temperatures below zero may inactivate polyphenol oxidase and avoid enzymatic activity. Intermittent drying using micro-waves is a promising alternative for cocoa bean drying as the reduced exposure to heat could minimise polyphenol degradation. Roasting causes the greatest degradation in polyphenols compared to other processing operations due to the use of high temperatures of up to 130 ℃. Alkalisation using NaOH compared to K_2CO_3 at low concentrations could minimise polyphenol degradation. The short extraction times and low temperatures used in the ultrasound-assisted extraction method are efficient in optimizing polyphenol extraction. Generally, processing temperatures and times cause chemical and biochemical changes that lead to the degradation of polyphenol compounds in cocoa beans. To minimise this degradation, further research should focus on developing and optimizing processing methods that can retain the polyphenol content of cocoa beans, through a detailed study of processing operations and modification of conventional methods. Overall, future research in this area can contribute to both the cocoa industry and a deeper understanding of the relationship between processing methods and the preservation of polyphenol compounds, which have important health and sensory implications.

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