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Research Article

Polyphenols in cocoa (*Theobroma cacao* L.)

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Abstract

Polyphenols have gained much interest recently due to its antioxidant capacity and possible benefits to human health such as anti-carcinogenic, anti-atherogenic, anti-ulcer, anti-thrombotic, anti-inflammatory, immune modulating, anti-microbial, vasodilatory and analgesic effects. Cocoa (Theobroma cacao L.) is a rich source of polyphenols and reported having high antioxidant activity than teas and red wines. Cocoa and its derived products (cocoa powder, cocoa liquor and chocolates) contain varied polyphenol contents and possess different levels of antioxidant potentials. The polyphenols in cocoa beans contribute to about 12-18% of the dry weight of the whole bean. Main classes of polyphenolic compounds identified are such as simple phenols, benzoquinones, phenolic acids, acetophenones, phenylacetic acids, hydroxycinnamic acids, phenylpropenes, coumarines, chromones, naphtoquinones, xanthones, stilbenes, anthraquinones, flavonoids, lignans and lignins. Three main groups of cocoa polyphenols can be distinguished namely the catechins (37%), anthocyanins (4%) and proanthocyanidins (58%). The main catechin is (-)-epicatechin with up to 35% of polyphenol content. Effects of processing could influence the polyphenols of cocoa and its products. The aim of this paper is to provide a review of some of the latest developments and studies reported for cocoa polyphenols and its contribution of this area for the betterment of human health.

Keywords: Antioxidants, Cocoa, Human health, Polyphenols, *Theobroma cacao*

Introduction

Cocoa is best known for its derived products, especially chocolates, rather than in its original botanical form i.e. fruits and beans. These products are consumed in great demand worldwide due to its unique flavour and aroma that cannot be replaced by other plant products. *Theobroma cacao* is the name given to the cocoa tree and belongs to the family Sterculiaceae. Cocoa trees are found wild in the rain forest of the western hemisphere from 18°N to 15°S, which is from Mexico to the southern edge of the Amazon forests [1]. *Theobroma cacao* is the only species cultivated commercially in major producing countries such as Ivory Coast, Ghana, Nigeria, Cameroon, Brazil, Ecuador, Indonesia and Malaysia. The Forastero and Criollo are the two main varieties planted. However, the criollo variety is mostly planted in the Central and South America and produces in small volume. This variety is conventionally referred to as 'fine' cocoa due to its unique flavour that cannot be found in the forastero types. Currently, Ivory Coast is the leader in cocoa production follows by Ghana and Indonesia. The current world production of cocoa beans is estimated at 3520,000 tonnes while the grinding is estimated at 3678,000 tonnes for year 2008/09 [2]. Figure 1 shows the production output from major producing countries.

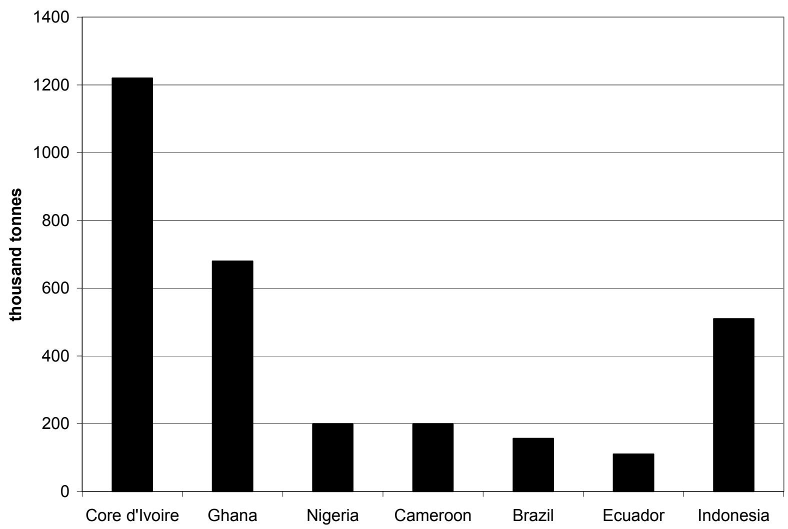


Figure 1. Major producers of cocoa beans (2008/09).

In recent years enormous research has been focused into cocoa polyphenols, especially the flavonoids, and its function as potent antioxidant in human health. Cocoa is a very rich source of dietary flavonoids and reported of having higher flavonoids per serving than teas and red wine [3]. Main classes of polyphenolic compounds identified are such as simple phenols, benzoquinones, phenolic acids, acetophenones, phenylacetic acids, hydroxycinnamic acids, phenylpropenes, coumarines, chromones, naphtoquinones, xanthones, stilbenes, anthraquinones, flavonoids, lignans and lignins. Some of the beneficial effects of polyphenols are such as anti-carcinogenic, anti-atherogenic, anti-ulcer, anti-thrombotic, anti-inflammatory, immune modulating, anti-microbial, vasodilatory and analgesic effects [4]. Cocoa and Chocolate have long play it parts as medicinal supplement as mentioned in some historical texts such as in the Badianus Manuscript, Florentine Codex and Princeton Codex [5]. In these texts some 150 uses of cocoa for medical treatments by using various parts of the plant such as the bean, bark, butter, flower, fruit pulp and leaf were documented. In the North America, medicinal use of chocolate could be dated back to the 16th century. Chocolate was used as 'patients diet' in the 19th century in the United States and it was not until after the 1930s, the consumption of chocolate is shifted from medicinal to confectionery [6].

In general, dietary sources of polyphenols can be found in variety of fruits, plant based beverages, vegetables, cereals, nuts, seeds and in manufactured food such as chocolate. The research on flavonoids and other polyphenols was believed to have truly begun after 1995 [7]. However, some reported studies had been documented in 30's by Adam et al. [8], Freudenberg et al. [9] and Knapp and Hearne [10] in the studies of catechin. Some established studies on cocoa polyphenols were mostly related to the biochemistry aspect during the curing process and flavour studies [11-17]. The research in cocoa polyphenols continued thereafter, with the advancement of analytical and instrumentation techniques, polyphenols in cocoa were identified and quantified and in this aspect Wollgast and Anklam [5] has reviewed and compiled the various methods used. Studies by Kim and Keeney [18,19] formed the basis of the analyses of (-)-epicatechin content in cocoa beans by using high performance liquid chromatography. Numerous studies were carried out to determine the nutritional contribution of cocoa polyphenols to human health. Various review papers can be cited that cover topics such as on health benefit aspects [20,21], prevention of cardiovascular disease [22-24], anti-inflammatory impact of flavanols [25] and issues on the exact contribution of polyphenols to human health [26, 27].

The objective of this review paper is focused on: (a) the quantity of polyphenols in various cocoa based products; (b) effects of processing on cocoa polyphenols; (c) the antioxidant capacity of cocoa polyphenols; and (d) summary of some latest published research findings on contribution of polyphenols to human health.

Processing of Cocoa Beans

A ripe mature cocoa fruit contains about 30-40 seeds which are covered in a sweet, slightly sour, mucilaginous pulp. The mucilaginous pulp is an important substrate during fermentation as the pulp sugars are consumed by the yeast and bacteria (Figure 2). The seed comprised of two main parts namely the testa and the cotyledon. The cotyledon, upon drying, formed the nib which is an essential raw component used in the downstream manufacturing process. Lopez and Dimick [28] presented the components and composition of the cocoa seed as tabulated in Table 1. Two types of parenchyma storage cells form the cotyledon namely the protein-lipid cells and the polyphenolic cells and contain polyphenols and alkaloids. During fermentation, the enzyme-substrate biological barriers break down and allowing free mixing of the enzymes and the substrates which produce the important flavour and aroma precursors of chocolate [29].

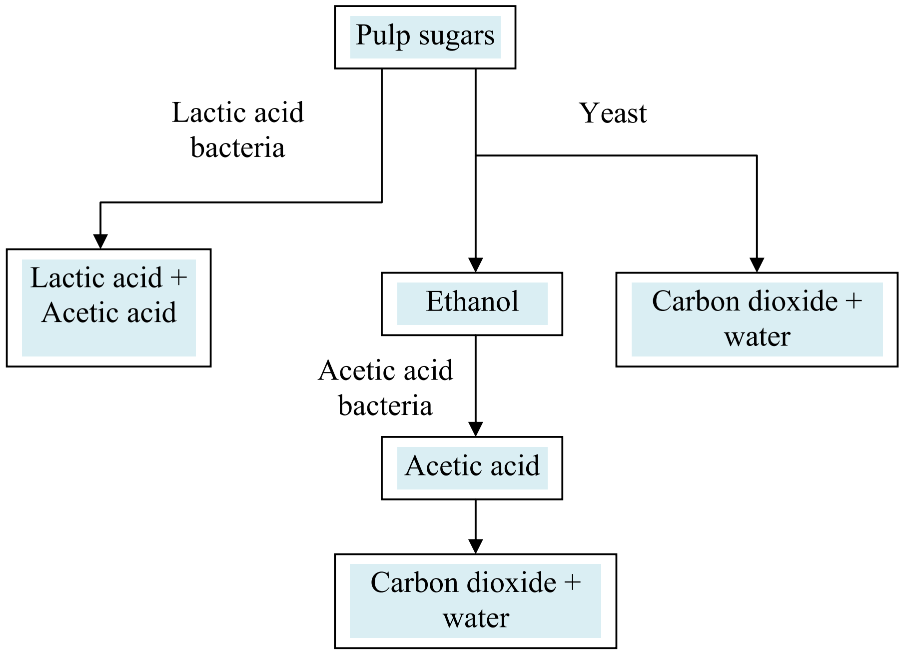


Figure 2. Chemical changes in the pulp during fermentation [30].

Table 1. The components and composition of cocoa seed [28].

Component	Composition
Pulp	
- Water	82-87%
- Sugars	10-13%
- Pentosans	2-3%
- Citric acid	1-2%
- Salts	8-10%
Cotyledon	
- Water	32-39%
- Cellulose	2-3%
- Starch	4-6%
- Pentosans	4-6%
- Sucrose	2-3%
- Fat	30-32%
- Proteins	8-10%
- Theobromine	2-3%
- Caffeine	1%
- Acids	1%
- Polyphenols	5-6%

Harvested cocoa beans are subject to combination of fermentation and drying treatments during processing. These treatment steps are also referred to as curing where it is defined as an operation which involves both the removal of water and complex biochemical changes upon which the end product quality depends [31]. Fermentation is usually carried out by using the box or heap methods while drying can be carried out using the natural or artificial drying methods. Sun drying uses sun light and wind to facilitate drying while hot air is used in the artificial method either by natural or forced convection. The effects of processing parameters on cocoa quality have been reported in various published literature [1, 32-35].

Upon drying the dried beans are usually stored in warehouse prior to shipment and supply to the grinding factories for subsequent processing. A summary of the processing steps involved are shown in Figure 3.

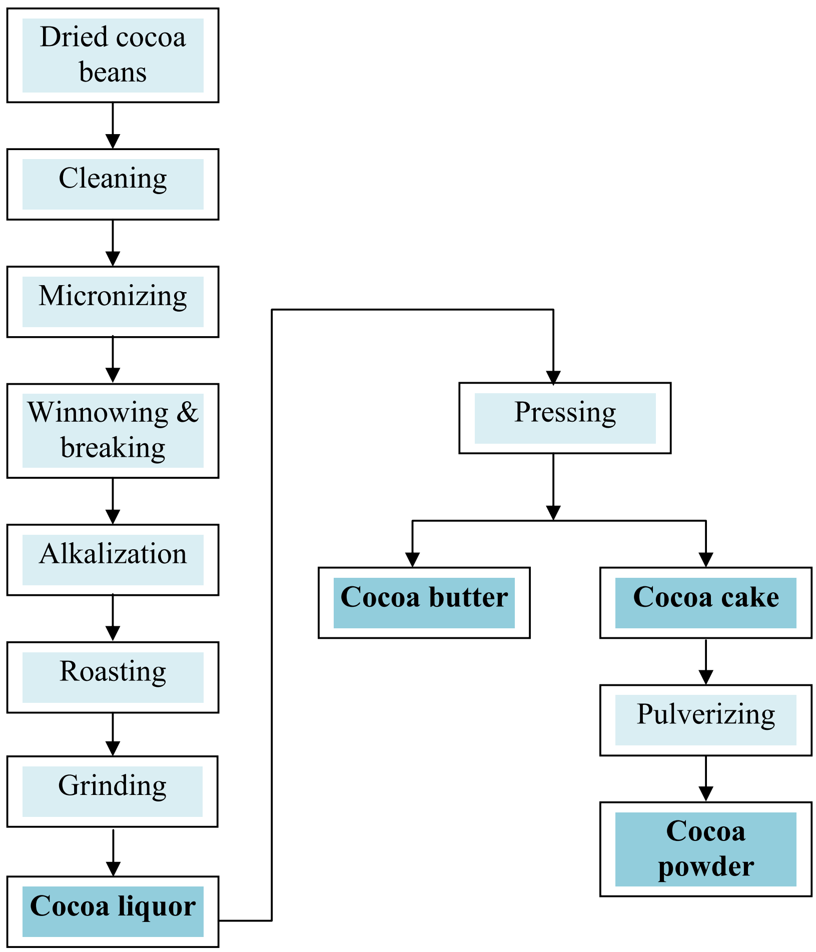


Figure 3. Manufacturing processes of cocoa beans into semi-finished products.

Alkalization and roasting are perhaps the two major steps that contribute to the flavour and colours of the resultant semi-finished products (except for cocoa butter). During alkalization alkali solution is added to the nibs and processed in a pressurerized vessel to change its colour (range from reddish to dark brown) and pH. In the roasting process the cocoa nibs (natural or alkali treated) are roasted at high temperatures (120-150°C) to further develop the aroma and the typical chocolate flavour.

Chocolate is manufactured by mixing ingredients such as cocoa liquor, cocoa butter, sugar, milk powder, emulsifiers and other flavouring additives. There are three basic types of chocolates available in the market namely milk, dark and white chocolate. The types of ingredients and recipes used will determined the type of chocolate produced and its associated flavour. Upon mixing the chocolate paste is usually refine using a five-roller refiner to reduce its particle size to ensure smooth texture consistency in the finished products. The refine chocolate paste is then conched at temperatures ranging from 50°C to 80°C to decrease the remaining moisture and evaporate off volatile substances. The final flavour and texture of the chocolate is determined during conching.

Polyphenols in Cocoa and Chocolate Products

The consumption of cocoa and its derived products has increased 2.0 millions tonnes per year from 1960 to 2004 [2]. The largest consumption of cocoa powder is by Spain (1.67 kg per person per year) followed by Norway (1.65 kg per person per year) and Sweden (1.29 kg per person per year) as cited by Andres-Lacueva et al. [36]. In terms of chocolate confectionery consumption the European countries remain among the top ranking with Switzerland being the highest (10.05 kg per head) followed by United Kingdom (9.16 kg per head) and Germany (9.16 kg per head) as reported by The Association of Chocolate, Biscuit and Confectionery Industries of the European Union [37]. Asian countries typically registered lower level of consumption. For example, Japan and China registered consumption level at 1.86 kg per head and 0.12 kg per head, respectively. This is because chocolate is not a typical food consumed in typical Asian diet.

In view of the high consumption of cocoa and chocolate products, various published literatures have reported the amount of polyphenols available in cocoa and its derived products. Table 2 shows the total polyphenol content reported for cocoa beans and Table 3 summarizes the various polyphenols reported in varieties of cocoa and chocolate products. It can be seen that the range of total polyphenols recorded (range from 40.0 mgGAE/g to 84.2 mgGAE/g) varies among geographical origins and also the planted varieties. The criollo variety generally shows lower total polyphenol content since it is lacking in anthocyanins, which is a type of polyphenol. Anthocyanins are hydrolyzed to anthocyanidins which polymerized with simple catechins to form complex tannins during fermentation. Kim and Keeney [19] have reported concentration of (-)-epicatechin ranging from 2.66 mg/g to 16.52 mg/g for cocoa beans obtained from various countries.

Table 3 shows that dark chocolates generally contain more polyphenols than milk chocolate due to the higher cocoa content. The contribution of polyphenols is mostly from the cocoa liquor content. Typical chocolate recipes in Table 3 [44] shows that cocoa liquor content in dark chocolate is at least 3 times higher than that in milk chocolate. Cocoa powder shows higher polyphenol content than milk and dark chocolates since it is purely the low fat solid component from the cocoa nib. However, processing affect the amount of polyphenols present in the cocoa powder. Natural cocoa powder shows higher polyphenols content as compared to alkalized cocoa powder. This could be due to the adjustment in pH by the alkali and the high temperature and pressure used. The data in Table 2 suggested that the consumption of different type or products could result in different amount of polyphenols absorbed into the human body system. The different amount of polyphenols could also contribute to different level of antioxidant capacity that is beneficial to human health.

Table 2. Total polyphenols content in cocoa beans.

Geographical origin	Variety	Total polyphenol content	Source
Ivory Coast	Forastero	81.5 mgGAE/g	
Columbia	Amazon	81.4 mgGAE/g	
Guinea Ecuatorial	Amazon Forastero	72.4 mgGAE/g	
Ecuador	Amazon hybrid	84.2 mgGAE/g	[38]
Venezuala	Trinitario	64.3 mgGAE/g	
Peru	Criollo	50.0 mgGAE/g	
Dominican Republic	Criollo	40.0 mgGAE/g	
Malaysia	Unknown	71.42-82.68 mgGAE/g	[39]
Cameroon	Unknown	86.6-143.6 mg epicatechin equivalent/g	[40]

Table 3. Polyphenols in various cocoa and chocolate products.

	Ref.[41]		Ref.[36]	Ref.[42]		Ref.[43]	
Product type	Catechins (mg/g)	Procyanidins (mg/g)	Total flavanols & flavonols (µg/g)	Total polyphenols (mg/g)	Catechin and epicatechin (mg/g)	Polyphenols (mgGAE/g)	Procyanidins (mg/g)
Milk chocolate	0.23-0.32	2.16-3.14	-	15.0	0.15-0.16	3.25-5.38	0.43-0.90
Dark chocolate	0.77-1.58	8.52-19.85	-	36.5	0.48-1.37	11.73-14.88	2.78-4.10
Baking chips(1)/Baking chocolate(2)/Semisweet chocolate chips(3)	1.01-1.33(1)	8.71-15.57(1)	-	-	-	26.91-27.18(2) 11.76-12.88(3)	12.57-15.84(2) 3.70-6.29(3)
Unsweetened chocolate	1.47-3.17	18.76-25.20		-	-	-	-
Natural powder	2.90-3.48	32.19-48.70	2109.00-3058.52	-	-	-	-
Alkalized cocoa powder	0.41-0.73	7.02-10.82	848.81-1148.32	-	-	-	-
Cocoa powder	-	-	-	65.0	2.96-3.27	45.30-60.20	19.28-23.71
Chocolate syrup	-	-	-	-	-	3.66-4.79	0.37-0.91

Table 3. Typical dark and milk chocolate recipes [44].

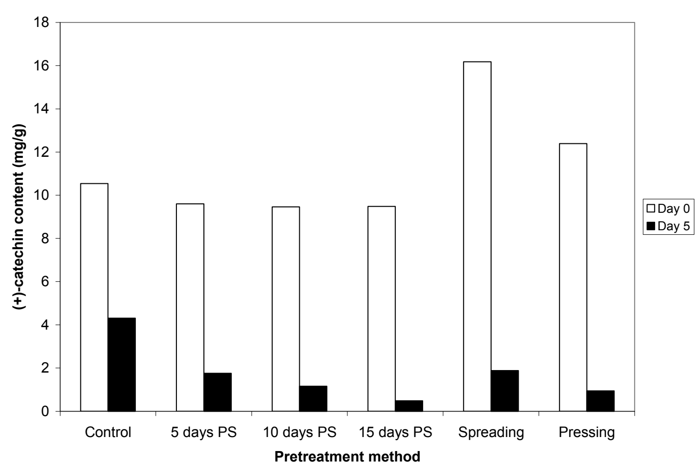
Ingredient	Dark (%)	Milk (%)
Cocoa liquor	39.62	11.78
Cocoa butter	11.75	19.98
Milk powder	-	19.08
Sugar	48.08	48.73
Lecithin	0.35	0.35
Vanillin	0.14	0.08
Salt	0.06	-

Effects of Processing on Cocoa Polyphenols

Primary processing

The polyphenols in cocoa beans could contribute to about 12-18% of the dry weight of the whole bean [45]. Three groups of polyphenols can be distinguished namely the catechins (or also known as flavan-3-ols), anthocyanins and proanthocyanidins. Fermentation and drying affect the concentration of these polyphenols mainly due to the enzymatic browning reactions. However, some pretreatment factors should be taken into account as this would affect the extent of the enzymatic reactions occurring during processing. Table 4 summarizes the effect of fermentation and drying on polyphenol content.

Studies of effect of various pretreatment effects on cocoa quality during fermentation have been carried out by many researchers [45-48]. Nazaruddin et al. [49] extended this study by analyzing the effects of these pretreatment methods on polyphenols content (Figure 4). The pod storage (PS) method caused reduction in pulp volume and might have facilitated the oxidation and polymerization of (-)-epicatechin and its oxidation products. Lower content of (-)-epicatechin and (+)-catechin in pressed bean was observed as compared to spread beans which could be due to loss as exudates during pressing.



(Day 0 = zero day pod storage and Day 5 = five days pod storage)

Figure 4. Effect of pretreatment methods on (+)- catechin content in cocoa beans.

The polyphenols in cocoa beans undergo oxidation reaction which is both non-enzymatic and enzymatic through the action of polyphenol oxidase. Hansen et al. [33] reported this enzyme is strongly inactivated during the first day of fermentation, remaining only 50 and 6% of enzyme activity after 1 and 2 days, respectively. Forsyth [11] reported loss of polyphenols caused by diffusion into the fermentation sweatings and confirmed by the microscopic studies carried out by de Brito et al. [50].

Table 4. Effects of fermentation and drying on cocoa polyphenols.

rocessing step	Findings	Source
Fermentation	Levels of (-)-epicatechin and (+)-catechin was affected by pre-treatment methods	[49]
	Pod storage treatments until 15 days have ificant effect in the reduction of polyphenols content as compared to the spreading and pressing methods	
	Effect of pod storage on the content of (+)-catechin was not significant	
	Fifteen days pulp preconditioning is the optimum condition	
	Loss of total phenols are due to diffusion out of ledons at 24% after 60 hours of fermentation, reaching 58% after 8 days	[11]
	Microscopic analysis showed reduction in asmic content of phenolic compounds during fermentation	[50]
	Polyphenols diffused throughout the whole cotyledon up to 48 hours	
Drying	Polyphenol oxidase was completely inactivated hin 24 hours at 65°C and that at 55°C more than 80% loss in activity was observed in 24 hours and over 95% in 48 hours	[17]
	The enzyme showed pH optimum at 6 at 35.5°C	
	After drying the level of phenolic compounds decreased by 32% compared to the fermented sample	[50]
	The concentration of polyphenols decline rapidly ring drying under air conditions of 40-60°C and 50-80% RH	[51]
	The higher the temperature and relative humidity, ver is the residual amount of polyphenols in the cocoa beans	
	Freeze drying retained the highest total polyphenol content as compared to hot air and sun drying	[39]
	The sun dried samples showed the lowest total enol content due to the lower temperature profile and longer drying process	

The enzymatic oxidation reactions that have begun during fermentation continues during drying provided sufficient moisture still exist. The evaporation of the moisture ensures continuous supply of gaseous oxygen to the inner vicinity of the cotyledon. Polyphenols in cocoa beans undergo oxidation to condense high molecular insoluble tannins. The polymeric brown pigments formed (melanin) at the end of the reaction give the typical brown colour of chocolate. Quesnel and Jugmohunsingh [17] investigated the activity of polyphenoloxidase and found that the enzyme showed pH optimum at 6 at 35.5°C. Kyi et al. [51] found that the reaction kinetics of polyphenol oxidation and condensation reactions fit a pseudo first order reaction. Reduction in polyphenols content was observed by De Brito et al.[50], Kyi et al. [51]

and Hii et al. [39] in their drying studies. Effects of drying methods showed that freeze drying was able to retain the most polyphenols in cocoa beans followed by hot air and sun drying [39]

Cocoa with too high polyphenols content is undesirable as this will impart high bitterness and astringency to the finished chocolate and masked the characteristic chocolate flavour. However, there still exist niche markets where high polyphenol content chocolate products are sought after by health conscious consumer.

Secondary processing

Semi-finished products (cocoa liquor, cocoa cake, cocoa butter and cocoa powder) are manufactured during secondary processing. The effects of various unit operations on cocoa polyphenols are not fully understood especially for operation that involves heat such as infrared micronizing, alkalization, roasting, grinding, butter pressing and pulverizing. Studies were reported on the effect of alkalization and roasting on polyphenols in cocoa beans as indicated in Table 5. Studies by Miller et al. [52] showed that alteration in pH due to the addition of alkali affects the total polyphenol content of the cocoa powder produced. It was found that natural cocoa powders (pH range 5.39-5.76) showed the highest levels of antioxidant capacity and total polyphenols as compared to the heavily processed (alkalized) powders (pH range 7.69-8.06). The data showed linear relationship with pH for both antioxidant capacity (based on ORAC) and total polyphenols. Destruction of total polyphenols was also reported by Andres-Lacueva et al. [36] where losses in total flavanoid content were observed. The author attributed this to the oxidation of phenolic compounds under basic pH condition, leading to brown pigments that are polymerized to different degrees. It was suggested secondary reactions involving o-quinones previously formed during fermentation are probably involved in further reactions during alkalization.

In general, roasting studies showed that reduction in polyphenols was observed especially at the high processing temperatures used. The reduction could probably due to the high redox-activity of polyphenols under such high oxygen environment. However, no exact details on the reaction mechanism were explained by the authors. It was concluded by Kealey et al. [53] that temperature is an important factor in the retention of cocoa polyphenols especially the higher oligomers.

Table 5. Effects of alkalization and roasting on cocoa polyphenols.

Processing step	Findings	Source
Alkalization	- Total polyphenols and antioxidant capacity decreased with increasing pH values of cocoa powder - Natural cocoa powders showed the highest total flavanols (22.86-40.25 mg/g) compared to lightly alkali (8.76-24.65 mg/g) and heavily alkali (1.33-6.05 mg/g) processed powders	[52]
	- Resulted in 60% loss in total flavonoid content - Among flavanols, (-)-epicatechin showed the largest decline (67%) than (+)-catechin (38%) - In terms of flavonols, quercetin showed the highest loss (86%) as compared to quercetin-3-glucuronide (58%), quercetin-3-arabinoside (62%) and isoquercitrin (61%)	[36]
Roasting	- Significant reduction ($p<0.05$) from 157 mg tannic acid/g (before roasting) to 131 mg tannic acid/g (after roasting)	[50]
	- High processing temperatures and/or longer processing time reduce the amount of cocoa polyphenols - As roasting temperature increased from 127 to 181 °C the level of polyphenols decreased from 24618 to 12786 μg/g and that of procyanidin pentamer from 1953 to 425 μg/g	[53]

Antioxidant Capacity of Cocoa and Chocolate Products

Upon knowing the polyphenols content of cocoa beans and various cocoa and chocolate products, researchers took a further step to investigate the antioxidant capacity of these products. The determination of antioxidant capacity is commonly carried out using Ferric reducing/antioxidant power assay (FRAP), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation assay (ABTS), 2,2,-diphenyl-1-picrylhydrazyl radical cation assay (DPHH) and Oxygen radical absorbance capacity assay (ORAC). Huang et al. [54] has compiled and review methods associated with the determination of antioxidant capacity according to assays involving hydrogen transfer reactions and assays involving electron transfer reaction.

Table 6 shows some selected studies of antioxidant capacity determined for cocoa and chocolate products including the raw material cocoa beans. Othman et al. [55] reported that geographical origin showed various level of antioxidant capacity depending on the type of extracting solvent used. The highest antioxidant and scavenging activity was observed for Ghanaian cocoa beans followed by Ivory Coast, Malaysian and Sulawesi cocoa beans.

In terms of finished products derived from cocoa, it can be seen that the antioxidant capacity of dark chocolate is higher than milk chocolate due to the presence of higher nonfat cocoa solid (NFCS). Flavanol compounds tend to be hydrophilic, therefore, they are found mostly in the nonfat fraction of cocoa and chocolate [43].Polyphenols are also able to associate with proteins and that the formation of milk protein-polyphenol complexes may explain why the antioxidant capacity of cocoa products is reduced by the addition of milk and milk polyphenols [56].

Among the various products examined, cocoa powder was found having the highest antioxidant capacity as compared to chocolates and other products based on the ORAC assay. Studies by Miller et al. [43] reported that cocoa powders showed the highest ORAC levels followed by baking chocolates, dark chocolates, semi-sweet chocolate chips, milk chocolates and chocolate syrups. Cocoa powder contains very high NFCS at 86-88% and 12-14% cocoa butter [57] as compared to baking chocolates (47-49%), dark chocolates (20-29.5%) and milk chocolates (4.9-7.2%). Miller et al. [43] observed a high degree of correlation between NFCS and ORAC (R^2 = 0.9849). High ORAC values were recorded higher for hydrophilic antioxidant capacity as compared to to lipophilic antioxidant capacity in cocoa powders [41].

Past studies by researchers have indicated that loss of flavanols and antioxidant activity can vary with the degree of dutching process or also know as the alkalization process [36, 52]. Gu et al. [41] attributed this loss due to the degradation of procyanidins as a result of alkali treatment and that flavanols are unstable under such basic condition. Therefore, the manufacturing recipes used to produce cocoa powders of various colours (from red to brown/black) could adversely affect the flavanol content of the semi-finished products. Inverse relationships were observed between ORAC, total polyphenols, total flavanols, flavanol dimmers, flavanol monomers, flavanol trimers and pH, respectively, during the dutching (alkalization) process [52].

Table 6. Antioxidant capacity of various cocoa and chocolate products.

Product	β-Carotene-linoleate bleaching	Scavenging activity on DPHH radicals	Ferric reducing activity based on FRAP assay	ABTS radical cation assay	Oxygen radical absorbance capacity (ORAC)	Source
Cocoa Beans Extracted by Ethanol:	(%)	(EC_{50} DPPH mg/ml)				[55]
Malaysia	67.6	1.3				
Ghana	74.1	1.3				
Ivory Coast	71.02	1.5				
Sulawesi	26.1	ND				
Extracted by water:						
Malaysia	72.7	2.4				
Ghana	74.9	1.7				
Ivory Coast	83.69	2.9				
Sulawesi	51.4	ND				
Extracted polyphenols:		(EC_{50} DPPH mg/ml)	(µmol TE/g)	(µmol TE/g)		[56]
Dark chocolate		7.04	149.87	78.80		
Milk chocolate		17.31	61.50	42.72		
Cocoa powder 1		13.23	71.83	42.37		
Cocoa powder 2		7.11	84.46	54.74		
Cocoa paste		1.68	606.14	290.29		

Table 6. Antioxidant capacity of various cocoa and chocolate products (continued).

Product	β-Carotene-linoleate bleaching	Scavenging activity on DPHH radicals	Ferric reducing activity based on FRAP assay	ABTS radical cation assay	Oxygen radical absorbance capacity (ORAC)	Source
Condensed tannins:			(μmol TE/g)	(μmol TE/g)		[56]
Dark chocolate			144.05	60.02		
Milk chocolate			84.31	27.12		
Cocoa powder 1			51.83	22.26		
Cocoa powder 2			60.15	25.88		
Cocoa paste			695.64	246.14		
					(μmol of TE/g)	[41]
Milk chocolate					68-86	
Dark chocolate					161-349	
Baking chips					148-242	
Unsweetened chocolate					450-523	
Natural powder					709-899	
Dutched (alkalized) powder					397-406	
					(μmol of TE/g)	[43]
Chocolate syrup					57.5-66.7	
Milk chocolate					41.7-72.3	
Semisweet chocolate chips					174.0-190.3	
Dark chocolate					151.7-246.0	
Baking chocolate					384.0-499.0	
Cocoa powder					720.0-875.0	

Contributions to Human Health

Wollgast and Anklam [5] has reviewed and compiled some reports on the health beneficial effects of polyphenols such as anti-carcinogenic, anti-atherogenic, anti-ulcer, anti-trombotic, anti-inflammatory, anti-allergenic, immune modulating, anti microbial, vasodilatory and analgesic effects. Some recent reviews on health benefit aspects of cocoa polyphenols have also been reported such as on blood pressure [58], anti-inglammatory impact of flavanols [25] and link between antioxidant properties and health [26].

Phenolic antioxidants are terminators of free radicals and chelators of metal ions that are capable of catalyzing lipid peroxidation [45]. The oxidation of lipids is interfered by rapid donation of a hydrogen atom to the radicals.

$$\mathrm{ROO\bullet + PPH \rightarrow ROOH + PP\bullet}$$
$$\mathrm{RO\bullet + PPH \rightarrow ROH + PP\bullet}$$

The stable phenoxyl radical intermediates also act as terminators of the propagation route by reacting with other free radicals.

$$\mathrm{ROO\bullet + PP\bullet \rightarrow ROOPP}$$
$$\mathrm{RO\bullet + PP\bullet \rightarrow ROPP}$$

Studies on the antioxidant activity of polyphenols have been carried out by using various *in vitro* and *in vivo* models. Buijsse et al. [59] reported the Zutphen elderly study which involved study population of 470 men and subject to various cocoa-containing food items. It was found that cocoa intake was inversely associated with systolic and diastolic blood pressure. The mean systolic and diastolic blood pressure in the highest tertile of cocoa intake (>2.30 g/d) was 3.7 mmHg and 2.1 mmHg lower than the lowest tertile of cocoa intake (<0.36 g/d). Cocoa intake was also found inversely related to cardiovascular mortality. The reduction in blood pressure (systolic and diastolic) and cholesterol (LDL and HDL) were reported by Fraga et al. [60] among young soccer players that consumed flavanol-containing milk chocolate (FCMC). Figure 5 illustrated the findings which show the changes in various physiological variables after 14 days of FCMC consumption. Other studies indicating lower blood pressure compared with the cocoa-free control was reported by Taubert et al. [58] suggesting that consumption of food rich in cocoa may reduce blood pressure and interestingly, it was found that tea intake appears to have no effect.

However, is there an exact contribution between cocoa polyphenols and human health? One reason is due to the bioavailability of the ingested polyphenols and that little information is available on the absorption, distribution, metabolism and excretion of polyphenols in human body. It was reported that non-extractable polyphenols (NEPP) shows non-availability as compared to extractable polyphenols (EPP). NEPP are high-molecular-weight phenols bound to dietary fibre or protein that remain insoluble in the usual solvents while EPP are low and intermediate-molecular-mass phenolics including some hydrolysable tannins and proanthocyanidins [45].

Compilation of studies by Scalbert and Williamson [61] on dietary polyphenols bioavailability generally showed that the rate and extent of intestinal absorption and the nature of the metabolites circulating in the plasma depends on the chemical structure of the polyphenols. The quantities of polyphenols found intact in urine was low for quercetin and rutin, but high for catechins, isoflavones, flavanones and anthocyanidins. It was suggested that repeated ingestion of polyphenols over time is required in order to maintain a high concentration in plasma.

Mhd Jalil and Ismail [26] summarized that most studies underline the effects of polyphenols but the question of whether the presence of methylxanthines enhances or reduces the health benefits of cocoa flavanoids remains unanswered satisfactory. The reason is that methylxanthines such as caffeine could exert pro-oxidant properties while caffeine, theobromine and theophylline could exert antioxidant activity and protective ability [62].

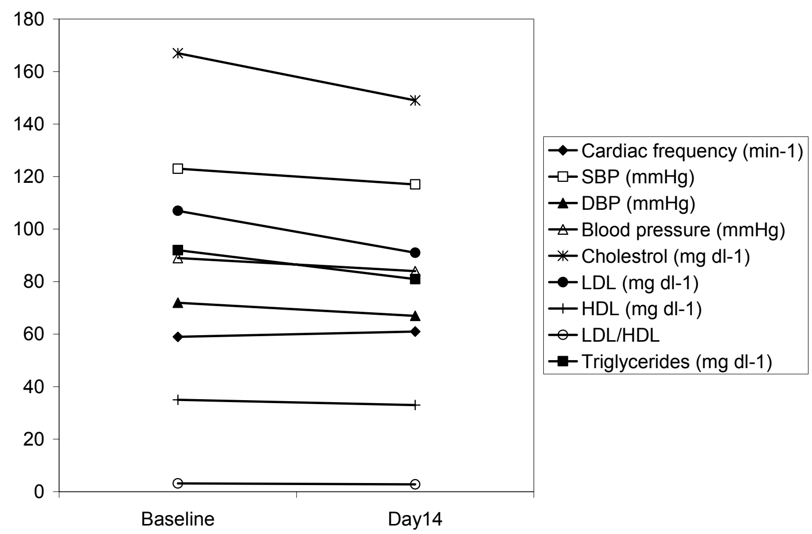


Figure 5. Changes in physiological variables after 14 days of FCMC consumption.

Conclusion

Studies have shown that the content of polyphenols and its associated antioxidant capacity vary among the wide range of cocoa and chocolate products available in the market. Processing can have a great impact on the level of polyphenols retained in the cocoa beans and its derived products. Favourable results have been reported on the contribution of cocoa polyphenols to human health. However, full understanding is still required in the bioavailability aspects of cocoa polyophenols with regards to the exact absorption, distribution, metabolism and excretion of polyphenols in human body. Future research should also investigate the effect of other active compounds presence in the products and its associative effect with cocoa polyphenols.

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