



Review

Theobroma cacao L., the Food of the Gods: A scientific approach beyond myths and claims

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ABSTRACT

Cocoa beans are rich source of polyphenols, contributing about 10% of the dry weight of the whole bean and its derivative chocolate, particularly dark chocolate, is considered one of the major contributors of antioxidants to the American diet after fruits and vegetables. At present the wide variation in cocoa processing and in the content and profile of polyphenols make it difficult to determine to what extent the findings about positive effects expressed in different studies, translate into tangible clinical benefits. Moreover, before claiming any healthy properties to a plant, natural product or food item on human subject, a basic research project approved by scientific and ethical commissions has to be performed. Until now the definition, composition, manufacturing specifications, packaging and labelling of cocoa and chocolate products in Europe, are regulated by "Directive 2000/36/EC of the European parliament and of the council". The definitions take changes in consumer tastes, chocolate composition and labelling into account, but do not consider the real potential of healthy, beneficial and nutraceutical effects. In fact, they fail to establish an official analytical methodology for the quantification of phenolic compounds in cocoa and chocolate. Moreover quantification of these compounds is not used in product classification.

This article reviews many qualitative differences of cocoa and chocolate, in particular dark chocolate, aiming to establish the different implications for public health through the use of the analyzed concentration of polyphenols in cocoa products.

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1. Introduction

Chromatographic analyses of residues extracted from pottery vessels show that cacao beverages were being made there before 1000 B.C., extending the confirmed use of cacao in Mesoamerica back at least 500 years. Cocoa came to Europe in the 16th century

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Table 1
Polyphenol content of different dark chocolates. Data reelaborated from Refs. [79] and [80].

Polyphenols (g/100 g)	Brand	Analytical method	Ref.
0.34–2.34 Mean 1.09	Dark chocolate n = 46	Colorimetric assay Std: epicatechin	[80]
1.10	Dark chocolate	Hplc	[81]
0.48	Dark chocolate	Not stated	[82]
1.23 ± 0.01 1.17 ± 0.03 1.48 ± 0.04	Dark chocolate n = 3	Colorimetric assay Std: gallic acid	[83]
2.38	Dark chocolate	Colorimetric assay Std Catechin	[84]
2.62	Dark chocolate	Hplc-Ms	[85]
0.36	Dark chocolate	Hplc	[86]
0.51	Dark chocolate	Hplc	[87]
1.59 1.52 2.13 1.23 2.11 1.71	Dark chocolate ^a n = 6	Prussian Blue Std: Epi	[88]
2.23	Dark chocolate	Colorimetric assay Std: gallic acid	[2]
0.84	Dark chocolate	Colorimetric assay	[89]
2.42 2.41 2.03 1.87 2.31	Dark chocolate N = 5	Colorimetric assay Std: gallic acid	Unpublished data

^a Each value represents the mean of duplicate measurements.

and in 1737, Linnaeus named the cocoa tree *Theobroma* (Food of God) [1,8,96].

In 1590, the Florentine Codex suggested a remedy made out of cocoa beans, maize and the herb *tlacoxochitl* (*Calliandra anomala*) to alleviate fever, shortness of breath and heart conditions and manuscripts produced in Europe and New Spain from the 16th to early 20th century revealed more than 100 medicinal uses for cocoa and/or chocolate [8].

Theobroma cacao L. is a small but economically important tree. It is an evergreen, 4–8 m tall, of the *Sterculiaceae* family, native to the tropical region of the Americas.

Each seed contains a significant amount of fat (40–50% as cocoa butter) and polyphenols which make up about 10% of the whole bean's dry weight (epicatechin: concentrations among freshly harvested beans of verified genetic origin ranged from 21.89 to 43.27 mg/g of dry defatted samples) [2,3]. When beans undergo the process of fermentation and drying, which are critical steps in cocoa processing, the walls of pigment cells break down and their contents are exposed to other constituents within the bean.

Fermentation of cocoa beans is crucial for the development of precursors for chocolate flavour. The colour of the bean changes from purple to brown in well-fermented beans. Moreover, polyphenols undergo a variety of reactions: epicatechin diffuses from its storage cells, undergoes oxidation and polymerization reactions to form complex tannins. The consequence in fermented cocoa beans is a decrease of epicatechin concentration to approximately 2–17 mg/g [2,4].

There are three main cultivar groups of cacao beans used to make cocoa and chocolate: Criollo, the cocoa tree used by the Mayas, is highly prized and rare, less bitter and more aromatic than other beans, from which only 5–10% of chocolate is made. Forastero trees, which include several sub-varieties, are significantly hardier than Criollo trees and produce cheaper cocoa beans, in fact they are used for 80% of world chocolate production. The Arriba

variety is considered the best one. Trinitario, a hybrid of Criollo and Forastero, is used in about 10–15% of chocolate production [1,5].

The cocoa beans used in the confectionery industry come from a wide range of geographical areas, and may have different chemical and organoleptic properties but manufacturers have to produce chocolates of constant flavour using raw material that is variable ([7]; Table 1). Kim and Keeney [4] for example, reported the differences in epicatechin content in defatted samples of cocoa beans from different sources and they report a minimum content of 2.66 mg/g in Jamaican beans and a maximum of 16.52 mg/g in Costa Rican beans.

The first human clinical study with chocolate was performed in 1996 by Kondo et al. [9] who found that 35 grams of delipidated cocoa decreased LDL oxidation between 2 and 4 h after ingestion. Since 1996 at least another 38 human studies involving the use of cocoa in different forms have been performed. A multitude of properties have been described. All of these may be summarized under three main headings: antioxidant, cardiovascular protector and antitumoral. At present the high level of variability in cocoa processing and polyphenols content and profile, make it difficult, to determine how far the findings about these positive effects translate into tangible clinical benefits ([10]; Tables 2 and 3). Moreover, it is obvious that before claiming any healthy properties for a plant or natural product, it is necessary to plan and perform appropriate scientific studies – basic, preclinical and clinical – *in vitro* and *in vivo* as well as to develop appropriate standardization methods as regards the active moieties, to afford accountability and reproducibility of the results.

The preclinical and clinical studies should be approved by ethics committee.

The authors of this review examine the qualitative differences of cocoa and dark chocolate. A related topic is discussed, regarding the effect of the polyphenols found in cocoa products on public health.

Table 2

Estimated quantity (mg) and percentage (%) of polyphenols assumed from different polyphenol containing dark chocolate: A = 3.4 mgECE/g and B = 23.4 mgGAE/g.

		CH	AU	IR	D	GB	B	A	USA	FR	NL	J	ES	P	BR
A	mg	94.5	85.3	82.2	76.2	73.8	55.4	54.7	48.9	47.9	44.5	16.4	15.9	11.2	9.5
	%	18	17	16	15	14	11	11	10	9	9	3	3	2	2
B	mg	650	585	566	524	508	382	377	337	330	306	112	109	74	65
	%	130	117	113	104	101	77	77	67	66	61	22	21	16	13

2. Cocoa and chocolate composition: the EU Directive

Cocoa and cocoa based products like chocolate are widely consumed in many countries and cultures (Table 3). For example in the Dutch population chocolate contributes up to 20% of the total flavonoids intake in adults, and in children the percentage is even higher [62]. Chocolate is considered the third highest contributor of antioxidants to American diet with 100–107 mg/day (fruits 255 mg/day, vegetables 233 mg/day) [84].

Indications on some functional cocoa constituents, with beneficial effects on human health have been published. Particularly in relationship with its high content of monomeric (epicatechin and catechin) and oligomeric (procyanidins) flavanols. Therefore, cocoa and chocolate may be considered functional foods but the functional significance of each component has not been equally clarified.

Cocoa contains approximately 380 known chemicals and 10 psychoactive compounds [11]: interactions between constituents must be considered in relation to, firstly, their efficacy and secondly, their safety.

The definition, composition, manufacturing specification, packaging and labelling of cocoa and cocoa derivative products are regulated by the “Directive 2000/36/EC of the European parliament and of the council”, effective since January 2007. To summarize, Directive 2000/36/EC takes changes in consumer taste, chocolate composition and labelling [12] – which is legally controlled in many countries – into account, but does not consider the real potential of healthy beneficial effects, for example, by establishing the minimum concentration of polyphenols in cocoa products.

In western and developed countries, but also in new emergent countries, even China and India, ever more people seem to have distastes from fruit and vegetables [106]. As a result the inhabitants of these countries fail to assume sufficient amounts of dietary polyphenols [13]. Although the consumption of fruits and vegetables is of more relevance, in this particular context a few pieces of a dark chocolate bar, containing cocoa polyphenols, with a standardized polyphenols content, could be more appreciated as a food supplement than a pill with flavonoids.

3. Cocoa polyphenols

Polyphenols constitute one of the most numerous and widely distributed groups of substances in the plant kingdom. More than 8000 phenolic structures are known [14] and dietary polyphenols represent the main source of antioxidants for humans use [15]. Based on the chemical structure polyphenols are classified into, at least, 10 different classes. Flavonoids, the most important single group, are divided into 13 classes, with more than 5000 compounds [16].

Cocoa beans are virtually inedible because of the high concentration of polyphenols and consequently they have an extremely

bitter flavour [2,3]. Polyphenols content might decrease from 100% to 10% in the final cocoa product (for example chocolate) throughout the different manufacturing processes (fermentation, roasting, dutching) [17,18]. According to Forsyth [19], losses of total phenol are due to diffusion out of the cotyledons and can be estimated as 24% after 60 h of fermentation, reaching 58% after 8 days.

In fact, polyphenols content and profile in food can be affected by several factors including environmental (sample origin, variety, degree of ripeness, climate, etc), food processing (heating, alkalisation) and food storage (refrigeration practice) [20–24].

Kim and Keeney [4] reported differences in epicatechin content on cocoa beans from shipments representing several countries of production ranging from 2.66 mg/g in Jamaican beans to 16.52 mg/g in Costa Rica's beans.

Fermentation is a critical step for flavour generation in cocoa and with a longer fermentation period is associated a higher reduction in flavanol content. Niemenak et al. [7] showed that the total content of polyphenolic compounds in freshly harvested beans, compared to similar beans subjected to fermentation, does not evolve regularly during fermentation: in some clones the total phenolic compound increased by about 25% after 2 days, in others there was a decrease of 14% and 25%, while in other cases, it did not change. The increase of polyphenols during the fermentation process, may reflect a formation of polymeric pro anthocyanidins.

The fermented cocoa beans were subsequently roasted and since flavanols are heat labile, significant losses of polyphenols can also occur during this crucial manufacturing process. The alkalising process further reduces flavanol concentration [94]. Moreover, possible interactions between many others chemical constituents present in cocoa and chocolate, such as milk proteins, may influence the bioactivity and bioavailability of flavanols in foods. At least, in the gastrointestinal tract, polyphenols from cocoa and chocolate are subject to absorption and their subsequent efficacy may vary for different reasons [59,60].

From a more general viewpoint, it could be assumed that the cocoa and chocolate products currently on the market show a variability in polyphenols content. As a result, it is very difficult to generalize any hypothetical health claims deriving from cocoa polyphenols.

Dark chocolate with high cocoa content (>35%) [12] is considered to have the richest polyphenols content within group of cocoa derivatives. However, each chocolate show a variability in polyphenol content and/or profile ([25]; Table 3).

Furthermore, to correlate beneficial effects, such as polyphenol-related antioxidant capacity in humans, it could be necessary to further examine the absorption, distribution and metabolites production of these substances [26].

Although the total polyphenols content may represent a useful indicator of potential human nutritional benefits, a specific and well-defined phenolic profile seems to be more important.

Table 3

Chocolate consumption in different countries.

	CH	AU	IR	D	GB	B	A	USA	FR	NL	J	ES	P	BR
kg/person/year	10.14	9.13	8.83	8.18	7.93	5.96	5.89	5.27	5.16	4.78	1.76	1.71	1.21	1.02
g/person/day	27.8	25.1	24.2	22.4	21.7	16.3	16.1	14.4	14.1	13.1	4.8	4.7	3.3	2.8

In fact, complex mixtures of plant polyphenols, of which some are chemically defined, have been shown to be absorbed in the gastrointestinal tract following different ways of metabolization. Little is known about their mutual interactions, interactions with hormones, neurotransmitters molecules related to the immune system, *in vivo* antioxidant activity and bioavailability. Therefore, to sum up, several metabolites identified in human plasma after consuming flavonoids need also to be tested for possible non-antioxidant activities. So far any claims on the biological and health protective effects of natural polyphenolic compounds in our diet are premature and unjustified [27].

3.1. Composition of cocoa polyphenols

Polyphenols in cocoa beans are stored in the pigment cells of the cotyledons. Depending on the amount of anthocyanins those pigment cells, also called polyphenol-storage cells, range in colour from white to deep purple.

Three groups of polyphenols can be distinguished:

- (1) catechins or flavan-3-ols (ca. 37%);
- (2) anthocyanins (ca. 4%); and
- (3) proanthocyanidins (ca. 58%).

The main catechin is (–)-epicatechin with up to 35% of polyphenol content [16].

Phenolic compounds make up 12–18% of the total weight of dried cocoa nibs (i.e., roasted cocoa beans) [14]. Approximately 35% of the total content of polyphenols in non-fermented cocoa nibs belonging to the Forastero variety is epicatechin. Epicatechin content in non-fermented cocoa nibs of different varieties range between 34.65 and 43.27 mg/g (defatted sample). This amount decreases during the process of fermentation and drying. Depending on the place of production, epicatechin content may change between 2.66 and 16.5 mg/g of defatted sample [28,29,4,16], while the amount of catechin increases [30].

Apart from flavan-3-ols, catechin, epicatechin and their dimers procyanidin B1 and procyanidin B2, which are major compounds in cocoa, the following polyphenols have been identified and quantified in cocoa beans or cocoa products: procyanidin B3 = catechin-(4 α → 8)-catechin, procyanidin B4 = catechin-(4 α → 8)-epicatechin, procyanidin B5 = epicatechin-(4 β → 6)-epicatechin, procyanidin C1 = epicatechin-(4 β → 8)-epicatechin-(4 β → 8)-epicatechin, procyanidin D = epicatechin-(4 β → 8)-epicatechin-(4 β → 8)-epicatechin-(4 β → 8)-epicatechin, higher oligo- and polymers, mostly homologues of epicatechin with 2–18 monomeric units, as well the following flavanols: quercetin, quercetin-3-O-glucoside (isoquercetin), quercetin-3-O-galactoside (hyperoside), quercetin-3-O-arabinoside. Moreover following flavones have been detected: apigenin, apigenin-8-C-glucoside (vitexin), apigenin-6-C-glucoside (isovitexin), luteolin and luteolin-7-O-glucoside, dihydroquercetin, dihydroxykaempferol, kaempferol-rutinoside, naringenin, naringenin-glucoside, myricetin-glucoside. Finally at least the following phenolic compounds are identified: caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, phenylacetic acid, phloretic acid, protocatechuic acid, syringic acid, vanillic acid, clovamide and dideoxyclovamide [16,31–33].

3.2. Bioavailability of cocoa polyphenols

In humans, the total intake of polyphenols is about 1 g/day [112] with some great differences. For example the mean daily intake of polyphenols in the Spanish diet was estimated between 2590 and 3016 mg/person/day [6]. Large uncertainties remain due to the lack of comprehensive data on the content of some of the main polyphenol classes in food.

The maximum concentration in plasma rarely exceeds 1 μ M after the consumption of 10–100 mg of a single phenolic compound [112]. The absorption and metabolism of polyphenol are determined by their chemical structure, which is related with the degree of glycosylation/acetylation/polymerization, their basic structure (i.e., benzene or flavone derivatives), conjugation with other phenolics, molecular size, and solubility [14]. Catechins and procyanidins can only be found as aglycones in plants and plant-derived food products and thus, molecular size and solubility might be the determining properties for absorption [93].

Cocoa polyphenols have been reported to present a low C_{max} in the plasma, a short half-life and a rapid excretion and therefore they have a relatively low bioavailability [34], while flavanols in cocoa products are present in monomeric form and, in a larger proportion, as oligomeric procyanidins [26].

Among all the different classes of flavonoids, procyanidins appear to be 10- to 100-fold less absorbed than their monomeric constituents [35].

Among studies devoted to polyphenols activity, one of them concluded that chocolate contain prevalently (–)-epicatechin and (–)-catechin, with only small amounts of (+)-epicatechin and (+)-catechin. Moreover, the form (+) was almost 10 times more absorbed than the form (–) [36–38]. However, epicatechin is much better absorbed than catechin [38,43] possibly due to stereochemical differences which results in different degrees of hydrophobicity [44].

On the contrary in cocoa beans the (+) form predominates [39].

3.2.1. Upon ingestion

Upon ingestion, flavanols first react with proline-rich proteins in the mouth to elicit an astringent response.

Clinical data have shown that flavanols and procyanidins are stable during gastric transit [51]. Spencer et al. [58] proposed that in the acidic environment of the gastric milieu, a significant proportion of flavanol oligomers are hydrolyzed into monomers and dimers, enhancing their absorption in the small intestine, but possibly modifying their biological activity.

In the mesenteric circulation, flavanols exist predominantly in a conjugated form and are absorbed from the jejunal lumen into the epithelial cell layer, where they are methylated and glucuronated [52,53].

Moreover these metabolites could be responsible, at least in part, for the effects observed on the central nervous system after cocoa and chocolate consumption. In fact, epicatechin metabolites (glucuronide and 3-O-methylglucuronide) can cross the blood–brain barrier and act at a cerebral level [54]

Rios et al. [51] demonstrated that high molecular weight flavanol oligomers seem to reach the small intestine intact and are available for absorption.

It was shown that absorption of epicatechin from chocolate was significantly less when consumed with milk or as milk chocolate. The hypothesis is that milk proteins bind to cocoa polyphenols, which in turn prevents their absorption in the gastrointestinal tract [59,60]. Studies after this have not found this reduction in epicatechin bioavailability when cocoa was consumed with milk [61], but also have not been able to definitively explain why the original paper found these results and the controversy remains open. In this context the possibility of matrix effect and interactions between two or more compounds present in cocoa or cocoa derivatives like chocolate, must be considered.

Manach et al. [34] hypothesizes that some individuals could have better absorption than others, possibly because of particular polymorphisms. This could be the factor that explains the high variability in the percentages of flavonoid absorption that have been published [62].

3.2.2. Intestine

Dimers and, probably, trimers are absorbed in the small intestine but less efficiently (under 0.5%) than epicatechin and catechin monomers, whose absorption level ranges between 22% and 55% [40–42].

Some glycosides are hydrolysed by enzymes such as lactase phloridzin hydrolase and cytosolic β -glucuronidase, in the small intestine [104].

The procyanidins that cross the intestinal barrier are conducted to the liver via the portal vein, where they further degrade into metabolites by methylation, glucorination and sulfation, which result in the potential antioxidants capacity of flavanols. These metabolites can possibly reach all tissues within hours following consumption as described in radiolabelled experiments with rats. Further modification in the colon gives rise to other bioactivities that are attributed to flavanols. In this same experiment the urine and feces analysis confirmed the presence of low molecular weight metabolites which indicates that polymeric procyanidins are absorbed through the intestinal barrier after degradation in low molecular weight metabolites, most probably by gut microflora [46–49].

Although it has been suggested that the metabolism of procyanidin polymers (specifically dimer B-3 and trimer C-2) by intestinal microflora is limited [42], human fecal microflora, grown under anaerobic conditions *in vitro*, have the ability to degrade procyanidins (mDP=6) to low molecular weight metabolites within 48 h.

In vitro studies showed that procyanidin oligomers (dimers and trimers) passed through the human epithelial Caco-2 cell monolayer, whereas polymers did not [3].

By investigating the metabolite excretion of healthy human subjects after ingestion of either chocolate or grape seed extract, Rios et al. [47] and Ward et al. [50], found an increase in different flavanol-derived acids in urine.

3.2.3. Kinetics, clearance, T_{max} , C_{max} , area under the curve (AUC), $t_{1/2}$ for elimination

The first human bioavailability trial on polyphenols from chocolate shows that with 40 g of dark chocolate (892 mg total polyphenols expressed as gallic acid equivalent and 82 mg epicatechin), epicatechin was indeed absorbed into the blood. Epicatechin was present in plasma as metabolites conjugated with glucuronide and sulphate groups. These compounds exhibited a T_{max} of 2 h in the plasma and C_{max} of 111 ng/ml. The compounds were still detectable after 8 h.

With 80 g dark chocolate (1783 mg total polyphenols expressed as gallic acid equivalent and 164 mg epicatechin), T_{max} shifted to 2.57 h and the C_{max} to 203 ng/ml.

Interestingly, the C_{max} and area under the curve (AUC) of the plasma epicatechin kinetics were proportional to the dose of chocolate ingested. The clearance of epicatechin from the plasma compartment was very fast ($t_{1/2}$ for elimination of 1.9 and 2.3 h for 40 and 80 g chocolate, respectively) [2].

In another study [45] performed with high-flavanol cocoa drink (hFCD; total flavanols: 917 mg), C_{max} range of about 2.75 nM while T_{max} were similar to those indicated after ingestion of 80 grams dark chocolate. It seems that matrix effects are very limited for the absorption of flavanol between cocoa drinks and dark chocolate and they demonstrated that pure epicatechin ingested by humans closely and quantitatively mimics the vascular effects of flavanol-rich cocoa and that chronic consumption of a high-flavanol diet is associated with a high urinary excretion of NO metabolites and consistent with an augmented NO production.

In the same study a significant correlation between flavanol metabolites and Flow Mediated Dilatation (FMD), a predictor for endothelial dysfunction, whose found with epicatechin ($r=0.486$; $P<0.01$) and other three metabolites (sum $r=0.432$; $P<0.01$) but no

significance was observed with catechin, suggesting that epicatechin is, at least in part, responsible for the improvement of vascular function.

Several studies have demonstrated that epicatechin is rapidly absorbed in humans, with plasma levels detected after 30 min of oral ingestion and reaching a peak after 2–3 h and returning to baseline values within 6–8 h after consumption of flavanol-rich chocolate. On a regular daily basis the overall effects may potentially accumulate [103]. Heiss et al. [55], demonstrated that the effects of cocoa polyphenols can be cumulative if taken in high doses (918 mg of flavanols/day for 1 week). An open question concerns polyphenol dose-response studies and it must also be considered that at the moment it is not possible to generalize and to suggest for example how much chocolate is needed for an antioxidant effect.

A *in vitro* study by Martin et al. [56], showed that pre-treatment for 2 or 20 h of HepG2 cultures with different doses (0.05–50 μ g/ml) of cocoa phenolic extract significantly reduced the t-BOOH-induced increase of LDH and prevented cell damage. This and other studies indicate the prevention potential of cocoa polyphenols also *in vivo*.

Other studies indicate that monomeric flavanols appear to be absorbed in a dose-dependent manner, whereby maximum plasma concentrations peak 2 h after ingestion and return to baseline within 24 h [2,57].

3.3. Potential adverse effect of cocoa polyphenols

Procyanidins have been considered antinutritional compounds because they can interact with proteins, starch, essential amino acids, carbohydrates and inhibit certain enzymes [65–67]. This binding depends on the degree of polymerization, the larger molecules tend to bind more efficiently [68]. However at the dose present in cocoa no adverse effect has been observed [62,109].

In addition, the level of flavonoids required to induce mutations and cytotoxicity may not be physiologically achievable through dietary sources; however the use of flavonoid supplements could result in exposure to potential toxic levels [69].

Reported flavanol-related health detrimental effects include activation of pro-carcinogens, pro-oxidant activity, haemorrhage formation, initiation of hepatotoxicity, genotoxic effects, interference with thyroid hormone biosynthesis alteration of pharmacokinetics of therapeutic drugs, increased estrogenic tumor formation, mutagenicity, modification of plasma biochemistry, instigation of gastroenteritis, antinutritive activity and weight loss [63,64,70,71].

Proanthocyanidin-rich products also possess the ability to cause cell toxicity to normal, healthy tissue [71] and although generally considered safe, ingestion of higher concentration of proanthocyanidins instigates destruction of mucosal lining of the digestive tract, gastroenteritis, and congestion of the intestinal wall in rats, hemorrhagic gastroenteritis in rabbits [72], and striking lesions in the digestive tract of sheep [73].

Structurally similar to endogenous steroid hormones, flavonoids may also promote estrogenic activity by initiating increased expression of aromatase (CYP19) which correlates with tumor initiation, promotion and progression [74,75].

Moreover it must be considered that mostly, the active compounds may not be the native polyphenols found in food, which are most often tested in *in vitro* studies; they are more likely to be metabolites [76].

At least flavonols give a bitter, astringent flavour to foodstuffs, frequently masked in chocolates and confections by aggressive processing and adulteration with other flavours. Extensive processing, dilution, and the addition of flavour modifiers may improve the palatability of chocolate, but could have negative nutritional and clinical benefits [77].

3.4. Potential adverse effects of other cocoa component

The concentration of theobromine in dry fat free cocoa is in the range of 2.4–3.2 g/100 g, while caffeine is between 0.3 and 1 g/100 g [108]. Moreover, theobromine has an LD₅₀ in rats of 837 mg/kg (oral), which is equivalent to 10,000 g of milk chocolate or 4500 g dark chocolate ingested by a 60 kg human [9].

Caffeine has an LD₅₀ in rats of 192 mg/kg (oral) [113] and fatal caffeine overdoses in adults are relatively rare and require the ingestion of a large quantity of the drug, typically in excess of 5 g [114].

Caffeine and theobromine are known to cross placental and blood brain barrier thus potentially inducing neurophysiological and foetus development effects [92,111]. Moreover their presence in cocoa could limit its potential as a nourishing food [92].

On this basis, FDA has been advising women since 1980 to avoid caffeine or consume it only moderately during pregnancy [99] so like many health professional organisations [100] and at the moment there is insufficient evidence to confirm or refute the effectiveness of caffeine avoidance on birth weight or other pregnancy outcomes [115].

On the other hand Giannandrea [101] in his epidemiological study suggest that exposure to cocoa during prenatal life or childhood may be associated to the risk of both hypospadias and testicular cancer in the offspring.

For these reasons, well-designed future research are needed to investigate and define the role of individual exposure to cocoa, particularly during prenatal and in the early life of human, and the real role of caffeine in foetal mortality. Besides, epidemiological studies could be in any case controversial [102].

Furthermore for the healthy adult population, moderate daily caffeine intake (6 mg/kg body weight/day in a 65 kg person) is not associated with adverse effects such as general toxicity, cardiovascular effects, effects on bone status and calcium balance (with consumption of adequate calcium), changes in adult behaviour, increased incidence of cancer and effects on male fertility. On the contrary, reproductive-aged women and children are potentially risk subgroups who may require specific advice on moderating their caffeine intake (reproductive-aged women: 4.6 mg/kg bw/day for a 65 kg person) while children should consume ≤2.5 mg kg(–1) bw/day [107].

4. Discussion

Interest in the biological activities of cocoa polyphenols is increasing steadily. In fact the high polyphenol content of cocoa, coupled with its widespread presence in many food items, render this food of particular interest from the nutritional and pharmacological viewpoints [90].

In 1996 an editorial of the authoritative scientific review *The Lancet* entitled “Secrets about drugs are not healthy” [95]. Today it must be considered that also some health claims attributed to a food cannot be healthy when the only reason why these claims are made is for marketing purposes. We can say health claims are not even healthy.

Nowadays, this could apply to chocolate.

In fact, the actual evidence for beneficial effects of polyphenols against various diseases has generated new expectations for improvements in health, with great interest from the food and nutritional supplement industry regarding promotion and development of polyphenols-rich products. But the health implications related to and derived from different chocolates within the same category, in this case dark chocolate, are theoretically different and depend on the polyphenols content and profile which is related to the bean origin and to the manufacturing processes and therefore it

is impossible to establish appropriate public health recommendations. Further, to date it is still impossible to evaluate the individual and social benefits that increases in polyphenols intake could have for the general population or for particular groups at specific disease risk. Furthermore, a significant increase in the consumption of polyphenols, as for many other phytochemicals, may not be without risk. Some hazards associated with the consumption of polyphenols are documented, but evaluation among humans is still very limited for many reasons. In vitro studies have demonstrated the lack of cytotoxicity of cocoa compounds and (–)-epicatechin on different cell lines [109].

The polyphenols content and profile is of more importance and it is essential that, in the future, all published trials give a full characterisation of the chocolate or cocoa used and the calculated dose. This characterisation should include a breakdown of the types of polyphenols, especially monomer content and the methodology behind the measurement, i.e., HPLC or colorimetry.

Polyphenols are extensively conjugated in the body, and non-conjugated metabolites most often account for a minor fraction of the circulating metabolites. Very little is currently known regarding the biological activities of these conjugated metabolites [78] and we are just now beginning to understand that the mechanism of action of polyphenols is not always an antioxidant one and most likely involves the metabolites rather than the original polyphenols [97,98].

Antioxidant capacities of foods measured by different methods represent different underlying mechanisms and result in different rankings of the same foods.

Antioxidant capacities measured by the same method in similar foods demonstrate variability in values perhaps accountable by factors such as application of analytical methods by different laboratories or to the cultivar, season etc. Moreover, it must be considered that other macronutrients and micronutrients, for example fructose, can directly or through their metabolites affect the total antioxidant capacity of plasma [98,105].

The bioavailability of procyanidins depends on their absorption and metabolism, which in turn are determined by their chemical structure, the vehicle of delivery, as well as the degree of their conjugation, glycosylation, sulfonation and acylation [14,57].

Cocoa is a rich source of flavonoids and several studies have demonstrated the positive effects of these compounds and cocoa on central nervous system following cell damage triggers. This effect is possibly linked with the antioxidant capacity of both cocoa extract and (–)-epicatechin. Interestingly the maximum reduction of ROS production was similar in both cocoa extract and epicatechin conditions [109] linking the neuroprotective effect with this compounds. At this moment no positive neuropsychological effects on healthy adults are suggested from other studies [110].

Despite the complex mixture of polyphenols in cacao, its antioxidant effect seem to be associated with the presence of (–)-epicatechin also in humans [45,109]. Other compounds need to be tested for its antioxidant properties. Of more importance is to consider the possible interactions between two or more compounds present in cocoa or cocoa derivatives, also at low concentration that would not cause an achievable effect if taken as single compound. This is particularly difficult with complex mixture like cocoa.

An epidemiological study conducted in 2008 [91] concluded that 6.7g dark chocolate diminish inflammation measured as CRP plasma concentration, but with the above consideration, to date it is not possible to correlate this hypothetical beneficial effect, with any compound like polyphenols present in the cocoa derivatives.

In our society where the consumers are even more informed, exacting and attentive over the quality of products, a greater trans-

parency for example by including on the label of cocoa derivatives information on polyphenols content, will augment the choice criteria of consumers and will done an augmented value for artisanal producers, limiting at the same time speculative health claims. But before the scientific community need to complete the hard work to provide a RDI of the principal polyphenols. This can give a notably advantage also in fruit and vegetables choice.

Author disclosures

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