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Review Article

Cocoa Polyphenols: Can We Consider Cocoa and Chocolate as Potential Functional Food?

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Chocolate has been consumed as confection, aphrodisiac, and folk medicine for many years before science proved its potential health benefiting effects. Main compounds of cocoa and chocolate which contribute to human health are polyphenols that act as antioxidants and have potential anti-inflammatory, cardioprotective, antihepatotoxic, antibacterial, antiviral, antiallergenic, and anticarcinogenic properties. This paper gives a short overview of scientific literature regarding cocoa polyphenols and influence of cocoa and chocolate on human health. Although research on health benefits of dark chocolate and cocoa is quite extensive nowadays and shows potentially beneficial effects of dark chocolate and cocoa, there are still lots of unknowns and some controversies. This is obviously an area that needs more research in order to determine factual influence of chocolate on health.

1. Introduction

Cocoa and chocolate are consumed by humans for thousands of years. To Mayan people, cocoa pods were symbols of fertility and life and food of gods. Aztecs believed that consumption of cocoa gave wisdom and power and used cocoa as currency. Aztecs and Mayas made dark, unsweetened drink based on cocoa, which was called *xocoatl*. They seasoned it with chili peppers and added corn meal, but sugar was unknown to them. In 1492 Columbus brought cocoa beans from America to Europe, but at that time they were not interesting to Europeans [1].

Hernan Cortez, in 1528, brought cocoa to Spain along with secret of making *Chocolatl*. In Spain, sugar, vanilla, nutmeg, cloves, allspice, and cinnamon were added to the original recipe and aphrodisiac shortly made breakthrough in Europe [1].

However, chocolate bars were not produced until the 18th century, when mechanical mills for squeezing cocoa butter from cocoa mass were produced, and milk chocolate was first produced in the 19th century by Daniel Peter and Henry Nestle. Rodolphe Lindt invented a process called conching, which enabled formation of smooth chocolate aroma and Milton Hershey was a pioneer of mass production of affordable chocolate bars.

For many years, chocolate was consumed purely for pleasure, but in the last 20 years researches have shown that dark chocolate and cocoa could have beneficial effect on human health due to high content of polyphenols.

Polyphenols are large and heterogeneous group of biologically active secondary metabolites in plants, where they act as cell wall support materials, colourful attractants for birds and insects, and defence mechanisms under different environmental stress conditions (wounding, infection, excessive light, or UV irradiation) [2]. Based on a number of phenolic rings and of the structural elements that link these rings, they are divided into four groups: phenolic acids, lignans (recognized as phytoestrogens; flaxseed and flaxseed oil are the main source), flavonoids (the most abundant polyphenols in human diets), and stilbenes (resveratrol is under investigation for its anticarcinogenic properties). Flavonoid group is subdivided into: anthocyanins, flavonols, flavanols (catechins in tea, red wine, and chocolate), flavanones (citrus fruit are the main source), flavones, and isoflavones (main source is soya) [3] (Figure 1).

2. Cocoa Polyphenols

Three main groups of polyphenols in unfermented cocoa bean are flavan-3-ols or catechins, anthocyanins, and proanthocyanidins, with average content of 120–180 g/kg [4, 5]. Main polyphenol compound in fresh cocoa bean is (−)-epicatechin (Figure 2), with average content of 21–43 mg/g of defatted sample, followed by (+)-catechin, and dimers and trimers of these compounds [6]. Complex alteration products of catechin and tannin give brown and purple colour to cocoa bean, and leucoanthocyanins are present as glycosides [6].

Research of Counet et al. (adopted from [7]) showed that genetic characterization influences polyphenol content in cocoa. Namely, they found that *Criollo* cultivars contained higher levels of procyanidins than *Forastero* and *Trinitario* beans. In addition, crop season and country of origin have impact on polyphenols in cocoa beans [7].

Cocoa bean processing highly affects polyphenol content. During fermentation, polyphenols diffuse with cell liquid from storage cells and are subjected to oxidation (both nonenzymatic and polyphenol-oxidase-catalyzed), polymerisation, and reactions with proteins [4, 8]. Anthocyanins are hydrolysed to anthocyanidins and sugar component, leucocyanidins are dimerised [5], and (−)-epicatechin and soluble polyphenol content are reduced to 10–20% [8]. Hurst et al. [9] studied levels of flavan-3-ol monomers during fermentation, drying, and roasting cacao bean. They reported that unripe and ripe cacao pods contain solely (−)-epicatechin and (+)-catechin. During fermentation, levels of both of these compounds were reduced, but (−)-catechin was formed due to heat-induced epimerization.

During drying, additional loss of polyphenol occurs, mainly due to nonenzymatic browning reactions [4, 5].

Roasting results in significant loss of polyphenols due to thermolabile flavanols [10] and oxidation of epicatechin and catechin to quinones which complex with amino acids and proteins and polymerize with other polyphenols [11]. According to research of Hurst et al. [9], in this processing step loss of (−)-epicatechin and (+)-catechin is partly attributed to heat-induced epimerization to (−)-catechin.

All these processes are needed to develop characteristic cocoa aroma. Polyphenols give astringent and bitter aroma to cocoa and contribute to reduced perception of “cocoa flavour” by sensory panel [5]. However, nowadays processes are conducted in such manner to preserve as much polyphenol as possible with maintaining satisfactory aroma.

Research of Crozier et al. [12] showed that cocoa powder had significantly higher content of polyphenols and higher antioxidant activity compared to pomegranate and blueberry powder. However, alkalization has been shown to destroy polyphenols and significantly reduce antioxidant activity of cocoa powder.

During process of chocolate making, composition and content of polyphenols are furtherly altered, mainly due to rather high temperatures and presence of oxygen [8]. Therefore, dark chocolate had similar antioxidant activity to pomegranate juice, despite higher content of polyphenols [12].

3. Bioavailability of Cocoa Polyphenols

Generally, bioavailability of polyphenols is affected by chemical structure of polyphenols, food matrix, factors related to food processing, and interactions with other constituents in diet, as well as with some host related factors (genetic aspects of individuals, gender and age, disorders and physiological condition, and microbiota metabolism and enzyme activity in the colon) [3, 13]. The most important food sources of polyphenols are vegetables and fruits, green and black tea, red wine, coffee, chocolate, olives, and some herbs and spices, as well as nuts and algae [14]. Besides, some polyphenols are specific to particular food and some are found in all plant products, so that, generally, food is considered to contain complex mixtures of polyphenols [13].

Isoflavones and phenolic acids have highest absorption, followed by catechins, flavanones, and quercetin glucosides, whereas proanthocyanidins, anthocyanidins, and galloylated tea catechins are poorly absorbed [15].

Once absorbed, polyphenols are conjugated to glucuronide, sulphate, and methyl groups in the gut mucosa and inner tissues, where epicatechin and epigallocatechin are mostly present as the glucuronide and sulfate conjugates. Absorption of epicatechin and catechin in the intestine averages between 22% and 55%, while dimers and trimers are poorly absorbed (less than 0.5%). Procyanidins cross intestinal barrier and are transported to liver, where they undergo methylation, glucuronidation, and sulfation which result in antioxidant capacity [15].

Polyphenols that reach colon are fermented by microflora to phenolic acids of low molecular weight [15].

Epicatechin from chocolate is rapidly absorbed by humans, with plasma levels detected after 30 min of oral digestion, peaking after 2-3 h and returning to baseline after 6–8 h. In addition, cumulative effect in high daily doses was recorded [10].

Generally, it can be stated that the smaller the polyphenol, the higher the concentration in blood and the higher the chance it will reach its target organ in the body [16]. Chirality might also influence bioavailability of polyphenols—(+)-form of catechin is almost 10 times more absorbed than (−)-form [16].

Presence of sugars and oils generally increases bioavailability of polyphenols, while proteins, on the other hand, decrease it [3]. Research of Neilson et al. [17] showed that milk proteins and sucrose modulate metabolism, plasma pharmacokinetics, and bioavailability of catechins from chocolate confections. They found that milk proteins reduce bioavailability of epicatechin in chocolate confectionary. Serafini et al. [18] reported inhibition of *in vivo* antioxidant activity of chocolate by addition of milk either during manufacturing process or during ingestion. However, this effect was not observed in chocolate beverages [17]. Study of interactions

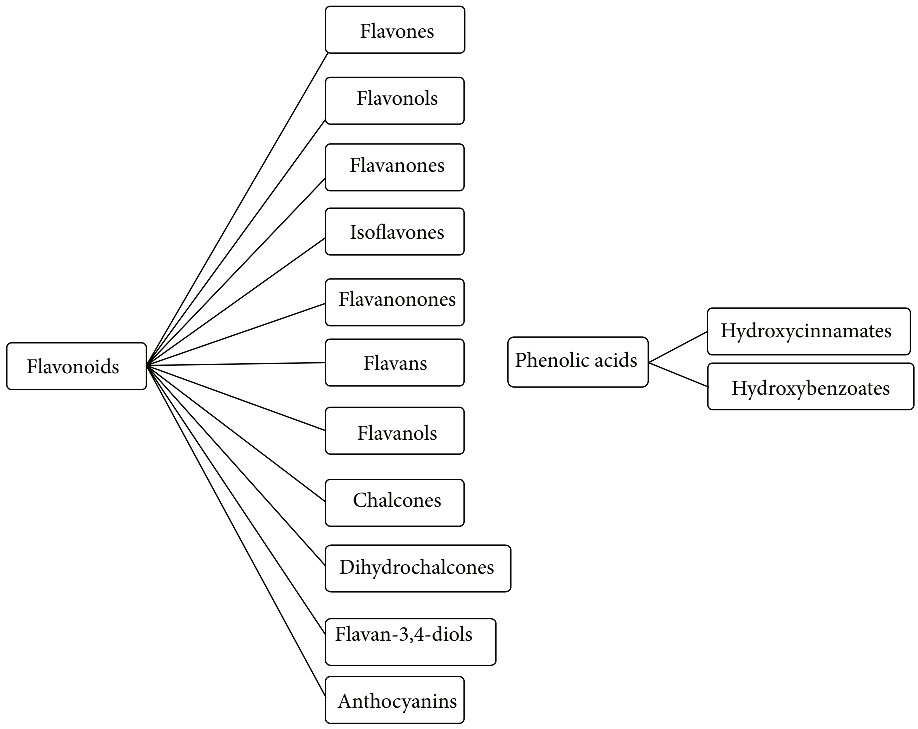

Figure 1: Basic classification of flavonoids and phenolic acids [59].

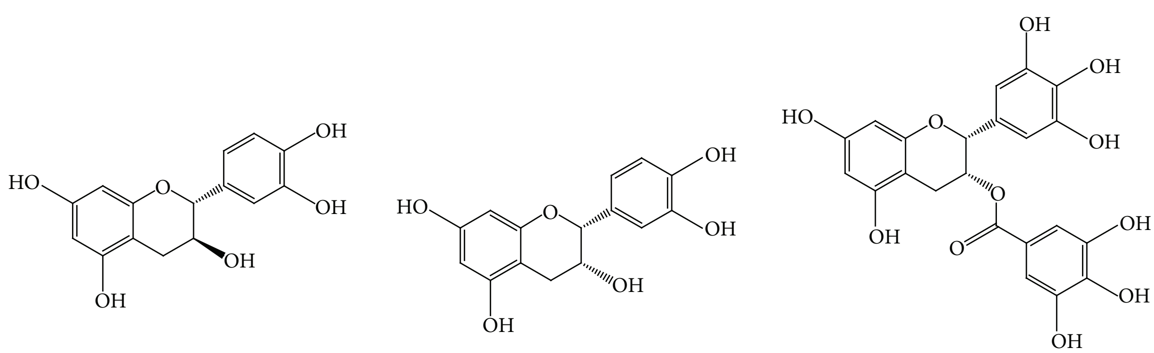

Figure 2: Structure of main cocoa polyphenols [60].

of cocoa polyphenols with milk proteins by proteomic techniques demonstrated that protein-polyphenol complex formation involves covalent binding of free SH-group of the free cysteine residue of protein. This was supported by the fact that alkylated form of peptide did not react with flavanols, while lactosylation did not prevent polyphenol binding. Since only small portion of protein interacts with polyphenol, bioavailability of polyphenols themselves is not significantly influenced [19]. This is supported by researches of Roura et al. [20] and Keogh et al. [21] who reported that milk does not affect bioavailability of cocoa powder flavonoids in healthy adults.

Sucrose increased bioavailability of polyphenols, but formulation also influenced the extent of sucrose impact. Schramm et al. [22] observed enhanced uptake of aglycone flavanols when they were consumed immediately after carbohydrate-rich meal. Peters et al. [23] concluded that sucrose addition to green tea resulted in delay of catechin absorption, partly due to viscosity increase, but it also improved catechin uptake by intestine.

4. Influence of Cocoa Polyphenols on Health

Unlike vitamins, polyphenols are not essential components of human diet. Nevertheless, they are consumed on daily basis due to their ubiquitous presence in fruits and vegetables. Many researches have shown that polyphenols and/or polyphenol-rich foods have an important role in health preservation due to antioxidant properties [15, 16, 24]. The antioxidant activity of cocoa and chocolate was shown to be correlated with their catechin and procyanidin contents [25].

Antioxidant properties of polyphenols highly depend on the arrangement of functional groups around the nuclear structure. Free radical scavenging capacity is primarily attributed to hydroxyl groups, and aglycones are more potent antioxidant than their responding glycosides [26].

Polyphenols can act as proton donor-scavenging radicals [27], inhibitors of enzymes that increase oxidative stress, chelate metals, bind carbohydrates, and proteins [26]. These properties enable them to act as anticarcinogenic, anti-inflammatory, antihepatotoxic, antibacterial, antiviral, and antiallergenic compounds [27–30].

This is supported by research of Hollenberg et al. [31], who established relationship between high consumption of cocoa beverages and very low blood pressure levels, reduced frequency of myocardial infarction, stroke, diabetes mellitus, and cancer in Kuna Indians residing in archipelago on the Caribbean Coast of Panama, unlike Kuna Indians residing on Mainland. Another study, conducted on elderly men free of chronic diseases in Zutphen, Netherlands, showed that consumption of cocoa reduced blood pressure and decreased risk of cardiovascular and all-cause death by 45–50% [32].

Grassi et al. observed decrease of blood pressure by short-term administration of dark chocolate in healthy [33] and glucose-intolerant, hypertensive subjects [34]. However, they investigated only 15 subjects per research and these findings should be taken with reserve. Djoussé et al. [35, 36] associated frequent consumption of dark chocolate with lower prevalence of cardiovascular diseases in men and women independently of traditional risk factors estimated based on health questionnaire. This association was perceived both in smokers and nonsmokers, as well as in subjects under and above 60 years of age. The research included large number of examinees, but data about consumption of chocolate were self-reported and there was no differentiation between dark and milk chocolate.

On the other hand, Ried et al. [37] did not find a blood pressure reducing effect of dark chocolate in 36 prehypertensive healthy adult volunteers with daily consumption of 50 g of dark chocolate.

Almoosawi et al. [38] investigated influence of 2-week consumption of polyphenol-rich dark chocolate on blood pressure of 14 obese and overweight individuals. Results of their research showed that both systolic and diastolic pressure decreased, along with reduction of fasting blood glucose levels and urinary free cortisone levels.

In addition to lowering blood pressure levels, cocoa polyphenols might be involved in cholesterol control. Waterhouse et al. (1996) reported polyphenols from chocolate inhibited LDL oxidation by 75%, compared to 37–65% of red wine (adopted from [16]). In addition, Vinson et al. [39] reported that dark chocolate had higher quality of phenol antioxidants expressed as IC_{50} for LDL + VLDL oxidation compared to red wine and black tea, with high lipoprotein bound antioxidant activity, which is very important in prevention of heart diseases. A survey implemented by a group of experts showed that in the case of similar absorption, about 50 g of dark chocolate should be eaten to provide equivalent flavonoids to about 200 mL of red wine, which has been shown to reduce heart attack risk for an average adult [40].

Mursu et al. [41] reported increase of HDL cholesterol after 3-week consumption of dark and polyphenol-rich dark chocolate. Total and LDL cholesterol were decreased after 15-day consumption of polyphenol-rich dark chocolate by 6.5% and 7.5%, respectively [33, 34]. Hamed et al. [42] reported 6% decrease of LDL cholesterol and 9% increase of HDL after 7-day consumption of regular dark chocolate.

Hot cocoa beverage was proven to successfully reduce LDL cholesterol, increase HDL cholesterol, and suppress LDL oxidation in research of Baba et al. [43]. Atherosclerotic cholesterol profile (cholesterol : HDL ratio) in patients with diabetes was improved after 8-week chocolate consumption without affecting weight, inflammatory markers, insulin control, or glycaemic control [44].

On the other hand, Kurlandsky and Stote [45] reported no significant difference in HDL and LDL cholesterol levels between "chocolate consuming" and control group. However, large age difference between control and "chocolate consuming" group may have influenced these results. Almoosawi et al. [38] also observed no significant change in total cholesterol level after consumption of dark chocolate.

Glucose blood levels could be reduced by consumption of dark chocolate; however, treatment duration and dark chocolate dose seem to significantly influence the effectiveness of treatment. Namely, as opposed to short-term treatments of Stote et al. [46, 47] and low-dose treatment of Taubert et al. [48], long-term high-dose treatment of Almoosawi et al. [38] proved to be effective. Researches of Grassi et al. showed that short-term administration of dark chocolate increases insulin sensitivity both in healthy [33] and in glucose-intolerant hypertensive people [34]. Davison et al. [49] reported significant insulinaemia and differential plasma glucose response after consumption of dark chocolate 2 h prior to exercise compared to fasting, but no significant effect on oxidative stress reduction.

Insulin response and blood pressure could be linked with the regulation of nitric oxide production by dark chocolate flavanols. Increased generation of nitric oxide (NO) and reactive oxygen species (ROS) in the vessel wall in response to dietary isoflavones enhances the activity of antioxidant defense enzymes in endothelial and smooth muscle cells (probably owing to estrogenic properties of isoflavones) by activation signaling pathways that increase NO bioavailability and regulate phase II and antioxidant enzyme expression via the redox sensitive transcription factor Nrf2 [50, 51]. Investigations carried out by Ried and coworkers showed that flavonol-rich chocolate may have a small but statistically significant effect on lowering blood pressure by 2-3 mm Hg in the short term [52, 53].

Flavanol-rich cocoa increases blood flow to key areas of brain increasing blood oxygenation level-dependent response to cognitive task switching paradigm in healthy young people [54] and could be useful in treatment of cerebrovascular flow (CBF) dementia [55], Alzheimer's disease [56], and stroke [57]. Chandranayagam et al. [58] reported that tannin-rich chocolate can be considered as functional food which effectively antagonizes adverse effects of arsenic intoxication. However, this research was conducted on Sprague Dawley rats and should yet be confirmed by research on humans.

5. Conclusion

Recent researches have shown that cocoa and dark chocolate could have beneficial impact on our health, mainly on cardiovascular system. However, part of the researches could be arguable, since either a small number of examinees were included or information about type of chocolate and consumption was scarce and/or ambiguous. In addition, since chocolate is a rich source of sugar and saturated fat, it is questionable whether chocolate consumption can be recommended in vascular health promotion because of its contribution to total calorie intake and impact on weight.

More systematic approaches should be applied in human studies to reduce possible misinterpretation of data—more examinees, longer test periods, and larger age differences should be involved in addition to controlled chocolate administration with specified polyphenol content and composition. Individual nutritive preferences which could have great impact on results should also be taken into consideration.

Chocolate and cocoa contain not only polyphenols but also methylxanthines which could additionally contribute to the health impact of these foods. There is need for additional researches that would elucidate the extent of polyphenols and methylxanthines health impact and possible synergy of these compounds in chocolate, with respect to energy contribution.

Obviously, elucidation of cacao and chocolate impact on human health is rather a complex problem and should be addressed as such data should not be lightly interpreted but closely examined and reassessed before withdrawing conclusions.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] S. T. Beckett, *Industrial Chocolate Manufacture and Use*, Blackwell Scientific Publications, London, UK, 3rd edition, 1999.

[2] S. Hakkinen, *Flavonols and Phenolic Acids in Berries and Berry Products [Ph.D. dissertation]*, Faculty of Medicine, Kuopio, Finland, 2000.

[3] F. A. Tomas-Barberan, "Types, food sources, consumption and bioavailability of dietary polyphenols nutrinsight," in *Proceedings of the Symposium 11th Nutrition Conference*, Kraft Foods, 2012.

[4] S. J. Misnawi, B. Jamilah, and S. Nazamid, "Effect of polyphenol concentration on pyrazine formation during cocoa liquor roasting," *Food Chemistry*, vol. 85, no. 1, pp. 73–80, 2004.

[5] S. J. Misnawi, B. Jamilah, and S. Nazamid, "Sensory properties of cocoa liquor as affected by polyphenol concentration and duration of roasting," *Food Quality and Preference*, vol. 15, no. 5, pp. 403–409, 2004.

[6] A. B. M. M. Jalil and A. Ismail, "Polyphenols in cocoa and cocoa products: is there a link between antioxidant properties and health?" *Molecules*, vol. 13, no. 9, pp. 2190–2219, 2008.

[7] R. Saltini, R. Akkerman, and S. Frosch, "Optimizing chocolate production through traceability: a review of influence of farming practices on cocoa bean quality," *Food Control*, vol. 29, pp. 167–187, 2013.

[8] J. Wollgast and E. Anklam, "Polyphenols in chocolate: is there a contribution to human health?" *Food Research International*, vol. 33, no. 6, pp. 449–459, 2000.

[9] W. J. Hurst, S. H. Krake, S. C. Bergmeier, M. J. Payne, K. B. Miller, and D. A. Stuart, "Impact of fermentation, drying, roasting and Dutch processing on flavan-3-ol stereochemistry in cacao beans and cocoa ingredients," *Chemistry Central Journal*, vol. 5, no. 1, article 53, 2011.

[10] M. Rusconi and A. Conti, "Theobroma cacao L., the Food of the Gods: a scientific approach beyond myths and claims," *Pharmacological Research*, vol. 61, no. 1, pp. 5–13, 2010.

[11] Y. Li, Y. Feng, S. Zhu, C. Luo, J. Ma, and F. Zhong, "The effect of alkalization on the bioactive and flavor related components in commercial cocoa powder," *Journal of Food Composition and Analysis*, vol. 25, no. 1, pp. 17–23, 2012.

[12] S. J. Crozier, A. G. Preston, J. W. Hurst et al., "Cacao seeds are a "Super Fruit": a comparative analysis of various fruit powders and products," *Chemistry Central Journal*, vol. 5, no. 1, article 5, 2011.

[13] M. D'Archivio, C. Filesi, R. Varì, B. Scazzocchio, and R. Masella, "Bioavailability of the polyphenols: status and controversies," *International Journal of Molecular Sciences*, vol. 11, no. 4, pp. 1321–1342, 2010.

[14] M. Quinones, M. Miguel, and A. Aleixandre, "Beneficial effects of polyphenols on cardiovascular disease," *Pharmacological Research*, vol. 68, pp. 125–131, 2013.

[15] X. Han, T. Shen, and H. Lou, "Dietary polyphenols and their biological significance," *International Journal of Molecular Sciences*, vol. 8, no. 9, pp. 950–988, 2007.

[16] K. A. Cooper, J. L. Donovan, A. I. Waterhouse, and G. Williamson, "Cocoa and health: a decade of research," *British Journal of Nutrition*, vol. 99, no. 1, pp. 1–11, 2008.

[17] A. P. Neilson, T. N. Sapper, E. M. Janle, R. Rudolph, N. V. Matusheski, and M. G. Ferruzzi, "Chocolate matrix factors modulate the pharmacokinetic behavior of cocoa flavan-3-ol phase II metabolites following oral consumption by Sprague-Dawley Rats," *Journal of Agricultural and Food Chemistry*, vol. 58, no. 11, pp. 6685–6691, 2010.

[18] M. Serafini, R. Bugianesi, G. Maiani, S. Valtuena, S. de Santis, and A. Crozier, "Plasma antioxidants from chocolate," *Nature*, vol. 424, no. 6952, p. 1013, 2003.

[19] M. Gallo, G. Vinci, G. Graziani, C. de Simone, and P. Ferranti, "The interaction of cocoa polyphenols with milk proteins studied by protomic techniques," *Food Research International*, vol. 54, pp. 406–415, 2013.

[20] E. Roura, C. Andrés-Lacueva, R. Estruch et al., "Milk does not affect the bioavailability of cocoa powder flavonoid in healthy human," *Annals of Nutrition and Metabolism*, vol. 51, no. 6, pp. 493–498, 2008.

[21] J. B. Keogh, J. McInerney, and P. M. Clifton, "The effect of milk protein on the bioavailability of cocoa polyphenols," *Journal of Food Science*, vol. 72, no. 3, pp. S230–S233, 2007.

[22] D. D. Schramm, M. Karim, H. R. Schrader et al., "Food effects on the absorption and pharmacokinetics of cocoa flavanols," *Life Sciences*, vol. 73, no. 7, pp. 857–869, 2003.

[23] C. M. Peters, R. J. Green, E. M. Janle, and M. G. Ferruzzi, "Formulation with ascorbic acid and sucrose modulates catechin bioavailability from green tea," *Food Research International*, vol. 43, no. 1, pp. 95–102, 2010.

[24] F. B. Awe, T. N. Fagmebi, B. Olawunmi, T. Ifesan, and A. A. Badejo, "Antioxidant properties of cold and hot water extract of cocoa, Hibiscus flower extract, ginger beverage blend," *Food Research International*, vol. 52, no. 2, pp. 490–495, 2013.

[25] Y. Wan, J. A. Vinson, T. D. Etherton, J. Proch, S. A. Lazarus, and P. M. Kris-Etherton, "Effects of cocoa powder and dark chocolate on LDL oxidative susceptibility and prostaglandin concentrations in humans," *American Journal of Clinical Nutrition*, vol. 74, no. 5, pp. 596–602, 2001.

[26] K. E. Heim, A. R. Tagliaferro, and D. J. Bobilya, "Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships," *Journal of Nutritional Biochemistry*, vol. 13, no. 10, pp. 572–584, 2002.

[27] C. A. Rice-Evans, N. J. Miller, and G. Paganga, "Antioxidant properties of phenolic compounds," *Trends in Plant Science*, vol. 2, no. 4, pp. 152–159, 1997.

[28] N. T. Zaveri, "Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications," *Life Sciences*, vol. 78, no. 18, pp. 2073–2080, 2006.

[29] I. C. W. Arts and P. C. H. Hollman, "Polyphenols and disease risk in epidemiologic studies," *The American Journal of Clinical Nutrition*, vol. 81, no. 1, pp. 317S–325S, 2005.

[30] J. A. Vita, "Polyphenols and cardiovascular disease: effects on endothelial and platelet function," *The American Journal of Clinical Nutrition*, vol. 81, no. 1, pp. 292S–297S, 2005.

[31] N. K. Hollenberg, N. D. L. Fisher, and M. L. McCullough, "Flavanols, the Kuna, cocoa consumption, and nitric oxide," *Journal of the American Society of Hypertension*, vol. 3, no. 2, pp. 105–112, 2009.

[32] B. Buijsse, E. J. M. Feskens, F. J. Kok, and D. Kromhout, "Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study," *Archives of Internal Medicine*, vol. 166, no. 4, pp. 411–417, 2006.

[33] D. Grassi, C. Lippi, S. Necozione, G. Desideri, and C. Ferri, "Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons," *American Journal of Clinical Nutrition*, vol. 81, no. 3, pp. 611–614, 2005.

[34] D. Grassi, G. Desideri, S. Necozione et al., "Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate," *The Journal of Nutrition*, vol. 138, no. 9, pp. 1671–1676, 2008.

[35] L. Djoussé, P. N. Hopkins, K. E. North, J. S. Pankow, D. K. Arnett, and R. C. Ellison, "Chocolate consumption is inversely associated with prevalent coronary heart disease: the National Heart, Lung, and Blood Institute Family Heart Study," *Clinical Nutrition*, vol. 30, no. 2, pp. 182–187, 2011.

[36] L. Djoussé, P. N. Hopkins, D. K. Arnett et al., "Chocolate consumption is inversely associated with calcified atherosclerotic plaque in the coronary arteries: the NHLBI Family Heart Study," *Clinical Nutrition*, vol. 30, no. 1, pp. 38–43, 2011.

[37] K. Ried, O. R. Frank, and N. P. Stocks, "Dark chocolate or tomato extract for prehypertension: a randomised controlled trial," *BMC Complementary and Alternative Medicine*, vol. 9, article 22, 2009.

[38] S. Almoosawi, L. Fyfe, C. Ho, and E. Al-Dujaili, "The effect of polyphenol-rich dark chocolate on fasting capillary whole blood glucose, total cholesterol, blood pressure and glucocorticoids in healthy overweight and obese subjects," *British Journal of Nutrition*, vol. 103, no. 6, pp. 842–850, 2010.

[39] J. A. Vinson, J. Proch, and L. Zubik, "Phenol antioxidant quantity and quality in foods: cocoa, dark chocolate, and milk chocolate," *Journal of Agricultural and Food Chemistry*, vol. 47, no. 12, pp. 4821–4824, 1999.

[40] F. A. Pimentel, J. A. Nitzke, C. B. Klipel, and E. V. D. Jong, "Chocolate and red wine—a comparison between flavonoids content," *Food Chemistry*, vol. 120, no. 1, pp. 109–112, 2010.

[41] J. Mursu, S. Voutilainen, T. Nurmi et al., "Dark chocolate consumption increases HDL cholesterol concentration and chocolate fatty acids may inhibit lipid peroxidation in healthy humans," *Free Radical Biology and Medicine*, vol. 37, no. 9, pp. 1351–1359, 2004.

[42] M. S. Hamed, S. Gambert, K. P. Bliden et al., "Dark chocolate effect on platelet activity, C-reactive protein and lipid profile: a pilot study," *Southern Medical Journal*, vol. 101, pp. 1203–1208, 2008.

[43] S. Baba, M. Natsume, A. Yasuda et al., "Plasma LDL and HDL cholesterol and oxidized LDL concentrations are altered in normo- and hypercholesterolemic humans after intake of different levels of cocoa powder," *The Journal of Nutrition*, vol. 137, no. 6, pp. 1436–1441, 2007.

[44] D. D. Mellor, T. Sathyapalan, E. S. Kilpatrick, S. Beckett, and S. L. Atkin, "High-cocoa polyphenol-rich chocolate improves HDL cholesterol in Type 2 diabetes patients," *Diabetic Medicine*, vol. 27, no. 11, pp. 1318–1321, 2010.

[45] S. B. Kurlandsky and K. S. Stote, "Cardioprotective effects of chocolate and almond consumption in healthy women," *Nutrition Research*, vol. 26, no. 10, pp. 509–516, 2006.

[46] K. S. Stote, B. A. Clevidence, and D. J. Baer, "Effect of cocoa and green tea consumption on glucoregulatory biomarkers in insulin resistant men and women," *The FASEB Journal*, vol. 21, p. 847.17, 2007.

[47] K. S. Stote, B. A. Clevidence, J. A. Novotny, T. Henderson, S. V. Radecki, and D. J. Baer, "Effect of cocoa and green tea on biomarkers of glucose regulation, oxidative stress, inflammation and hemostasis in obese adults at risk for insulin resistance," *European Journal of Clinical Nutrition*, vol. 66, pp. 1153–1159, 2012.

[48] D. Taubert, R. Roesen, C. Lehmann, N. Jung, and E. Schömig, "Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide: a randomized controlled trial," *The Journal of the American Medical Association*, vol. 298, no. 1, pp. 49–60, 2007.

[49] G. Davison, R. Callister, G. Williamson, K. A. Cooper, and M. Gleeson, "The effect of acute pre-exercise dark chocolate consumption on plasma antioxidant status, oxidative stress and immunoendocrine responses to prolonged exercise," *European Journal of Nutrition*, vol. 51, pp. 69–79, 2012.

[50] G. E. Mann, D. J. Rowlands, F. Y. L. Li, P. de Winter, and R. C. M. Siow, "Activation of endothelial nitric oxide synthase by dietary isoflavones: role of NO in Nrf2-mediated antioxidant gene expression," *Cardiovascular Research*, vol. 75, no. 2, pp. 261–274, 2007.

[51] R. C. M. Siow and G. E. Mann, "Dietary isoflavones and vascular protection: activation of cellular antioxidant defenses by SERMs or hormesis?" *Molecular Aspects of Medicine*, vol. 31, no. 6, pp. 468–477, 2010.

[52] K. Ried, T. Sullivan, P. Fakler, O. R. Frank, and N. P. Stocks, "Does chocolate reduce blood pressure? A meta-analysis," *BMC Medicine*, vol. 8, article 39, 2010.

[53] K. Ried, T. R. Sullivan, P. Fakler, O. R. Frank, and N. P. Stocks, "Effect of cocoa on blood pressure," *Cochrane Database of Systematic Reviews*, vol. 8, Article ID CD008893, 2012.

[54] S. T. Francis, K. Head, P. G. Morris, and I. A. Macdonald, "The effect of flavanol-rich cocoa on the fMRI response to a cognitive task in healthy young people," *Journal of Cardiovascular Pharmacology*, vol. 47, no. 2, pp. S215–S220, 2006.

[55] N. D. L. Fisher, F. A. Sorond, and N. K. Hollenberg, "Cocoa flavanols and brain perfusion," *Journal of Cardiovascular Pharmacology*, vol. 47, no. 2, pp. S210–S214, 2006.

[56] A. K. Patel, J. T. Rogers, and X. Huang, "Flavanols, mild cognitive impairment, and Alzheimer's dementia," *International Journal of Clinical and Experimental Medicine*, vol. 1, pp. 181–191, 2008.

[57] F. A. Sorond, L. A. Lipsitz, N. K. Hollenberg, and N. D. L. Fisher, "Cerebral blood flow response to flavanol-rich cocoa in healthy elderly humans," *Neuropsychiatric Disease and Treatment*, vol. 4, no. 2, pp. 433–440, 2008.

[58] C. Chandranayagam, G. Veeraraghavan, A. Subash, and H. R. Vasanthi, "Restoration of arsenite induced hepato-toxicity by crude tannin rich fraction of Theobroma cacao in Sprague Dawley rats," *Food Research International*, vol. 50, pp. 46–54, 2013.

[59] L. Jakobek, *Characterization of polyphenols in fruits and their influence on antioxidant activity of fruits [Ph.D. thesis]*, Faculty of Food Technology Osijek, Osijek, Croatia, 2007.

[60] S. T. Saito, A. Welzel, E. S. Suyenaga, and F. Bueno, "A method for fast determination of epigallocatechin gallate (EGCG), epicatechin (EC), catechin (C) and caffeine (CAF) in green tea using HPLC," *Ciencia e Tecnologia de Alimentos*, vol. 26, no. 2, pp. 394–400, 2006.