

# Dark polyphenols-rich chocolate and gut microbiota: a literature review

## Polifenóis do chocolate amargo e microbiota intestinal: uma revisão da literatura

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### Abstract

Dark chocolate has been widely used in research due to its beneficial effects in a variety of organ systems and antioxidant capacity in function of polyphenol content derived from cocoa (*Theobroma cacao*). There is strong evidence that life stress impacts directly on gastrointestinal function in animals and humans via modulation of key physiological parameters, such as intestinal permeability and secretion and release of biological mediators. Changes of gastrointestinal functional ecology are intimately linked to microbial populations and activities, and abnormal microbiota composition is often observed in the development of irritable bowel syndromes. Studies show that dark chocolate plays a role in various human diseases, which can be by modulation of intestinal microbial species as demonstrated in recent studies published. This study aimed to evaluate research on dark chocolate intake effects on intestinal microbiota through scientific literature, focusing on reciprocal interactions between the intestinal microbiota and polyphenols, mechanisms of action and the consequences of these interactions on human health.

**Keywords:** Chocolate. Cacao. Microbiota. Gastrointestinal Microbiome.

### Resumo

O chocolate amargo tem sido amplamente utilizado em pesquisas devido a seus efeitos benéficos numa variedade de sistemas de órgãos e capacidade antioxidante em função do teor de polifenóis derivados do cacau (*Theobroma cacao*). Há fortes

evidências de que o impacto do estresse atue diretamente sobre a função gastrointestinal em animais e humanos através da modulação dos principais parâmetros fisiológicos, tais como a permeabilidade intestinal e secreção e liberação de mediadores biológicos. Alterações da ecologia funcional gastrointestinal estão intimamente ligadas às populações e atividades microbianas, e a composição da microbiota anormal é frequentemente observada no desenvolvimento da síndrome do intestino irritável. Estudos mostram que o chocolate escuro desempenha um papel em várias doenças e distúrbios humanos, que pode ser por modulação das espécies microbianas intestinais, como demonstrado em estudos recentes publicados. O objetivo deste estudo foi investigar os efeitos da ingestão de chocolate amargo sobre a microbiota intestinal por meio da literatura científica, com foco em interações recíprocas entre a microbiota intestinal e polifenóis, mecanismos de ação e as consequências dessas interações sobre a saúde humana.

**Palavras-chave:** Chocolate. Cacau. Microbiota. Microbioma Gastrointestinal.

## Introduction

Chocolate is a food industry product formed by ingredients combination as cocoa liquor, cocoa butter, sugar, milk and emulsifier, and can be classified in “milk”, “semisweet” and “bitter or dark” according to ingredients proportion. In the dark chocolate (DC) classification, fat solids of cocoa beans in its processing is not considered, featuring the bitter and astringent taste. The bittersweet chocolate contains little sugar and may or may not have added milk. Due to its high cocoa concentration, dark chocolate has been highlighted as a food source of polyphenols.<sup>1</sup>

DC has been studied due to its beneficial effects in several organ systems. Its moderate consumption has been associated with improved lipid profile, insulin resistance, endothelial function, body composition and cognitive performance, as well as cancer prevention.<sup>2,3</sup> This capacity is due to the high content of polyphenols in cocoa beans (*Theobroma cacao*), the main ingredient of DC.<sup>1</sup>

Polyphenols constitute a group of compounds with biological properties that can be naturally found in plants. However, their concentration in food can be impaired in processing and, in the case of chocolate, can be influenced by cocoa beans source and processing, as well as conservation methods. In this case, total content can be reduced up to 90% when compared with the polyphenol content in seeds. In addition, most polyphenols are not absorbed in the gut (90%) and follow

through colon, where are metabolized by intestinal bacteria, producing short chain fatty acids (SCFA) that are able to modulate intestinal microbiota composition.<sup>4</sup>

Several studies have showed the effects of dark chocolate in the gut microbiota, leading to positive changes in its composition and, hence, resulting in an increase in beneficial bacteria population such as *Lactobacillus* and *Bifidobacterium* genus. These effects on gut microbiota modulation are reciprocal. During digestion and absorption, intestinal microbiota is responsible for polyphenols hydrolysis and absorption, also influencing bacterial composition.<sup>5</sup>

This study aimed to gather findings of the literature on dark chocolate ability to modulate gut microbiota.

## Methods

This is a narrative bibliographic review based on full scientific articles published on the internet about the dark chocolate impact on intestinal microbiota. The main question of the study was: “Can dark chocolate consumption positively modulate the gut microbiota?” The search was carried out through bibliographic databases, PubMed and “Periodicos CAPES” portal, from May 1, 2016 to August 1, 2016, using the following descriptors: dark chocolate, cocoa, microbiota and gastrointestinal microbiome. Articles published in Portuguese and English have been selected from the last 10 years. After each database search, duplicate references were deleted. Studies carried out in humans, animals and “*in vitro*” were considered for the article elaboration.

## Human microbiota: overview of microbial colonization

The range of bacteria present in intestinal microbiota is responsible for the nondigestible compounds fermentation, as fibers, synthesis of vitamin K, protection against food pathogen, epithelial maturation, generation of compounds with antioxidant activity, lipids with biologic activity (Lipopolysaccharides - LPS), short-chain fatty acids, gases (H<sub>2</sub>, CO<sub>2</sub>, CH<sub>4</sub> and H<sub>2</sub>S), and stool formation.<sup>6</sup>

However, to develop these functions accurately, it is important to keep a balance between bacterial population, to avoid dysbiosis. Among important risks factors that alter microbiota composition, dietary habits have a major effect. Diets rich in vegetable-derived polysaccharides, for example, have been associated with high prevalence of genus *Prevotella*. On the other hand, in subjects with high meat consumption, genus *Bacteroides* prevail.<sup>7</sup>

It is well established in the literature that dietary pattern influences colonic bacteria population profile, which are identified as low gene count (LGC) or high gene count (HGC). Individuals with

low gene count present *Bacteroides* genus as dominant, which is usually associated with obesity and metabolic disturbances. However, when treated with focus on weight loss, these individuals tend to have a greater genetic diversity with improvement in symptoms of metabolic syndrome.<sup>6</sup>

Besides dietary habits, comorbid conditions can lead to a bacteria population imbalance, requiring the adoption of special care, as increased fiber consumption, use of probiotics or symbiotics, as well as using SCFA. The latter serve as source of energy for intestinal cells, and promote cell integrity maintenance. Therefore, the balance among bacterial groups on gut microbiota promotes SCFA production necessary to keep intestinal health. It occurs through presence of main bacteria phylum involved in SCFA synthesis. Phylum *Bacteroidetes* and phylum *Firmicutes* constitute the main bacteria group, producing propionate and butyrate, respectively. Among the bacteria found in smaller proportions, but with high intestinal health impact, one can mention *Bifidobacterium*, *Lactobacillus* and *Escherichia coli*, belonging to *Actinobacteria*, *Firmicutes* and *Proteobacteria* phylum, respectively.<sup>6</sup>

*Bifidobacterium* and *Lactobacillus* genus bacteria are able to suppress proinflammatory cytokine IL-6 and IL-17 expression, and to promote major tight junction expression, reducing visceral hypersensitivity.<sup>8</sup> Thus, they are effective in reducing inflammatory bowel disease symptoms and improving individuals' well-being.<sup>9</sup> Recent studies showed probiotics, including *Bifidobacterium longum* and *Lactobacillus acidophilus*, with hypocholesterolemic effects in both rat and human. Mechanisms involved may be the following: (1) fermentation products of lactic acid bacteria inhibit cholesterol synthesis enzymes and reduce cholesterol production; (2) bacteria facilitate cholesterol elimination in feces; (3) and inhibit cholesterol absorption back into the body by binding with cholesterol; (4) and interfere with bile salt recycling (a metabolic product of cholesterol) and facilitate its elimination, raising bile salt demand made from cholesterol and resulting in body cholesterol consumption; and (5) lactic acid assimilation.<sup>6,9,10</sup>

Moreover, *bifidobacteria* acetate production could enhance intestinal epithelial cells mediated defense, protecting the host against *E. coli* O157:H7 infection.<sup>11</sup> *E. coli* strains are found in relatively lower numbers than other major commensal bacteria. However, it is the most common cause of intestinal and extra-intestinal disease. Pathogenic *E. coli* strains may carry several virulence factors directly involved in these bacteria pathogenesis, although commensal strains might also cause disease in immunocompromised hosts.<sup>12</sup>

Colonization is not restricted to gut, however. Some studies point out to *Streptococcus* colonization throughout the digestive tract, in esophagus distal portion and stomach. Esophagus microbial diversity, as genera *Prevotella*, *Actinomyces*, *Lactobacillus*, and *Staphylococcus*, is related to chronic inflammation and dysplasia. Bacterial population limitation is also present in the stomach due to low gastric pH acid. The pH favors *H. pylori* colonization, which can remain many years without symptoms, but it can lead to gastric cancer cases, gastritis and peptic ulcers. Nevertheless, the

stomach most commonly found bacteria are *Prevotella*, *Streptococcus*, *Veillonella* and *Rothia*. Intestinal colonization is also able to promote behavioral changes through the gut-brain axis. Intestinal dysbiosis condition with increased *Ruminococcus gnavus* and *Ruminococcus torques* genera is associated with autism in children.<sup>13</sup> So microbiota modulation would be able to increase or decrease the exploratory behavior of this group.

Another study with mice inoculated with *Salmonella* submitted to stress condition by food deprivation presented *Lactobacillus* genus reduction. In addition, stress itself led to *Verrucobacteria* and *Clostridium spp* imbalance. However, this situation could be reversed through the introduction of a probiotics diet.<sup>14</sup>

## Dark chocolate as functional food

Functional foods are classified as food or food ingredient consumed as part of the normal diet that contains bioactive compounds able to produce beneficial health effects.<sup>15</sup> In this scenario, a market for functional foods has been developed, and nowadays plays an important role in food industry, due to the increasing demand for healthier products with beneficial properties to the body. Recently, compounds in chocolate have been found to bring health benefits, and chocolate has been included in the group of functional foods. In addition, chocolate is frequently associated with pleasure and stress reduction, as well as other properties discussed throughout this review.<sup>16,17</sup>

Cocoa polyphenols represent about 5-6% of its dry weight, and are subdivided in 6 groups: flavanols, flavonols, anthocyanins, flavones, flavanones e isoflavones. Cocoa contains flavanols in monomer (epicatechin and catechin), oligomer, and polymer (proanthocyanidins) forms, as well as anthocyanins, flavonol glycosides, clovamide, dideoxyclovamide, phenylethylamine, N-oleoyl- and N-linoleoylethanolamine, and theobromin. However, catechin, epicatechin and their oligomers are the main active compounds from the flavonoids group, being (-) - epicatechin the most abundant, representing 35% of total phenolic compounds content.<sup>17</sup> The latter can be absorbed completely in gut, with peak about 2 hours after ingestion and may be detectable in blood for up to 8h.<sup>18,19</sup>

Instead of white chocolate, which has cocoa butter in its composition (containing at least 20% [g / 100 g] of total solids of cocoa butter), research has shown dark chocolate promoting systolic and diastolic blood pressure reduction, flow-mediated dilation increase, improved insulin sensitivity and cognitive performance, innate and adaptive immune system stimulation, as well as reduced cerebral oxidative stress.<sup>20</sup>

Concerning cardioprotective effects, a meta-analysis using randomized controlled trials associated dark chocolate or cocoa drinks intake with a significant reduction in total and LDL cholesterol in participants after intervention.<sup>21</sup> Another study performed with Swedish women, using regular chocolate consumption (1-2 per serving / week), reduced chances of developing

heart failure by 32%. These results can be explained by Fraga *et al.*, who related cocoa and its derivatives, such as chocolate, with blood pressure reduction capacity in animals and humans through nitric oxide (NO) production.<sup>22</sup>

Regarding antioxidant capacity, one study found cocoa powder with significantly higher antioxidant capacity compared to acai, blueberry powder, cranberry or pomegranate powder. Furthermore, flavonols content was significantly higher in cocoa powder, compared with other kinds of fruit.<sup>23</sup> These findings justified the use of DC as functional foods in clinical practice.

## Dark chocolate consumption and microbiota modulation

### In vitro studies

Besides stimulating beneficial bacteria growth, in vitro studies have found that cocoa powder is related to pathogenic species foodborne inhibition, as *Escherichia coli* O157: H7 (EHEC), *Salmonella typhimurium*, and *Listeria monocytogenes*; it also reduces these pathogens invasiveness to host cells.<sup>24</sup>

Phenolic compounds fermentation of DC 90% cocoa, as epicatechin, catechin, and procyanidin B-type polymers resulted in metabolites production of 5-(3',4'-dihydroxyphenyl)- $\gamma$ -valerolactone, (3,4-dihydroxyphenyl) acetic acid and protocatechuic acid. These are already detectable in the blood up to 24 hours after colonic fermentation, making these metabolites potential flavan-3-ol consumption markers in humans.<sup>25-27</sup> Moreover, the literature associates catechin fermentation with an increase in *Clostridium coccooides*, *Eubacterium rectale*, *Bifidobacterium spp.* and *Escherichia coli* bacteria group, and inhibition of bacteria *C. histolyticum*.<sup>26</sup>

Colonic bacteria metabolic SCFA production activity appears to be stimulated by DC fermentation, with polydextrose addition. Most SCFA ( $\geq 95\%$ ) are represented by ethyl (C2), propionate (C3) and butyrate (C4), and are rapidly absorbed by colonocytes, acting as cell energy source, in addition to beneficial effects such as intestinal barrier improvement function, stimulating cancer cells apoptosis and anti-inflammatory effects.<sup>28</sup>

### Animal studies

A study with female Wistar rats on AIN-93M diet pattern foods with standard diet with 10% cocoa (10.62 mg / g polyphenols) for six weeks was conducted. Even when there was no significant difference in total bacterial counts in groups with or without supplementation, the group fed diet with 10% cocoa showed a reduction in the proportion of *Bacteroides*, *Staphylococcus* and *Clostridium* genus bacteria ( $p < 0, 05$ ).<sup>29</sup>

In relation to microbiota modification and generation of specific metabolites, phenolic compounds can alter immune response through lymphoid tissues functionality engagement in rats, such as gut associated lymphoid tissue (GALT), resulting in a loss in IgA secretion. This reduction appears to be associated with reduced bacteria proportion of *Clostridium* and *Streptococcus* genus. Diet rich in cocoa polyphenols is also capable of inducing toll-like receptors expression (TLR) change in small intestine, reducing TLR9 receptor and tendency of decreased TLR2 and TLR7 expression. TLRs are responsible for microbial pathogens recognition through molecular patterns structures associated with the pathogen (PAMPs). Thus, differentiation in these expressions generate TLR subclasses, allowing changes in intestinal microbiota composition.<sup>29</sup>

Also regarding the immunological effect, a randomized study of pigs submitted to different cocoa powder concentrations (0g, 2.5g, 10g, 20g) for 27 days, observed a TLR9 reduction in ileum Peyer's patches and mesenteric lymph of pigs fed 2.5-20 g cocoa powder / d , compared with pigs not supplemented. Concerning microbiota modulation, significant increase of *Lactobacillus* genus was observed (7 fold, P = 0.005) in feces when compared to unsupplemented pigs. Nevertheless, there was a increased *Bidifubacterium* genus (9 fold, P = 0.01) in the contents of the proximal colon.<sup>30</sup>

In addition to changes related to microbiota modulation, a study performed with male C5BL / 6 mice randomized into high fat (HF) diet, HF diet with 8% cocoa powder and low fat (LF) diet groups followed for 18 weeks, observed a metabolic endotoxemia improvement, as well as intestinal function, by increased glucagon-like peptide 2 (GLP-2) plasma levels in HF diet group (8% cacao powder), when compared with HF and HF /LF diet groups, respectively.<sup>31</sup>

Another study, male Sprague Dawley rats was randomized in 3 groups : standard diet, standard diet supplemented with 10% of dark chocolate and standard diet supplemented with 10% of dark chocolate presenting "fat bloom" (whitish layer formed on the surface due to lipid oxidation) for 6 weeks. It was observed that regular consumption of dark chocolate and chocolate featuring "fat bloom" reduced colonic crypts proliferation significantly, suggesting benefits of dark chocolate in colorectal cancer treatment.<sup>32</sup>

## Human studies

Martin et al. showed that 40g of chocolate consumption (74% cocoa) during two weeks in subjects treated for anxiety was able to reduce urinary excretion of phenyl-acetyl-glutamine and p-cresol sulfate, suggesting an adaptation of the microbiota intestinal to dark chocolate active compounds. Urinary excretion of trimethylamine (TMA) and hippurate increase was also observed. These data suggest phenolic compounds presence, indoles and derivatives, benzoic, methylamines, short chain fatty acid and its metabolites, providing indirect microbial composition, allowing changes in microbial activity identification.<sup>33,34</sup>

Concerning bacterial composition changes, dark chocolate consumption was reported in the literature predominantly by *Bifidobacterium* and *Lactobacillus* growing populations, and inhibition of *Clostridium* group.<sup>34,35</sup> *Clostridium* group, however, appears to be involved in the synthesis of p-cresol sulfate, a potentially toxic compound produced in the colon from the tyrosine and tryptophan.<sup>36</sup>

A double-blind study with chocolate intake modified containing sugar substitutes (maltitol, maltitol and polydextrose, and maltitol associated with resistant starch) for 14 days, showing significant increase in *Lactobacillus* and *Bifidubacteria* population in all chocolate tests. However, increase in *Lactobacillus* and greater production of propionate and butyrate were more pronounced in the polydextrose group. *Bacteroides* population was significantly higher in groups receiving modified chocolate. These data suggest that replacement of sugar in chocolate, while reducing total calories, may also modulate microbiota positively, improving gut health biomarkers.<sup>37</sup>

## Conclusion

Despite the lack of studies using dark chocolate, data suggest that its consumption may be encouraged in moderation and combined with a diet rich in fiber, in order to maintain healthy gut microbiota.

Concerning its use as prebiotic, the chocolate market seems to be promising due to increased demand for healthier options, coupled with its ability to maintain integrity of microorganisms and palatability.

This review shows that consumption of dark chocolate or cocoa flavanols can significantly affect selected gut microbiota growth, suggesting potential prebiotic benefits associated with dietary inclusion of flavanol-rich foods.

## Contributors

All authors contributed worked in all steps, which consisted in study design; collection, analysis and interpretation of data, manuscript writing and proof-reading of final copy.

Conflict of Interest: The authors declare no conflict of interest.

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