

## Insight on the Health Benefits of Functional Food: Dark Chocolate

Deepan Sundararaj<sup>1</sup>, Vishnuvanditha Vuppuluri<sup>2</sup>, Venkatachalam Bharathi<sup>3</sup>, Sakthivel Muthu<sup>4</sup>, Nallusamy Duraisamy<sup>5\*</sup>

<sup>1</sup>Department of Biotechnology, Vivekanandha Arts and Science College for Women, Salem, Tamil Nadu, India

<sup>2</sup>Department of Anesthesiology, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu, India

<sup>3</sup>Department of Microbiology, Vivekanandha Arts and Science College for Women, Salem, Tamil Nadu, India

<sup>4</sup>Center for Global Health Research, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu, India

<sup>5</sup>Department of Research, Meenakshi Academy of Higher Education and Research, K.K. Nagar, Chennai, Tamil Nadu, India

### Abstract

Dark chocolate is known for its rich content of bioactive compounds such as polyphenols and flavonoids, which possess potent antioxidant properties that counteract oxidative stress and lower the risk of chronic diseases like cardiovascular diseases and certain cancers. Studies have shown that dark chocolate consumption can improve cardiovascular health by enhancing endothelial function and reducing blood pressure. Mendelian randomization studies have further indicated a potential decrease in the risk of essential hypertension and venous thromboembolism. Additionally, dark chocolate may favour lipid metabolism, weight management, and inflammation in individuals with chronic kidney disease. There is also emerging evidence suggesting that dark chocolate could positively influence cognitive function and mood and offer protection against neurodegenerative diseases like Alzheimer's and Parkinson's. Researchers are exploring innovative fortification methods to enhance the nutritional profile of dark chocolate and maximize its health benefits. In this review, we have summarized the investigation of the association between dark chocolate consumption and its health-promoting effects.

**Keywords:** Cardiovascular Diseases, Cognitive Function, Dark Chocolate, Health-Promoting Effects, Mood.

### Introduction

Dark chocolate, with its unique blend of bioactive compounds such as polyphenols, flavonoids, and procyanidins, stands out for its numerous health benefits [1]. These compounds, known for their antioxidant properties, play a crucial role in cellular protection and overall health, significantly reducing oxidative stress and inflammation [2]. The most well-documented benefit of dark

chocolate is its positive impact on cardiovascular health [1]. Studies have shown that the flavonoids in dark chocolate can improve cardiovascular function, reduce the risk of essential hypertension, and potentially decrease the risk of venous thromboembolism [2]. Additionally, dark chocolate has been linked to improved cognitive performance and mood enhancement, likely due to its ability to increase blood flow to the brain and its content

of mood-boosting compounds like theobromine and caffeine. Dark chocolate, a versatile health enhancer, has been found to aid in blood glucose regulation, making it a favourable option for improving glycemic control compared to white chocolate, which is high in sugar [1]. It also contains copper, which helps synthesize neurotransmitters like endorphins, serving as a natural analgesic and sedative, thus reducing menstrual pain in adolescents with dysmenorrhea [3]. The nutritional profile of dark chocolate varies depending on the cocoa variety and geographical origin, with some varieties, like those from Peru, showing higher total phenolic content and antioxidant capacity [4]. Despite its high energy density, moderate consumption of dark chocolate is associated with health benefits such as improved lipid metabolism, vascular function, and overall quality of life [5, 6]. Dark chocolate's potential benefits, backed by scientific evidence, extend to bone health, with its high flavonoid and mineral content supporting bone density and reducing the risk of osteoporosis, particularly in adolescents. Furthermore, dark chocolate's immune-modulating properties help safeguard against cardiovascular diseases, certain cancers, and neurodegenerative disorders like Alzheimer's and Parkinson's [1, 6]. Fortifying dark chocolate with additional nutrients such as fruits, spices, and probiotics can enhance its health benefits, increasing its antioxidant content and improving its physical properties and consumer acceptance [1, 7-9]. Probiotic dark chocolate, for instance, has shown high viability of beneficial bacteria, making it an effective delivery system for probiotics and offering additional health benefits [9]. Hence, dark chocolate emerges as a functional food with a wide array of health benefits, including cardiovascular protection, cognitive enhancement, pain relief, blood glucose regulation, bone health support, and immune system modulation, making it a scientifically supported choice for those seeking a healthful

indulgence. In our review, we have delineated research on the correlation between the consumption of dark chocolate and its associated health advantages.

### **Nutritional Content of Dark Chocolate**

Dark chocolate is a nutritionally rich food product known for its high content of bioactive compounds and essential nutrients. It is particularly abundant in antioxidants, such as polyphenols and flavonoids, which contribute to its health benefits, including cardiovascular protection and cognitive enhancement [1, 10]. The total polyphenol content in dark chocolate can vary significantly, with some samples containing up to 39.82 mg/g GAE and flavonoid content reaching up to 37.85 mg/g CE, which correlates with its antioxidant activity [10]. However, the content and type of polyphenols in dark chocolate can vary significantly based on the geographical and genetic origins of the cocoa beans, a testament to the rich diversity and complexity of this beloved treat. The processing methods used also play a crucial role. For instance, chocolates from hybrid cocoa cultivated in Bahia, Brazil, showed that higher levels of theobromine, caffeine, anthocyanins, and gallic acid were associated with more significant bitterness and astringency. In comparison, lower levels of these compounds highlighted fruity, sweet, and caramelized notes, enhancing consumer acceptance [11]. The extraction methods also play a crucial role in determining the polyphenol content. Green solvents like Deep Eutectic Solvents (DES) are more effective and environmentally friendly than conventional solvents, achieving higher yields of total polyphenols and flavonoids in dark chocolate [12]. Additionally, incorporating raw cocoa during the final refining stage of chocolate production can significantly increase the antioxidant capacity and total polyphenol content, with a linear increase in antioxidant activity and an exponential increase in polyphenol content as

the dose of raw cocoa increases [13]. The health benefits of polyphenols in dark chocolate extend to improving cognitive function and maintaining performance during cognitively demanding tasks, as high concentrations of cacao polyphenols have been shown to sustain cognitive performance and concentration better than lower concentrations [14].

Furthermore, dark chocolate consumption has been linked to reduced inflammation in patients with chronic kidney disease, as evidenced by decreased plasma levels of TNF- $\alpha$  after a two-month intervention with 70% cocoa dark chocolate [14]. The phenolic profiles of dark chocolates also vary, with black chocolates being decadent in glycosylated flavonols and A-type procyanidin dimers and trimers. These A-type procyanidins are known for their antioxidant properties and are believed to contribute to the health benefits of dark chocolate [14]. In contrast, brown chocolates contain higher levels of B-type procyanidins, which may influence their color and sensory properties. B-type procyanidins also have antioxidant properties, but they are structurally different from A-type procyanidins and may have different health effects [10]. The antioxidant potential of dark chocolate, measured through various assays, correlates well with the cocoa content, indicating that higher cocoa percentages generally result in more excellent antioxidant activity [10].

Further, dark chocolate is also a good source of essential minerals like magnesium (252.2 mg/100 g), iron (10.9 mg/100 g), zinc (3.5 mg/100 g), and selenium (0.1 mg/100 g), particularly in varieties with higher cocoa content, such as those containing 90% cocoa [15]. Additionally, dark chocolate contains significant amounts of theobromine and caffeine, contributing to its stimulant effects and potential health benefits [4]. The nutritional profile of dark chocolate can be further enhanced through fortification with various ingredients. For instance, adding

unripe banana peel powder increases its antioxidant, antibacterial, and antibiotic properties, along with iron, folic acid, and vitamin B12 content [16]. Similarly, incorporating lyophilized pequi mesocarp boosts the total phenolic content and antioxidant capacity without significantly altering its physical properties [17]. Baobab flour enhances vitamin C, calcium, potassium, phosphorus, chlorine, and sulfur content, improving its nutritional value [17]. Virgin coconut oil (VCO) addition also increases the phenolic content and antioxidant activity, making chocolate more nutritionally beneficial [18]. Dark chocolate's health-promoting factors, such as polyphenols, flavonoids, procyanidins, and vitamins, contribute to its role as a functional food with anti-diabetic, anti-inflammatory, and anti-microbial properties [9]. Moreover, dark chocolate is rich in saturated fatty acids and theobromine, which have beneficial effects against metabolic disorders and atherosclerosis when combined with a diet rich in vegetables and fruits [19]. The variability in the nutritional content of dark chocolate is influenced by the type of cocoa used, its geographical origin, and the specific processing methods, which can affect the levels of bioactive compounds and essential nutrients [4]. Overall, dark chocolate is a nutritionally dense food with a wide range of health benefits, making it a valuable addition to a balanced diet.

### **Health Benefits of Dark Chocolate**

Dark chocolate, rich in cocoa and bioactive compounds, has been extensively studied for its potential benefits to cardiovascular health. The antioxidants in dark chocolate, such as procyanidins, neutralize free radicals, reducing oxidative stress, a significant contributor to cardiovascular diseases (CVDs) [1]. A key finding from a Mendelian randomization study is the significant association between dark chocolate intake and a reduced risk of essential hypertension (EH) and venous

thromboembolism (VTE), suggesting a causal relationship between dark chocolate consumption and lower risks of these conditions [2]. The high cocoa content in dark chocolate is also linked to improved blood pressure, lipid metabolism, and vascular functions, essential for maintaining cardiovascular health [2, 20]. The phytochemicals in dark chocolate, such as catechins and epicatechin isomers, modulate nitric oxide levels, leading to vasodilation and improved blood flow while also reducing the formation of reactive oxygen species and activating antioxidant enzymes [21]. Clinical trials have demonstrated that dark chocolate can reduce inflammatory markers like TNF- $\alpha$  in patients with chronic kidney disease, which benefits cardiovascular health as inflammation is a key factor in developing CVDs [20, 22].

Moreover, the bioactive components of dark chocolate have been shown to positively modulate the immune system and lower blood cholesterol levels, thereby reducing the risk of heart attacks, strokes, and other cardiovascular events [9, 23]. The cardioprotective effects of dark chocolate, attributed to its ability to decrease oxidative stress and inflammation, are crucial in preventing atherosclerosis and other cardiovascular dysfunctions [24]. However, it is important to note that while dark chocolate has these beneficial effects, the processing of chocolate can lead to the loss of some nutrients. Thus, fortification with additional ingredients can enhance its health benefits. Despite these promising findings, some studies indicate that the impact of dark chocolate on lipid profiles and anthropometric variables may vary, possibly due to differences in cocoa concentration and population characteristics [25]. This underscores the need for further research to confirm these effects, engaging the audience in the ongoing scientific exploration of dark chocolate's potential. Overall, the consumption of dark chocolate, particularly those with high cocoa content, can be encouraged as part of a balanced diet to

support cardiovascular health, but with caution regarding its caloric content and the form of chocolate production.

### **Antioxidant and Anti-Inflammatory Properties**

Dark chocolate is renowned for its significant antioxidant properties, primarily attributed to its rich content of polyphenols, flavonoids, and other bioactive compounds [10]. These antioxidants play a crucial role in neutralizing free radicals, reducing oxidative stress, and thereby mitigating the risk of various chronic diseases such as cancer, diabetes, and cardiovascular diseases [10]. Studies have shown that dark chocolate's antioxidant capacity is influenced by its cocoa content, with higher cocoa percentages correlating with greater antioxidant activity [26]. The incorporation of honey powder, particularly honey/maltodextrin (H/M), into dark chocolate has been found to enhance its antioxidant capacity, as evidenced by increased 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) values [26]. Additionally, fortification with various ingredients such as fruits, spices, and probiotics has been explored to further boost the antioxidant content of dark chocolate. For instance, the addition of elderberries, elderflowers, and chokeberries significantly increased the total phenolic content and antioxidant capacity of dark chocolates, as measured by multiple assays including DPPH, ABTS, CUPRAC, and FRAP. Similarly, the enrichment of dark chocolate with white tea and jujube extracts, both in free and microencapsulated forms, resulted in a notable increase in total phenolic content and antioxidant properties [27]. The use of raw cocoa during the final refining stage of chocolate production has also been shown to significantly enhance the antioxidant capacity and polyphenol content, with a linear increase in antioxidant activity observed with higher doses of raw cocoa [13]. Furthermore,

dark chocolate formulated from unfermented dry cocoa beans exhibited high antioxidant activity due to its substantial polyphenol content, which is preserved through minimal processing [28]. Clinical studies have demonstrated that regular consumption of dark chocolate can reduce inflammation and oxidative stress markers, such as TNF- $\alpha$ , in patients with chronic kidney disease, highlighting its potential therapeutic benefits [22]. The antioxidant properties of dark chocolate are not only beneficial for cardiovascular health, as they help lower blood cholesterol and reduce the risk of heart attacks and strokes, but also for cognitive health, potentially enhancing cognitive performance and mood [1]. Moreover, innovative approaches like the incorporation of zinc oxide nanoparticles into dark chocolate have shown promising results in enhancing its free radical scavenging ability and inhibitory effects on cytochrome P450 enzymes, further underscoring the multifaceted antioxidant potential of dark chocolate [23]. Overall, the diverse methods of fortification and processing, along with the inherent properties of cocoa, make dark chocolate a potent source of antioxidants with wide-ranging health benefits.

### **Dark Chocolate: Reducing Inflammation and Oxidative Stress**

Dark chocolate, with its renowned anti-inflammatory properties, is a beacon of hope for high-risk populations. Its high content of polyphenols, flavonoids, and other bioactive compounds exert significant antioxidant and anti-inflammatory effects by modulating various biochemical pathways. For instance, cocoa polyphenols activate endothelial nitric oxide synthase, releasing nitric oxide, which induces vasodilation and offers cardioprotective benefits [29]. Additionally, dark chocolate's phytochemicals, such as catechins and epicatechin isomers, modulate nuclear factor kappa B (NF- $\kappa$ B), reducing the

production of inflammatory markers like tumour necrosis factor-alpha (TNF- $\alpha$ ) and interleukins (IL), and decrease the formation of reactive oxygen species (ROS) [21]. Clinical trials have demonstrated that dark chocolate consumption can reduce plasma levels of TNF- $\alpha$  in patients with chronic kidney disease (CKD) on hemodialysis, highlighting its potential to mitigate inflammation in high-risk populations [30]. Dark chocolate's unique role in reducing oxidative stress, a significant contributor to cardiovascular diseases (CVDs) and other chronic conditions, is worth highlighting [31]. For instance, cocoa flavanols have been shown to decrease adhesion molecules and leukotrienes in serum and activate NF- $\kappa$ B in leukocytes, thereby lowering inflammation. The anti-inflammatory potential of dark chocolate is also evident in its ability to modulate the immune system, as seen in studies where cocoa polyphenols inhibited cytokine release and NF- $\kappa$ B activation in human monocytes [32].

Moreover, the benefits of dark chocolate are not limited to specific populations; its versatility is truly intriguing. It has been shown to reduce oxidative bursts in neutrophils and monocytes, particularly in overweight and obese individuals with higher oxidative and inflammatory stress [33]. Fortifying dark chocolate with additional bioactive ingredients, such as unripe banana peel powder, further enhances its nutritional value and anti-inflammatory properties, although it may affect its colour [16]. Despite the processing losses of some nutrients, fortification with fruits, spices, and probiotics can increase the overall antioxidant content and improve consumer acceptance [9]. While dark chocolate can reduce exercise-induced oxidative stress, its effects on exercise-related inflammation are mixed, suggesting further research to clarify these outcomes [34]. Overall, the anti-inflammatory properties of dark chocolate are well-supported by various

studies, making it a valuable functional food for improving health outcomes, particularly in individuals with chronic inflammatory conditions.

### **Dark chocolate Effects on Cognitive Performance and Mood**

Dark chocolate Effects on Cognitive Performance and Mood Dark chocolate has been extensively studied for its potential benefits on cognitive function and mood enhancement. Research indicates that dark chocolate consumption can improve cognitive performance, particularly in tasks requiring response inhibition and selective attention, and maintain concentration during cognitively demanding activities such as problem-solving, studying, or working on complex tasks [14]. This is attributed to increased cerebral blood flow and bioactive compounds such as catechin, epicatechin, and procyanidins, which have strong antioxidant properties [35]. Additionally, dark chocolate has been shown to reduce mental and physical fatigue, thereby indirectly enhancing cognitive functions such as executive function, memory, and overall brain health [36]. Studies involving both acute and sub-chronic consumption of dark chocolate have demonstrated improvements in verbal episodic memory and mood, with significant effects observed two hours post-consumption and sustained benefits after 30 days of daily intake [37, 38]. The mood-enhancing effects of dark chocolate are thought to be mediated by its flavanols and methylxanthines, which influence brain activation patterns and neurotrophin levels, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) [38, 39]. Furthermore, dark chocolate consumption under stress conditions has shown significant improvements in memory consolidation and locomotor activity, providing reassurance about its stress-relieving properties [40]. The positive effects on mood, including increased calmness and contentedness, have been

observed in healthy individuals, providing a rationale for exploring its benefits in clinical populations with anxiety or depression [41]. Despite some inconsistencies in the literature, the evidence supports that dark chocolate can confer cognitive and mood benefits, likely due to its complex composition of flavonoids, methylxanthines, and other minor constituents [42]. Therefore, incorporating dark chocolate into the diet may offer a tasty and functional approach to enhancing cognitive function and mood, particularly during periods of high cognitive demand or stress.

Importantly, the combination of dark chocolate and exercise has artistically affected cognitive function and mood enhancement in older adults, supported by various studies. Dark chocolate, rich in polyphenols, has been shown to increase cerebral blood flow and improve cognitive function, particularly in tasks requiring response inhibition and selective attention, as well as maintaining performance and concentration during cognitively demanding tasks [14]. Chronic chocolate consumption has also been associated with improved executive function, memory, and global cognitive performance, suggesting that daily cocoa intake can enhance cognitive abilities in learning, memory, and attention [43]. Furthermore, dark chocolate has been found to reduce mental and physical fatigue, which indirectly enhances vitality, executive function, memory, and grey matter volume, thereby improving overall brain health and quality of life [36]. On the other hand, physical exercise alone has been well-documented to improve cognitive domains such as global cognition, memory, and executive function/attention, particularly in older adults with mild cognitive impairment (MCI). When combined, cognitive interventions and physical exercise yield additive and synergistic effects on cognition, showing more significant improvements in global cognition, memory, and executive function compared to either intervention alone,

encouraging the audience about the potential of these interventions[36]. This synergistic effect is further supported by studies indicating that combined interventions, such as MEMO (a cognitive training program) with physical activity or psychoeducation, result in significant cognitive gains and reductions in depressive symptoms in older adults [44].

Moreover, the concurrent use of acute dark chocolate consumption and dual-site transcranial direct current stimulation (a-tDCS) has been shown to improve cognitive and endurance performance, suggesting that dark chocolate can enhance the effects of other cognitive interventions [45]. While some randomized clinical trials (RCTs) have shown no significant overall benefits of cocoa extract (CE) supplementation on global or domain-specific cognitive function over two years, subgroup analyses suggest potential cognitive benefits among those with poorer baseline diet quality, indicating that the effects of dark chocolate may be more pronounced in specific populations [46]. Additionally, systematic reviews have highlighted that chocolate or its constituents can influence cognitive function and mood, with some studies showing clear evidence of cognitive enhancement and mood improvement following cocoa flavanols and methylxanthine intake [42]. Therefore, integrating dark chocolate consumption with regular physical exercise offers a comprehensive approach to enhancing cognitive function and mood in older adults, reassuring the audience of the thoroughness of the research and the potential benefits of the interventions.

### **Optimal Daily Servings of Dark Chocolates for Health Benefits**

The recommended daily servings of dark chocolate vary based on the health outcomes being targeted and the specific population under study. Research indicates that dark chocolates, rich in polyphenols and flavonoids, offer numerous health benefits, including

cardiovascular protection, improved lipid metabolism, and anti-inflammatory effects. However, it's crucial to remember that each person's health journey is unique. For instance, a study on male Balb/c mice exposed to cigarette smoke demonstrated that a daily dose of 0.05 grams of dark chocolate significantly increased spermatozoa count, suggesting its potential to mitigate oxidative stress-induced damage [47]. In human studies, 100 grams of dark chocolate daily for 15 days significantly reduced blood pressure in patients with essential hypertension, highlighting its antihypertensive properties [48]. However, other studies suggest more moderate consumption. For example, a study involving patients with metabolic syndrome found that consuming 20 to 40 grams of dark chocolate daily for two months did not significantly alter lipid profiles, indicating that while dark chocolates have health benefits, their effects can vary based on the dosage and individual health conditions [49]. Another study on hypertensive patients aged 45-55 showed that consuming 100 grams of 80% dark chocolate daily for seven days significantly reduced systolic blood pressure, reinforcing its cardiovascular benefits [50]. Additionally, a longitudinal clinical trial with chronic kidney disease patients on hemodialysis found that 40 grams of dark chocolate per dialysis session (totalling 120 grams per week) for two months helped modulate inflammation markers without increasing phosphorus plasma levels, suggesting a safe and beneficial dosage for this population [30]. Despite these findings, some studies call for caution due to potential confounding factors and the need for larger-scale trials to confirm these benefits and establish standardized recommendations [51].

Moreover, the high magnesium content in dark chocolate (327 mg/100g) could contribute to its cardiovascular benefits, as magnesium supplementation has been linked to reduced blood pressure and systemic inflammation [48]. Therefore, while moderate consumption

of dark chocolate, typically around 20-40 grams per day, is generally recommended for its health benefits, specific dosages may vary based on individual health conditions and targeted outcomes. It is also essential to consider the potential caloric and cadmium content when consuming dark chocolate regularly [52]. However, when incorporated thoughtfully into a balanced diet, dark chocolate can offer significant health benefits. It's not just a treat, but a part of a healthy lifestyle. Still, it should be done thoughtfully and in moderation to maximize its positive effects while minimizing potential risks.

### **Alternative Sweeteners and Fortification in Dark Chocolates for Better Health**

Sugar and calorie considerations significantly impact the nutritional value of dark chocolate, influencing its health benefits and suitability for various dietary needs [16]. While rich in antioxidants, polyphenols, and flavonoids, traditional dark chocolate often contains high levels of sucrose, a factor that contributes to its calorie density and can lead to potential adverse health effects when consumed in excess [4, 16]. This underscores the importance of exploring alternative sweeteners and fortification methods to address these concerns. For instance, replacing sucrose with coconut and palm sugar enhances the antioxidant activity and total polyphenol content, providing a protective effect for probiotics and making the chocolate more functional without significantly altering its calorie value [53]. Similarly, substituting sucrose with honey powder, particularly honey/maltodextrin, has been shown to increase antioxidant capacity and alpha-amylase inhibition, which is beneficial for managing blood sugar levels without affecting the overall calorie content [26]. Low-calorie sweeteners like stevioside, acesulfame-k, sucralose, and inulin have also been used to create dark chocolate suitable for people with diabetes and those aiming to reduce calorie

intake, with inulin showing the most significant calorie reduction, albeit with some sensory drawbacks [54]. Additionally, sugar alcohols such as maltitol, sorbitol, and xylitol have been tested, with xylitol providing the highest sensory acceptability and maintaining functional nutrients like polyphenols and flavonoids [55]. The use of palm sap-based sugars has also been investigated, showing potential for maintaining desirable physical properties and enhancing the aroma profile of dark chocolate while offering additional health benefits [56]. Fortification with ingredients like unripe banana peel powder and lyophilized pequi mesocarp further enhances the nutritional profile by adding antioxidants, vitamins, and minerals. However, these additions can affect the product's color and viscosity [57]. Moreover, including guar gum and palm oil in semi-dark chocolate formulations has improved organoleptic properties and reduced calorie content while increasing phenolic and antioxidant levels, making it a healthier option [58]. Overall, these modifications not only improve the health benefits of dark chocolate by enhancing its antioxidant and anti-diabetic properties but also cater to specific dietary needs, such as lower calorie intake and better glycemic control, thereby broadening its appeal and utility as a functional food [9].

### **Allergen Risks in Dark Chocolate: Challenges and Advances in Detection**

Dark chocolate, while celebrated for its health benefits and rich bioactive compounds, poses significant challenges for individuals with allergies and sensitivities. The presence of allergenic proteins in processed chocolate products can trigger severe reactions in susceptible individuals, with components such as polyphenols, tannins, and fat complicating the extraction and detection of these proteins [59]. Studies have shown that the chocolate matrix can negatively impact the recovery of allergens like peanuts. At the same time,



tempering processes can reduce the recovery of milk allergens, though egg allergens remain unaffected by these factors [59]. Additionally, the introduction of a rapid size-exclusion solid-phase extraction step before liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS2) analysis has significantly improved the sensitivity for detecting nut allergens in dark chocolate. This step enhances the separation of allergenic proteins from other components, achieving high recoveries and good precision [60]. Despite these advancements, the lack of standardized assays and quantitative requirements for flavanol content in commercial dark chocolate brands complicates the establishment of clear dietary recommendations for individuals with sensitivities [9, 61].

Moreover, the fortification of dark chocolate with various ingredients such as fruits, spices, and probiotics to enhance its nutritional profile and antioxidant content can introduce additional allergens, underscoring the need for caution and vigilance among those with food sensitivities [9]. The well-documented health benefits of dark chocolate, including its anti-inflammatory, anti-diabetic, and cardiovascular protective properties, must be carefully weighed against the potential risks for allergic individuals [35]. For instance, while dark chocolate can reduce inflammation and oxidative stress markers in patients with chronic kidney disease, it is crucial to be proactive and monitor for any adverse reactions due to its bioactive components [22]. The development of dark chocolate with added functional ingredients like passion fruit seeds and orange peels, which increase dietary fibre and antioxidant properties, also requires careful allergen management to ensure consumer safety [62]. The heterogeneity in cocoa varieties and their bioactive compound content further complicates the allergen landscape, as different geographical origins of cocoa can result in varying levels of allergens

and bioactive compounds [4]. Therefore, while dark chocolate offers numerous health benefits, it is essential for individuals with allergies and sensitivities to approach its consumption with caution, considering the potential for hidden allergens and the variability introduced by different processing and fortification methods.

## **Conclusion and Future Perspective**

With its wealth of bioactive compounds like polyphenols and flavonoids, dark chocolate is lauded for its health benefits. With their antioxidant properties, these compounds combat oxidative stress and reduce the risk of chronic diseases such as cardiovascular diseases and certain cancers. The consumption of dark chocolate has been linked to improved cardiovascular health, as it enhances endothelial function and reduces blood pressure. This is supported by a Mendelian randomization study that showed a decreased risk of essential hypertension and venous thromboembolism. Dark chocolate also has the potential to positively impact lipid metabolism, aid in weight management, and reduce inflammation in patients with chronic kidney disease. Moreover, it may enhance cognitive function and mood and offer protection against neurodegenerative diseases like Alzheimer's and Parkinson's. The ongoing research into innovative fortification methods to improve its nutritional profile and further boost its health advantages is a beacon of hope for the future of preventive healthcare.

The future of dark chocolate research is poised to explore several promising avenues, driven by its rich composition of bioactive compounds and the potential health benefits they confer. One significant area of focus will be the cardiovascular benefits of dark chocolate, particularly its ability to reduce blood pressure, improve arterial function, and enhance lipid profiles by increasing HDL cholesterol and reducing LDL cholesterol levels. However, more research is needed to

identify the specific compounds responsible for these effects. The antioxidant properties of cocoa polyphenols, which contribute to improved endothelial function and reduced oxidative stress, will also be a critical area of study, especially in populations under significant oxidative stress or with antioxidant deficiencies. Additionally, the role of dark chocolate in modulating the immune system and its potential protective effects against diseases such as Alzheimer's, Parkinson's, and certain cancers will be further investigated, with a particular interest in its anti-inflammatory, anti-diabetic, and anti-microbial properties. The manufacturing process of dark chocolate, including tempering and conching, will continue to be refined to enhance the physical and sensory properties of the final product. The research will likely focus on optimizing the tempering process to achieve the desired hardness, snap, and gloss and understanding the impact of particle size distribution and fat content on the tempering behaviour. Innovations in processing techniques, such as coarse conching, will be explored to improve the efficiency and quality of dark chocolate production, particularly emphasizing the type of cocoa raw material used and its impact on the final product. Fortifying dark chocolate with additional nutrients and bioactive compounds will be another critical research area. This includes incorporating fruits, spices, phytosterols, probiotics, and prebiotics to enhance the antioxidant and overall nutritional profile while potentially reducing the calorie content and improving consumer acceptance by modifying the bitter taste. The application of

## References

[1]. Dehghani, P., Taheri, F., Asgary, S., 2024, Decoding the delights: Unraveling the health benefits of dark chocolate in comparison to white chocolate. *Functional Food Science*. 4:119-133.

microencapsulation technology to enhance the bioavailability of cocoa flavonoids and other bioactive ingredients will also be a significant focus, addressing the current lack of standardized assays and quantitative requirements for flavanol content in commercial dark chocolate products.

Furthermore, the sustainability and resilience of cacao cultivation will be critical, with research efforts aimed at bolstering the cacao tree through selective breeding, pest management, and farmer education to meet the increasing global demand for cocoa. Finally, long-term, randomized clinical trials will be essential to establish clear dietary recommendations for cocoa flavonoid consumption and to conclusively link dark chocolate intake with health benefits such as reduced risk of cardiovascular diseases, insulin resistance, and memory dysfunction. Overall, the future of dark chocolate research holds great potential for enhancing both the health benefits and the quality of this beloved confectionery.

## Conflict of Interest

All authors have no conflict of interest.

## Source of Funding

None.

## Acknowledgement

The authors gratefully acknowledge the Department of Research, Meenakshi Academy of Higher Education and Research, K.K. Nagar, Chennai., for providing research facilities for our study.

[2]. Yang, J., Zhou, J., Yang, J., 2024, Dark chocolate intake and cardiovascular diseases: A Mendelian randomization study. *Scientific Reports*. 14(1):968. Doi:10.1038/s41598-023-50351-6

[3]. Ferina, F., Hadiani, D.N., Fatimah, Y.U., 2023, Dark chocolate as a non-

- pharmacological alternative to reduce dysmenorrhea in adolescents. *Healthcare in Low-resource Settings*. 11.
- [4]. Cartas, J., Alvarenga, N., Partidário, A., 2024, Influence of geographical origin in the physical and bioactive parameters of single origin dark chocolate. *European Food Research and Technology* 250(10):2569-2580. Doi:10.1007/s00217-024-04558-0
- [5]. Kerimi, A., Williamson, G., 2015, The cardiovascular benefits of dark chocolate. *Vascular Pharmacology*. 71:11-15. Doi:10.1016/j.vph.2015.05.011
- [6]. Zugravu, C., Otelea, M.R., 2019, Dark chocolate: To eat or not to eat? A review. *Journal of AOAC International*. 102(5):1388-1396. Doi:10.1093/jaoac/102.5.1388
- [7]. Pierce, E.A., 2024, The Health Effects of Chocolate: A Literature Review.
- [8]. Singh, P.K., Khedkar, R.D., Chandra, S., 2024, Chocolate: An overview of functional potential and recent trends in fortification. *Brazilian Journal of Food Technology*. 27. Doi:10.1590/1981-6723.11823
- [9]. Samanta, S., Sarkar, T., Chakraborty, R., 2022, Dark chocolate: An overview of its biological activity, processing, and fortification approaches. *Current Research in Food Science*. 5:1916-1943. Doi: 10.1016/j.crf.2022.10.017
- [10]. Jaćimović, S., Popović-Djordjević, J., Sarić, B., Krstić, A., Mickovski-Stefanović, V., Pantelić, N.Đ., 2022 Antioxidant activity and multi-elemental analysis of dark chocolate. *Foods*. 11(10):1445. Doi:10.3390/foods11101445
- [11]. Martins LM, de Santana LRR, Maciel LF, Soares SE, Ferreira ACR, et al. 2023. Phenolic compounds, methylxanthines, and preference drivers of dark chocolate made with hybrid cocoa beans.
- [12]. Vinci, G., Maddaloni, L., Prencipe, S.A., Orlandini, E., Sambucci, M., 2023 Simple and reliable eco-extraction of bioactive compounds from dark chocolate by Deep Eutectic Solvents. A sustainable study. *International Journal of Food Science and Technology*. Doi:10.1111/ijfs.16315
- [13]. Mori Culqui, P.L., Quintana, C., 202, 1Antioxidantes y polifenoles totales de chocolate negro con incorporación de cacao (Theobroma cacao L.) crudo. *Revista de Investigaciones Altoandinas*. 23:266-273.
- [14]. Sasaki, A., Mizuno, K., Morito, Y., 2024, The effects of dark chocolate on cognitive performance during cognitively demanding tasks: A randomized, single-blinded, crossover, dose-comparison study. *Heliyon*. 10(2): e24430. Doi: 10.1016/j.heliyon.2024.e24430
- [15]. Cinquanta, L., Di Cesare, C., Manoni, R., Piano, A., Roberti, P., Salvatori, G., 2016, Mineral essential elements for nutrition in different chocolate products. *International Journal of Food Sciences and Nutrition*. 67(7):773-778. Doi:10.1080/09637486.2016.1199664
- [16]. Singh, R., Deshpande, A.S., Pallavi, A., Kamboj, V., 2023, Sustainable Use of Waste Banana Peel (*Musa sapientum* L.) Powder for Enhancement of Nutritional Properties of Dark Chocolate. *Agro Environmental Sustainability is AgroEnviron*. 1(1):22-28.
- [17]. Monteiro, S., Dias, J., Lourenço, V., 2023, Development of a functional dark chocolate with baobab pulp. *Foods*. 12(8). Doi:10.3390/foods12081711
- [18]. Rashid, A., Misson, S., Yaakob, M., Latiff, H., Sarmidi, N.A., 2017, Addition of virgin coconut oil: Influence on the nutritional value and consumer acceptance of dark chocolate. *Journal Science China Technological Sciences*. 4(3-3):426-431.
- [19]. Berroukche, A., Terras, M., Dellaoui, H., 2018, Theobromine dark chocolate extract and vitamin A antagonist effects on biochemical parameters in rats fed with high-fat diet. *Biochemistry and Molecular Biology Journals*. 04(03). Doi:10.21767/2471-8084.100071
- [20]. Tan, T.Y.C., Lim, X.Y., Yeo, J.H.H., Lee, S.W.H., Lai, N.M., 2021, The health effects of chocolate and cocoa: A systematic

- review. *Nutrients*. 13(9):2909. Doi:10.3390/nu13092909
- [21]. De, P., Silva, T., Silva, A.A., Toffolo, M., De Aguiar, A.S., 2022, The action of phytochemicals present in cocoa in the prevention of vascular dysfunction and atherosclerosis. *Journal of Clinical and Translational Research*. 8(6).
- [22]. Ribeiro, M., Fanton, S., Paiva, B.R., 2023, Dark chocolate (70% cocoa) attenuates the inflammatory marker TNF- $\alpha$  in patients on hemodialysis. *Clinical Nutrition ESPEN*. 53:189-195. Doi: 10.1016/j.clnesp.12.009
- [23]. Prenetha, R., Parameswari, R., Lakshmi, T., In vitro antioxidant and cytochrome p450 inhibitory activity of dark chocolate mediated zinc oxide nanoparticles.
- [24]. García, L.C., Hernández, A.N.M., 2020, Beneficial effects of cocoa and dark chocolate polyphenols on health. Federation of American Societies for Experimental Biology. 34(S1):1-1. Doi:10.1096/fasebj.2020.34.s1.07187
- [25]. Alvarez, L., Contreras, J., Giraldo, M., 2020, An individual patient data meta-analysis with Colombian studies on the effect of dark chocolate consumption on cardiovascular risk parameters. *Journal of Nutrition and Metabolism*. 2020:3419598. Doi:10.1155/2020/3419598
- [26]. Rusli, A.A., Mohamad, N.J., Mahmood, A., Ibrahim, N.H., 2023, Antioxidant Capacity, Alpha Amylase Inhibition, and Calorie Value of Dark Chocolate Substituted with Honey Powder. *Pertanika Journal of Tropical Agricultural Science*. 46(4).
- [27]. Shahbazi, S., Didar, Z., Vazifedoost, M., Naji-Tabasi, S., 2022, Enrichment of dark chocolate with free and microencapsulated white tea and jujube extracts: Impacts on antioxidant, physicochemical, and textural properties. *Quality Assurance and Safety of Crops & Foods*. 14(4):188-201.
- [28]. Tafurt, G., Suarez, O., Lares, M. del. C., Álvarez, C., Liconte, N., 2020, Capacidad antioxidante de un chocolate oscuro de granos cacao orgánico sin fermentar Revista Digital de Postgrado. 10(1). Doi:10.37910/rdp.2021.10.1.e280
- [29]. Magrone, T., Russo, M.A., Jirillo, E., 2017, Cocoa and dark chocolate polyphenols: From biology to clinical applications. *Frontiers in Immunology*. 8:677. Doi:10.3389/fimmu.2017.00677
- [30]. Mafra, D., M Ribeiro, M., Fanton, S., 2022, MO570: Effects of dark chocolate on inflammation and oxidative stress in patients with chronic kidney disease on hemodialysis. Nephrology, Dialysis, Transplantation. 37. Doi:10.1093/ndt/gfac074.01531.
- [31]. Ellinger, S., Stehle, P., 2016, Impact of cocoa consumption on inflammation processes-A critical review of randomized controlled trials. *Nutrients*. 8(6):321. Doi:10.3390/nu8060321
- [32]. Zeng, H., Locatelli, M., Bardelli, C., 2011, Anti-inflammatory properties of clovamide and Theobroma cacao phenolic extracts in human monocytes: evaluation of respiratory burst, cytokine release, NF- $\kappa$ B activation, and PPAR $\gamma$  modulation. *Journal of Agricultural and Food Chemistry*. 59(10):5342-5350. Doi:10.1021/jf2005386
- [33]. Ioannone, F., Sacchetti, G., Serafini, M., 2017, Effect of dark chocolate extracts on phorbol 12-myristate 13-acetate-induced oxidative burst in leukocytes isolated by normo-weight and overweight/obese subjects. *Frontiers in Nutrition*. 4:23. Doi:10.3389/fnut.2017.00023
- [34]. Massaro, M., Scoditti, E., Carluccio, M.A., Kaltsatou, A., Cicchella, A., 2019, Effect of cocoa products and its polyphenolic constituents on exercise performance and exercise-induced muscle damage and inflammation: A review of clinical trials. *Nutrients*. 11(7):1471. Doi:10.3390/nu11071471
- [35]. Patel, N., Jayswal, S., Maitreya, B.B., 2019, Dark Chocolate: Consumption for human health. *Journal of Pharmacognosy and Phytochemistry*. 8(3):2887-2890.

- [36]. Nemoto, K., Kokubun, K., Ogata, Y., Koike, Y., Arai, T., Yamakawa, Y., 2022, Dark chocolate intake may reduce fatigue and mediate cognitive function and gray matter volume in healthy middle-aged adults. *Behavioural Neurology*. 2022:6021811. Doi:10.1155/2022/6021811
- [37]. Lamport, D.J., Christodoulou, E., Achilleos, C., 2020, Beneficial effects of dark chocolate for episodic memory in healthy young adults: A parallel-groups acute intervention with a white chocolate control. *Nutrients*. 12(2):483. Doi:10.3390/nu12020483
- [38]. Sumiyoshi, E., Matsuzaki, K., Sugimoto, N., 2019, Sub-chronic consumption of dark chocolate enhances cognitive function and releases nerve growth factors: A parallel-group randomized trial. *Nutrients*. 11(11):2800. Doi:10.3390/nu11112800
- [39]. Tuenter, E., Foubert, K., Pieters, L., 2018, Mood components in cocoa and chocolate: The mood pyramid. *Planta Medica*. 84(12-13):839-844. Doi:10.1055/a-0588-5534
- [40]. Kalantarzadeh, E., Radahmadi, M., Reisi, P., 2020, Effects of different dark chocolate diets on memory functions and brain corticosterone levels in rats under chronic stress. *Journal of physiology and pharmacology*. 24(3):185-196. Doi:10.32598/ppj.24.3.40
- [41]. Pase, M.P., Scholey, A.B., Pipingas, A., 2013, Cocoa polyphenols enhance positive mood states but not cognitive performance: a randomized, placebo-controlled trial. *Journal of Psychopharmacology*. 27(5):451-458. Doi:10.1177/0269881112473791
- [42]. Scholey, A., Owen, L., 2013, Effects of chocolate on cognitive function and mood: a systematic review. *Nutrition Reviews*. 71(10):665-681. Doi:10.1111/nure.12065
- [43]. Shateri, Z., Kooshki, A., Hormoznejad, R., Hosseini, S.A., Mousavi, R., Foroumandi, E., 2023, Effects of chocolate on cognitive function in healthy adults: A systematic review and meta-analysis on clinical trials. *Phytotherapy Research*. 37(9):3688-3697. Doi:10.1002/ptr.7896
- [44]. Sacramento, A.M., Silva, H.S. da., Melo, G.F. de., Tavares, G.S., Abreu, J.N.S., Chariglione, I.P.F.S., 2022, Benefits of combined interventions for cognitive enhancement in older adults. *Journal of Gerontology and Geriatrics*. 16. Doi:10.53886/gga.e0220018
- [45]. Banaei, P., Tadibi, V., Amiri, E., Machado, D.G. da. S., 2023, Concomitant dual-site tDCS and dark chocolate improve cognitive and endurance performance following cognitive effort under hypoxia: a randomized controlled trial. *Scientific Reports*. 13(1):16473. Doi:10.1038/s41598-023-43568-y
- [46]. Vyas, C.M., Manson, J.E., Sesso, H.D., 2024, Effect of cocoa extract supplementation on cognitive function: results from the clinic subcohort of the COSMOS trial. *American Journal of Clinical Nutrition*. 119(1):39-48. Doi: 10.1016/j.ajcnut.2023.10.031
- [47]. Anggi, A.R., Herlina, E.C., Pengaruh, Pemberian., 2016, Dark Chocolate Terhadap Jumlah Spermatozoa Mencit Balb/C Jantan yang Dipapar Asap Rokok. *Jurnal Kedokteran Diponegoro (Diponegoro Medical Journal)*. 5(4):475-484.
- [48]. Eslami, O., 2017, Dark Chocolate and Metabolic Syndrome; Do Polyphenols All that Matter? *Journal of Nutrition and Food Security*. 2(2):139-140.
- [49]. Naghdipour, B.A., Mozaffari, K.H., Poursoleiman, F., Zavar, R.J., Rahmanian, M., Dehghani, A., 2014, The Effect of Dark Chocolate Consumption on Lipid Profile in Patients with Metabolic Syndrome: A Randomized Clinical Trial.
- [50]. Novianto, E., Maria, L., Mumpuni, R.Y., 2022, Pengaruh Dark Chocolate Terhadap Penurunan Tekanan Darah Sistolik Pada Penderita Hipertensi Usia 45-55 Tahun di RW. 04 Desa Banjarejo Kecamatan Pakis Kabupaten Malang: The Effect of Dark Chocolate on Controlling Systolic Blood

Pressure in Hypertensive Patients. *Medica Hospitalia: Journal of Clinical Medicine*. 9(2):122-129.

[51]. Haritha, K., Kalyani, L., Rao, A.L., 2014, Health benefits of dark chocolate. *Journal of Advanced Drug Delivery*. 1(4):184-195.

[52]. Belz, G., Mohr-Kahaly, S., 1946, Cocoa and dark chocolate in cardiovascular prevention? *Deutsche Medizinische Wochenschrift*. Deutsche Medizinische Wochenschrift. 136:2657-2663.

[53]. Nurhayati, R., Zulfa, N., Herawati, E.R.N., Laila, U., 2022, Physicochemical, and microbiological characteristics of probiotic dark chocolate bar sweetened with palm sugar and coconut sugar. *Food Research*. 6(5):97-107. Doi:10.26656/fr.2017.6(5).591

[54]. Ali, Laila, A.S., Nahed, M.E., Magda, S.S., 2021, Producing and quality attributes of low calories dark chocolate using different intense sweeteners and wheat fiber isolate. *American Journal of Food Technology*. 9(1):1-7. Doi:10.12691/ajfst-9-1-1

[55]. Kim, S.Y., 2014, Department of Food Engineering, Dankook University, Zu G, Kim BK. Quality characteristics of single origin bean-to-bar dark chocolate prepared with sugar alcohols. *Food Engineering Progress*. 18(3):194-202.

Doi:10.13050/foodengprog.2014.18.3.194

[56]. Saputro, A.D., Van, de. Walle. D., Aidoo, R.P., 2017, Quality attributes of dark chocolates formulated with palm sap-based sugar as nutritious and natural alternative

sweetener. *European Food Research and Technology*. 243(2):177-191.

Doi:10.1007/s00217-016-2734-9

[57]. Lorenzo, N.D., Ovd, S., Scds, L., 2022, Structure and nutrition of dark chocolate with pequi mesocarp (*Caryocar villosum* (Alb.) Pers.). *Food Science and Technology*. 42.

[58]. Nazira, S., Azada, Z., 2016, Development of a novel calorie controlled and sugar-free dark chocolate enriched with guar gum. *Journal of Food Research*. 4(02):39-46.

[59]. Khuda, S.E., Williams, K.M., 2015, Effect of Processing on Dark Chocolate Composition: A Focus on Allergens. Processing and Impact on Active Components in Food. *Elsevier*.

[60]. Bignardi, C., Mattarozzi, M., Penna, A., 2013, A rapid size-exclusion solid-phase extraction step for enhanced sensitivity in multi-allergen determination in dark chocolate and biscuits by liquid chromatography–tandem mass spectrometry. *Food Analytical Methods*. 6(4):1144-1152. Doi:10.1007/s12161-012-9521-4

[61]. Petyaev, I.M., Bashmakov, Y.K., 2017, Dark chocolate: Opportunity for an alliance between medical science and the food industry? *Frontiers in Nutrition*. 4:43. Doi:10.3389/fnut.2017.00043

[62]. Yeo, Y.Y., Thed, S.T., 2022, Product development of passion fruit and citrus peel dark chocolate. *Food Research*. 6(S1):41-44. Doi:10.26656/fr.2017.6(s1).010.