



Review

Functional chocolate: exploring advances in production and health benefits

Sümeyye Sarıtaş,¹ Hatice Duman,¹ Burcu Pekdemir,¹ João Miguel Rocha,^{2,3,4} Fatih Oz⁵  & Sercan Karav^{1*} 

1 Department of Molecular Biology and Genetics, Çanakkale Onsekiz Mart University, Çanakkale 17000, Turkey

2 Universidade Católica Portuguesa, CBQF—Centro de Biotecnologia e Química Fina—Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

3 LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto (FEUP), Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal

4 ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto (FEUP), Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal

5 Department of Food Engineering, Faculty of Agriculture, Ataturk University, Erzurum 25240, Turkey

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Summary Chocolate has been a part of human consumption for millennia, serving as a confection, medicine and aphrodisiac. Chocolate consumption is increasing worldwide, and independent of the age or social background. The substantial content of chocolate may provide consumers with antioxidant, anti-inflammatory, antimicrobial, antiallergenic, and anticarcinogenic benefits. Beyond such properties, diverse bioactive ingredients are utilised in the creation of functional chocolate products aiming at promoting health and meeting the modern consumers' demands and market orientations. These products are primarily focused on enhancing nutraceutical effects, such as antioxidant activity, protein content and prebiotic effects. Additionally, the use of A2 milk powder in chocolate production holds promising expectations towards enhancing the digestibility of the products. Due to the superior affinity of proteolytic enzymes, A2 milk can be digested more easily than A1 milk. In this way, with the addition of A2 milk to chocolate, it may become more easily digestible. The objectives of this review are a comprehensive understanding of the evolution of chocolate consumption, its health benefits, and the contemporary innovations in chocolate production. Additionally, the potential for developing easily digestible, functional chocolates made from A2 milk, which could rejuvenate functional chocolate production, is discussed in this article.

Keywords A2 beta-casein, A2 milk, bioactive components, cocoa, digestibility, functional chocolate, health.

Introduction

Chocolate consumption has increased extremely in the world in the 21st century. The allure of this food is mostly due to its ability to induce pleasant sensations and uplifting feelings. From a nutritional and pharmacological perspective, chocolate and cocoa have been connected to the prevention and treatment of various diseases, especially obesity, owing to their antioxidant, anti-inflammatory, cardiovascular and metabolic effects (Loffredo *et al.*, 2016; Godočiková *et al.*, 2017). With the growing recognition of the connection between nutritious eating and wellness, the food industry has started producing functional foods, a relatively new

category that offers health advantages beyond basic nutrition (Büker *et al.*, 2021). The main purpose of the food industry is to increase the protein content, the antioxidant activity and the prebiotic and probiotic effects of the products (Hossain *et al.*, 2021a; Jovanović *et al.*, 2022). In this regard, chocolate attracts an enormous interest in the functional food sector due to its high level of nutritional content, health benefits and popularity (Tolve *et al.*, 2018).

The deep-rooted history of chocolate began with the cultivation of the *Theobroma*, known as the cocoa tree, by the Maya people in South America (Muhammad *et al.*, 2019; Monteiro *et al.*, 2023). For the Maya, the beverage consisting of cocoa mixed with hot water and sweetened by adding spices is described as chocolate (Lippi, 2015). Among the Aztecs, cocoa and chocolate were described as the 'Food of the Gods'

*Correspondent: Fax: +905513866466; e-mail: sercankarav@comu.edu.tr

and were consumed as a beverage for ceremonial purposes. Additionally, they used chocolate as a therapeutic drug because they believed it had medicinal properties (Monteiro *et al.*, 2023). Cocoa, brought to Europe with an understanding of its commercial value in the 16th century, has been described as ‘brown gold, and, consequently, the consumption of chocolate prepared from cocoa rapidly became widespread for different purposes such as treatment of some diseases (Lippi, 2015)’.

The composition of cocoa beans may differ as a result of genetic and environmental variables (Żyżelewicz *et al.*, 2021). According to different cocoa samples, they are known to contain 0.9%–8.1% of moisture, 0.9%–7.3% of ash and 3.1%–19.4% of fibre (Aremu *et al.*, 1995; Lippi, 2015; Djikeng *et al.*, 2018; de Melo *et al.*, 2020; Grassia *et al.*, 2021). Cocoa beans contain 8%–54% carbohydrates, comprising monosaccharides, disaccharides, oligosaccharides, sugar alcohols, starch and dietary fibres (Liendo *et al.*, 1997; de Melo *et al.*, 2020). Due to its high carbohydrate content, it is well recognised to have bioactive characteristics (Barišić *et al.*, 2019). The composition of carbohydrates is significant for the development of taste in the manufacture of chocolate (Jean-Marie *et al.*, 2022). Proteins constitute 7.9%–22.5% of the dry weight of cocoa beans (Agus *et al.*, 2018; de Melo *et al.*, 2020). They contain mainly albumin and globulin proteins (Rawel *et al.*, 2019; Jean-Marie *et al.*, 2022) containing high levels of lysine, glutamic acid, aspartic acid, arginine and leucine amino acids (Barišić *et al.*, 2019; Jean-Marie *et al.*, 2022). The determination of protein-based flavourings may be crucial for flavour development in chocolate manufacturing (Rawel *et al.*, 2019). In addition, cocoa beans contain 33%–62% cocoa butter, almost all of which are composed of triacylglycerols (TAG's) (de Melo *et al.*, 2020). TAG's contain varying amounts of palmitic, stearic and oleic acids, ranging from 24% to 38%, and low amounts of linoleic acid (Barišić *et al.*, 2019; Febrianto *et al.*, 2022). The composition of cocoa butter, extractability, melting point and crystallisation properties are significant criteria for chocolate production (Barišić *et al.*, 2019). These factors significantly influence the texture, flavour and overall quality of chocolate products, highlighting the importance of understanding TAG composition and cocoa butter characteristics in the chocolate-producing process.

There are approximately 30–50 olive-sized seeds in the shell of the cocoa fruit, and these beans are referred to as cocoa, which is pivotal as the primary ingredients in chocolate (Jean-Marie *et al.*, 2022). The transformation of cocoa into chocolate occurs as a result of a series of processes that begin with the cocoa bean (Muhammad *et al.*, 2020; Bólek *et al.*, 2022). These processes can be listed as the fermentation of the cocoa bean, drying, roasting and then, grinding

the bean to obtain cocoa liquor, followed by the production of various chocolates by adding other ingredients (Żyżelewicz *et al.*, 2021; Angor *et al.*, 2023). The components in cocoa vary greatly depending on the bean variety and origin as well as the agricultural and processing processes; this process may lead to alterations in the composition of chocolate (Barišić *et al.*, 2019; Rawel *et al.*, 2019). The main commercial categories of chocolate are milk, dark and white chocolate, which are manufactured using different proportions of ingredient in chocolate production (Rawel *et al.*, 2019; Jovanović *et al.*, 2022). Moreover, other seeds may be found to produce chocolate-like products using similar processes as cocoa, for example, the *cupuacu* seeds (Souza *et al.*, 2020).

Chocolate has been enhanced with a variety of useful ingredients since it is not only seen as a sweet delight but also as a product that promotes a healthy way of living. People are now gravitating towards nutrient-dense, naturally occurring resources due to the quickly growing trend of healthy eating and conscientious consumerism (Sik *et al.*, 2021; Shahbazi *et al.*, 2022). During the COVID-19 epidemic, consumer interest in functional foods has increased significantly. In light of this, the food industry has started to develop novel foods that can be enhanced with a variety of bioactive substances in order to promote healthy living and well-being, as well as prevent and treat degenerative and chronic illnesses (Sik *et al.*, 2021; Darwish *et al.*, 2023). In summary, the chocolate industry is always changing to meet the needs of consumers and the latest trends in healthy living, which results in a constant stream of new, useful products being introduced. This transformational process repositions chocolate as a smart option for people who are health-conscious, going beyond the conventional view of it as a delicious indulgence. The advantages of chocolate as well as the investigations and analyses of the present functional chocolates are outlined in this review. Furthermore, it highlights how A2 milk can be used in this situation as a creative way to improve the functional qualities of chocolate.

Chocolate production and its variations

Chocolate production consists of basic steps such as the harvesting and processing of cocoa beans, followed by the transformation of the resulting product into various types of chocolate. Cocoa beans, the raw material for chocolate production, are known as the most significant source of phenolic compounds. Considering the importance of the nutritional and health values of the products obtained after production, the conditions and parameters of the methods and technological processes used in chocolate production are of great importance (Kowalski *et al.*, 2023).

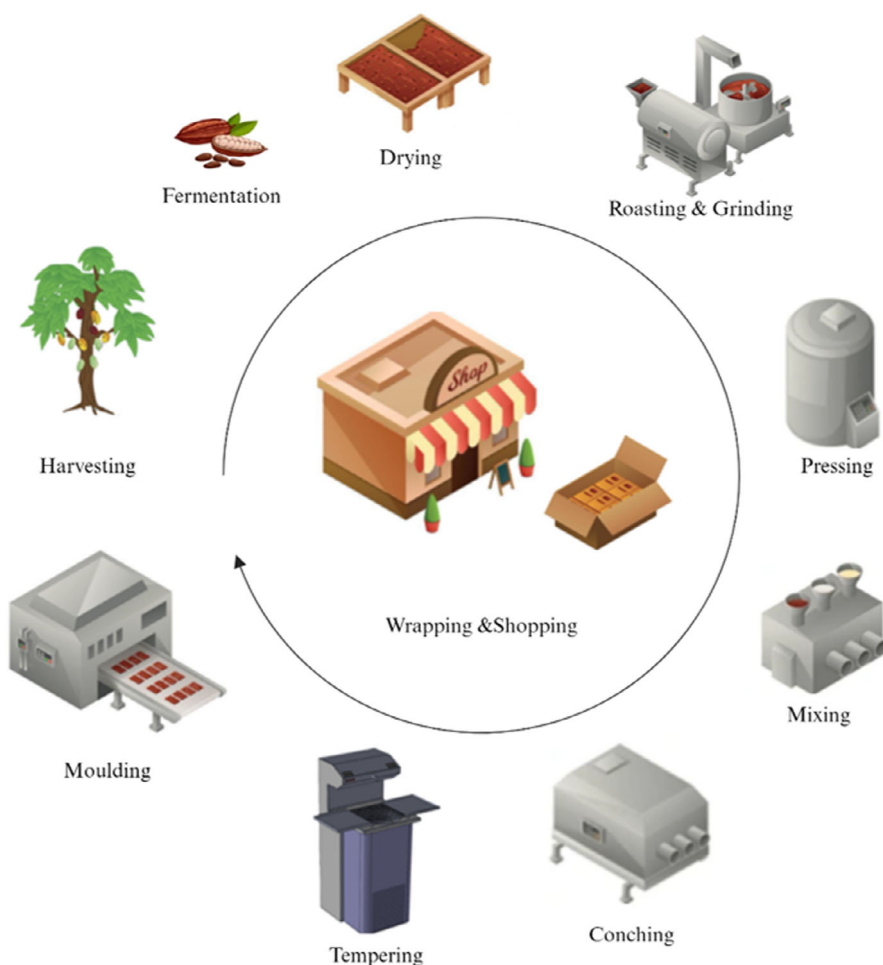


Figure 1 Schematic representation of the basic chocolate production process.

The fermentation process, which is the next step after harvesting cocoa beans for chocolate production, is known as one of the main factors that affect flavour formation and bitterness removal, as well as the chemical and bioactive composition of cocoa beans. In a study, it has been shown that the total phenolic content and antioxidant level of cocoa beans increase in the later days of the fermentation process (Tee *et al.*, 2022). Following the fermentation process, various methods such as sun drying, air drying, mechanical drying, microwave drying and freeze-drying are used in the drying process to prevent mould formation, stabilise aroma compounds and reduce moisture content in the beans (Fig. 1). As the moisture content decreases, the water activity of the food also decreases. Temperature and drying time are key parameters directly affecting the quality of cocoa beans. Additionally, it is important to prefer any drying method that minimises the exposure of the food to ultraviolet light,

oxidation and heat to preserve the bioactive components necessary for high product quality (Dzelagha *et al.*, 2020). After the drying process, the beans move on to the roasting process to fully develop their flavours through caramelization and enhance their taste. This process affects the sensory parameters of the final product, chocolate. As a result of applying high temperatures during roasting, cocoa beans are sterilised, but a decrease in total phenolic content and total flavonoid content is observed after this process, which takes place for 5–120 min in the range of 120–150 °C (Kowalski *et al.*, 2023). Following the roasting process, cocoa beans are ground into a fine paste called cocoa mass or cocoa liquor using various types of equipment such as ball mills, conche machines or roller refiners (Bolenz & Langer, 2020).

After the production of cocoa mass, it can be further processed by pressing to produce cocoa powder and cocoa butter. Cocoa solids are used to make

cocoa powder, while cocoa butter is used to add fat content to chocolate products and create a smooth texture. A study has shown that the pressing time affects the brightness of cocoa powder and the fat content, but not the temperature (Anoraga *et al.*, 2019). Once cocoa powder and cocoa butter are produced through grinding and pressing, the chocolate-making process can begin. Chocolate liquor is refined in a machine called a conche, which mixes and aerates the chocolate to develop its texture and flavour (Toker *et al.*, 2019). After refining the chocolate mixture, it undergoes a tempering stage based on heating and cooling to specific temperatures to ensure it has the correct crystal type and texture. This process, which gives chocolate a shiny appearance, also helps prevent the formation of air bubbles. Tempered chocolate is then moulded into bars, bonbons, or various shapes and packaged as chocolate products (Quiñones-Muñoz *et al.*, 2011).

Differences in ready-to-consume chocolate varieties are diversified based on their flavour profiles and utilisation in culinary applications, leading to each chocolate type acquiring distinct characteristics of its own. One type of chocolate, bitter chocolate, is made from cocoa solids, cocoa butter and sugar, with minimal or no milk solids added. It boasts a rich, intense flavour profile and higher cocoa content (Nouri *et al.*, 2019). Compared to bitter chocolate, milk chocolate presents a creamier texture and a sweeter taste, comprising cocoa solids, cocoa butter, sugar and milk powder or condensed milk for incorporation (Liang & Hartel, 2004). White chocolate, on the other hand, is made from cocoa butter, sugar, and milk solids but does not contain cocoa solids. Compared to dark or milk chocolate, it exhibits a milder and creamier taste profile (Verdet, 2003). Ruby chocolate, characterised by its natural pink hue, is a newer variety made from cocoa beans. It features a fruity, slightly tangy flavour profile (Tuenter *et al.*, 2021). Filled chocolates are chocolates containing various fillings such as caramel, nuts, fruits or cream, thereby offering a variety of textures and flavours. The occurrence of bloom in filled chocolates is contingent upon the interplay between liquid oil and solid fat (De Clercq *et al.*, 2014). Each type of chocolate has its unique characteristics, flavour profile and uses in culinary applications.

The recommended daily consumption of chocolate and its derivatives varies depending on factors such as age, overall health status and specific dietary requirements. Generally, consuming moderate amounts of dark chocolate with high cocoa content (at least 70% cocoa solids) has been associated with potential health benefits due to its rich antioxidant content, particularly flavonoids. These benefits may include improved cardiovascular health, cognitive function and mood regulation (Montagna *et al.*, 2019; Lampport *et al.*, 2020). For adults, consuming approximately 20–30 g (about

1–2 small squares) of dark chocolate per day may be considered as part of a balanced diet. However, it is imperative to be mindful of portion sizes and overall calorie intake, as chocolate is calorically dense, and excessive consumption may contribute to weight gain and other health issues (Buitrago-Lopez *et al.*, 2011; Nehlig, 2013; West *et al.*, 2014). In contrast, milk chocolate and white chocolate contain higher amounts of sugar and saturated fat and lower levels of cocoa solids compared to dark chocolate. Therefore, their consumption should be limited (Wollgast & Anklam, 2000). In summary, incorporating moderate amounts of dark chocolate into a balanced diet rich in fruits, vegetables, whole grains and lean proteins can contribute to overall health and well-being. However, maintaining moderation and balance is crucial, and individuals with specific health concerns or dietary restrictions should seek personalised recommendations from healthcare professionals or registered dietitians.

Health benefits of chocolate

Polyphenols, a source of nutritional and therapeutic value, make up to 10% of the dry weight of cocoa beans (Komes *et al.*, 2013; Carvalho *et al.*, 2018). The polyphenols are characterised as free radical scavengers, demonstrating antioxidant and anti-inflammatory effects that contribute to the improvement of immune function (Nambiar *et al.*, 2018; Zohreh, 2020). Researches indicate that the increase in plasma polyphenols after chocolate consumption leads to improved plasma antioxidant capacity and a reduction in plasma lipid oxidation (Wang *et al.*, 2000; Di Mattia *et al.*, 2017; Leyva-Soto *et al.*, 2018). Furthermore, it has been established that the consumption of polyphenol-rich cocoa and chocolate under conditions requiring high physical exertion enhances the antioxidant status and the oxidative stress response (Table 1) (Faria *et al.*, 2011; Gadhiya *et al.*, 2018). It is believed to affect the oxidative and inflammatory states, which may be connected to chocolate's bioactive composition's capacity to modify the microbiome (Fraga *et al.*, 2005; Gadhiya *et al.*, 2018).

The non-flavonoids and flavonoids found in cocoa include procyanidins, epicatechins, catechins and phenolic acid (Laličić-Petronijević *et al.*, 2017; Hossain *et al.*, 2022a). It is known that cocoa flavonoids cause a bitter taste, notably in chocolate types containing high cocoa (Febrianto *et al.*, 2022). In healthy adults, the consumption of flavonoid-rich dark chocolate improves endothelial and platelet functions, which may be associated with higher plasma epicatechin concentrations (Table 1) (Ostertag *et al.*, 2013; West *et al.*, 2014; Loffredo *et al.*, 2018). In addition, chocolate polyphenols have some protective effects on the cardiovascular system (Nambiar *et al.*, 2018; Pereira *et al.*, 2019). The consumption of foods high in

Table 1 Health impact related to consumption of chocolate

Health impact	Chocolate type	Outcome	References
Cardiovascular diseases and chronic diseases related	Dark chocolate	<ul style="list-style-type: none"> Exhibit a reduction in risk of essential hypertension Exhibit a reduction in plasma levels of tumour necrosis factor-α 	Yang <i>et al.</i> (2024) Ribeiro <i>et al.</i> (2023)
	Sugar-free dark chocolate	<ul style="list-style-type: none"> Exhibit an improvement in blood glucose level 	Oliveira <i>et al.</i> (2022)
	Milk and dark chocolate	<ul style="list-style-type: none"> Exhibit an improvement in endothelial functions 	Dural <i>et al.</i> (2022)
	Dark chocolate	<ul style="list-style-type: none"> Exhibit a reduction in blood pressure 	Matsumoto <i>et al.</i> (2020)
	Polyphenol-rich dark chocolate	<ul style="list-style-type: none"> Exhibit a reduction in postprandial plasma glucose elevations 	Kawakami <i>et al.</i> (2021)
	Cocoa-rich chocolate	<ul style="list-style-type: none"> Exhibit improvement in vascular function 	Pereira <i>et al.</i> (2019)
	Flavanol-enriched dark chocolate	<ul style="list-style-type: none"> Exhibit a reduction in genotoxic stress effect 	Leyva-Soto <i>et al.</i> (2018)
	Dark chocolate	<ul style="list-style-type: none"> Exhibit an improvement in endothelial functions 	Loffredo <i>et al.</i> (2018)
	†	<ul style="list-style-type: none"> Exhibit a reduction in the risk of type 2 diabetes mellitus 	Mathew <i>et al.</i> (2017)
	Dark chocolate	<ul style="list-style-type: none"> Exhibit an improvement in lipid profile 	Lee <i>et al.</i> (2017)
		<ul style="list-style-type: none"> -Exhibit an improvement in lipid profile Exhibit an improvement in high-density lipoprotein cholesterol 	Souza <i>et al.</i> (2017)
		<ul style="list-style-type: none"> Exhibit an improvement in high-density lipoprotein cholesterol Exhibit an improvement in arterial elasticity 	da Teixeira <i>et al.</i> (2017)
	Epicatechin-rich dark chocolate	<ul style="list-style-type: none"> Exhibit an improvement in exercise capacity Exhibit an improvement in high-density lipoprotein cholesterol 	Taub <i>et al.</i> (2016)
	Dark chocolate	<ul style="list-style-type: none"> Exhibit an antioxidant activity due to NOX₂ down-regulation 	Loffredo <i>et al.</i> (2016)
		<ul style="list-style-type: none"> Exhibit an improvement in vascular function 	Esser <i>et al.</i> (2014)
		<ul style="list-style-type: none"> Exhibit an improvement in endothelial function and arterial stiffness 	West <i>et al.</i> (2014)
		<ul style="list-style-type: none"> Exhibit reduction risk of heart failure 	Petrone <i>et al.</i> (2014)
	Flavanol-enriched dark chocolate	<ul style="list-style-type: none"> Exhibit an improvement in platelet function 	Ostertag <i>et al.</i> (2013)
	Dark chocolate	<ul style="list-style-type: none"> Exhibit an improvement in high-density lipoprotein cholesterol Exhibit reduction in abdomen circumference 	Di Renzo <i>et al.</i> (2013)
		<ul style="list-style-type: none"> No significant difference in the short-term consumption of dark chocolate on the blood pressure 	Chan <i>et al.</i> (2012)
	Cocoa product	<ul style="list-style-type: none"> Exhibit a reduction in the blood cholesterol 	Jia <i>et al.</i> (2010)
	Dark chocolate	<ul style="list-style-type: none"> Exhibit an increase in the plasma antioxidant capacity Exhibit an improvement in the oxidative stress markers 	Spadafranca <i>et al.</i> (2010)

Table 1 (Continued)

Health impact	Chocolate type	Outcome	References
	Flavonoid-rich dark chocolate	• Exhibit an improvement in endothelial function	Shiina <i>et al.</i> (2009)
	Dark chocolate	• Exhibit a reduction of serum C-reactive protein levels • Exhibit a reduction of inflammation	di Giuseppe <i>et al.</i> (2008)
	High-polyphenol dark chocolate	• Exhibit a reduction in blood pressure • Exhibit an improvement in the insulin sensitivity	Grassi <i>et al.</i> (2008)
	Polyphenol-rich dark chocolate	• Exhibit a reduction in blood pressure • Exhibit an improvement in the insulin sensitivity	Grassi <i>et al.</i> (2005)
	Flavanol-rich milk chocolate	• Exhibit an improvement in the oxidative stress markers	Fraga <i>et al.</i> (2005)
	Procyanidin-rich chocolate	• Exhibit an increase in the plasma antioxidant capacity • Exhibit a decrease in plasma lipid oxidation	Wang <i>et al.</i> (2000)
Performance related	White, milk and dark chocolate	• Exhibit a reduction in induced pain intensity	Hajati <i>et al.</i> (2023)
	Dark chocolate	• Modulate obesity-induced inflammation	Eskandari <i>et al.</i> (2020)
		• Exhibit a reduction in oxidative stress markers • Exhibit a reduction in markers of muscle damage	Cavarretta <i>et al.</i> (2018)
		• Modulate muscle carbohydrate partitioning • No significant difference in cycling time-trial	Stellingwerff <i>et al.</i> (2014)
		• Exhibit an increase in antioxidant capacity	Davison <i>et al.</i> (2012)
		• Exhibit a reduction in oxidative stress markers • Maintenance mobilisation of plasma-free fatty acids	Allgrove <i>et al.</i> (2011)
Psycho-pharmacological activities related		• Modulate metabolic changes	Djarova <i>et al.</i> (2009)
	Polyphenol-rich dark chocolate	• Exhibit improvement in cognitive performance and concentration during continuous and demanding tasks	Sasaki <i>et al.</i> (2024)
	The combination of chocolate with fish oil and probiotics	• Exhibit improvement in spatial memory and learning	Faccinnetto-Beltrán <i>et al.</i> (2022)
	Dark chocolate	• Exhibit a reduction in fatigue • Exhibit improvement in cognitive function	Nemoto <i>et al.</i> (2022)
		• Exhibit improvement in memory, memory consolidation and locomotor activity	Kalantarzadeh <i>et al.</i> (2020)
		• Exhibit improvement in cognitive performance • Exhibit an increase in nerve growth factor in plasma	Sumiyoshi <i>et al.</i> (2019)
		• Exhibit a reduction in absolute power of the Delta and Theta bands, and improvement in absolute power of the Alpha and Beta bands in most brain regions, especially in the temporo-occipital leads	Santiago-Rodríguez <i>et al.</i> (2018)

Table 1 (Continued)

Health impact	Chocolate type	Outcome	References
Gut health related	Flavanol-rich chocolate	• Exhibit improvement in working memory following sleep deprivation	Grassi <i>et al.</i> (2016)
	Dark chocolate	• Modulate exercise-induced intestinal permeability	Nocella <i>et al.</i> (2023)
		• Exhibit improvement in gut microbial metabolic activity	Martin <i>et al.</i> (2012)
		• Exhibit improvement in high-density lipoprotein cholesterol	
Dental health		• Modulate energy metabolism and intestinal microbial activities	Martin <i>et al.</i> (2009)
	Milk chocolate	• Exhibit an increase in the level of gingival inflammation	Fahmi <i>et al.</i> (2023)
	Probiotic chocolates	• Exhibit a reduction in acidogenic properties	Janani <i>et al.</i> (2019)
	White, milk, dark and caramel chocolate	• Exhibit alterations in dental plaque pH	Vasanthakumar <i>et al.</i> (2016)

†Not identified.

flavonoids is inversely related to the risk of cardiovascular disease (Shiina *et al.*, 2009; Esser *et al.*, 2014; Magrone *et al.*, 2017). In the study conducted by Yang *et al.* (2024), the effect of dark chocolate on various cardiovascular risk factors was investigated. According to the results of the study, it was revealed that dark chocolate may have potential benefits for the cardiovascular system, including improving endothelial function, coronary artery vasodilation, preventing platelet adhesion and reducing blood lipid levels (Yang *et al.*, 2024). Another study conducted by Ribeiro *et al.* (2023) aimed to evaluate the effects of dark chocolate consumption containing 70% cocoa on inflammation and oxidative stress markers in patients with chronic kidney disease on haemodialysis. According to this study, while chocolate consumption caused a decrease in plasma tumour necrosis factor- α levels of participants, no clear effect was determined on oxidative stress markers.

In a study conducted by Allgrove *et al.* (2011), the effects of regular consumption of dark chocolate before extensive long-term exercise on plasma metabolites, hormones and oxidative stress markers were investigated. The research results suggest that consuming dark chocolate may help reduce oxidative stress in the body during physical activity (Allgrove *et al.*, 2011). Furthermore, it has been demonstrated that dark chocolate consumption can contribute to increased mobilisation of plasma-free fatty acids, which are significant for energy production during exercise (Allgrove *et al.*, 2011). Despite these findings, the consumption of dark chocolate did not significantly affect the plasma concentrations of the

measured hormones or cytokines (Allgrove *et al.*, 2011). Elite football athletes face high oxidative stress and muscle injuries due to intense physical exercise (Djarova *et al.*, 2009; Cavarretta *et al.*, 2018). Consistent with other studies on athletes (Davison *et al.*, 2012; Hajati *et al.*, 2023), according to a study conducted by Cavarretta *et al.* (2018), regular supplementation with dark chocolate appeared to enhance antioxidant capacity and decrease markers of muscle damage. In another study involving healthy volunteers, the effects of consuming dark chocolate and white chocolate on plasma epicatechin levels, DNA resistance to oxidative stress, and total antioxidant activity were compared (Spadafranca *et al.*, 2010). The results indicate that while consumption of dark chocolate improves DNA resistance against oxidative stress and enhances antioxidant activity, no significant effects were observed with white chocolate consumption (Spadafranca *et al.*, 2010). Additionally, the consumption of flavonoid-rich chocolate has a significant impact on diseases including diabetes, stroke and heart diseases (Petrone *et al.*, 2014; Lee *et al.*, 2017; Maskarinec *et al.*, 2019).

Several studies have examined the relationship between type 2 diabetes (T2D) and chocolate consumption (Mathew *et al.*, 2017; Oliveira *et al.*, 2022). According to a study, findings indicate that sugar-free dark chocolate sweetened with stevia, erythritol and inulin leads to a decrease in postprandial blood sugar levels in individuals with diabetes and that sugar-free chocolates can be used as a potential benefit for glucose control in these individuals (Oliveira *et al.*, 2022). Another study published in 2022 revealed that

chocolate consumption acutely reduces N-terminal pro-brain natriuretic peptide values in heart failure (Dural *et al.*, 2022). It has also been determined that the consumption of dark chocolate improves endothelial functions by increasing dilation values (Dural *et al.*, 2022).

Chocolate is considered to have a beneficial impact on lipid metabolism, potentially increasing levels of high-density lipoprotein (HDL) cholesterol and decreasing levels of low-density lipoprotein (LDL) cholesterol (Table 1) (Jia *et al.*, 2010; Di Renzo *et al.*, 2013; Vargas-rechia *et al.*, 2015; Taub *et al.*, 2016; da Teixeira *et al.*, 2017; Souza *et al.*, 2017). In a study conducted by Taub *et al.* (2016), the consumption of dark chocolate was found to be associated with an increase in HDL levels and a decrease in triglyceride levels among sedentary subjects (Taub *et al.*, 2016). Additionally, the research demonstrated a decrease in oxidative stress indicators in skeletal muscle, indicating an improvement in exercise efficiency and general well-being (Taub *et al.*, 2016). A recent study suggests that dark chocolate supplementation, combined with jump rope exercise, may positively affect inflammatory markers, adipokines and body composition in obese adolescent boys (Eskandari *et al.*, 2020).

Some chocolate varieties have been linked to lower blood pressure and increased insulin sensitivity in healthy people (Grassi *et al.*, 2005, 2008; Matsumoto *et al.*, 2020; Kawakami *et al.*, 2021). According to a randomised crossover study involving 20 middle-aged Japanese volunteers, the consumption of dark chocolate was associated with blood pressure-lowering effects (Matsumoto *et al.*, 2020). Another study aimed to evaluate short-term changes in children's blood pressure by providing dark chocolate to schools. The results of the study indicated that there were no significant differences in the short-term blood pressure levels of the children (Chan *et al.*, 2012).

The study by di Giuseppe *et al.* (2008) aimed to investigate the relationship between consuming dark chocolate and serum C-reactive protein (CRP) levels (di Giuseppe *et al.*, 2008). C-reactive protein serves as a marker of inflammation in the body, and elevated levels are associated with an increased risk of chronic diseases such as cardiovascular disease (di Giuseppe *et al.*, 2008). According to the study's findings, an inverse relationship was observed between dark chocolate consumption and CRP concentrations (di Giuseppe *et al.*, 2008), suggesting that regular consumption of dark chocolate may reduce inflammation (di Giuseppe *et al.*, 2008). The bioactive compounds in chocolate may help prevent and treat certain types of cancer (Lippi, 2015; Saadatdoust *et al.*, 2015). With a potent antiproliferative function, these bioactive compounds may inhibit cancer cell growth and tumour development (Saadatdoust *et al.*, 2015).

The intense physical activity performed by football athletes can cause increased intestinal permeability, allowing harmful substances such as lipopolysaccharides (LPS) to enter the bloodstream and cause damage (Nocella *et al.*, 2023). In a study conducted by Nocella *et al.*, the effect of consuming dark chocolate containing 85% cocoa on the intestinal permeability biomarkers of elite football athletes was examined (Nocella *et al.*, 2023). According to the results of the study, it was revealed that the consumption of dark chocolate may help modulate exercise-induced intestinal permeability among elite football athletes (Nocella *et al.*, 2023). In a study conducted by Martin *et al.*, the effect of dark chocolate consumption on energy metabolism, intestinal microbiota, and stress-related metabolism was investigated (Martin *et al.*, 2009). According to the results of the study, it was revealed that consumption of dark chocolate had beneficial effects on stress-related alterations in energy metabolism and intestinal microbial activities (Martin *et al.*, 2009).

Cocoa products contain theobromine and caffeine, two significant methylxanthines/alkaloids with remarkable health benefits (Table 1) (Karača *et al.*, 2020; Sik *et al.*, 2021; Kongor *et al.*, 2024). Their psycho-pharmacological properties indicate that they can influence the mood that occurs with the consumption of cocoa and chocolate (Yeo & Thed, 2022). On the other hand, chocolate has several other psycho-pharmacological activities that increase cognitive performance, including preventing hearing loss, increasing contrast sensitivity, improving visual acuity and decreasing mental tiredness (Santiago-Rodríguez *et al.*, 2018; Sumiyoshi *et al.*, 2019). The study conducted by Sasaki *et al.*, aimed to determine whether consuming chocolate with high polyphenol content helps maintain cognitive performance during challenging tasks (Sasaki *et al.*, 2024). According to the results of the study, it was revealed that the consumption of chocolate containing high polyphenols protected cognitive performance and improved concentration (Sasaki *et al.*, 2024). A recent study conducted by Nemoto *et al.* aimed to determine whether the consumption of dark chocolate could reduce fatigue and subsequently improve cognitive function and brain structure (Nemoto *et al.*, 2022). The results showed that the consumption of dark chocolate led to a reduction in both mental and physical fatigue in participants (Nemoto *et al.*, 2022). It has been demonstrated that some chocolate polyphenols reach the brain by crossing the blood-brain barrier and may accumulate in the brain, thus having the possibility of affecting the central nervous system (Faria *et al.*, 2011). The study conducted by Kalantarzadeh *et al.* aimed to investigate the effects of different dark chocolate diets on memory functions and brain corticosterone levels in rats under chronic

stress (Kalantarzadeh *et al.*, 2020). Various dark chocolate diets showed different effects on brain functions under chronic stress (Kalantarzadeh *et al.*, 2020). According to the results of the study, while restricted and compulsory dark chocolate diets appeared to positively affect memory under stress, memory consolidation, locomotor activity, serum and hippocampal corticosterone levels improved only in the compulsory dark chocolate diet (Kalantarzadeh *et al.*, 2020). The raw material of chocolate, cocoa, has the potential to be used as a preventive agent for some neurodegenerative diseases, including Alzheimer's disease (Faria *et al.*, 2011; Cerit *et al.*, 2016).

Additionally, cocoa is rich in magnesium, selenium, zinc, iron elements and vitamins providing individuals with essential daily vitamins and minerals through chocolate consumption (Febrianto *et al.*, 2022). Other beneficial effects of chocolate consumption can include a suppression of appetite and the maintenance of circadian fitness under conditions of shift work or jet-lag (Lippi, 2015; Succi *et al.*, 2017).

In addition to the beneficial health effects associated with incorporating chocolate into the diet, there are several health concerns linked to excessive chocolate consumption (Ren *et al.*, 2019; Sanlier *et al.*, 2022). These include weight gain, dental issues and diabetes (Greenberg & Buijsse, 2013; Vasanthakumar *et al.*, 2016; Sanlier *et al.*, 2022; Fahmi *et al.*, 2023). Such problems typically arise from the overconsumption of chocolates high in sugar and fat content (Ren *et al.*, 2019). A recent study conducted by Fahmi *et al.*, involving a total of 120 children aged 6–12, aimed to investigate the relationship between milk chocolate consumption habits, chronotype, and gingivitis in children during the mixed dentition period (Fahmi *et al.*, 2023). The findings revealed that both milk chocolate consumption habits and chronotype could potentially influence the development or severity of gingivitis in children during the mixed dentition period (Fahmi *et al.*, 2023). In another study examining the effect of chocolate on oral health in children, it was determined that probiotic chocolates were less acidogenic than other chocolates (Janani *et al.*, 2019). These findings suggest that chocolate can be used as a probiotic carrier and promote oral health (Janani *et al.*, 2019). This study offers a new approach to the development of chocolate formulations that can contribute to oral health for children (Janani *et al.*, 2019).

In the context of diabetes, body weight is positively correlated with T2D (Oliveira *et al.*, 2022). At this point, it is crucial to consider consuming chocolates with high sugar and fat content (Oliveira *et al.*, 2022).

Furthermore, consuming large quantities of chocolate can lead to insomnia and an increased heart rate due to its caffeine and theobromine content (Latif & Majeed, 2020; Hernández-González *et al.*, 2021). A

recent study showed that the timing of chocolate intake in the morning or evening/night affected sleep patterns and temperature rhythms (Hernández-González *et al.*, 2021). However, another study aimed to investigate the effects of consuming chocolate rich in flavanols on cognitive abilities and cardiovascular parameters following sleep deprivation (Grassi *et al.*, 2016). According to the results of the study, the consumption of flavanol-rich chocolate may enhance cognitive performance, particularly working memory, after sleep deprivation (Grassi *et al.*, 2016). It is also hypothesized that sleep deprivation may mitigate the negative effects on cardiovascular parameters (Grassi *et al.*, 2016).

On the other hand, in a study conducted by Latif & Majeed (2020), the relationship between chocolate consumption frequency and heart rate variability in young women was investigated. The study divided participants into three groups: 'No regular chocolate consumption', '2–4 servings/week' and '5 or more servings/week' (Latif & Majeed, 2020). However, no significant difference in heart rate variability parameters was observed among these groups (Latif & Majeed, 2020).

Additionally, some individuals may experience migraines or allergic reactions as a result of excessive chocolate intake (Khuda & Williams, 2015; Aladdin *et al.*, 2022). Consequently, the consumption of dark chocolate with a higher cocoa content is considered a favourable approach to health, owing to its antioxidant properties and reduced sugar content.

Current functional chocolate products

Today, the rapidly increasing trend of healthy eating and conscious consumption has driven people towards nutritious, natural and healthy resources (Sik *et al.*, 2021; Shahbazi *et al.*, 2022). Consumer's interest in functional foods has particularly accelerated during the COVID-19 pandemic. Under this framework, the food industry has begun to develop new foods that can be fortified with various bioactive ingredients to prevent and treat chronic and degenerative diseases as well as to support healthy living and well-being (Sik *et al.*, 2021; Darwish *et al.*, 2023). These foods are known as functional foods and they may be manufactured by the addition of bioactive compounds to food compositions (Fig. 2) (Gültekin-Özgülven *et al.*, 2016). Additionally, the varieties of chocolate – including white, dark and milk chocolate – exhibit varying effects on the production process of functional chocolate (Kavitha *et al.*, 2016; Sik *et al.*, 2021). The selection of chocolate variety represents an important aspect that impacts how functional advantages are delivered in the product.

It is crucial to pinpoint bioactive elements that ensure health advantages, whether by improving

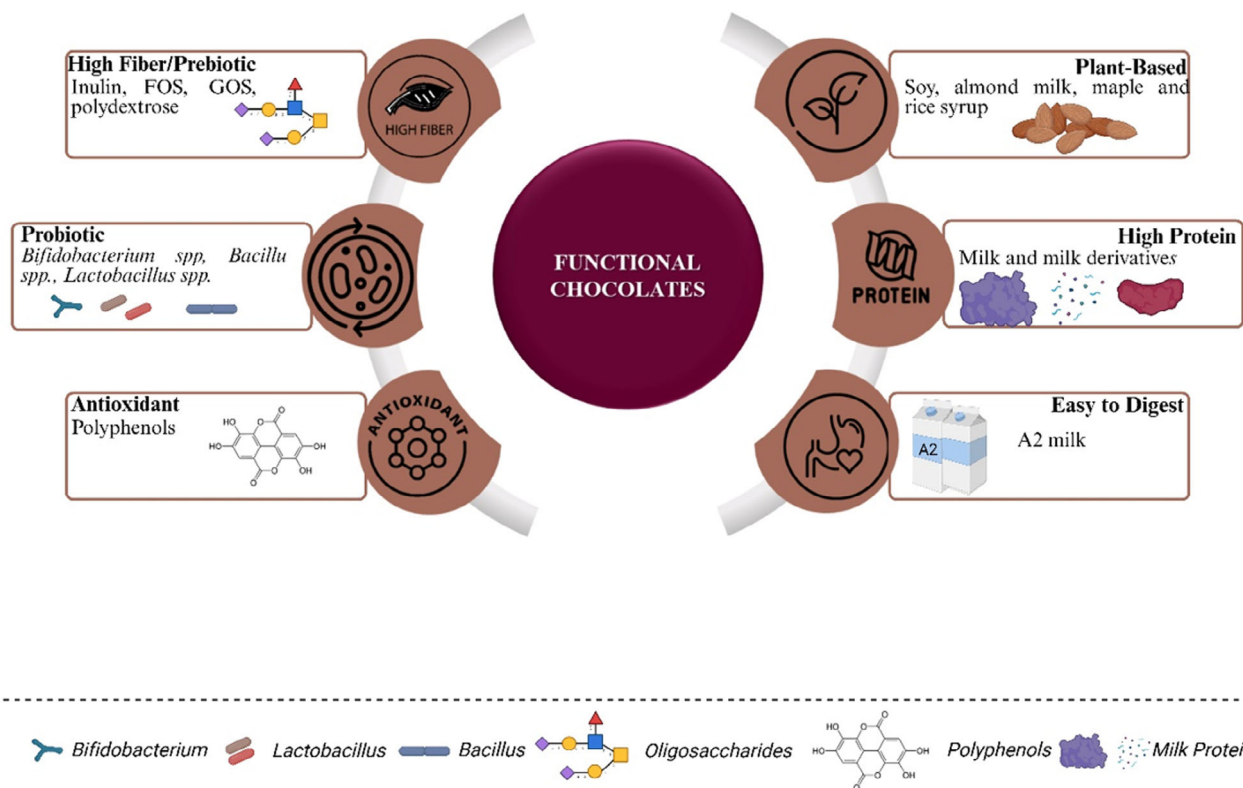


Figure 2 Different types of functional chocolates.

essential nutrition or triggering known healthy effects when crafting functional foods (Table 2) (Sik *et al.*, 2021). Various bioactive substances can be employed for this purpose (Erdem *et al.*, 2014). The principal components that can be utilised as food replacements or enrichment agents include soluble and insoluble fibres, prebiotics, probiotics, postbiotics, paraprobiotics, psychobiotics, parapsychobiotics, synbiotics, minerals, vitamins, herbal extracts and other phytochemicals (Duman *et al.*, 2021; Zarić *et al.*, 2023). These bioactive substances may include essential fatty acids. Fatty acids, carotenoids, tocopherols, polyphenols, phytosterols and oil-soluble vitamins are only a few examples of the hydrophobic nature of most bioactive substances (Muhammad *et al.*, 2021). An essential component of bioactive substances, omega-3 fatty acids, are a subclass of polyunsaturated fatty acids (Gómez-Fernández *et al.*, 2021; Faccinnetto-Beltrán *et al.*, 2022). Several medical diseases, such as coronary heart disease, blood platelet aggregation, high cholesterol levels and many carcinomas, have been shown to be helped by eicosapentaenoic acid and docosahexaenoic acid in both prevention and treatment (Faccinnetto-Beltrán *et al.*, 2022).

High-fibre/Prebiotic-based chocolates

Dietary fibres have gained popularity as useful food additives due to their strong capacity to absorb water, low digestible energy content and claim that they aid the digestive system, leading to their inclusion in food compositions (Bolenz *et al.*, 2006; Lončarević *et al.*, 2021). These health advantages underscore the importance of dietary fibre in terms of nutrition and have encouraged consumers to choose foods with high content in fibre (Lončarević *et al.*, 2021). Various studies have established the value of dietary fibre, with cereal bran, especially wheat bran, being the most popular source of fibrous fibres added to functional food products (Matecki *et al.*, 2020; Lončarević *et al.*, 2021). Numerous research on the addition of dietary fibre to functional food items, including baked goods, drinks, confectionery, dairy, frozen dairy, meat, pasta and soups, have been published (Bolenz *et al.*, 2006; Rezende *et al.*, 2015; Bouaziz *et al.*, 2017). Thus, prebiotics and probiotics can be seen as a promising approach to enhancing the nutritional profile of chocolate and expanding the functional food market (Konar *et al.*, 2014; Silva *et al.*, 2017).

Table 2 Current functional chocolate studies

Functional property	Content	Transmission strategy or delivery system	Chocolate variety	References
Probiotic	<i>Streptococcus thermophilus</i>	Spray-dried	Dark and milk chocolate	Nebesny <i>et al.</i> (2005)
	<i>Lactocaseibacillus casei</i>	Freeze-dried	Dark chocolate	Nebesny <i>et al.</i> (2007)
	<i>Lactocaseibacillus paracasei/casei</i>			
	<i>Lactocaseibacillus casei</i>		Milk chocolate	Żyżelewicz <i>et al.</i> (2010)
	<i>Lactobacillus helveticus</i>	Microencapsulated		Possemiers <i>et al.</i> (2010)
	<i>Bifidobacterium longum</i>			
	<i>Lactiplantibacillus plantarum</i>	–	Dark chocolate	Foong <i>et al.</i> (2013)
	<i>Lactobacillus acidophilus</i>	Freeze-dried	Milk and dark chocolates	Laličić-Petronijević <i>et al.</i> (2015)
	<i>Bifidobacterium lactis</i>		Milk chocolate	Rouhi <i>et al.</i> (2015)
	<i>Lactobacillus acidophilus</i>			
	<i>Lactocaseibacillus casei</i>			
	<i>Lactobacillus brevis</i> subsp. <i>coagulans</i>		Chocolate	Yonejima <i>et al.</i> (2015)
	<i>Lactobacillus brevis</i>			
	<i>Lactobacillus acidophilus</i>		Milk chocolate	Zarić <i>et al.</i> (2016)
	<i>Lactocaseibacillus rhamnosus</i>			
	<i>Bifidobacterium lactis</i>			
	<i>Lactocaseibacillus casei</i>		White, milk and dark chocolates	Kemsawasd <i>et al.</i> (2016)
	<i>Lactobacillus acidophilus</i>		Milk and dark chocolates	Klindt-Toldam <i>et al.</i> (2016)
	<i>Lactobacillus acidophilus</i>		Milk chocolate	Kavitha <i>et al.</i> (2016); Oliveira & Deliza (2021)
	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>			Silva <i>et al.</i> (2017)
	<i>Lactobacillus acidophilus</i>		Chocolate	
	<i>Bifidobacterium animalis</i> subsp. <i>Lactis</i>			
	<i>Lactocaseibacillus paracasei</i>		Milk chocolate	Toker <i>et al.</i> (2017)
	<i>Lactobacillus acidophilus</i>			
	<i>Lactocaseibacillus rhamnosus</i>		Dark chocolate	Succi <i>et al.</i> (2017)
	<i>Lactocaseibacillus paracasei</i>			
	<i>Lactocaseibacillus casei</i>			
	<i>Limosilactobacillus reuteri</i>			
	<i>Lactobacillus acidophilus</i>	Microencapsulated	Milk, semisweet and dark chocolate	Laličić-Petronijević <i>et al.</i> (2017)
	<i>Streptococcus thermophilus</i>			
	<i>Bifidobacterium breve</i>			
	<i>Lactobacillus acidophilus</i>	Freeze-dried	White chocolate	Konar <i>et al.</i> (2018)
	<i>Lactocaseibacillus paracasei</i>			
	<i>Lactiplantibacillus plantarum</i>	Spray-dried	Dark chocolate Milk chocolate	Mirković <i>et al.</i> (2018) Nambiar <i>et al.</i> (2018)
				Gadhiya <i>et al.</i> (2018)
	<i>Lactobacillus helveticus</i>	Freeze-dried	Dark chocolate	Kobus-Cisowska <i>et al.</i> (2019)
	<i>Bacillus coagulans</i>		Chocolate	Hossain <i>et al.</i> (2021b)
	<i>Lactocaseibacillus rhamnosus</i>			Hossain <i>et al.</i> (2021a)
	<i>Lactobacillus acidophilus</i>			
	<i>Lactocaseibacillus rhamnosus</i>			
	<i>Lactobacillus sanfranciscensis</i>			
	<i>Lactiplantibacillus plantarum</i>			
	<i>Lactocaseibacillus casei</i>			
	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>			
	<i>Streptococcus thermophilus</i>			
	<i>Lactiplantibacillus plantarum</i>	Spray-dried	Milk chocolate	Gómez-Fernández <i>et al.</i> (2021); Faccineto-Beltrán <i>et al.</i> (2022)
	<i>Lactocaseibacillus rhamnosus</i>			
	<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	Freeze-dried	Chocolate	Hossain <i>et al.</i> (2022b)
	<i>Lactobacillus acidophilus</i>	–	Milk chocolate	Islam <i>et al.</i> (2022)
	<i>Lactocaseibacillus rhamnosus</i>	Freeze-dried	Chocolate	Hossain <i>et al.</i> (2022a)
	<i>Lactocaseibacillus casei</i>			

Table 2 (Continued)

Functional property	Content	Transmission strategy or delivery system	Chocolate variety	References
Prebiotic	<i>Lactiplantibacillus plantarum</i> <i>Lactobacillus acidophilus</i> <i>Lactobacillus sanfranciscensis</i> <i>Bifidobacterium animalis</i> subsp. <i>lactis</i> Inulin	–	Milk chocolate	Farzanmehr & Abbasi (2009); Konar (2013)
	Fructooligosaccharides	Dried powder		Konar <i>et al.</i> (2014)
Prebiotic and antioxidant	Goji berry (<i>Lycium barbarum</i>)	Dried	White chocolate	Morais Ferreira <i>et al.</i> (2017)
Synbiotic	<i>Lactocaseibacillus casei</i> Inulin	Freeze-dried	Milk chocolate	Mandal <i>et al.</i> (2013)
	<i>Bacillus indicus</i> <i>Lactocaseibacillus paracasei</i> <i>Lactobacillus acidophilus</i> Inulin		Dark chocolate	Erdem <i>et al.</i> (2014) Konar <i>et al.</i> (2017)
Antioxidant activity	<i>Lactobacillus acidophilus</i> Inulin	Dried powder Freeze-dried	Milk chocolate	Toker <i>et al.</i> (2018)
	Baobab Pulp Chilli pepper Orange Raspberry	Powder Dried	Dark chocolate	Monteiro <i>et al.</i> (2023) Jaćimović <i>et al.</i> (2022)
	White tea (<i>Camellia sinensis</i>) Jujube fruit	Free and microencapsulated		Shahbazi <i>et al.</i> (2022)
	Pomegranate extracts (<i>Punica granatum</i>) Chlorogenic acids		White chocolate Dark chocolate	Didar (2020) Zohreh (2020)
	Mulberry molasses (<i>Morus alba</i> L.) Cinnamon barks (<i>Cinnamomum burmannii</i> Blume)	Microencapsulated Nano-encapsulated	White chocolate	Didar (2023) Muhammad <i>et al.</i> (2021)
	Cinnamon barks (<i>Cinnamomum burmannii</i> Blume)	Essential oil		Muhammad <i>et al.</i> (2020)
	Raspberry leaves (<i>Rubus idaeus</i> L.)	Freeze-dried	Milk, semisweet, and dark chocolates	Belščak-Cvitanović <i>et al.</i> (2012)
	Cinnamon powder (<i>Cinnamomum burmannii</i> Blume)	Nanoparticle	–	Muhammad <i>et al.</i> (2019)
	Muscadine grapes (<i>Vitis rotundifolia</i>) <i>Santolina chamaecyparissus</i> L.	Freeze-dried Essential oil	Dark chocolate	Darwish <i>et al.</i> (2023) Bölek <i>et al.</i> (2022)
	Black mulberry (<i>Morus nigra</i> L.) Sea buckthorn (<i>Hippophae rhamnoides</i> L.) <i>Salvia Lavandulaefolia</i> <i>Salvia officinalis</i>	Extracts Powder		Godočiková <i>et al.</i> (2017) Zarić <i>et al.</i> (2023)
	Acerola (<i>Malpighia glabra</i>) Matcha green tea (<i>Camellia sinensis</i> L.) Moringa (<i>Moringa oleifera</i>) leaf	Spray-dried Powder	White chocolate	Poliński <i>et al.</i> (2022)
	Blueberries Raspberries Blackberries Pomegranates Beetroots	Freeze-dried	Milk and dark chocolates	Żyżelewicz <i>et al.</i> (2021)
	Elderberry (<i>Sambucus nigra</i> L.) Black chokeberry (<i>Aronia melanocarpa</i>) Passion fruit	Vacuum-dried extracts Freeze-dried	Dark chocolate	Poliński <i>et al.</i> (2021) Yeo & Thed (2022)
	Oranges Cornelian cherry (<i>Cornus mas</i> L.) Spinach (<i>Spinacia oleracea</i>) Bee pollen		White chocolate	Cerit <i>et al.</i> (2016)
		Dried powder		

Table 2 (Continued)

Functional property	Content	Transmission strategy or delivery system	Chocolate variety	References
Dietary fibre	Blackberry juice	Spray-dried		Lončarević <i>et al.</i> (2018)
	Green tea (<i>Camellia sinensis</i> L., <i>Theaceae</i>) extract			Lončarević <i>et al.</i> (2019)
	Peanut extract		Milk chocolate	Dean <i>et al.</i> (2016)
	Kale (<i>Brassica oleracea</i> var. <i>acephala</i>)	Freeze-dried		Carvalho <i>et al.</i> (2018)
	Grape (<i>Vitis vinifera</i>)			
	Prunes	Dried	Milk and dark chocolate	Komes <i>et al.</i> (2013)
	Papaya			
	Apricots			
	Raisin			
	Cranberries			
	Nettle (<i>Urtica dioica</i> L.)	Freeze-dried	Milk, semisweet and dark chocolate	Belščak-Cvitanović <i>et al.</i> (2015)
	Beetroot (<i>Beta vulgaris</i> L.)	Dried powder	Dark chocolate	Kongor <i>et al.</i> (2024)
	Darjeeling Green tea	Powder	White chocolate	Godočiková (2019)
	Matcha Green tea			
	Earl Grey Black tea			
	Goji berry (<i>Lycium barbarum</i>)	Dried		Morais Ferreira <i>et al.</i> (2016)
	Phytosterols	Spray-dried	Dark chocolate	Tolve <i>et al.</i> (2018)
	Black mulberries (<i>Morus nigra</i>) extract			Gültekin-Özgüven <i>et al.</i> (2016)
	Lemon balm (<i>Melissa officinalis</i> L.) extract	Freeze-dried		Sik <i>et al.</i> (2021)
	Blueberry juice	Spray-dried	White chocolate	Jovanović <i>et al.</i> (2022)
	Opuntia cactus stems	Dried powder	Dark chocolate	Angor <i>et al.</i> (2023)
	Resistant starch	Powder	White chocolate	Lončarević <i>et al.</i> (2021)
	Apple pomace	Dried	Milk chocolate	Büker <i>et al.</i> (2021)
	Inulin	Powder	Milk chocolate	Rezende <i>et al.</i> (2015)
	β-glucan			

Prebiotics have been defined more precisely as ‘selectively fermented ingredients that allow certain changes, both in the composition and/or activity in the gastrointestinal microbiota, that impart advantages’, according to Roberfroid (Roberfroid, 2008). In the food industry, numerous foods can be fortified with prebiotics, and the use of nutrients like probiotics and prebiotics – that support well-being, health and a decreased risk of diseases – has increased globally. Since 2005, the market has seen the introduction of more than 500 new prebiotic-enriched products (da Silveira *et al.*, 2015). Prebiotics’ nutritional and practical advantages have drawn the attention of researchers as well as the food industry (Scheid *et al.*, 2013). Prebiotic compounds are beneficial because of their nutritional benefits and the potential to improve some sensory features of food formulations (Sousa *et al.*, 2011; Morris & Morris, 2012).

Inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), and polydextrose (PDX) are extensively researched and employed prebiotics (Morris & Morris, 2012). The use of these advantageous elements for enriching the appropriate material applies to chocolate (Konar *et al.*, 2018). Inulin satisfies a range of

consumer expectations because it is prebiotic, rich in fibre, and low in calories (Konar, 2013). It can modulate physiological functions, such as lipid metabolism and the composition of gut bacteria. These functions enable improvements in the intestinal function and may help to reduce risk of colon cancer (Kalyani Nair *et al.*, 2010; Ilievska *et al.*, 2019). Prebiotic effects of inulin are associated with promoting the proliferation of beneficial bacteria in the colon, inhibiting the growth of harmful microorganisms, and potentially treating colonic dysfunctions (Kalyani Nair *et al.*, 2010). Due to its non-digestibility by the intestinal microbiota, inulin can be used as an ingredient in diabetic foods in the food industry (Fan *et al.*, 2016).

FOS are bifidogenic oligosaccharides that are nondigestible carbohydrates and dietary fibres (Kumar *et al.*, 2018). According to research, FOS’ pharmacological effects, which are mostly similar to other prebiotics, may be listed as dietary fibre impact, mineral absorption, dental caries prevention, antidiabetic activity, anticancer activity, and reduce the risk of cardiovascular diseases (Kumar *et al.*, 2018; Kherade *et al.*, 2021). It is an ingredient of essential functional food due to its functional, organoleptic and nutritional

qualities (Kherade *et al.*, 2021). Products made of FOS, which primarily contain short-chain fatty acids (SCFA), improve flavour and sweetness and are used to partially replace sugar in foods (Morais Ferreira *et al.*, 2017; Hossain *et al.*, 2021b).

The other major nondigestible carbohydrate that serves a prebiotic role is GOS. They have the ability to promote health, such as facilitating normal digestive activities, stimulating mineral absorption and decreasing blood lipid levels (Sangwan *et al.*, 2011; Lamsal, 2012). Researchers have evidence that GOS have an effect on immune modulation and reduces the risk of cancer (Sangwan *et al.*, 2011). While the prebiotic effect of GOS promotes the growth of beneficial bacteria, they confer resistance to pathogen colonisation, which is remarkable for its potential use in the treatment of inflammatory bowel disease (Laparra & Sanz, 2010; Sangwan *et al.*, 2011; Lamsal, 2012). The remarkable health benefits of GOS allow for a primary focus on prebiotic functional food development with GOS (Lamsal, 2012).

The prebiotic effect of PDX serves as a carbon source for microorganisms throughout the colon (Röytiö & Ouwehand, 2014; do Carmo *et al.*, 2016). After consumption of PDX, it is not digested in the small intestine and reaches the large intestine, where it is fermented into SCFA and gas by colonic microorganisms in the colon (Hernot *et al.*, 2009; Röytiö & Ouwehand, 2014; Hossain *et al.*, 2022b). In general, PDX has been shown in several studies to have an effect on postprandial benefits, such as energy intake, gastrointestinal function, nutrient absorption and immunological modulation (do Carmo *et al.*, 2016). PDX, the soluble fibre synthesised from glucose, is used to enhance the fibre content of foods (Costabile *et al.*, 2012). In the production of functional chocolate with prebiotics, the potential exists to reduce calories and sugar while simultaneously increasing fibre content. Several studies have been conducted to explore the production of high-fibre/prebiotic chocolates, emphasising the ongoing efforts in this area (Farzaneh & Abbasi, 2009; Morais Ferreira *et al.*, 2017).

Probiotic-based chocolates

Similar to prebiotics, probiotics alter the composition of the gut microbiota, and as a result, it has been demonstrated that they have an impact on both intestinal and overall body functions (Nebesny *et al.*, 2005, 2007). Its significance in terms of carrying probiotics has come to light when considering the popularity of chocolate eating (Yonejima *et al.*, 2015; Hossain *et al.*, 2022a). Consumers' quest for savoury foods that will meet their nutritional demands is driving a continued rise in the popularity of functional foods. Probiotics are one of these nutrients that may provide a positive effect on the

composition of the gut microbiota and general health (Hossain *et al.*, 2022a). Some probiotics have been linked to functional features, including lowering the symptoms of digestive system diseases (Klindt-Toldam *et al.*, 2016; Islam *et al.*, 2022). However, bacterial cultures must still be alive and active at the time of intake in order to be effective (Żyżelewicz *et al.*, 2010; Foong *et al.*, 2013).

Chocolate and chocolate-based products are appropriate probiotic delivery systems, protecting the survival of various bacteria strains (Table 2) (Possemiers *et al.*, 2010; Islam *et al.*, 2022). From this information, researchers and food companies have developed functional chocolate and chocolate-based products using different probiotic strains, such as *Bacillus coagulans*, *Bacillus indicus*, *Bifidobacterium animalis* subsp. *lactis*, *Bifidobacterium breve*, *Bifidobacterium lactis*, *Bifidobacterium longum*, *Enterococcus faecium*, *Fructolactobacillus sanfranciscensis*, *Lactobacillus acidophilus*, *Lactocaseibacillus casei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus*, *Lactocaseibacillus paracasei*, *Lactiplantibacillus plantarum*, *Lactocaseibacillus rhamnosus*, *Levilactobacillus brevis*, *Limosilactobacillus reuteri* and *Streptococcus thermophilus* (Table 2) (Laličić-Petronijević *et al.*, 2015; Zarić *et al.*, 2016; Toker *et al.*, 2018).

Synbiotic is a combination of probiotics and prebiotics designed to work in harmony, fostering a balanced and healthy gut microbiome (Mandal *et al.*, 2013). Taking this concept a step further, synbiotic chocolate emerges as a unique and innovative product that seamlessly integrates the goodness of probiotics and prebiotics with the delightful experience of indulging in chocolate (Rouhi *et al.*, 2015). This novel approach involves incorporating specific probiotic strains and prebiotic components into the chocolate formulations (Mandal *et al.*, 2013; Toker *et al.*, 2017). Manufacturers, by doing so, can craft functional foods that not only deliver the satisfying taste of chocolate but also provide additional health benefits (Konar *et al.*, 2017). Synbiotic chocolate, therefore, becomes a pleasurable treat that goes beyond mere indulgence, actively contributing to the promotion of gut health and overall well-being (Rouhi *et al.*, 2015). The combined action of probiotics and prebiotics in this chocolate variant creates a harmonious environment in the digestive system, supporting the growth of beneficial bacteria and optimising the overall microbial balance (Konar *et al.*, 2018).

Antioxidant-based chocolates

Cacao is a shelf-stable food matrix that has a medium or long shelf-life food product (Godočiková, 2019). The antioxidant potential of chocolate depends on the raw material, its origin, genetics, cultivation and post-harvest processes, and the production processes of the

chocolate (Mirković *et al.*, 2018). In functional food production, polyphenol-enriched chocolate has an enhanced antioxidant capacity (Table 2) (Belščak-Cvitanović *et al.*, 2012; Angor *et al.*, 2023). There are various strategies to boost chocolate's polyphenol content. One of these is the production of chocolate from polyphenol-enriched cacao (Jačimović *et al.*, 2022). Another way is the flavonoid content – which is a class of polyphenols present in chocolate – can be increased after the addition of the form of plant extracts and marine-derived food, which are potential sources of antioxidants (Dean *et al.*, 2016; Didar, 2020; Jovanović *et al.*, 2022). Different phenolic extracts with the correct source and percentage of phenolic may be considered ideal sources for improving antioxidant activity when added to chocolate formulations (Poliński *et al.*, 2022; Didar, 2023). The addition of these antioxidant-rich functional ingredients may significantly modify the sensory characteristics and nutraceutical properties of the base product into which they are incorporated (Belščak-Cvitanović *et al.*, 2015; Angor *et al.*, 2023). In general, the functional products that enrich the polyphenol content demonstrate a greater antioxidant capacity due to their higher absorption in the gastrointestinal system (Kemsawasd *et al.*, 2016).

Plant-based chocolates

On the other hand, global consumption of plant-based functional foods is growing quickly in the food marketing industry as a result of their rising popularity. The term 'plant-based' refers to a contemporary consumer trend that involves eschewing animal-based items in favour of plant-based alternatives, consuming fewer animal-based meals overall, or else adhering into dietary regimens that place a greater or even sole emphasis on plant-based products (Aschemann-Witzel *et al.*, 2021). Considering the crucial roles that plant-based elements, particularly proteins, play in the production of functional foods for human consumption that promote health and well-being (Lončarević *et al.*, 2019). Numerous researchers have investigated the potential impact of substances derived from various plant sources on a variety of disorders, including those with cardiovascular risk factors, obesity, metabolic syndromes, *etc.* (Lippi, 2015). The results of these studies suggest that plant-based foods should be included in the human diet to reduce the risk factors for certain metabolic illnesses. In this direction, there are several applications of plant-based functional chocolates in the food sector (Morais Ferreira *et al.*, 2016; Poliński *et al.*, 2021). By substituting components of plant origin for those of animal origin in the chocolate production process, food companies can make vegan functional chocolates. Soy, almond milk, maple and rice syrup are examples of plant sources that can be

chosen for the production of plant-based functional chocolates (Wang *et al.*, 2008).

High protein-based chocolates

The significant interest in high-protein products on the market has become part of the current trends in healthy and functional nutrition. A broad group of consumers, especially physically active people, would rather be used to products enriched in protein or in which protein is the main ingredient (Małecki *et al.*, 2020). The type of proteins used can alter the nutritional values, textural parameters or physicochemical parameters of the product (Małecki *et al.*, 2020). High-protein content functional chocolate, including bars, can be produced by adding microalgae, protein-enriched fractions from plants and milk components (Barišić *et al.*, 2019; Kobus-Cisowska *et al.*, 2019; Małecki *et al.*, 2020). Milk and milk derivatives, with their bioactive constituents, are highly preferred components for the production of protein-rich chocolate (Attaie *et al.*, 2003; Karav, 2019; Arslan *et al.*, 2021; Kaplan *et al.*, 2022b).

Easy to digest chocolates

The primary nutrients for human nutrition are milk and dairy products, which are among the most produced and consumed foods worldwide. Milk plays a crucial role in several physiological functions, particularly those related to immune system development (Duman & Karav, 2023). This is achieved by various proteins and peptides, including growth factors, immunoglobulins, enzymes, inhibitors, hormones and antibacterial substances (Karav *et al.*, 2017; Bolat *et al.*, 2022).

Despite the beneficial effects of milk on health, a lot of people cannot consume milk and its derivatives because of health problems. Studies have demonstrated that these problems are closely related to A1 milk consumption (Jianqin *et al.*, 2015; Ramakrishnan *et al.*, 2020). In this situation, the A2 milk approach has drawn attention to the production of functional chocolate. It is noteworthy that A1 milk is characterised by a higher concentration of A1 β -casein protein, whereas A2 milk is distinguished by a greater prevalence of A2 β -casein protein (Fig. 3) (Kaplan *et al.*, 2022a).

These variants display dissimilarities in their biological properties, protein structure, charges, digestion points and products generated after digestion. One significant difference between the A1 and A2 variants of β -casein is the type of digestive products they produce. When the A1 variant is digested through gastrointestinal proteolysis, it creates a bioactive peptide called β -casomorphin-7 (BCM-7), which activates μ -opioid receptors of the body (Singh *et al.*, 2023). These

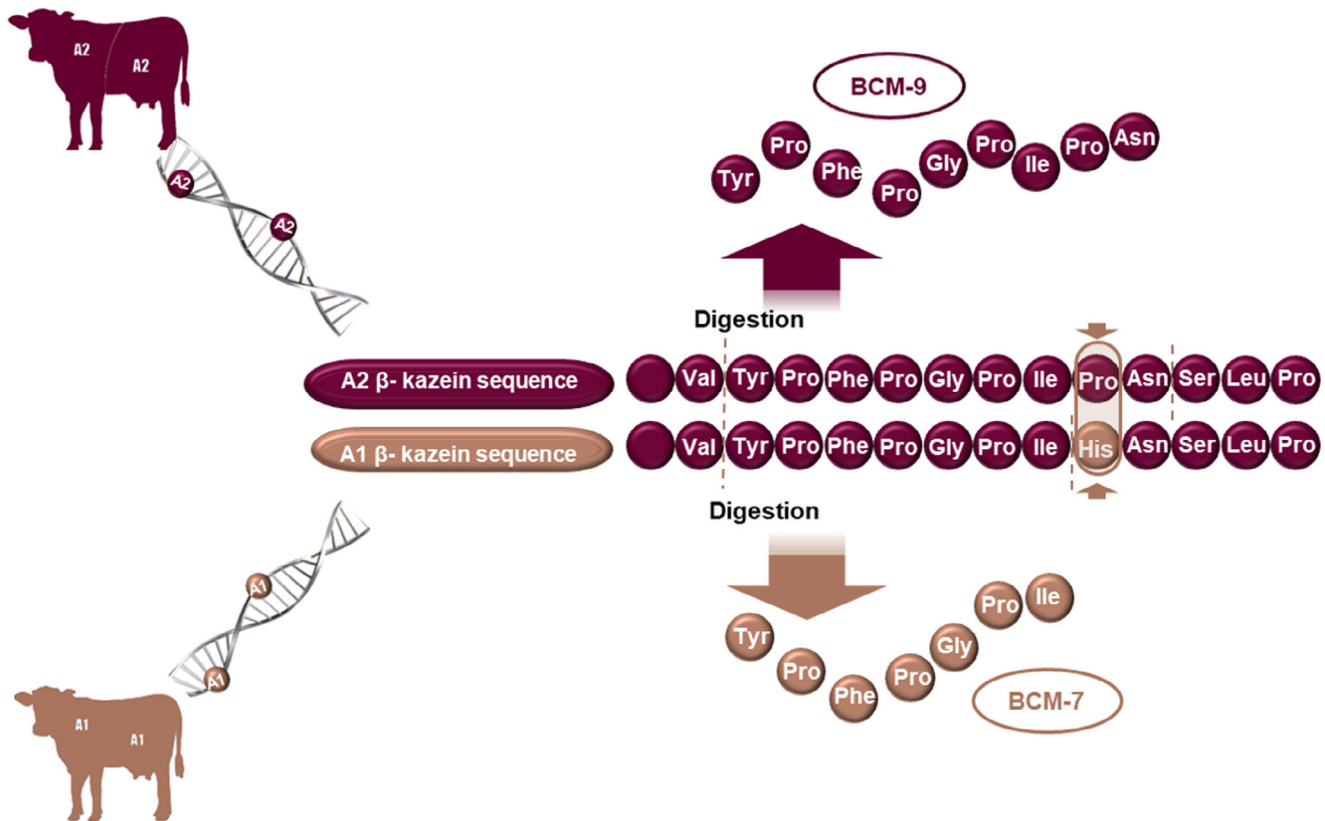


Figure 3 The distinction between beta-casein types A1 and A2 as well as the release of BCM-7 and BCM-9 molecules.

receptors regulate the body's response to pain. On the other hand, the digestion of the A2 variant leads to the formation of β -casomorphin-9 (BCM-9) (Kamiński *et al.*, 2007). After consuming regular milk with the mutated form of β -casein, the BCM7 metabolite is formed. This form of milk is difficult for human-associated digestive enzymes to breakdown and has been proven to be the main cause of digestive and health disorders related to the intake of milk and its derivatives. On the other hand, A2 milk containing β -casein is not associated with a trigger to cow's milk protein allergy, activation of μ -opioid receptors of the body or any health issues (Sun *et al.*, 1999). A potential solution for individuals experiencing gastrointestinal discomfort after consuming milk has been suggested to lie in the consumption of A2 milk. A2 milk is believed to be devoid of the protein that is responsible for such digestive issues, rendering it a suitable option for those who experience such problems (Gustavsson *et al.*, 2014; Milan *et al.*, 2020). Recent studies examining the effects of A1 and A2 milk on digestive discomfort have yielded noteworthy findings (Milan *et al.*, 2020). Specifically, these studies have demonstrated that individuals unable to tolerate A1 milk did not experience any

gastrointestinal issues after consuming A2 milk (Ho *et al.*, 2014; Jianqin *et al.*, 2015). Furthermore, recent research suggests that A2 milk is effective in reducing gastrointestinal symptoms associated with lactose intolerance, while A1 milk decreases lactase activity and increases gastrointestinal-related symptoms (Kaplan *et al.*, 2022a).

Overall, switching to A2 milk may hold promise as a solution for individuals with milk sensitivities. A2 milk is easily digested and might not cause serious diseases. In this way, A2 milk is becoming widespread in the global market in numerous food products, especially milk and dairy products, infant formulas, chocolate varieties, snacks and food supplements. Therefore, A2 milk is seen as a functional dairy product due to its natural health benefits (Bentivoglio *et al.*, 2020; Kaplan *et al.*, 2022c).

Conclusion

This article focuses on various aspects highlighting how chocolate, consumed from the past to the present, has been equipped with functional features to adapt to the changing demands of consumers. At this point, the

emphasis is on different elements added to chocolate, such as various plant extracts, probiotics, prebiotics, protein and health-beneficial ingredients, to meet consumer demands and to create diverse consumer portfolios. Chocolate is not just perceived as a sweet treat, but as a product that supports a healthy lifestyle, leading to its enrichment with various functional components. For instance, plant extracts added to chocolate are known for their antioxidant properties, providing consumers with a healthy option. The inclusion of probiotic and prebiotic ingredients creates a product friendly to the digestive system, while added protein can enhance the nutritional value of chocolate and provide energy. Milk chocolate is the most preferred type of chocolate worldwide. In this context, the use of A2 milk as the milk powder in the production of milk chocolate contributes significantly to the production of easily digestible functional chocolate. A2 milk is particularly suitable for consumers with digestive problems due to its ability to be digested by proteolytic enzymes. This highlights a specific market segment targeting individuals with a focus on healthy eating and digestive sensitivities when consuming chocolate.

In conclusion, the chocolate industry undergoes continuous evolution to align with consumer demands and trends in healthy living, consistently introducing numerous new products with functional features. This transformative process extends beyond the traditional perception of chocolate as a delectable indulgence, repositioning it as a sensible choice for health-conscious consumers. As we look to the future, the sector is well-positioned to explore more deeply into customised diets, taking advantage of developments in personalised nutrition to customise chocolate recipes to meet particular dietary demands and digestive sensitivity. With a more meaningful connection being made between enjoyment and well-being, this individualised approach has the potential to completely change how consumers view and use chocolate in their daily lives. Because of this, functional chocolate has a bright future ahead of it, thanks to its capacity to improve and maintain general health and vitality in addition to its exquisite flavour.

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Author contributions

Sümeyye Saritaş: Conceptualization; investigation; writing – original draft; writing – review and editing; project administration. **Hatice Duman:** Conceptualization; investigation; project administration; writing – original draft; writing – review and editing. **Burcu Pekdemir:** Investigation; conceptualization; project administration; writing – original draft; writing – review and editing. **João Miguel Rocha:** Conceptualization; investigation; writing – original draft; writing – review and editing; supervision; project administration. **Fatih Oz:** Supervision; investigation; conceptualization; writing – review and editing; project administration; writing – original draft. **Sercan Karav:** Writing – review and editing; supervision; project administration; writing – original draft; conceptualization; investigation.

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The authors declare no conflicts of interest.

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This paper does not require ethical approval.

Informed consent statement

Not applicable.

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Data availability statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

References

- Agus, B.A.P., Mohamad, N.N. & Hussain, N. (2018). Correction to: composition of unfermented, unroasted, roasted cocoa beans and cocoa shells from Peninsular Malaysia. *Journal of Food Measurement and Characterization*, **12**, 2590.
- Aladdin, Y.S., Alsharif, R., Mattar, W. *et al.* (2022). Migraine prevalence and analysis of dietary habits in relation to headache in the female population: a single-center study from Jeddah, Saudi Arabia. *Cureus*, **14**, e24848.
- Allgrove, J., Farrell, E., Gleeson, M., Williamson, G. & Cooper, K. (2011). Regular dark chocolate consumption's reduction of oxidative stress and increase of free-fatty-acid mobilization in response to prolonged cycling. *International Journal of Sport Nutrition and Exercise Metabolism*, **21**, 113–123.
- Angor, M., al Khalailah, N., al-Marazeeq, K., al-Rousan, W. & Ajo, R. (2023). Investigating chemical, antioxidant, and sensory

- properties of chocolate fortified with cactus stems powder. *Frontiers in Sustainable Food Systems*, **7**, 1204403.
- Anoraga, S.B., Wijanarti, S. & Sabarisman, I. (2019). Effect of extraction time and pressing temperature on characteristic of cocoa powder quality. *IOP Conference Series: Earth and Environmental Science*, **355**, 012050.
- Aremu, C.Y., Agiang, M.A. & Ayatse, J.O.I. (1995). Nutrient and antinutrient profiles of raw and fermented cocoa beans. *Plant Foods for Human Nutrition*, **48**, 217–223.
- Arslan, A., Kaplan, M., Duman, H. et al. (2021). Bovine colostrum and its potential for human health and nutrition. *Frontiers in Nutrition*, **8**, 651721.
- Aschemann-Witzel, J., Gantriis, R.F., Fraga, P. & Perez-Cueto, F.J.A. (2021). Plant-based food and protein trend from a business perspective: markets, consumers, and the challenges and opportunities in the future. *Critical Reviews in Food Science and Nutrition*, **61**, 3119–3128.
- Attaie, H., Breitschuh, B., Braun, P. & Windhab, E.J. (2003). The functionality of milk powder and its relationship to chocolate mass processing, in particular the effect of milk powder manufacturing and composition on the physical properties of chocolate masses. *International Journal of Food Science & Technology*, **38**, 325–335.
- Barišić, V., Kopjar, M., Jozinović, A. et al. (2019). The chemistry behind chocolate production. *Molecules*, **24**, 3163.
- Belščak-Cvitanović, A., Komes, D., Benković, M. et al. (2012). Innovative formulations of chocolates enriched with plant polyphenols from *Rubus idaeus* L. leaves and characterization of their physical, bioactive and sensory properties. *Food Research International*, **48**, 820–830.
- Belščak-Cvitanović, A., Komes, D., Durgo, K., Vojvodić, A. & Bušić, A. (2015). Nettle (*Urtica dioica* L.) extracts as functional ingredients for production of chocolates with improved bioactive composition and sensory properties. *Journal of Food Science and Technology*, **52**, 7723–7734.
- Bentivoglio, D., Finco, A., Bucci, G. & Staffolani, G. (2020). Is there a promising market for the A2 Milk? Analysis of Italian consumer preferences. *Sustainability*, **12**, 6763.
- Bolat, E., Eker, F., Kaplan, M. et al. (2022). Lactoferrin for COVID-19 prevention, treatment, and recovery. *Frontiers in Nutrition*, **9**, 992733.
- Bölek, S., Tosya, F. & Akçura, S. (2022). Effects of *Santolina chamaecyparissus* essential oil on rheological, thermal and antioxidative properties of dark chocolate. *International Journal of Gastronomy and Food Science*, **27**, 100481.
- Bolenz, S. & Langer, M. (2020). Feasibility of a coarse conching process for dark chocolate. *European Food Research and Technology*, **246**, 125–138.
- Bolenz, S., Amtsberg, K. & Schäpe, R. (2006). The broader usage of sugars and fillers in milk chocolate made possible by the new EC cocoa directive. *International Journal of Food Science & Technology*, **41**, 45–55.
- Bouaziz, M.A., Abbes, F., Mokni, A., Blecker, C., Attia, H. & Besbes, S. (2017). The addition effect of Tunisian date seed fibers on the quality of chocolate spreads. *Journal of Texture Studies*, **48**, 143–1501.
- Buitrago-Lopez, A., Sanderson, J., Johnson, L. et al. (2011). Chocolate consumption and cardiometabolic disorders: systematic review and meta-analysis. *BMJ*, **343**, d4488.
- Büker, M., Angin, P., Nurman, N. et al. (2021). Effects of apple pomace as a sucrose substitute on the quality characteristics of compound chocolate and spread. *Journal of Food Processing and Preservation*, **45**, 1–10.
- do Carmo, M., Walker, J.C., Novello, D. et al. (2016). Polydextrose: physiological function, and effects on health. *Nutrients*, **8**, 553.
- Carvalho, J.C.S., Romoff, P. & da Lannes, S.C.S. (2018). Improvement of nutritional and physicochemical properties of milk chocolates enriched with kale (*Brassica oleracea* var. *acephala*) and grape (*Vitis vinifera*). *Food Science and Technology*, **38**, 551–560.
- Cavarretta, E., Peruzzi, M., del Vescovo, R. et al. (2018). Dark chocolate intake positively modulates redox status and markers of muscular damage in elite football athletes: A randomized controlled study. *Oxidative Medicine and Cellular Longevity*, **2018**, 1–10.
- Cerit, İ., Şenkaya, S., Tulukoğlu, B., Kurtulus, M., Seçilmişoğlu, Ü.R. & Demirkol, O. (2016). Enrichment of functional properties of white chocolates with cornelian cherry, spinach and pollen powders. *GIDA/The Journal of Food*, **41**, 311–316.
- Chan, E.K., Quach, J., Mensah, F.K., Sung, V., Cheung, M. & Wake, M. (2012). Dark chocolate for children's blood pressure: randomised trial. *Archives of Disease in Childhood*, **97**, 637–640.
- Costabile, A., Fava, F., Røytiö, H. et al. (2012). Impact of polydextrose on the faecal microbiota: a double-blind, crossover, placebo-controlled feeding study in healthy human subjects. *British Journal of Nutrition*, **108**, 471–481.
- Darwish, A.G., el-Sharkawy, I., Tang, C., Rao, Q. & Tan, J. (2023). Investigation of antioxidant and cytotoxicity activities of chocolate fortified with muscadine grape pomace. *Foods*, **12**, 3153.
- Davison, G., Callister, R., Williamson, G., Cooper, K.A. & Gleeson, M. (2012). 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*, **51**, 69–79.
- de Clercq, N., Depypere, F., Delbaere, C., Nopens, I., Bernaert, H. & Dewettinck, K. (2014). Influence of cocoa butter diacylglycerols on migration induced fat bloom in filled chocolates. *European Journal of Lipid Science and Technology*, **116**, 1388–1399.
- Dean, L.L., Klevorn, C.M. & Hess, B.J. (2016). Minimizing the negative flavor attributes and evaluating consumer acceptance of chocolate fortified with peanut skin extracts. *Journal of Food Science*, **81**, S2824–S2830.
- Di Mattia, C.D., Sacchetti, G., Mastrocola, D. & Serafini, M. (2017). From cocoa to chocolate: the impact of processing on in vitro antioxidant activity and the effects of chocolate on antioxidant markers in vivo. *Frontiers in Immunology*, **8**, 1207.
- Di Renzo, L., Rizzo, M., Sarlo, F. et al. (2013). Effects of dark chocolate in a population of normal weight obese women: a pilot study. *European Review for Medical and Pharmacological Sciences*, **17**, 2257–2266.
- Didar, Z. (2020). Characterization of white chocolate enriched with free or encapsulated pomegranate extract. *Journal of Nutrition, Fasting and Health*, **8**, 302–309.
- Didar, Z. (2023). Effect of mulberry molasses microcapsules as a sugar substitute in white chocolate formulation on physicochemical, thermal, textural, and functional properties. *Journal of Food Measurement and Characterization*, **17**, 1997–2009.
- Djarova, T., Andreeva, L., Stefanova, D. et al. (2009). Influence of dark chocolate administration on uric acid, liver enzymes, lactate and glucose changes induced by submaximal exercise in athletes. *South African Journal for Research in Sport Physical Education and Recreation*, **32**, 1–8.
- Djikeng, F.T., Teyomnou, W.T., Tenyang, N. et al. (2018). Effect of traditional and oven roasting on the physicochemical properties of fermented cocoa beans. *Heliyon*, **4**, e00533.
- Duman, H. & Karav, S. (2023). Bovine colostrum and its potential contributions for treatment and prevention of COVID-19. *Frontiers in Immunology*, **14**, 1–17.
- Duman, H., Kaplan, M., Arslan, A. et al. (2021). Potential applications of endo- β -N-acetylglucosaminidases from *Bifidobacterium longum* subspecies *infantis* in designing value-added, next-generation infant formulas. *Frontiers in Nutrition*, **8**, 646275.
- Dural, I.E., Onrat, E., Çelik, S. et al. (2022). The relationships between chocolate consumption and endothelial dysfunction in patients with heart failure. *Türk Kardiyoloji Derneği Arşivi*, **50**, 334–339.
- Dzelagha, B.F., Ngwa, N.M. & Nde Bup, D. (2020). A review of cocoa drying technologies and the effect on bean quality parameters. *International Journal of Food Science*, **2020**, 1–11.

- Erdem, Ö., Gültekin-Özgüven, M., Berktaş, I. et al. (2014). Development of a novel synbiotic dark chocolate enriched with *Bacillus indicus* HU36, maltodextrin and lemon fiber: optimization by response surface methodology. *LWT – Food Science and Technology*, **56**, 187–193.
- Eskandari, M., Hooshmand Moghadam, B., Bagheri, R. et al. (2020). Effects of interval jump rope exercise combined with dark chocolate supplementation on inflammatory adipokine, cytokine concentrations, and body composition in obese adolescent boys. *Nutrients*, **12**, 3011.
- Esser, D., Mars, M., Oosterink, E., Stalmach, A., Müller, M. & Afman, L.A. (2014). Dark chocolate consumption improves leukocyte adhesion factors and vascular function in overweight men. *The FASEB Journal*, **28**, 1464–1473.
- Faccineto-Beltrán, P., Aguirre-López, L.O., Bañuelos-Pineda, J. et al. (2022). Fish oil and probiotics supplementation through milk chocolate improves spatial learning and memory in male Wistar rats. *Frontiers in Nutrition*, **9**, 1–18.
- Fahmi, M.I.W., Cilmiaty, R. & Nurwati, I. (2023). Correlation between milk chocolate consumption habit and chronotype with gingivitis in children with mixed tooth period. *Jurnal Aisyah: Jurnal Ilmu Kesehatan*, **8**. <https://doi.org/10.30604/jika.v8i2.1759>
- Fan, C.H., Cao, J.H. & Zhang, F.C. (2016). The prebiotic inulin as a functional food – a review. *European Review for Medical and Pharmacological Sciences*, **20**, 3262–3265.
- Faria, A., Pestana, D., Teixeira, D. et al. (2011). Insights into the putative catechin and epicatechin transport across blood-brain barrier. *Food & Function*, **2**, 39–44.
- Farzanmehr, H. & Abbasi, S. (2009). Effects of inulin and bulking agents on some physicochemical, textural and sensory properties of milk chocolate. *Journal of Texture Studies*, **40**, 536–553.
- Febrianto, N.A., Wang, S. & Zhu, F. (2022). Chemical and biological properties of cocoa beans affected by processing: a review. *Critical Reviews in Food Science and Nutrition*, **62**, 8403–8434.
- Foong, Y.J., Lee, S.T., Ramli, N., Tan, Y.N. & Ayob, M.K. (2013). Incorporation of potential probiotic *Lactobacillus plantarum* isolated from fermented cocoa beans into dark chocolate: bacterial viability and physicochemical properties analysis. *Journal of Food Quality*, **36**, 164–171.
- Fraga, C.G., Actis-Goretta, L., Ottaviani, J.I. et al. (2005). Regular consumption of a flavanol-rich chocolate can improve oxidant stress in young soccer players. *Clinical and Developmental Immunology*, **12**, 11–17.
- Gadhiya, D., Shah, N.P., Patel, A.R. & Prajapati, J.B. (2018). Preparation and shelf life study of probiotic chocolate manufactured using *Lactobacillus helveticus* MTCC 5463. *Acta Alimentaria*, **47**, 350–358.
- di Giuseppe, R., di Castelnuovo, A., Centritto, F. et al. (2008). Regular consumption of dark chocolate is associated with low serum concentrations of C-reactive protein in a healthy Italian population. *The Journal of Nutrition*, **138**, 1939–1945.
- Godóčiková, L. (2019). The impact of addition of different tea powders on the biological value of white chocolates. *Journal of Microbiology, Biotechnology and Food Sciences*, **9**, 396–399.
- Godóčiková, L., Ivanišová, E. & Kačániová, M. (2017). The influence of fortification of dark chocolate with sea buckthorn and mulberry on the content of biologically active substances. *Advanced Research in Life Sciences*, **1**, 26–31.
- Gómez-Fernández, A.R., Faccineto-Beltrán, P., Orozco-Sánchez, N.E. et al. (2021). Physicochemical properties and sensory acceptability of sugar free dark chocolate formulations added with probiotics. *Revista Mexicana de Ingeniería Química*, **20**, 697–709.
- Grassi, D., Lippi, C., Necozione, S., Desideri, G. & Ferri, C. (2005). Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons. *The American Journal of Clinical Nutrition*, **81**, 611–614.
- Grassi, D., Desideri, G., Necozione, S. et al. (2008). 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*, **138**, 1671–1676.
- Grassi, D., Socci, V., Tempesta, D. et al. (2016). Flavanol-rich chocolate acutely improves arterial function and working memory performance counteracting the effects of sleep deprivation in healthy individuals. *Journal of Hypertension*, **34**, 1298–1308.
- Grassia, M., Messina, M.C., Marconi, E. et al. (2021). Microencapsulation of phenolic extracts from cocoa shells to enrich chocolate bars. *Plant Foods for Human Nutrition*, **76**, 449–457.
- Greenberg, J.A. & Buijsse, B. (2013). Habitual chocolate consumption may increase body weight in a dose-response manner. *PLoS One*, **8**, e70271.
- Gültekin-Özgüven, M., Karadağ, A., Duman, Ş., Özkal, B. & Özçelik, B. (2016). Fortification of dark chocolate with spray dried black mulberry (*Morus nigra*) waste extract encapsulated in chitosan-coated liposomes and bioaccessibility studies. *Food Chemistry*, **201**, 205–212.
- Gustavsson, F., Buitenhuis, A.J., Johansson, M. et al. (2014). Effects of breed and casein genetic variants on protein profile in milk from Swedish Red, Danish Holstein, and Danish Jersey cows. *Journal of Dairy Science*, **97**, 3866–3877.
- Hajati, A., Brondani, M., Angerstig, L. et al. (2023). Chocolate intake and muscle pain sensation: A randomized experimental study. *PLoS One*, **18**, e0284769.
- Hernández-González, T., González-Barrio, R., Escobar, C. et al. (2021). Timing of chocolate intake affects hunger, substrate oxidation, and microbiota: a randomized controlled trial. *The FASEB Journal*, **35**, e21649.
- Hernot, D.C., Boileau, T.W., Bauer, L.L. et al. (2009). In vitro fermentation profiles, gas production rates, and microbiota modulation as affected by certain fructans, galactooligosaccharides, and polydextrose. *Journal of Agricultural and Food Chemistry*, **57**, 1354–1361.
- Ho, S., Woodford, K., Kukuljan, S. & Pal, S. (2014). Comparative effects of A1 versus A2 beta-casein on gastrointestinal measures: a blinded randomised cross-over pilot study. *European Journal of Clinical Nutrition*, **68**, 994–1000.
- Hossain, M.N., Ranadheera, C.S., Fang, Z. & Ajlouni, S. (2021a). Impact of encapsulating probiotics with cocoa powder on the viability of probiotics during chocolate processing, storage, and in vitro gastrointestinal digestion. *Journal of Food Science*, **86**, 1629–1641.
- Hossain, M.N., Ranadheera, C.S., Fang, Z., Hutchinson, G. & Ajlouni, S. (2021b). Protecting the viability of encapsulated *Lactobacillus rhamnosus* LGG using chocolate as a carrier. *Emirates Journal of Food and Agriculture*, **33**, 647.
- Hossain, M.N., Ranadheera, C.S., Fang, Z. & Ajlouni, S. (2022a). Interaction between chocolate polyphenols and encapsulated probiotics during in vitro digestion and colonic fermentation. *Fermentation*, **8**, 253.
- Hossain, M.N., Senaka Ranadheera, C., Fang, Z., Masum, A.K.M. & Ajlouni, S. (2022b). Viability of *Lactobacillus delbrueckii* in chocolates during storage and in-vitro bioaccessibility of polyphenols and SCFAs. *Current Research in Food Science*, **5**, 1266–1275.
- Ilievska, N., Pavlova, V., Kirovska, V., Ilievska, J. & Pavlovska, M. (2019). Nutritional and health benefits of inulin as functional food and prebiotic. *Journal of Hygienic Engineering and Design*, **27**, 45–48.
- Islam, M.Z., Masum, A.K.M. & Harun-ur-Rashid, M. (2022). Milk chocolate matrix as a carrier of novel *Lactobacillus acidophilus* LDMB-01: physicochemical analysis, probiotic storage stability and in vitro gastrointestinal digestion. *Journal of Agriculture and Food Research*, **7**, 100263.
- Jačimović, 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**, 1445.
- Janani, R.G., Asokan, S. & Geetha Priya, P.R. (2019). Effect of custom-made probiotic chocolates on *Streptococcus mutans*, plaque

- pH, salivary pH and buffering capacity in children – A randomised controlled trial. *Oral Health & Preventive Dentistry*, **17**, 7–15.
- Jean-Marie, E., Jiang, W., Bereau, D. & Robinson, J.C. (2022). *Theobroma cacao* and *Theobroma grandiflorum*: botany, composition and pharmacological activities of pods and seeds. *Foods*, **11**, 3966.
- Jia, L., Liu, X., Bai, Y.Y. *et al.* (2010). Short-term effect of cocoa product consumption on lipid profile: a meta-analysis of randomized controlled trials. *The American Journal of Clinical Nutrition*, **92**, 218–225.
- Jianqin, S., Leiming, X., Lu, X., Yelland, G.W., Ni, J. & Clarke, A.J. (2015). Effects of milk containing only A2 beta casein versus milk containing both A1 and A2 beta casein proteins on gastrointestinal physiology, symptoms of discomfort, and cognitive behavior of people with self-reported intolerance to traditional cows' milk. *Nutrition Journal*, **15**, 35.
- Jovanović, P., Pajin, B., Lončarić, A. *et al.* (2022). Whey as a carrier material for blueberry bioactive components: incorporation in white chocolate. *Sustainability*, **14**, 14172.
- 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. *Physiology and Pharmacology*, **24**, 185–196.
- Kalyani Nair, K., Kharb, S. & Thompson, D.K. (2010). Inulin dietary fiber with functional and health attributes—A review. *Food Reviews International*, **26**, 189–203.
- Kamiński, S., Cieślińska, A. & Kostyra, E. (2007). Polymorphism of bovine beta-casein and its potential effect on human health. *Journal of Applied Genetics*, **48**, 189–198.
- Kaplan, M., Baydemir, B., Günar, B.B., Arslan, A., Duman, H. & Karav, S. (2022a). Benefits of A2 Milk for sports nutrition, health and performance. *Frontiers in Nutrition*, **9**, 935344.
- Kaplan, M., Arslan, A., Duman, H. *et al.* (2022b). Production of bovine colostrum for human consumption to improve health. *Frontiers in Pharmacology*, **12**, 796824.
- Kaplan, M., Şahutoğlu, A.S., Saritaş, S. *et al.* (2022c). Role of milk glycane in prevention, treatment, and recovery of COVID-19. *Frontiers in Nutrition*, **9**, 1033779.
- Karača, S., Trifković, K., Martinić, A. *et al.* (2020). Development and characterisation of functional cocoa (*Theobroma cacao* L.)-based edible films. *International Journal of Food Science & Technology*, **55**, 1326–1335.
- Karav, S. (2019). Application of a novel endo- β -N-acetylglucosaminidase to isolate an entirely new class of bioactive compounds: N-glycans. In: *Enzymes in Food Biotechnology* (edited by M. Kuddus). Pp. 389–404. Amsterdam, Netherlands: Elsevier. <https://doi.org/10.1016/B978-0-12-813280-7.00022-0>
- Karav, S., German, J., Rouquié, C., le Parc, A. & Barile, D. (2017). Studying lactoferrin N-glycosylation. *International Journal of Molecular Sciences*, **18**, 870.
- Kavitha, P., Banumathi, M. & Sindhuja, D. (2016). Isolation of *Lactobacillus* sps from yoghurt and its application in probiotic chocolate. *International Journal of Science and Research*, **5**, 1696–1697.
- Kawakami, Y., Watanabe, Y., Mazuka, M. *et al.* (2021). Effect of cacao polyphenol-rich chocolate on postprandial glycemia, insulin, and incretin secretion in healthy participants. *Nutrition*, **85**, 111128.
- Kemsawasd, V., Chaikham, P. & Rattanasena, P. (2016). Survival of immobilized probiotics in chocolate during storage and with an in vitro gastrointestinal model. *Food Bioscience*, **16**, 37–43.
- Kherade, M., Solanke, S., Tawar, M. & Wankhede, S. (2021). Fructooligosaccharides: a comprehensive review. *Journal of Ayurvedic and Herbal Medicine*, **7**, 193–200.
- Khuda, S.E. & Williams, K.M. (2015). Effect of processing on dark chocolate composition. In: *Processing and Impact on Active Components in Food*. Pp. 667–674. Amsterdam, Netherlands: Elsevier. <https://doi.org/10.1016/B978-0-12-404699-3.00080-9>
- Klindt-Toldam, S., Larsen, S.K., Saaby, L. *et al.* (2016). Survival of *Lactobacillus acidophilus* NCFM® and *Bifidobacterium lactis* HN019 encapsulated in chocolate during in vitro simulated passage of the upper gastrointestinal tract. *LWT*, **74**, 404–410.
- Kobus-Cisowska, J., Szymanowska, D., Maciejewska, P. *et al.* (2019). Enriching novel dark chocolate with *Bacillus coagulans* as a way to provide beneficial nutrients. *Food & Function*, **10**, 997–1006.
- Komes, D., Belščak-Cvitanović, A., Škrabal, S., Vojvodić, A. & Bušić, A. (2013). The influence of dried fruits enrichment on sensory properties of bitter and milk chocolates and bioactive content of their extracts affected by different solvents. *LWT – Food Science and Technology*, **53**, 360–369.
- Konar, N. (2013). Influence of conching temperature and some bulk sweeteners on physical and rheological properties of prebiotic milk chocolate containing inulin. *European Food Research and Technology*, **236**, 135–143.
- Konar, N., Özhan, B., Artık, N., Dalabasmaz, S. & Poyrazoglu, E.S. (2014). Rheological and physical properties of inulin-containing milk chocolate prepared at different process conditions. *CyTA Journal of Food*, **12**, 55–64.
- Konar, N., Palabiyik, I., Toker, O.S. *et al.* (2017). Effect of inulin DP on various properties of sugar-free dark chocolates containing *Lactobacillus paracasei* and *Lactobacillus acidophilus*. *International Journal of Food Engineering*, **13**, 20170045.
- Konar, N., Palabiyik, I., Toker, O.S. *et al.* (2018). Conventional and sugar-free probiotic white chocolate: effect of inulin DP on various quality properties and viability of probiotics. *Journal of Functional Foods*, **43**, 206–213.
- Kongor, J.E., de Pascual-Teresa, S., Owusu, M., Kyei-Baffour, V.O. & Oduro-Yeboah, C. (2024). Investigating the effect of red beetroot powder concentration and processing time on the bioactive compounds composition and antioxidant capacity of beetroot dark chocolate. *Journal of the Science of Food and Agriculture*, **104**, 184–195.
- Kowalski, R., Rosochacki, M., Wyrostek, J. & Islam, M.T. (2023). Evaluating the quality of raw chocolate as an alternative to commercial products. *Applied Sciences*, **13**, 1274.
- Kumar, C.G., Sripada, S. & Poornachandra, Y. (2018). Status and future prospects of fructooligosaccharides as nutraceuticals. In: *Role of Materials Science in Food Biotechnology*. Pp. 451–503. Amsterdam, Netherlands: Elsevier. <https://doi.org/10.1016/B978-0-12-811448-3.00014-0>
- Laličić-Petronijević, J., Popov-Raljić, J., Obradović, D. *et al.* (2015). Viability of probiotic strains *Lactobacillus acidophilus* NCFM® and *Bifidobacterium lactis* HN019 and their impact on sensory and rheological properties of milk and dark chocolates during storage for 180 days. *Journal of Functional Foods*, **15**, 541–550.
- Laličić-Petronijević, J., Popov-Raljić, J., Lazić, V., Pezo, L. & Nedović, V. (2017). Synergistic effect of three encapsulated strains of probiotic bacteria on quality parameters of chocolates with different composition. *Journal of Functional Foods*, **38**, 329–337.
- 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**, 483.
- Lamsal, B.P. (2012). Production, health aspects and potential food uses of dairy prebiotic galactooligosaccharides. *Journal of the Science of Food and Agriculture*, **92**, 2020–2028.
- Laparra, J.M. & Sanz, Y. (2010). Interactions of gut microbiota with functional food components and nutraceuticals. *Pharmacological Research*, **61**, 219–225.
- Latif, R. & Majeed, F. (2020). Association between chocolate consumption frequency and heart rate variability indices. *Explore*, **16**, 372–375.
- Lee, Y., Berryman, C.E., West, S.G. *et al.* (2017). Effects of dark chocolate and almonds on cardiovascular risk factors in overweight and obese individuals: A randomized controlled-feeding trial. *Journal of the American Heart Association*, **6**, e005162.
- Leyva-Soto, A., Chavez-Santoscoy, R.A., Lara-Jacobo, L.R., Chavez-Santoscoy, A.V. & Gonzalez-Cobian, L.N. (2018). Daily

- consumption of chocolate rich in flavonoids decreases cellular genotoxicity and improves biochemical parameters of lipid and glucose metabolism. *Molecules*, **23**, 2220.
- Liang, B. & Hartel, R.W. (2004). Effects of milk powders in milk chocolate. *Journal of Dairy Science*, **87**, 20–31.
- Liendo, R., Padilla, F.C. & Quintana, A. (1997). Characterization of cocoa butter extracted from Criollo cultivars of *Theobroma cacao* L. *Food Research International*, **30**, 727–731.
- Lippi, D. (2015). Sin and pleasure: the history of chocolate in medicine. *Journal of Agricultural and Food Chemistry*, **63**, 9936–9941.
- The article conducted by Lippi (2015), which includes the history of chocolate, discusses the nutritional values and benefits of using chocolate as a medical product. For our review study on functional chocolate, this article has served as a source for us to address the historical narrative of chocolate.
- Loffredo, L., del Ben, M., Perri, L. *et al.* (2016). Effects of dark chocolate on NOX-2-generated oxidative stress in patients with non-alcoholic steatohepatitis. *Alimentary Pharmacology & Therapeutics*, **44**, 279–286.
- Loffredo, L., Baratta, F., Ludovica, P. *et al.* (2018). Effects of dark chocolate on endothelial function in patients with non-alcoholic steatohepatitis. *Nutrition, Metabolism, and Cardiovascular Diseases*, **28**, 143–149.
- Lončarević, I., Pajin, B., Fištes, A. *et al.* (2018). Enrichment of white chocolate with blackberry juice encapsulate: impact on physical properties, sensory characteristics and polyphenol content. *LWT*, **92**, 458–464.
- Lončarević, I., Pajin, B., Tumbas Šaponjac, V. *et al.* (2019). Physical, sensorial and bioactive characteristics of white chocolate with encapsulated green tea extract. *Journal of the Science of Food and Agriculture*, **99**, 5834–5841.
- Lončarević, I., Pajin, B., Petrović, J. *et al.* (2021). White chocolate with resistant starch: impact on physical properties, dietary fiber content and sensory characteristics. *Molecules*, **26**, 5908.
- Magrone, T., Russo, M.A. & Jirillo, E. (2017). Cocoa and dark chocolate polyphenols: from biology to clinical applications. *Frontiers in Immunology*, **8**, 677.
- Małecki, J., Tomasevic, I., Djekic, I. & Sołowiej, B.G. (2020). The effect of protein source on the physicochemical, nutritional properties and microstructure of high-protein bars intended for physically active people. *Foods*, **9**, 1467.
- Mandal, S., Hati, S., Puniya, A.K., Singh, R. & Singh, K. (2013). Development of synbiotic milk chocolate using encapsulated *Lactobacillus casei* NCDC 298. *Journal of Food Processing and Preservation*, **37**, 1031–1037.
- Martin, F.-P.J., Rezzi, S., Peré-Trepas, E. *et al.* (2009). Metabolic effects of dark chocolate consumption on energy, gut microbiota, and stress-related metabolism in free-living subjects. *Journal of Proteome Research*, **8**, 5568–5579.
- Martin, F.-P.J., Montoliu, I., Nagy, K. *et al.* (2012). Specific dietary preferences are linked to differing gut microbial metabolic activity in response to dark chocolate intake. *Journal of Proteome Research*, **11**, 6252–6263.
- Maskarinec, G., Jacobs, S., Shvetsov, Y. *et al.* (2019). Intake of cocoa products and risk of type-2 diabetes: the multiethnic cohort. *European Journal of Clinical Nutrition*, **73**, 671–678.
- Mathew, A.C., Kishan, S., Joy, M., Manoj, D.A., Amirthvarshan, A. & Senthil Kumar, R. (2017). Chocolate consumption and its relation to risk of type 2 diabetes mellitus. *International Journal of Advances in Medicine*, **4**, 1473.
- Matsumoto, C., Tomiyama, H., Kimura, K. *et al.* (2020). Modulation of blood pressure-lowering effects of dark chocolate according to an insulin sensitivity-randomized crossover study. *Hypertension Research*, **43**, 575–578.
- de Melo, C.W.B., de Bandeira, M.J., Maciel, L.F., da Bispo, E.S., de Souza, C.O. & Soares, S.E. (2020). Chemical composition and fatty acids profile of chocolates produced with different cocoa (*Theobroma cacao* L.) cultivars. *Food Science and Technology*, **40**, 326–333.
- Milan, A.M., Shrestha, A., Karlström, H.J. *et al.* (2020). Comparison of the impact of bovine milk β -casein variants on digestive comfort in females self-reporting dairy intolerance: A randomized controlled trial. *American Journal of Clinical Nutrition*, **111**, 149–160.
- Mirković, M., Seratlić, S., Kilcawley, K., Mannion, D., Mirković, N. & Radulović, Z. (2018). The sensory quality and volatile profile of dark chocolate enriched with encapsulated probiotic *Lactobacillus plantarum* bacteria. *Sensors*, **18**, 2570.
- Montagna, M.T., Diella, G., Triggiano, F. *et al.* (2019). Chocolate, “Food of the Gods”: history, science, and human health. *International Journal of Environmental Research and Public Health*, **16**, 4960.
- Monteiro, S., Dias, J., Lourenço, V. *et al.* (2023). Development of a functional dark chocolate with baobab pulp. *Foods*, **12**, 1711.
- The article aims to evaluate the effect of baobab flour concentration on the development of functional dark chocolate, focusing on physical, chemical, nutritional, and sensory evaluations. In our article, which generally discusses the products that can be added to functionalize chocolate, the inclusion of this article, which discusses the parameters of adding baobab flour in the production of functional chocolate, has introduced a different perspective.
- Morais Ferreira, J.M., Azevedo, B.M., Silva, F.G.D., Luccas, V. & Bolini, H.M.A. (2016). Isosweetness concentrations of sucrose and high-intensity sweeteners and antioxidant activity in white chocolate with functional properties. *International Journal of Food Science & Technology*, **51**, 2114–2122.
- This study explores the potential of adding prebiotic component fructooligosaccharides (FOS) to white chocolate to create a functional chocolate with health benefits. FOS, a significant compound, supports gut health and reduces risks associated with excessive sugar consumption. Within the scope of the article submitted for review, FOS, one of the preferred prebiotics in functional chocolate production, is comprehensively described, and sample studies are presented in Table 1.
- Morais Ferreira, J.M., Azevedo, B.M., Luccas, V. & Bolini, H.M.A. (2017). Sensory profile and consumer acceptability of prebiotic white chocolate with sucrose substitutes and the addition of goji berry (*Lycium barbarum*). *Journal of Food Science*, **82**, 818–824.
- Morris, C. & Morris, G.A. (2012). The effect of inulin and fructooligosaccharide supplementation on the textural, rheological and sensory properties of bread and their role in weight management: a review. *Food Chemistry*, **133**, 237–248.
- Muhammad, D.R.A., Gonzalez, C.G., Doost, A.S., van de Walle, D., van der Meeren, P. & Dewettinck, K. (2019). Improvement of antioxidant activity and physical stability of chocolate beverage using colloidal cinnamon nanoparticles. *Food and Bioprocess Technology*, **12**, 976–989.
- Muhammad, D.R.A., Lemarcq, V., Alderweireldt, E. *et al.* (2020). Antioxidant activity and quality attributes of white chocolate incorporated with *Cinnamomum burmannii* Blume essential oil. *Journal of Food Science and Technology*, **57**, 1731–1739.
- Muhammad, D.R.A., Tuenter, E., Patria, G.D., Foubert, K., Pieters, L. & Dewettinck, K. (2021). Phytochemical composition and antioxidant activity of *Cinnamomum burmannii* Blume extracts and their potential application in white chocolate. *Food Chemistry*, **340**, 127983.
- Nambiar, R.B., Sellamuthu, P.S. & Perumal, A.B. (2018). Development of milk chocolate supplemented with microencapsulated *Lactobacillus plantarum* HM47 and to determine the safety in a Swiss albino mice model. *Food Control*, **94**, 300–306.
- Nebesny, E., Żyżelewicz, D., Motyl, I. & Libudzisz, Z. (2005). Properties of sucrose-free chocolates enriched with viable lactic acid bacteria. *European Food Research and Technology*, **220**, 358–362.
- Nebesny, E., Żyżelewicz, D., Motyl, I. & Libudzisz, Z. (2007). Dark chocolates supplemented with *Lactobacillus* strains. *European Food Research and Technology*, **225**, 33–42.

- Nehlig, A. (2013). The neuroprotective effects of cocoa flavanol and its influence on cognitive performance. *British Journal of Clinical Pharmacology*, **75**, 716–727.
- 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**, 1–8.
- Nocella, C., Cavarretta, E., Fossati, C. et al. (2023). Dark chocolate intake positively modulates gut permeability in elite football athletes: a randomized controlled study. *Nutrients*, **15**, 4203.
- Nouri, B., Mohtasebi, S.S. & Jahanbakhshi, A. (2019). Application of an olfactory system to detect and distinguish bitter chocolates with different percentages of cocoa. *Journal of Food Process Engineering*, **42**, e13248.
- Oliveira, D. & Deliza, R. (2021). Comparison of consumer-based methodologies for optimizing the development of new products: a case study with probiotic chocolate flavored milk. *Food Science and Technology International*, **27**, 539–553.
- Oliveira, B., Falkenhain, K. & Little, J.P. (2022). Sugar-free dark chocolate consumption results in lower blood glucose in adults with diabetes. *Nutrition and Metabolic Insights*, **15**, 117863882210769.
- Ostertag, L.M., Kroon, P.A., Wood, S. et al. (2013). Flavan-3-ol-enriched dark chocolate and white chocolate improve acute measures of platelet function in a gender-specific way—a randomized-controlled human intervention trial. *Molecular Nutrition & Food Research*, **57**, 191–202.
- Pereira, T., Bergqvist, J., Vieira, C., Grüner Sveälv, B., Castanheira, J. & Conde, J. (2019). Randomized study of the effects of cocoa-rich chocolate on the ventricle–arterial coupling and vascular function of young, healthy adults. *Nutrition*, **63–64**, 175–183.
- Petrone, A.B., Gaziano, J.M. & Djoussé, L. (2014). Chocolate consumption and risk of heart failure in the Physicians' Health Study. *European Journal of Heart Failure*, **16**, 1372–1376.
- Poliński, S., Kowalska, S., Topka, P. & Szydłowska-Czeriak, A. (2021). Physicochemical, antioxidant, microstructural properties and bioaccessibility of dark chocolate with plant extracts. *Molecules*, **26**, 1–14.
- Poliński, S., Topka, P., Tańska, M., Kowalska, S., Czaplicki, S. & Szydłowska-Czeriak, A. (2022). Impact of bioactive compounds of plant leaf Powders in white chocolate production: changes in antioxidant properties during the technological processes. *Antioxidants*, **11**, 752.
- Possemiers, S., Marzorati, M., Verstraete, W. & van de Wiele, T. (2010). Bacteria and chocolate: a successful combination for probiotic delivery. *International Journal of Food Microbiology*, **141**, 97–103.
- Quiñones-Muñoz, T., Gallegos-Infante, J.A., Rocha-Guzmán, N.E. et al. (2011). Mixing and tempering effect on the rheological and particle size properties of dark chocolate coatings Efecto del mezclado y temperado sobre las propiedades reológicas y de tamaño de partícula de coberturas de chocolate oscuro. *CyTA Journal of Food*, **9**, 109–113.
- Ramakrishnan, M., Eaton, T.K., Sermet, O.M. & Savaiano, D.A. (2020). Milk containing A2 β -casein only, as a single meal, causes fewer symptoms of lactose intolerance than milk containing A1 and A2 β -caseins in subjects with lactose maldigestion and intolerance: a randomized, double-blind, crossover trial. *Nutrients*, **12**, 3855.
- Rawel, H., Huschek, G., Sagu, S.T. & Homann, T. (2019). Cocoa bean proteins—characterization, changes and modifications due to ripening and post-harvest processing. *Nutrients*, **11**, 428.
- Ren, Y., Liu, Y., Sun, X.Z. et al. (2019). Chocolate consumption and risk of cardiovascular diseases: a meta-analysis of prospective studies. *Heart*, **105**, 49–55.
- Rezende, N.V., Benassi, M.T., Vissotto, F.Z., Augusto, P.P.C. & Grossmann, M.V.E. (2015). Effects of fat replacement and fibre addition on the texture, sensory acceptance and structure of sucrose-free chocolate. *International Journal of Food Science & Technology*, **50**, 1413–1420.
- The article aims to investigate the effect of various factors including composition, processing conditions, and formulation on the sensory properties and quality of chocolate. Additionally, the article aims to discuss the potential use of inulin, a dietary fiber, as a substitute for fats and sugars in food products, leading to the development of products with a more balanced nutritional composition without compromising texture. Within the scope of the study submitted for your evaluation, the properties of dietary fibers and their utilization in studies on the production of functional chocolate have been examined. In addition, changes in texture and sensory properties have also been discussed.
- Ribeiro, M., Fanton, S., Paiva, B.R. et al. (2023). Dark chocolate (70% cocoa) attenuates the inflammatory marker TNF- α in patients on hemodialysis. *Clinical Nutrition ESPEN*, **53**, 189–195.
- Robberfroid, M.B. (2008). Prebiotics: concept, definition, criteria, methodologies, and products. In: *Handbook of Prebiotics*. Pp. 49–78. Boca Raton, FL: CRC Press.
- Rouhi, M., Mohammadi, R., Mortazavian, A.M. & Sarlak, Z. (2015). Combined effects of replacement of sucrose with d-tagatose and addition of different probiotic strains on quality characteristics of chocolate milk. *Dairy Science & Technology*, **95**, 115–133.
- Röytiö, H. & Ouwehand, A.C. (2014). The fermentation of polydextrose in the large intestine and its beneficial effects. *Beneficial Microbes*, **5**, 305–313.
- Saadatdoust, Z., Pandurangan, A.K., Ananda Sadagopan, S.K., Mohd. Esa, N., Ismail, A. & Mustafa, M.R. (2015). Dietary cocoa inhibits colitis associated cancer: a crucial involvement of the IL-6/STAT3 pathway. *The Journal of Nutritional Biochemistry*, **26**, 1547–1558.
- Sangwan, V., Tomar, S.K., Singh, R.R., Singh, A.K. & Ali, B. (2011). Galactooligosaccharides: novel components of designer foods. *Journal of Food Science*, **76**, R103–R111.
- Sanlier, N., Açıklım, B., Eroglu, E., Kılınç, F. & Celik, B. (2022). Chocolate craving: does it affect eating attitude and body mass index? *Nutrition & Food Science*, **52**, 943–957.
- Santiago-Rodríguez, E., Estrada-Zaldívar, B. & Zaldívar-Urbe, E. (2018). Effects of dark chocolate intake on brain electrical oscillations in healthy people. *Foods*, **7**, 187.
- Sasaki, A., Mizuno, K., Morito, Y. et al. (2024). The effects of dark chocolate on cognitive performance during cognitively demanding tasks: a randomized, single-blinded, crossover, dose-comparison study. *Heliyon*, **10**, e24430.
- Scheid, M.M.A., Moreno, Y.M.F., Maróstica Junior, M.R. & Pastore, G.M. (2013). Effect of prebiotics on the health of the elderly. *Food Research International*, **53**, 426–432.
- 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 & Safety of Crops and Food*, **14**, 188–201.
- Shiina, Y., Funabashi, N., Lee, K. et al. (2009). Acute effect of oral flavonoid-rich dark chocolate intake on coronary circulation, as compared with non-flavonoid white chocolate, by transthoracic Doppler echocardiography in healthy adults. *International Journal of Cardiology*, **131**, 424–429.
- Sik, B., Lakatos, E.H., Kapcsándi, V., Székelyhidi, R. & Ajtony, Z. (2021). Exploring the rosmarinic acid profile of dark chocolate fortified with freeze-dried lemon balm extract using conventional and non-conventional extraction techniques. *LWT*, **147**, 111520.
- Silva, M.P., Tulini, F.L., Marinho, J.F.U. et al. (2017). Semisweet chocolate as a vehicle for the probiotics *Lactobacillus acidophilus* LA3 and *Bifidobacterium animalis* subsp. *lactis* BLC1: evaluation of chocolate stability and probiotic survival under in vitro simulated gastrointestinal conditions. *LWT*, **75**, 640–647.
- da Silveira, E.O., Neto, J.H.L., da Silva, L.A., Raposo, A.E.S., Magnani, M. & Cardarelli, H.R. (2015). The effects of inulin combined with oligofructose and goat cheese whey on the

- physicochemical properties and sensory acceptance of a probiotic chocolate goat dairy beverage. *LWT – Food Science and Technology*, **62**, 445–451.
- Singh, M.K., Kumar, A., Rai, D.C., Aggarwal, A. & Malik, M. (2023). Identification of β -casein phenotypes (A^1/A^2) in the milk of the Indian Jersey crossbreed bovine using the high-resolution accurate mass spectrometer. *International Journal of Food Science and Technology*, **59**, 4263–4267.
- de Sousa, V.M.C., dos Santos, E.F. & Sgarbieri, V.C. (2011). The importance of prebiotics in functional foods and clinical practice. *Food and Nutrition Sciences*, **2**, 133–144.
- Souza, S.J., Petrilli, A.A., Teixeira, A.M. *et al.* (2017). Effect of chocolate and mate tea on the lipid profile of individuals with HIV/AIDS on antiretroviral therapy: a clinical trial. *Nutrition*, **43–44**, 61–68.
- Souza, J.M.L., Rocha, J.M., Cartaxo, C.B.C. *et al.* (2020). Monitoring and optimization of Cupuaçu seed fermentation, drying and storage processes. *Microorganisms*, **8**, 1–34.
- Spadafranca, A., Martinez Conesa, C., Sirini, S. & Testolin, G. (2010). Effect of dark chocolate on plasma epicatechin levels, DNA resistance to oxidative stress and total antioxidant activity in healthy subjects. *British Journal of Nutrition*, **103**, 1008–1014.
- Stellingwerff, T., Godin, J.P., Chou, C.J. *et al.* (2014). The effect of acute dark chocolate consumption on carbohydrate metabolism and performance during rest and exercise. *Applied Physiology, Nutrition, and Metabolism*, **39**, 173–182.
- Succi, M., Tremonte, P., Pannella, G. *et al.* (2017). Survival of commercial probiotic strains in dark chocolate with high cocoa and phenols content during the storage and in a static in vitro digestion model. *Journal of Functional Foods*, **35**, 60–67.
- Sumiyoshi, E., Matsuzaki, K., Sugimoto, N. *et al.* (2019). Sub-chronic consumption of dark chocolate enhances cognitive function and releases nerve growth factors: a parallel-group randomized trial. *Nutrients*, **11**, 2800.
- Sun, Z., Cade, J.R., Fregly, M.J. & Privette, R.M. (1999). β -Casomorphin induces Fos-like immunoreactivity in discrete brain regions relevant to schizophrenia and autism. *Autism*, **3**, 67–83.
- Taub, P.R., Ramirez-Sanchez, I., Patel, M. *et al.* (2016). Beneficial effects of dark chocolate on exercise capacity in sedentary subjects: underlying mechanisms. A double blind, randomized, placebo controlled trial. *Food & Function*, **7**, 3686–3693.
- Tee, Y., Bariah, K., Hisyam Zainudin, B., Samuel Yap, K.C. & Ong, N.G. (2022). Impacts of cocoa pod maturity at harvest and bean fermentation period on the production of chocolate with potential health benefits. *Journal of the Science of Food and Agriculture*, **102**, 1576–1585.
- da Teixeira, A.M.N.C., Luzia, L.A., de Souza, S.J. *et al.* (2017). The impact of dark chocolate intake on arterial elasticity in individuals with HIV/AIDS undergoing ART: a randomized, double-blind, crossover trial. *Food & Function*, **8**, 2212–2219.
- Toker, O.S., Genc Polat, D., Gulfidan, O.G. *et al.* (2017). Stability of lactic acid bacteria in synbiotic sugared and sugar-free milk chocolates. *International Journal of Food Properties*, **20**(sup2), 1354–1365.
- Toker, Ö.S., Pirouzian, H.R., Konar, N. & Polat, D.G. (2018). β v seeding as an alternative pre-crystallization technique in synbiotic milk chocolate production. *GIDA*, **43**, 422–431.
- Toker, O.S., Palabiyik, I. & Konar, N. (2019). Chocolate quality and conching. *Trends in Food Science & Technology*, **91**, 446–453.
- Tolve, R., Condelli, N., Caruso, M.C., Barletta, D., Favati, F. & Galgano, F. (2018). Fortification of dark chocolate with microencapsulated phytosterols: chemical and sensory evaluation. *Food & Function*, **9**, 1265–1273.
- Tuenter, E., Sakavitsi, M.E., Rivera-Mondragón, A. *et al.* (2021). Ruby chocolate: a study of its phytochemical composition and quantitative comparison with dark, milk and white chocolate. *Food Chemistry*, **343**, 128446.
- Vargas-rechia, C.G., Vargas-rechia, C.G., Reicher, F. *et al.* (2015). A profile of the south African table grape market value chain. *Bioresource Technology*, **6**, 1–14.
- Vasanthakumar, A.H., Sharan, J. & Cruz, A.M.D. (2016). Plaque pH and dental retention after consumption of different types of chocolates. *International Journal of Clinical Preventive Dentistry*, **12**, 97–102.
- Vercet, A. (2003). Browning of white chocolate during storage. *Food Chemistry*, **81**, 371–377.
- Wang, J.F., Schramm, D.D., Holt, R.R. *et al.* (2000). A dose-response effect from chocolate consumption on plasma epicatechin and oxidative damage. *The Journal of Nutrition*, **130**, S2115–S2119.
- Wang, Y.F., Jr, W.S.Y., Yu, D., Champagne, C., Appel, L.J. & Lin, P.H. (2008). The relationship between dietary protein intake and blood pressure: results from the PREMIER study. *Journal of Human Hypertension*, **22**, 745–754.
- West, S.G., McIntyre, M.D., Piotrowski, M.J. *et al.* (2014). Effects of dark chocolate and cocoa consumption on endothelial function and arterial stiffness in overweight adults. *British Journal of Nutrition*, **111**, 653–661.
- Wollgast, J. & Anklam, E. (2000). Polyphenols in chocolate: is there a contribution to human health? *Food Research International*, **33**, 449–459.
- Yang, J., Zhou, J., Yang, J. *et al.* (2024). Dark chocolate intake and cardiovascular diseases: a mendelian randomization study. *Scientific Reports*, **14**, 968.
- Yeo, Y.Y. & Thed, S.T. (2022). Product development of passion fruit and citrus peel dark chocolate. *Food Research*, **6**(S1), 41–44.
- Yonejima, Y., Hisa, K., Kawaguchi, M. *et al.* (2015). Lactic acid bacteria-containing chocolate as a practical probiotic product with increased acid tolerance. *Biocatalysis and Agricultural Biotechnology*, **4**, 773–777.
- Zarić, D.B., Bulatović, M.L., Rakin, M.B., Krnić, T.Ž., Lončarević, I.S. & Pajin, B.S. (2016). Functional, rheological and sensory properties of probiotic milk chocolate produced in a ball mill. *RSC Advances*, **6**, 13934–13941.
- Zarić, D., Rakin, M., Bulatović, M. *et al.* (2023). Influence of added extracts of herbs (*Salvia lavandulifolia*, *Salvia officinalis*) and fruits (*Malpighia glabra*) on rheological, textural, and functional (AChE-inhibitory and antioxidant activity) characteristics of dark chocolate. *Journal of Food Measurement and Characterization*, **18**, 772–782.
- Zohreh, D. (2020). Properties of dark chocolate enriched with free and encapsulated chlorogenic acids extracted from green coffee. *Brazilian Journal of Food Technology*, **23**, e2019118.
- Żyżelewicz, D., Nebesny, E., Motyl, I. & Libudzisz, Z. (2010). Effect of milk chocolate supplementation with lyophilised *Lactobacillus* cells on its attributes. *Czech Journal of Food Sciences*, **28**, 392–406.
- Żyżelewicz, D., Oracz, J., Bilicka, M., Kulbat-Warycha, K. & Klewicka, E. (2021). Influence of freeze-dried phenolic-rich plant powders on the bioactive compounds profile, antioxidant activity and aroma of different types of chocolates. *Molecules*, **26**, 7058.