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Dark chocolate: An overview of its biological activity, processing, and fortification approaches

Sharmistha Samanta [a,1], Tanmay Sarkar [b,1], Runu Chakraborty [a,**], Maksim Rebezov [c,d,e], Mohammad Ali Shariati [d,e], Muthu Thiruvengadam [f], Kannan R.R. Rengasamy [g,*]

[a] Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata, 700032, India
[b] Department of Food Processing Technology, Malda Polytechnic, West Bengal State Council of Technical Education, Government of West Bengal, Malda, 732102, India
[c] Department of Scientific Research, V. M. Gorbatov Federal Research Center for Food Systems, Moscow, 109316, Russian Federation
[d] Department of Scientific Research, K.G. Razumovsky Moscow State University of Technologies and Management (The First Cossack University), 73 Zemlyanoy Val, Moscow, 109004, Russian Federation
[e] Department of Scientific Research, Russian State Agrarian University - Moscow Timiryazev Agricultural Academy, Moscow, 127550, Russian Federation
[f] Department of Crop Science, College of Sanghuh Life Sciences, Konkuk University, Seoul, 05029, Republic of Korea
[g] Laboratory of Natural Products and Medicinal Chemistry (LNPMC), Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, 600077, India

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ABSTRACT

Dark chocolate gets popularity for several decades due to its enormous health benefits. It contains several health-promoting factors (bioactive components - polyphenols, flavonoids, procyanidins, theobromines, etc, and vitamins and minerals) that positively modulate the immune system of human beings. It confers safeguards against cardiovascular diseases, certain types of cancers, and other brain-related disorders like Alzheimer's disease, Parkinson's disease, etc. Dark chocolate is considered a functional food due to its anti-diabetic, anti-inflammatory, and anti-microbial properties. It also has a well-established role in weight management and the alteration of a lipid profile to a healthy direction. But during the processing of dark chocolate, several nutrients are lost (polyphenol, flavonoids, flavan 3 ol, ascorbic acid, and thiamine). So, fortification would be an effective method of enhancing the overall nutrient content and also making the dark chocolate self-sufficient. Thus, the focus of this review study is to gather all the experimental studies done on dark chocolate fortification. Several ingredients were used for the fortification, such as fruits (mulberry, chokeberries, and elderberries), spices (cinnamon), phytosterols, peanut oil, probiotics (mainly *Lactobacillus*, *bacillus* spices), prebiotics (inulin, xanthan gum, and maltodextrin), flavonoids, flavan-3-ols, etc. Those fortifications were done to raise the total antioxidant content as well as essential fatty acid content simultaneously reducing total calorie content. Sometimes, the fortification was done to improve physical properties like viscosity, rheological propertiesand also improve overall consumer acceptance by modifying its bitter taste.

1. Introduction

Chocolate is a popular, lip-smacking sweet stuff among all age groups. Its consumption rate continues to grow around the world year after year. According to the consumption statistics, Switzerland was the leading country in chocolate consumption. According to a survey done in 2017, Swiss people have long affairs with the consumption of chocolate. Austria was ranked 2nd after Switzerland in per-capita consumption of chocolate. Germany, Ireland, Great Britain, Sweden, Estonia, Norway, and Poland are popular examples of per-capita chocolate consumption in the world (Hapsari and Yuniasih, 2020).

The consumer importance of chocolate is very high due to its delicacy and health benefits. The use of chocolate was 1st started in Meso America. Old Aztecs used chocolate as a valuable drug for the prevention of different ailments. Chocolate is also effective to increase overall longevity, sexual appetite, and fertility. These health benefits are due to

* Corresponding author.
** Corresponding author.
E-mail addresses: crunu@hotmail.com (R. Chakraborty), ragupathir.sdc@saveetha.com (K.R.R. Rengasamy).
[1] Marked authors contributed equally.

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the presence of cocoa in chocolate (Zugravu and Otelea, 2019). Cocoa was 1st cultivated 5300 years ago in Central America by the Mayo-Chin chipe people (Fanton et al., 2021). The main antioxidant phytonutrients present in the cocoa bean are polyphenols and flavonoids. 10% dry weight of the cocoa bean is composed of polyphenol. The most abundant flavonoids in cocoa powder are catechins, anthocyanins, and proanthocyanidins (Zugravu and Otelea, 2019). Cocoa beans (*Theobroma cacao)* contain 50–57% lipid, predominantly known as cocoa butter (Hannum and Erdman, 2000). Cocoa butter is one of the principal components of dark chocolate. The major constituents of cocoa butter are 33% oleic acid, 25% palmitic acid, and 33% stearic acid (Tokede et al., 2011; Didar, 2021). Cocoa is very much popular due to its extensive health benefits. Cocoa consumption is related to the reduction of cardiovascular mortality by about 50% (Araujo et al., 2016). Due to this characteristic, chocolate is considered a functional food; it has a positive relationship with the prevention of degenerative diseases such as diabetes, obesity, Alzheimer's disease, etc. (Araujo et al., 2016; Gianfredi et al., 2018). When health issues come to our mind, then selection should be proper. Dark chocolate instead of milk chocolate will be a better option for its high polyphenol and flavonoid content than milk chocolate. The total polyphenol and flavonoid content in dark chocolate is five times higher than in milk and white chocolate. The fat (30 g fat per 100 g) and sugar (52 g per 100 g) content is also quite higher in milk and white chocolate (Fanton et al., 2021). Henderson and Hudson (2019) first identified the usefulness of cocoa beverages before 1000 B.C (Henderson and Hudson, 2019) Chocolate-based drinks enhance the overall vitamin D and calcium status in human beings. It also strengthens the immune system by increasing IFN-γ levels. The chocolate drink also increases the BMI. Thus it can prevent the problem of being underweight and can be used successfully in the malnourished community for the prevention of malnutrition (Taslim et al., 2020).

Manufacturing of chocolate is a multistep process consisting of fermentation, drying, roasting, nib grinding and refining, conching, and tempering cocoa beans. During this prolonged procedure, the nutrient loss is quiet notable. Polyphenol content drops almost 10 times from its original form. Some flavonoids are completely diminished in time of formation of Maillard products. For this reason, the antioxidant compounds vary from raw materials to finished products (Mattia et al., 2017). Approximately 50% of epicatechin is lost during drying. A higher rate of drying leads to the production of acetic acid which imparts undesirable flavors and too slow drying generates high mold growth and an absence of optimal colour in the chocolate (Cheng et al., 2015). Degradation of anthocyanin occurs during fermentation due to hydrolysis and further polymerization of condensed tannins (Mattia et al., 2017). During the time of fermentation, the content of epicatechin, polyphenol and procyanidins are drastically decreased. Even, anthocyanidins become non-detectable after fermentation (Zugravu and Otelea, 2019). To compensate for these large losses of phytonutrients, fortification is one of the effective ways. If dark chocolate is fortified with other valuable micro-nutrients; its therapeutic importance will be raised.

Micronutrient deficiency is a very common scenario in India. So, fortification is an effective way to solve this problem because it leads to a higher intake of micronutrients (Clewes, 2013). To the best of our knowledge similar review emphasizing the role of fortified dark chocolate on human health is not available. The current review article covers the experimental studies which focus on different approaches to fortification and also present the positive impact of dark chocolate fortification with different micro-nutrients for raising the overall immunity and nutritional status by preventing micronutrient deficiency diseases in the human community. Dark chocolate itself is a nutritious food item frequently consumed as a desert by people of all age groups. So, dark chocolate could be a good candidate for fortification. After processing the losses of nutrients should be compensated as well as to make it a more affluent source of crucial nutrients, fortification is the crucial procedure. Fortification of dark chocolate was already done with several micronutrients and bioactive compounds. The main aim of the current work is to explore the positive relationship between dark chocolate fortification and the alleviation of different diseases. Finally, to raise the therapeutic importance of newly developed dark chocolate and its effective utilization for the prohibition of different diseases.

2. Methodology

This review study is performed on the collaboration of different aspects of dark chocolate fortification. This study is dealt with the health benefits of dark chocolate, the different bioactive components present in dark chocolate, and also the processing steps and nutrient losses during the processing. There are several recent experimental studies which have discussed the bioactive components present in dark chocolate (Virgens et al., 2021; Faccinetto-Beltrán et al., 2021). Researchers have focused on the health benefits of dark chocolate since 2008 (McShea et al., 2008). The processing of dark chocolate and the effect of dark chocolate fortification on nutrient composition is a commonly discussed topic (Charoenngam and Holick, 2020). There are numerous research articles available that have discussed the processing of dark chocolate and the effect of processing on the stability of several phytonutrients (Wong, 2018). This review covers those studies, but the main focus of this review is to identify different approaches to dark chocolate fortification and enrichment of overall health benefits and physical properties of newly developed dark chocolate and ultimately to buildup a positive relationship between newly developed dark chocolate and disease prevention. Fortification of dark chocolate started in 2007 (Nebesny et al., 2007). But during the last 5–7 years the trends of dark chocolate fortification has been increased. There are 20 studies from 2018 to 2022 and 13 studies from 2013 to 2017. Searching relevant keywords indicates that, the health benefits of dark chocolate was a commonly discussed topic, and several recent studies are available on this topic (Bartkiene et al., 2021; Lamport et al., 2020; Zugravu and Otelea, 2019). So, searching for relevant keywords related to this topic clearly reflect that dark chocolate becomes a compulsive topic of discussion among researcher.

3. Bioactive components present in cocoa

Bioactive components are the secondary metabolites that contribute additional protection to the cocoa tree from UV lights, parasites, and pathogens (Lechtenberg et al., 2012; Salvador et al., 2019). Those bioactive components are responsible for the organoleptic properties of cocoa and cocoa-derived products, for example, dark chocolate (Aprotosoaie et al., 2016; Brglez Mojzer et al., 2016). Dark chocolate is rich in total phenolic compounds, catechin, caffeine, epicatechin, flavonoids, etc., that provide higher intensity bitterness, acid taste, cocoa flavor, astringency, and sometimes negatively affect the acceptance of chocolate (Virgens et al., 2021). A brief elaboration is given below.

3.1. Polyphenols

Polyphenol is one of the major components in cocoa beans. Polyphenols found in the plant are of different types, e.g. phenolic acids, stilbenes, flavonol and flavan-3-ols, and anthocyanins. Total polyphenol content in cocoa is partially dependent on the cultivators of cocoa beans. The amount of polyphenol varies among different cocoa-containing foods. It gives the plant protection against different herbivores and pathogens (Manach et al., 2004). In the natural form, cocoa is inedible because of the high amount of polyphenol content in the raw cocoa bean. It contributes secondary metabolites and is responsible for extreme bitterness, and astringency (Marie et al., 2021). Fresh cocoa bean contains 5–6% polyphenol (Urbanska and Kowalska, 2019). Cocoa polyphenol has a preventive role against cancer, cardiovascular diseases, metabolic disorders, and anti-inflammatory diseases. Lipid peroxidation is inhibited by polyphenols and also reduces LDL cholesterol oxidation. Cocoa modulates the glycemic response, platelet functioning, and inflammation along with systolic and diastolic arterial pressure. Intestinal inflammation is also delayed by cocoa polyphenol by reducing neutrophil infiltration and the expression of the different transcription factors, thus, the production of pro-inflammatory cytokines is getting lowered. Cocoa polyphenol also possesses chemoprotective effects, antimutagenic effects, anticarcinogenic and antiproliferative effects, etc. (Andújar et al., 2012), (Jalil and Ismail., 2008).

3.1.1. Flavonoids

Cocoa contains 12–18% of flavonoids on a dry basis. Cocoa contains a higher amount of flavonoids than apple, onion, and wine. The structure of flavonoids consists of 15 carbon atoms with 2 aromatic rings (Ring A and Ring B), which are connected through a three-carbon bridge (RingC). Flavonoids can be further classified into different subcategories such as flavonols, flavones, anthocyanin, flavanone, flavan 3-ols, anthocyanins, etc. (Tsao, 2010). The bitterness of the chocolate is attributed to high levels of flavonols. This mainly represents the palatability and organoleptic properties of dark chocolate (Hurst et al., 2008). Flavonol contributes better healing capacity and photoprotection to the skin and also promotes better dermal blood flow (Heinrich et al., 2006; Neukam et al., 2007). Flavonoids are effective against certain types of cancers and cardiovascular diseases. Cocoa flavonoids have greater potency in brain functioning. Its neurobiological action gives protection to vulnerable neurons and also stimulates neuronal capabilities. It can regenerate neuronal action through the neuronal intracellular signaling pathways (such as phosphoinositide 3-kinase (PI3-kinase/Akt) signaling cascades, mitogen-activated protein kinase, and extracellular signal-regulated kinase, etc.) and finally leads to strong memory and long term potentiation (Spencer, 2008). Flavonoids restrain neuronal degeneration by interacting with signaling proteins which are essential in pro-survival pathways. Thus flavonoids permit protection against Alzheimer's, diseases, and Parkinson's diseases (Spencer, 2009). Cocoa flavonol is also improved brain function. It also improves insulin resistance and blood pressure and positively modulates endothelial functions and glucose sensitivity (Desideri et al., 2012; Ballard and Junior., 2018; Barrio et al., 2020).

3.1.1.1. Flavonols. Flavonol is one of the important candidates of cocoa flavonoids, which comprises kaempferol and quercetin derivatives. Cocoa beans and their derived products generally contain a glycosylated form of the flavonol. Quercetin and its derivatives are frequently occurring derivatives in cocoa and its derived products (Almatroodi et al., 2021). Quercetin-3-O-galactoside, quercetin-3-O-arabinoside, quercetin-3-Oglucoside and quercetin-3-O-rhamnoside, quercetin-3-O-rutinoside etc are the examples of quercetin derivatives present in cocoa beans (Sánchez-Rabaneda et al., 2003). According to Karakaya et al. the absorption of quercetin glycoside is higher than the quercetin aglycones due to low water solubility (Karakaya, 2004). Cytosolic β-glucosidase is the enzyme which involves in the hydrolysis of quercetin glycosides in the enterocytes. In mammals cytosolic β-glucosidase is synthesized in the small intestine, kidney, and liver. Quercetin glycosides are absorbed after deglycosylation and ultimately converted into quercetin aglycones. Quercetin aglycones are further translocated with the help of intestinal mucosa by passive diffusion (Day et al., 2003; Németh et al., 2003).

3.1.1.2. Anthocyanin. In raw cocoa beans, 4% of the total polyphenol is composed of anthocyanin. Chemically it is glycosides of the anthocyanidin aglycones. That is a derivative of flavylium (2-phenyl benzopyrylium) salts (Akkarachiyasit et al., 2010). Cyanidin-3-O-galactoside and cyanidin-3- O-arabinoside is mainly present in Cocoa beans that are derivatives of cyanidin (Blanco-Montenegro et al., 2007). Anthocyanin is poorly absorbed in the intestine among all the flavonoids (Czank et al., 2013). Among all forms of anthocyanin, O-glycosyl is most easily absorbed, and it occurs by distributing it into the systemic circulation (Xie et al., 2016). The absorption of anthocyanin is dependent on epithelial transporters, such as sodium-dependent glucose transporter 1 (SGLT1) (Ader et al., 2001). It has an effective role in cardiovascular diseases. It also reduces oxidative stress by reducing the amount of ROS and by increasing the synthesis of antioxidant enzymes like superoxide dismutase and catalase (Chiang et al., 2006).

3.1.1.3. Procyanidins. It is another type of cocoa polyphenol which have an antioxidant effect by reducing the production of mRNA expression interleukin 2. Thus, it can reduce acute inflammation. It also enhances the production of pro-inflammatory cytokines and interleukin 1β. The investigation was done at the level of transcription and protein secretion to detect both the resting and phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (Mao et al., 2000). The absorption of cocoa procyanidins is poor in the GI tract. The bioavailability of Procyanidins is influenced by both degree of polymerization and the presence of A-type linkages (Appeldoorn et al., 2009; Ou et al., 2012). The location and stereochemistry of the interflavan linkage between the monomeric units, as well as their molecular size, is the greater influential factor in the bioavailability of procyanidins. The degree of polymerization can decrease the absorption capacity of procyanidins (Aprotosoaie et al., 2016).

3.1.2. Non flavonoids

3.1.2.1. Stilbenes. Another commonly occurring compound in the cocoa bean is resveratrol. The chemical name of this compound is 3,5,40 -trihydroxystilbene. It can be classified into two forms, the Cis form and the transform. In those two forms, the cis form is the active form (Hurst et al., 2008). Due to the similarity of the structure with synthetic estrogendiethylstilbestrol, sometimes it is also called phytoestrogen. Resveratrol and its all derivatives elicit different types of biological activities such as antiproliferative, cardioprotective activities, chemopreventive and anti-inflammatory, antioxidant activities, etc. Resveratrol enhances NO production and reduces the pressure of the blood vessel wall. It can enhance the amount of HDL cholesterol and also inhibits the aggregation of blood platelets (Rauf et al., 2018).

3.1.2.2. Phenolic acid. Phenolic acid is found in fewer amounts in the cocoa bean. Hydroxybenzoic acids (protocatechuic acid, gallic acid (GA), vanillic acid (VA), syringic acid (SA)) or hydroxycinnamic acids (coumaric acid (CuA), caffeic acid (CA), chlorogenic acid (CHA) and ferulic acid (FA)) are the commonly occurring derivatives of phenolic acid. Among all mentioned derivatives of phenolic acids, gallic acid (GA) is the highest occurring compound in cocoa beans. In the upper part of the gastrointestinal tract, phenolic acid is easily absorbed by passive paracellular diffusion (Adam et al., 2002). In gastric mucosa active absorption of phenolic acid takes place. In the stomach FA, GA, CuA, and CA, CHA is absorbed (Konishi et al., 2006). Among all the derivatives of phenolic acids, the absorption of GA is the highest. Its metabolization rate is very fast and rapidly excreted after ingestion (Mennen et al., 2006). The bioavailability of esterified phenolic acid is 0.3–0.4%. Hydrolisation of esterified phenolic acids is occurred in the enterocytes before reaching the bloodstream. The intestinal enzymes are not capable of breaking the esterified bond. Esterified phenolic acids enter the colon where metabolized by colon microflora (Azuma et al., 2000).

3.1.3. Non-polyphenols

3.1.3.1. Methylxanthine. Methylxanthine is one of the important bioactive compounds present in cocoa beans. Methylxanthine can be classified into theobromine (TB), caffeine (CF), and theophylline (TP). Theobromine and caffeine is the most commonly occurring compound in the cocoa bean whereas, theophylline is found in a lesser amount (Hurst et al., 2008). Methylxanthine is rapidly absorbed in the GI tract and metabolized in the liver and then excreted in the urine (Burgos et al., 2014). Commercially available dark chocolate bars contain 188 mg of TB and 26 mg of caffeine to increase the amount of theobromine in saliva, plasma, and urine, oral administration of 376 mg of cocoa is needed. The amount of TB in blood serum gets higher after 2 h of dark chocolate intake (Richelle et al., 1999). About 90% of CF is distributed and absorbed in its undissociated form throughout the whole body. Its concentration in blood plasma is raised after 30–70 min of ingestion (Beach et al., 1986).

3.1.3.2. Theobromine. Cocoa contains a significant amount of methylxanthine compounds, in which caffeine and theobromine are the predominant ones. Theobromine falls under the category of a group of purine alkaloids that provide numerous physiological benefits (Ashihara et al., 2011). Its bioavailability is quite high than procyanidin (Neufingerl et al., 2013). Theobromine increases the level of HDL cholesterol in the blood. It also elicits its activity on both heart and lung muscles. It can stimulate heart muscle and also relaxes bronchial smooth muscles in the lungs that play a major role in the transmission of intracellular signals (Blinks et al., 1972). Its amazing antioxidant role is used to treat depressive disorders (Scapagnini et al., 2012). It might have a measurable amount of neurocognitive effect. Its satisfactory amount of antioxidant effect is quite helpful for scavenging the free radicals, which are produced in the skin in the presence of UV light. Those ROS can negatively alter the signaling pathways in the skin (Saraf and Kaur, 2010). The health benefits of bioactive components present in dark chocolate are summarised in Fig. 1.

4. Health benefits of dark chocolates

4.1. Protection against vascular endothelial disorders

According to previous studies, regular consumption of dark chocolate will lower the occurrence of high blood pressure. Thus, it delays the occurrence of Cardio Vascular Diseases (Grassi et al., 2005b, 2008). Vascular endothelial disorder is the most potent key event of atherosclerosis. The main cause of vascular endothelial dysfunction is reduced bioactivity of nitric oxide (NO), which dictates impaired flow-mediated vasodilatation (FMD). Cocoa flavonols can enhance the circulating NO levels that ultimately raise the response of FMD in conduit arteries. This effect results in a great increase in microcirculation. Epicatechin-7-O-glucuronide is an independent predictor of vascular effects when flavonol-rich cocoa is ingested (Schroeter et al., 2006). It also ameliorates the function of the vascular endothelial system by improving platelet function in healthy smokers 2–8 h after ingestion (Engler et al., 2004; Wang-Polagruto et al., 2006; Nogueira et al., 2012).

4.2. Protection from diabetes

It improves insulin sensitivity by reducing insulin resistance. Dark chocolate keeps blood vessels healthy and makes the blood flow unimpaired (Grassi, 2005a). Reduced production of NO by the NOS enzyme is also responsible for defective insulin formation. That can lead to the generation of insulin resistance. Epicatechin present in the cocoa augmented the level of endogenous NO production that stimulates PI3K signaling. PI3K signaling pathways can influence insulin activation and glucose transportation in the metabolic tissues till the step of Akt activation. The hemodynamic effect of insulin is characterized by the recruitment of capillaries leading to the induction of glucose uptake. Cocoa polyphenol has a dual strategy (Akt/PI3K and ERK1/2 pathways) to stimulate insulin production from pancreatic β cells. Procyandine is another beneficial component present in dark chocolate that can reduce post-prandial blood glucose levels. Though the bioavailability of procyanidin is poor, it can still interact with glucose transporters. Translocation of GLUT4 to the muscle can facilitate central glucose clearance and enhance insulin signaling (Kerimi and Williamson, 2015; Grassi et al., 2005b).

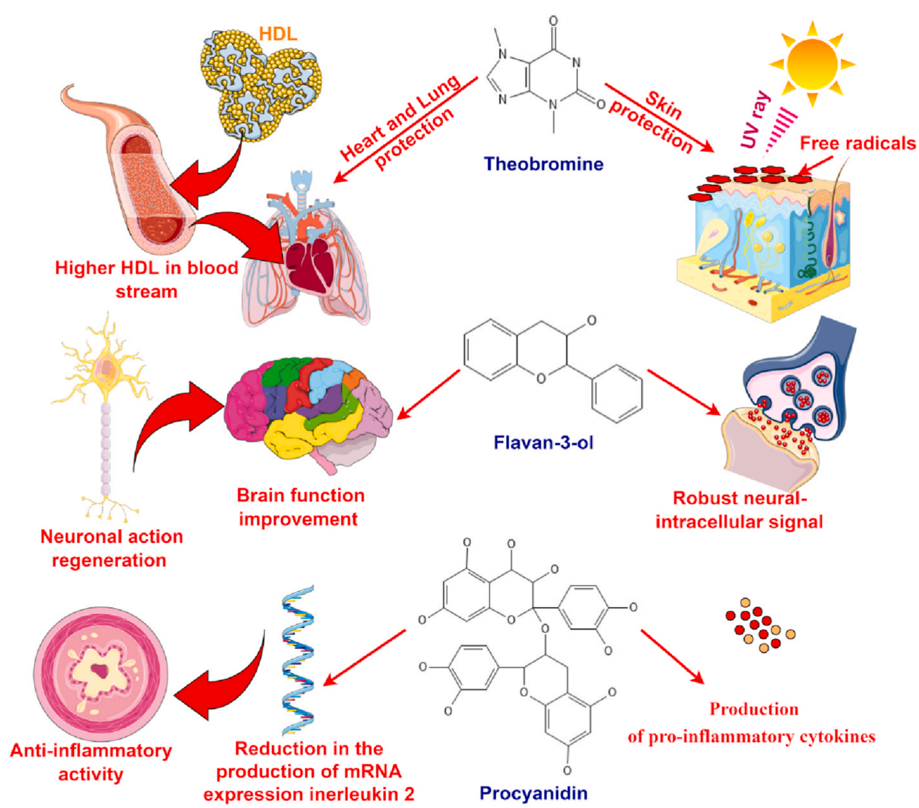

Fig. 1. Bioactive components present in dark chocolate and their health benefits.

4.3. Protection from oxidative stress

It gets scientific attention for its amazing antioxidant properties and reduction of cancer (Wan et al., 2001). Free radicals are continuously generated through metabolic processes. Those free radical damages healthy cells and dark chocolate consumption lowers the unnecessary cell damage (Baba et al., 2000). Antioxidants remove free radicals and protect the body from the early occurrence of cancer and aging (Davison et al., 2012). Dark chocolate stimulates mitochondrial activity as detected by citrate synthase activity and enhances glutathione levels which give protection from oxidative stress. Epicatechin present in dark chocolate influences mitochondrial production by increasing the level of nitric oxide that activates co-activator 1-alpha (PCG-1α), which is a stimulator of microbial biogenesis. These results improved mitochondrial function, gave protection against oxidative stress, enhanced cellular metabolism, and ultimately improved cellular metabolism. Mitochondrial function improves the level of ATP that also stimulates skeletal function. Epicatechin has another role in mitochondria. It directly inhibits the mitochondrial permeability transition pore (mPTP) and gives protection against cell death (Pinilla et al., 2015; Decroix et al., 2017).; (Munoz et al., 2013)

4.4. Amelioration of obesity

Dark chocolate is also used for weight management due to its low-calorie content. Regular intake of dark chocolate can reduce obesity (Farhat et al., 2014). Primary stages of adiposeness in preadipocytes are hampered by cocoa polyphenols. According to in vitro studies, cocoa polyphenol extract can resist diet-induced adipogenesis inside the cell by decreasing mitotic clonal expansion, which is an important step in DNA remodeling for gene expression atthe time of the development of fat cells. Mainly 2 types of transcription factors (peroxisome proliferator-activated receptor gamma and CCAAT enhancer-binding proteins alpha) are inhibited, which ultimately inhibits the mRNA expression of fatty acid synthase in these cells (Farhat et al., 2014; Kord-Varkaneh et al., 2019).

4.5. Maintenance of healthy lipid profile

A moderate amount of dark chocolate consumption will lead maintenance of blood cholesterol levels in a healthier direction. Cocoa resists the absorption and biosynthesis of cholesterol by decreasing the number of cholesterol receptors. The cocoa product reduces the amount of LDL and total cholesterol but has no significant effects on HDL levels. Cocoa polyphenol can reduce lipid peroxidation in the liver and serum, which lower levels of malondialdehyde production. But the liver glutathione level is not altered by cocoa fiber (Lecumberri et al., 2007).

4.6. Stimulate brain function

Cocoa is also believed to energize the human body. So, the cocoa-containing product is generally consumed before engaging in hard work (Toplar, 2017). Another prime importance of dark chocolate is to enhance brain function. DC (Dark Chocolate) is believed to improve the blood flow in both the heart and brain. The different chemical compounds present in dark chocolate stimulate cognitive function and also positively modulate mood swings (Lippi et al., 2009). Dark chocolate positively influences the nerve growth factor and theobromine levels in plasma (Sumiyoshi et al., 2019).

4.7. Anti-inflammatory effect

Several experimental studies found that Dark chocolate also has an anti-inflammatory effect. It enhances the expression of mRNA, especially anti-inflammatory cytokines IL10, by diminishing the pro-inflammatory stress response. Cocoa also has some direct influence on immune cells. Therefore, it can modulate innate and acquired immunity. Secretions of inflammatory substances from macrophages and other leukocytes are regulated by the ingestion of cocoa (Dugo et al., 2017). The health benefits of dark chocolate are summarised in Fig. 2.

The beneficial role of dark chocolate is entirely dependent on the characteristics of cocoa beans. Cocoa beans are rich in cocoa butter, but they also contain proteins and methylxanthines (caffeine and theobromine), minerals (zinc, phosphorus, potassium, iron, copper, and magnesium), as well as significant amounts of antioxidants (catechins, dietary polyphenols, proanthocyanidins, and anthocyanidins). 100 g cocoa contains 836.8 KJ or 200 kcal. The exact nutrient composition of Cocoa beans is summarised in Table 1.

5. Processing of dark chocolate

Chocolate is processed from cocoa beans after undergoing several steps (Table 2). At the initial stage, uniformly ripe cocoa pods are collected from the farmers. Harvested cocoa pods should be fermented within 3 days. Fermentation involved smashing the cocoa pods by breaking them down with an object on a concrete surface. Then cocoa beans are scooped by hand, and then these are taken for the fermentation step.

5.1. Fermentation

It is done by the basket method. This step consists of 3 stages. The 1st stage of the fermentation is dominated by yeast. It takes 24–36 h. The oxygen content and pH both are low in this phase. This stage is fully anaerobic. In the 2nd stage, Lactic acid bacteria are the predominant ones. The third stage is dominated by acetic acid bacteria. The alcohol present in cocoa is converted to acid at a high temperature (50 °C). This is an exothermic reaction. The chocolate aroma is formed by the fermentation step (Adeyeye et al., 2010; Afoakwa et al., 2008b).

5.2. Drying

The fermentation process is followed by drying. After completion of fermentation, beans are weighed and dried in an oven less than 70 °C. Moisture content is reduced to 6–8%. This process is important for the preservation of cocoa beans. Low moisture content reduces mold growth which makes the cocoa beans stable for transportation. After overnight drying, the beans are passed through a 20-mesh sieve screen for milling. At the end of milling, the finished product is stored in a refrigerator for further preparations. Storage should be done in an air-tight black polythene bag because bioactive components are very sensitive to air and light (Schwan, 1998).

5.3. Roasting

This process is mainly done by the chocolate factory. 120–140 °C temperature is applied for the development of the Maillard reaction. Roasting is essential for the reduction of undesirable compounds. This process is needed for the decontamination of cocoa beans and also for the generation of chocolate flavor and aroma. The precursors for the above steps react and generate new components (Afoakwa et al., 2008b; Barišić et al., 2019).

5.4. Grinding

Proper grinding and mixing are required to achieve the actual particle size of the chocolate. In this step, all the ingredients (cocoa liquor, cocoa butter, sugar, etc.) of chocolate are mixed properly to get a uniform mixture. The texture of the chocolate is also dependent on this step.

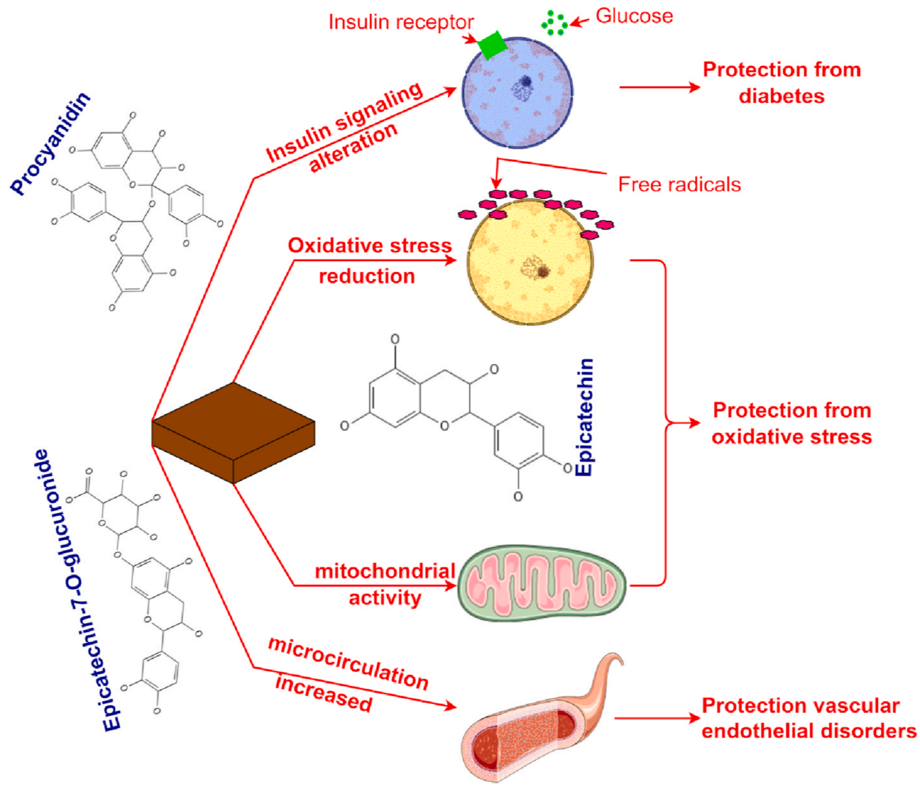

Fig. 2. Health benefits of dark chocolate.

Table 1
Nutrient composition of Cocoa bean (100 g).

Name of the Nutrients	(Abt et al., 2020)	Caprioli et al. (2016)	Aremu et al. (1995)	Afoakwa et al. (2013)	Poveda et al. (2020)
Energy (Kcal)	536	504.6	–	–	122
Moisture	–	3.53	5.0	4.2	3.60
Fiber (g)	–	–	5.9	–	3.60–13.13
Protein (g)	10.71	12.16	17.5	21.6	10.30–27.40
Carbohydrates (g)	–	34.53	–	–	7.85–70.25
Fat(g)	53.57	35.34	–	55.2	1.50–.49
Saturated	32.14	–	–	–	–
Sodium (g)	–	–	–	3.4	16.00–192.20
Calcium (mg)	–	–	29.2	140.2	0.23–0.44
Potassium (g)	–	–	–	2313.1	1.25–1.82
Iron (mg)	2.57	–	–	2.7	27.60–80.50
Phosphorus (mg)	–	–	201.0	236.6	58–100
Copper (mg)	–	–	–	11.1	2.35–6.62
Zinc (mg)	–	–	–	9.7	2.75–19.00
Magnesium (mg)	–	–	–	286.8	48–129

5.5. *Conching and tempering*

Final mixing and heating are done in this step. The higher temperature leads to the formation of liquid chocolate. In this step, all the solid particles are coated with fat, and volatile acids are evaporated. After conching, proper viscosity is achieved, excess moisture is removed from the cocoa, and the desired color is developed. After conching, tempering is applied. This process is done to obtain a stable product. Application of high temperature helps in the formation of consistently sized crystals of cocoa butter which ultimately leads to the formation of a stable crystalline network during cooling (Afoakwa et al., 2008b; Aprotosoaie et al., 2016). Above mentioned steps are common for all types of chocolates. These processing steps are required to retain the cocoa favor unaltered and are followed over the centuries. This process increases the overall palatability of the cocoa beans by changing physical and chemical characteristics. In their native form, cocoa beans are quite unpleasant in taste. So, many steps are involved in breaking down the shelled seed (Cotylendon) to neutralize unpleasant flavor. Fermentation was carried out when piles of harvested cocoa seeds were spontaneously inoculated. In the 1st 24 h of fermentation, the process is dominated by yeast, but in the 2nd half, the no. of Lactic Acid Bacteria is getting higher than yeast. For the preparation of dark chocolate, every ingredient should be present in the proper amount, for example, cocoa butter (45%), cocoa powder (15%), sugar (39.52%), Soy lecithin (0.3%), Sodium Carbonate (0.15%), and vanilla extract (0.03%). The sequential procedure for dark chocolate preparation is summarised in Fig. 3.

6. Significance of dark chocolate fortification

Dark chocolate is a natural source of antioxidants. Its antioxidant nature is entirely dependent on polyphenol, especially proanthocyanidins, flavan-3-ols (Epicatechin, catechin), and anthocyanins. The quantity of polyphenol present in dark chocolate is 12–15 mg/g. Its flavonoid content is comparatively higher than tea and wine. Biological availability is also greater in dark chocolate because its absorption capacity is much better than in milk chocolate. (Murga et al., 2011).

6.1. *Effect of roasting*

During the processing of dark chocolate, cocoa beans undergo various stages. So, the nutrient and antioxidant content may vary from the original one to the finished product. One of the important parts of cocoa processing is roasting, which can alter the composition of Nutrients that already exist in the cocoa beans. Roasting is responsible for the characteristic taste, color, aroma, and texture (Krysiak, 2006). Most of the time, 120–150 °C temperature is used for roasting and the total time taken for this process is 5–120 min. This condition exaggerates the loss

Table 2
Effect of processing on nutrients.

Processing Step	Key features	Effect on nutritional properties of dark chocolate	References
Roasting	Chocolate is treated at a high temperature (120–140 °C) temperature. Maillard reaction occurs.	Monomeric flavonols are reduced. Flavonoid content is reduced. Total antioxidant capacity was reduced by 44% and 45% at 190 °C for 15mins. FRAP decreases 51% and 45% at 125 °C and 145 °C. At 125 °C TRAP is reduced by 20%. The amount of fructose and glucose is decreased. 10% arabinose lost.	Ioannone et al. (2015) Redgwell et al. (2003) Moreno et al. (2012) (Vicker., 2017) (Vicker., 2017) Redgwell et al. (2003) Redgwell et al. (2003)
Fermentation	It is done by the Basket method. 3 step fermentation is applied. 1st phase is dominated by yeast. 2nd stage is dependent on Lactic acid bacteria. 3rd stage is predominant by *acetobacter aceti.*	Procyanidins and catechins are decreased. Phenolic compounds decreased than the raw materials. On the 9th day and 12th day of fermentation, protein content is reduced. After the 12th day, inorganic phosphate was reduced from 201.0 to 102.0 mg/100 g. Calcium content is increased 29.2 mg/100 g-60.4 mg/100 g. After the 6thday total fat content is starting to decrease. The amount of crude fiber is lost. Total antioxidant power is reduced. The composition of procyanidins and flavan-3-ols is altered. The amount of anthocyanin is reduced.	Aremu et al. (1995) Aremu et al. (1995) (Aremu et al., 1995) (Aremu et al., 1995) (Aremu et al., 1995) (McShea et al., 2008) McShea et al. (2008) (Vicker., 2017) (Moreno et al., 2012), (Djikeng et al., 2018) (Moreno et al., 2012), (Djikeng et al., 2018)
Conching	Solid particles are coated with fat and volatile acids are evaporated. Desirable color is developed. Proper viscosity is achieved.	In the Short time, conching process higher amount of monomer is produced. In Long term, conching process polyphenols undergo thermal alterations that are followed by a condensation reaction. The reduction of polyphenol is 3%.	Mattia et al. (2014) (Mattia et al., 2014) (Schumacher et al., 2009)
Drying	The temperature for drying is approximately 7 °C. Moisture content is reduced to 6–8%. Mold growth is significantly reduced.	Polyphenol content is reduced by 44–77% Flavan-3-ol content is reduced by 80%. Thermolabile vitamins (Thiamin, pantothenic acid, ascorbic acid, pyridoxine, riboflavin, etc) are reduced. Vitamin A and carotenoid undergo geometric isomerization and ultimately reduced their action. The amount of vitamin K is not much affected during drying.	Jawad et al. (2013) Mattia et al. (2013) Wong (2018) (Wong, 2018) (Wong, 2018) (Wong, 2018)

of total polyphenol content and flavonoids (Krysiak, 2011). The monomeric flavonols are lost during normal roasting temperature (Vicker., 2017; Ioannone et al., 2015). The rate of polyphenol degradation is reduced by maintaining 5% humidity during roasting. For a carbohydrate, for example, the amount of fructose and glucose is decreased, but the concentration of non-reducing sugar (stachyose, sucrose, verbascose, and raffinose) remains unaltered. In polysaccharides, approximately 10% arabinose is lost, but the overall level of pectic and hemicellulosic polymers is not changed after finishing roasting. The level of acetylation and esterification of pectic polysaccharides are not hampered by roasting. Polyphenolic compounds, polysaccharides, and Maillard products interact with each other by roasting (Redgwell et al., 2003). Traditional roasting causes higher nutrient loss than oven roasting. Antioxidant capacity is lost due to the destruction of the polyphenolic compounds (Reddy and Love, 1999). The high roasting temperature will increase the rate of polyphenol degradation, but sometimes it has been found that a high roasting temperature will lower the rate of polyphenol degradation due to low roasting time (Ioannone et al., 2015). Catechin is one of the important thermolabile antioxidant compounds, which is partially destroyed by roasting. Flavonoid content is also decreased during fermentation, but this is entirely dependent on the type of roasting procedure chosen by the industry (McShea et al., 2008). The total antioxidant capacity is reduced by 44% and 50% at a roasting temperature of 190 °C for 15 min (Moreno et al., 2012). The amount of FRAP decreases by 51% and 45% at 125 and 145 °C roasting temperatures, respectively. At 125 °C the number of TRAP increases by 7%. At the end of roasting, at 145 °C, temperature TRAP decreases by about 20% (Vickers, 2017).

6.2. *Effect of fermentation*

On the other hand, the duration of fermentation also plays an important role. On the 3rd day of fermentation, the total protein content does not differ from the raw one, but after 6 days of fermentation, this value is getting higher, while on the 9th and 12th days, the value is less than the raw content. The mineral content also differs from the original cocoa bean to dry matter. After 12 days of fermentation, the amount of inorganic phosphate varies from 201.0 to 102.0 mg/100 g. But the level of calcium increased from 29.2 mg/100 g to 60.4 mg/100 g (Aremu et al., 1995). The total lipid content is starting to decrease from day 3 of fermentation and the loss is getting significant from the 6th to day 9th onwards. Ash content is also lowered from the 6th day to the 9th day. The amount of crude fiber loss is also inversely related to the length of fermentation time. The antioxidant content of under-fermented cocoa beans is much higher and has an astringent flavor (McShea et al., 2008). The combined effect of heat (50 °C) and acid leads to the breakdown of cocoa seeds. During a time of fermentation process, several organic acids are generated. Phosphoric, oxalic, malic, and succinic acids are the organic acids that are responsible for the breakdown of cotyledons. This imparts the characteristic flavor of the chocolate, but the fermentation

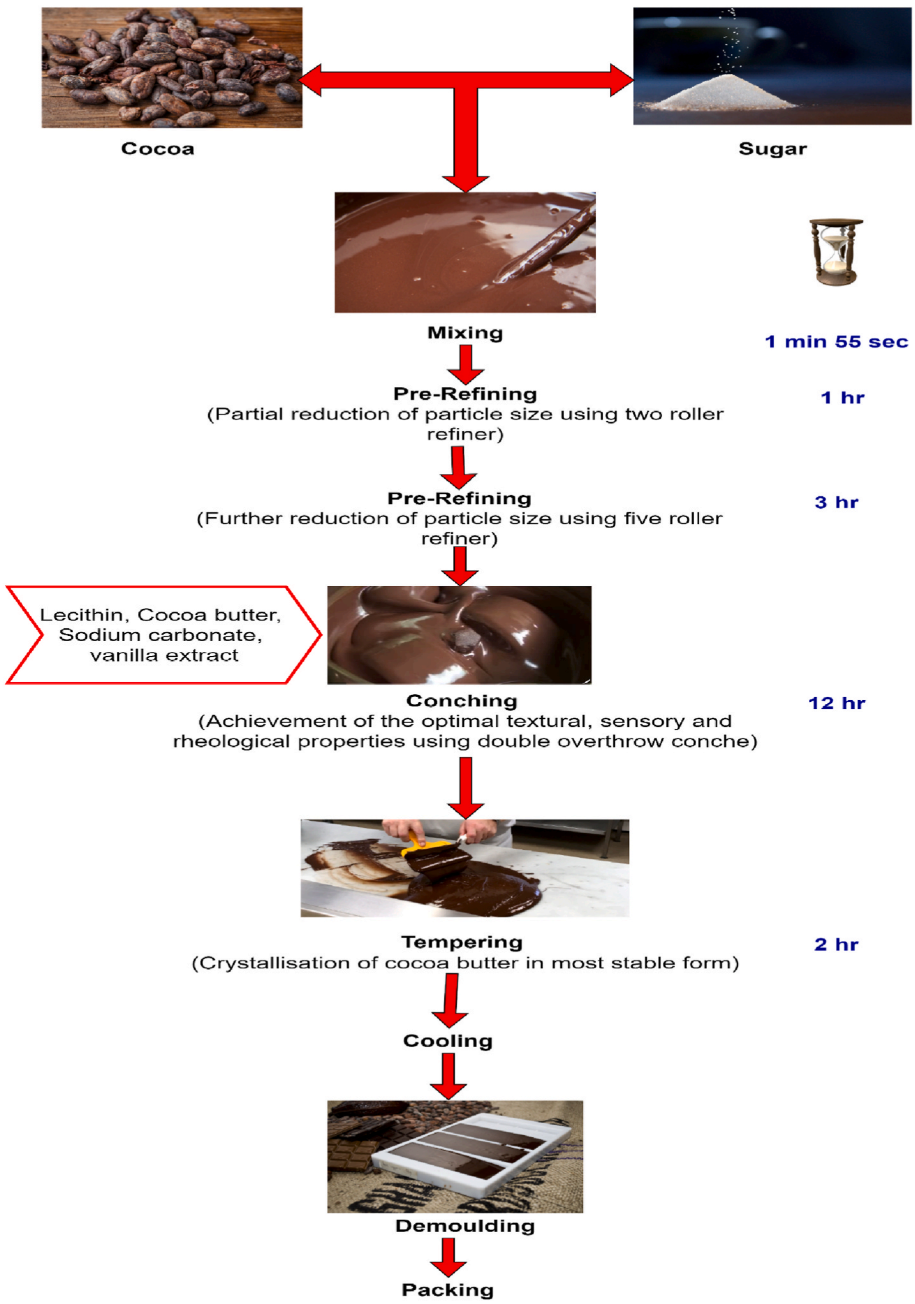

Fig. 3. Preparation steps for dark chocolate.

step is an uncontrolled and nonsterile process. Some variables interfere with the final flavor of chocolate. Mold colonization sometimes negatively affects the characteristics and flavor of chocolate. The composition and concentration of the flavan-3-ols and procyanidins are altered during the time of fermentation. According to several researchers' observations, fermented cocoa beans contain a higher amount of antioxidants with astringent flavor. The type of colonization and length of fermentation also result in the breakdown of flavonol content. Anthocyanin is an essential antioxidant component present in raw beans, but this content is reduced in fermented cocoa beans due to polymerization and hydrolysis of condensed tannin (Djikeng et al., 2018; Moreno et al., 2012). 20–40% reduction of total antioxidant capacity was observed during fermentation. Antioxidant capacity was measured by DPPH, ABTS, and FRAP (Vickers, 2017; Mattia et al., 2013). Recently one of the simplest and easiest methods of fermentation was found which was utilized in the formulation of kombucha tea by using SCOBY gel (Symbiotic Culture of Bacteria and Yeast). It can be used for the fermentation of cocoa beans because polyphenols and antioxidant properties will be enriched after fermentation. The addition of SCOBY gel will increase the polyphenol and antioxidant content of dark chocolate. The addition of SCOBY gel will increase the polyphenol and antioxidant content of dark chocolate (Permatasari et al., 2021, 2022a, 2022b).

6.3. Effect of conching

Conching is the mandatory step for attaining the actual viscosity, flavor, and texture of dark chocolate (Afoakwa et al., 2007). In maximum time, 70°C–90 °C was used for the dark chocolate preparation. When the temperature and condition applied for the conching varies from normal, then the flavor and texture also alter (Konar, 2013; Owusu et al., 2012). Grinding is the step when cocoa beans are converted into fine particles. Grinding also affects the nutrient content in chocolate. During the time of grinding, the fat and carbohydrate content of the chocolate is decreased. Conching is another essential part of processing. Conching is mainly of two types Short Time Conching process (STC) and Long Time conching process (LTC). Procyanidin content is significantly differing by the conching process. The composition of procyanidin varies in chocolate produced by LTC and STC. In the STC method higher amount of monomer is produced. In LTC, the polyphenols undergo thermal alteration that leads to a condensation reaction. On the other hand, a hydrolytic reaction occurs during the STC process. After considering two methods of conching, the yield of procyanidin is 70% in its bio-available form (Mattia et al., 2014). The polyphenol content and total antioxidant content of dark chocolate do not significantly change during this stage. The reduction of polyphenol is a maximum of 3% (Ozguven et al., 2016a; Albak and Takin, 2016; Schumacher et al., 2009).

6.4. *Effect of drying*

Drying is also responsible for the destruction of polyphenolic compounds. According to the researcher, sun drying reduces the total polyphenolic content by about 44–77% (Jawad et al., 2013). Sun drying is an uncontrolled process so it affects not only polyphenol content but also total antioxidant capacity. Flavan-3-ol is reduced by about 80% (Mattia et al., 2013). Apart from these antioxidant components, some types of vitamins are destroyed to some extent during processing due to their sensitivity to light, heat, and high exposure to oxygen. Certain types of fat-soluble vitamins, such as vitamin A and carotenoid, undergo geometric isomerization in time of thermal processing that may cause the degradation of vitamin content (Wong, 2018). Except for vitamin K, other types of fat-soluble vitamins are lost during thermal processing. The stability of the fat-soluble vitamins is dependent on the moisture content and water activity during processing and storage (Reid, 2020). Water-soluble vitamins such as Ascorbic acid, Thiamin, folacin, Niacin, Biotin, and Pantothenic acid are stable in acidic pH but lost in alkaline pH during manufacturing. Pyridoxin and riboflavin are lost photochemically during processing. Riboflavin biotin and niacin is stable in thermal processing. Thiamin, pantothenic, and ascorbic acid degradation occurs more easily due to their high sensitivity to temperature (Reddy and Love, 1999).

Minerals are much more stable component components, but the bioavailability of minerals is affected during milling, fermentation, and thermal processing (Reddy and Love, 1999). So, Fortification is needed to compensate for this large nutrient loss during processing because antioxidant plays a vital role in our body. Antioxidants are very much helpful in scavenging free radicals from our bodies. Those free radicals are the markers of early aging and also some types of cancers. If dark chocolate is fortified with antioxidant-rich nutrients and other vital nutrients, then it could play a vital role in delaying the occurrence of early aging and also preventing some types of cancers. Fortification also has multiple roles in enriching dark chocolate to enhance its nutritional aspects and also immunity-boosting capacity. The concept of fortification started successfully over several decades. Now it has become an emerging concern in every sector of the food industry. Thus, enriching those antioxidant components which are lost during processing fortification is a must. So, Fortification is an important step in chocolate manufacturing to gain its complete health benefits. The significance of dark chocolate fortification is summarised in the following Fig. 4.

7. *In vivo* antioxidant effect of dark chocolate

Chocolate is the most popular confectionary item throughout the world. But among all the chocolate varieties, dark chocolate is the healthiest option. Among all health effects, the anti-oxidant property is one of the most prominent ones. Dark chocolate is rich in polyphenol, a flavonoid. Epicatechin, flavan-3-ol monomers, catechin, and oligomeric procyanidins are the flavonoids commonly present in dark chocolate (Moutou et al., 2004). Flavonoids are very effective against oxidative stress. After all, they can scavenge free radicals from our bodies because they can chelate metal ions (Nicole, 2005). Procyanidins and their monomer, catechin, and epicatechin present in cocoa also have a protective role against DNA destruction and also reduce LDL oxidation as well as overall lipid peroxidation. Cocoa provides a safeguard against arterial plaque formation and maintains arterial flexibility, thus preventing atherosclerosis. Polyphenols and flavonoids are commonly present in dark chocolate and play dual actions in the treatment of cardiovascular disease and cancer (Kruger et al., 2014). Not only cancer, but dark chocolate also delays the generation of AIDS, Alzheimer's diseases, and alopecia and is helpful for the recovery of premenstrual syndrome (Baron et al., 1999).

Equally, it prevents unnecessary cell damage. In certain conditions, flavonoids and polyphenols also take part in some vital biological actions, for example, antithrombotic, anti-inflammatory, vasodilatory, antibacterial, antiviral (against influenza and HIV), antiplaque, and anticarcinogenic functions. Major polyphenols present in dark chocolate are Catechin, quercetin, Procyanidin B2, Protocatechuic acid,

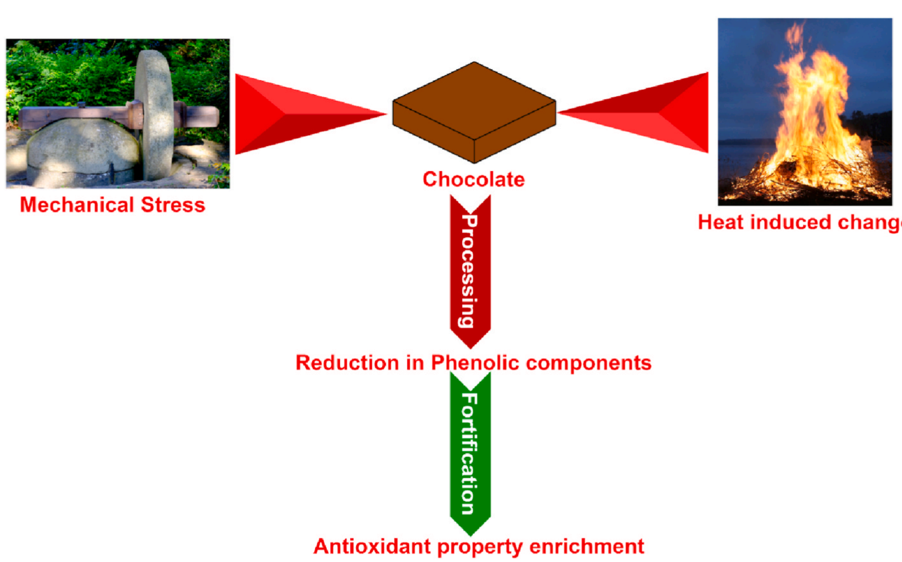

Fig. 4. Significance of dark chocolate fortification.

epicatechin, etc. Cocoa flavonoids are believed to improve insulin resistance by changing the vascular endothelial function; flavonoids can modify glucose metabolism for the reduction of oxidative stress. Compared to white chocolate, dark chocolate consumption increases beta cell functioning. The effect is markedly shown in that dark chocolate consumption is continued for a longer period (Hollenberg and Norman, 2006). It has also been observed that dark chocolate consumption reduces the fasting blood glucose level and maintains a healthy blood glucose profile. Flavonoid present in dark chocolate is responsible for the lowering of insulin resistance in blood and improves the sensitivity to the insulin receptor by inhibiting angiotensin-converting enzymes (Engler and Englaer, 2006). This ultimately maintains a healthy blood sugar level (Nogueira et al., 2012).

Flavonols in dark chocolate can dominate the growth of bacteria, especially gram-positive. Sometimes, several substances present in the flavonols form hydrogen peroxide (Vickers, 2017), which negatively affects the growth of gram-positive bacteria. It can also be effective against the toxins produced by *H. pylori* infections by suppressing the activity of urease. Flavonols can damage the bacterial cell wall. They are also proved to be effective in the prevention of some types of gut cancer, but the exact mechanism is still unknown.

Researchers have found that dark chocolate has a beneficial effect on arterial stiffness after doing some intervention studies. An intervention study was done on 35 individuals, where individuals were treated with dark chocolate for one week, and it was proved that dark chocolate improves arterial blood flow performance (Curtis et al., 2012). Flavan 3-ol has a direct effect on the prevention of cardiovascular diseases (Hooper et al., 2012). It prevents the hardening of arteries and also decreases the process of sticking white blood cells to the arteries (Fisher and Hollenberg, 2005; Mink et al., 2007). Flavonoid reduces oxidative stress by increasing the release of prostacyclins (Erdman et al., 2008). The main alkaloid present in cocoa is theobromine which shows a similar type of activity to caffeine. 40 g of dark chocolate contains approximately 240 mg of theobromine. The role of theobromine in dark chocolate is still under research. According to some limited findings, it can be said that theobromine cannot produce any satisfactory results in terms of anti-oxidant capacity (Cooper et al., 2008).

Dark chocolate is also popular for encouraging brain function. It influences the blood flow to the heart and brain so improves cognitive function. Several chemical compounds (tyramine, theobromine, phenylalanine, caffeine, etc.) are present in dark chocolate, responsible for the stimulating action on the brain. Phenylalanine is an aromatic compound that plays the main role in the secretion of endorphin, which makes a human being alert. Dark chocolate interacts with several neurotransmitters like serotonin and dopamine, which directly or indirectly regulate reward, mood regulation, and appetite. It enhances cognitive performance because it removes free radicals from the body, which causes memory loss and cognitive decline in old age. Several experimental studies have found that regular intake of flavonol-rich cocoa will increase cerebral blood flow. Thus dark chocolate could be used for the treatment of cognitive insufficiency, dementia, and stroke (Parker et al., 2006).

Theobromine also has a role in managing oral problems. It hardens the tooth enamel and prevents dental decay. Theobromine is not as strong as caffeine, but it also suppresses vagus nerves so it can cure cough (Martínez et al., 2015). Dark chocolate is a rich source of micronutrients, for example, vitamins B1, B2, B3, B9, K, Calcium, phosphorus, magnesium, manganese, iron, selenium, copper, potassium, and zinc. Potassium and copper have a cardioprotective role, especially in stroke and cardiovascular ailments. Iron is helpful for the prevention of anemia and magnesium has a role in pancreatic beta-cell functioning. Due to the presence of magnesium, dark chocolate has a preventive role against Type II Diabetes (Haritha et al., 2014). Cocoa product intake can correct magnesium deficiency problems in rats, but enough evidence was not to support this statement in the case of humans (Planells et al., 1999). So, At last, it can be well defined that dark chocolate has multiple therapeutic roles in human health. So, in the future dark chocolate could be an alternative medicine for the prevention of various degenerative diseases.

8. Approaches to fortify dark chocolate

The main motivation of the fortification is not only to make the product self-sufficient and complement the food product but also to make it nutritionally enriched. There are different approaches to fortifying dark chocolate. Several experimental studies have been done on dark chocolate fortification. For example, fruits and nuts, phytosterols, eicosapentaenoic acid, docosahexaenoic acid, etc., were fortified with dark chocolate. This review study includes different types of dark chocolate fortification. The detailed approaches are explained below (Table 3).

8.1. Dark chocolate fortified with fruits and nuts

Several researchers worked on fortification with fruits Ozguven et al. did fortification of dark chocolate with spray-dried black mulberry. Mulberry contains isoquercitrin, rutin, and 3-(- malonylglucoside), which reduces copper-induced LDL oxidation. It also plays an effective role in the prevention of atherosclerosis and Diabetes. Black mulberry is a rich source of flavonoids, especially anthocyanin, but during processing, storage, and distribution or in time of passing through the GI tract, the concentration of anthocyanin is lost to some greater extent. So, the main concern of this study is to store the existing amount of anthocyanin present in the black mulberry. So, the Fortification of mulberry in dark chocolate should be done in encapsulated form. The extract of black mulberry was combined with a fine dispersed anionic liposome, and the process was done by homogenization at 25,000 psi. Cationic chitosan was used to cover the primary liposome by using layer by layer depositing method. Maltodextrin was added before spray drying of mulberry. The fortification was done in the pH range of 4.5, 6, and 7.5 at conching temperatures of 40 °C, 60 °C, and 80 °C. The use of liposomes in time of fortification gave better protection against the loss of anthocyanin content, even though the sharp fluctuation in temperature and pH. Encapsulation also enhances the in-vitro bioaccessibility of anthocyanin (Ozguven et al., 2016b).

Godocikova et al. discussed the dark chocolate fortified with Sea buckthorn and mulberry. After fortification, the number of bioactive components was compared with the plain chocolate. Dark chocolate contained more polyphenolic compounds compared with milk chocolate and white chocolate. Milk solid reduced the availability of polyphenolic compounds. After fortification of dark chocolate with mulberry and sea buckthorn had a higher amount of polyphenol content than the control sample. The greater amount of polyphenol was mainly due to the presence of mulberry, even when a low amount of buckthorn was present. This study also included some other research works done on fruits and vegetables fortified with dark chocolate. Fruits are a rich source of polyphenols. Dark chocolate was fortified with different fruits like dried mulberry, prunes, papaya, cranberry apricot, and raisins. Especially fortification with prunes and cranberries has shown better polyphenol content than the control group. The total flavonoid content was almost double that of the original chocolate. The highest flavonoid content was observed again in mulberry fortified dark chocolate. Phenolic acid content was inversely related to the fortification with buckthorn. Total phenolic acid content was less than the normal chocolate. In terms of total antioxidant capacity measured by the DPPH free radical scavenging method best result was observed in the mulberry fortified product (4.39 mg $TEACa^{-1}$). Even the reducing power analysis also showed the best result in mulberry fortified dark chocolate (16.79 mg $TEACa^{-1}$) (Godočiková et al., 2017).

Here sik et al. had done another different type of fortification. In this experimental study, dark chocolate was fortified with lemon balm extract and rosmarinic acid level was checked. Lemon balm extract was

Table 3
Dark chocolate fortification approaches.

Fortification approach	Fortifying agent	Aim of fortification	Method of fortification	References
fortification with Fruits and nuts	Black Mulberry	To enrich the amount of anthocyanin content in dark chocolate.	The black mulberry was added in the encapsulated form to restore the efficiency of anthocyanin when it will be passing through the GI tract.	Ozguven et al. (2016b)
	Sea buckthorn and mulberry	To enhance the number of bioactive components, present in dark chocolate after fortification.	The fruit extracts were directly added to the dark chocolate after extracting bioactive components from it. After fortification, the total polyphenol, flavonoids, and total antioxidant capacities were checked.	(Godočiková et al., 2017)
	Lemon balm extract	To enhance the amount of rosmarinic acid content by fortifying with lemon balm extract.	This lemon balm extract was added in the form of freeze-dried after fortification rosmarinic acid content was checked.	Sik et al. (2021)
	Pequi mesocarp (pulp)	To improve the physical properties and nutritional properties for successful industrial production.	pequi mesocarp (pulp) was added in lyophilized form.	Lorenzo et al. (2022)
	elderberries (EFrE), elderflowers (EFlE), and chokeberries (ChFrE) plant extract	To enrich the functional properties of dark chocolate.	The dried plant extract was used for fortification.	Poliński et al. (2021)
	Dates and palms	To increase the amount of natural sugar extracted from dates and palms and also to reduce the total sucrose content of dark chocolate.	The palm and dates were added in the form of syrup. This syrup was added atthe time of the formation of dark chocolate. This was done by the double broiler method.	Ibrahim et al. (2020)
fortification with spices and herbs	Cinnamon oil	To increase the amount of total antioxidant content in dark chocolate.	The cinnamon was added in different percentage (0.25%, 0.50%, and 0.75%). The best possible concentration of cinnamon oil used for fortification was analyzed by checking sensory evaluation. The total antioxidant capacity was also checked after fortification.	Dwijatmokoa (2016)
	Cinnamon powder	To improve the dark chocolate sensorially, aromatically, and analytically.	Cinnamon was added in the form of powder. Gas chromatography-mass spectroscopy was used to analyze bioactive components and sensory properties were checked by using olfactometry and trained panelists.	Albak and Takin (2015)
	Cinnamon bark	To make the dark functionally and nutritionally enrich by adding cinnamon bark to the dark chocolate.	This fortification was done by adopting the process of encapsulation. Encapsulation was done to improve the functionality and bioavailability of the product.	Praseptiangga et al. (2019)
	Sakura green tea leaves and turmeric	Increasing the nutrient and antioxidant content of dark chocolate thus can prevent CVD and other degenerative diseases successfully.	The fortification was done in powdered form. Several bioactive components were checked after fortification for eg.ferulic acid, epicatechin, and epigallocatechin level were measured. FRAP and ABTS were applied to evaluate total antioxidant capacity.	Martini et al. (2018)
Fortification with Prebiotic and probiotic	Inulin (Prebiotic)	To design the chocolate in such a way that this can be used for the therapeutic purpose of the Prevention of gastrointestinal problems.	Inulin was added in powdered form. The sensory properties were checked by the trained panelists.	Norhayati et al. (2013)
	Bacillus coagulans	To make a novel type of dark chocolate that contains live microorganisms which could be helpful for the reduction of pathogenic bacteria in the intestine?	The bacteria were added after lyophilization. The stability of the new variant of chocolate was analyzed. The sensory properties were also checked after fortification.	Kobus-Cisowska et al. (2019)
	*Lactobacillus casei*01 *and Lactobacillus acidophilus* 5	To enhance the functional property of dark chocolate by adding an important variant of probiotic bacteria.	After adding lyophilized strain, the viability of live microorganisms was checked during preservation. The sensory properties were analyzed after fortification and storage.	Kemsawasd et al. (2016)
	Lactobacillus casei and Lactobacillus paracasei	To make healthy chocolate for diabetics by adding *Lactobacillus casei and Lactobacillus paracasei* strains and assess the overall acceptability during preservation.	After adding lyophilized strain, the viability of live micro-organisms was measured even during preservation. This chocolate was specially developed for diabetic patients. So, sucrose was replaced with Aspertame. To increase the volume of the finished isomaltose was added to the dark chocolate.	Nebesny et al. (2007)
	Lactobacillus plantarum	To make the dark chocolate suitable transporter for probiotic bacteria.	After the addition of lyophilized strain. The number of volatile components and aromatic profiles were evaluated. The stability was also checked even after 60days and 180days after preservation.	Mirković et al. (2018)
	L. plantarum 299v (L299v) and *L. acidophilus* La 3 (DSMZ 17742	Probiotic dark chocolate was made with low-calorie sweetener to use successively for diabetic individuals.	Microencapsulated strain was added in maltodextrin and then spray dried form was added to the dark chocolate.	Fernández et al. (2021)
	Leuconostocmessenteroids	To produce symbiotic chocolate to gain combined benefits from prebiotics and probiotics.	Dark chocolate was fortified with sterile flax seeds and fresh probiotic culture. After	Waghmode et al. (2020)

(*continued on next page*)

Table 3 (*continued*)

Fortification approach	Fortifying agent	Aim of fortification	Method of fortification	References
			fortification overall antioxidant capacity was checked.	
	Bacillus indicus HU36, maltodextrin and lemon fiber	Enrichment was done to produce functional dark chocolate.	The lyophilized strain was added to the dark chocolate. The lemon fiber and malto dextrin were added in powdered form.	Erdem et al. (2014)
Fortification with leaf	Raspberry leaf	To analyze the effect of raspberry addition to the three types of chocolate example dark chocolate, milk chocolate, and semi-sweet plain chocolate.	Freeze-dried leaf extract was added to make the healthy chocolate, After fortification physical, sensory and biochemical properties were measured.	Cvitanovic and Bauman (2012)
Fortification with polysaccharide	Poly dextrose and inulin	To make the chocolate less calorie dense by replacing sugar with polysaccharides and inulin.	Polydextrose and inulin were directly added. Mechanical and rheological properties were checked after fortification.	Aidoo et al. (2017)
	Xanthan gum, corn starch, and glycerine blends	The main focus of this study was to make the chocolate healthier by replacing the cocoa butter with a mixture of polysaccharides in dark chocolate.	Ingredients were directly added atthe time of formulation of chocolate. Physical properties (flow parameter, overall viscosity) were measured after fortification.	Syafiq et al. (2014)
	Polyglycerol polyricinoleate: water: gum Arabic: invert syrup	To improve the molding stability during the storage of dark chocolate.	Polyglycerol polyricinoleate: water: gum Arabic: invert syrup this combination was added in the form of emulsification.	Lillah et al. (2017)
	Wheat germ	To study the nutritional and physical characteristics of wheat germ fortified dark chocolate.	Both grounds and intact wheat germ were added during the time of chocolate mounding. After fortification, the proximate analysis was done to check the total nutrient content. Physical, microbiological, and sensory properties were checked.	Marazeeq (2018)
	D-tagatose and inulin	Production of fortified dark chocolate by using D-tagatose and inulin to reduce overall calorie content and glycemic index.	Fortifying agents were added in the powder form and then the physiochemical properties were checked.	Shourideh et al. (2012)
Fortification algae	Astaxanthin	To ensure if newly fortified dark chocolate could reduce the oxidative stress parameters in the human body.	The experiment was done in 4 groups. After applying the astaxanthin fortified dark chocolate oxidative stress biomarkers (Malonicdialdehyde and oxidized LDL level) were measured.	Petyaev et al. (2018)
	Spirulina platensis	To compare the effectiveness of dark chocolate and milk chocolate when fortified with *Spirulina platensis.*	The fortification was done by the encapsulation method. The color, texture, and aroma were compared with the control and that newly developed chocolate. The shelf life of those chocolates was also measured.	Asti and Ekantari (2020)
	Spirulina platensis	To make the dark chocolate calorie and vitamin A-rich, betacarotene was extracted from *Spirulina platensis* for fortification.	Spirulina was added in encapsulated form. To get a better result the researchers applied the analytical hierarchical method. The survey was carried out on 100 respondents.	Ekantari et al. (2019)
	Arthrospira carotenoid	To enhance the antioxidant content and check the acceptability of dark chocolate after the addition of arthospira carotenoid.	The project mapping method was applied to analyze the consumer response after fortification. Colour, texture, and flavor were evaluated after fortification.	Ramadhanti et al. (2021)
Fortification with lipid and related components	Phytosterols	Phytosterol has multiple roles in CVD prevention. So, the main focus of this study was to increase the unsaturated fatty acid content in dark chocolate by fortification.	Phytosterol was esterified with canola oil fatty acid and then it was added. After fortification measure the amount of unsaturated fatty acids in finished products.	Botelho et al. (2014)
	Peanut oil	To increase the composition of healthy fat in dark chocolate. So fortification with peanut oil could improve the composition because peanut oil was rich in tocopherol, phytosterol, and monounsaturated fatty acids.	The fortification was done by adopting the micro-encapsulation method. A spray drying technique was involved to encapsulate peanut oil.	Cooper et al. (2007)
	DHA, EPA	To enrich dark chocolate with DHA and EPA. DHA and EPA are very good components for brain development.	EPA was added in the form of micro-encapsulated powder from fish gelatin and DHA was added in the form of free-flowing micro-algal powder.	Toker et al. (2018a)
	Santolina chamaecyparissus	To make the dark chocolate essential fatty acid rich as well as the antioxidant content.	After extraction of essential oil by hydrodistillation method from this leaf, the oil was added to the chocolate in molding process.	Bölek et al. (2022)
Fortification with Protein	Whey Protein Isolate and erythritol	To measure the effect of whey protein isolate (WPI) and erythritol addition on physicochemical properties of untempered high-protein (16%, 20% and 24% of protein), sugar-free dark chocolates intended for athletes and diabetics.	WPI and Erythritol were directly added to the melted chocolate. Then molded in a proper shape.	Nastaj et al. (2022)
Fortification with bioactive components	Flavonol	To enhance the overall antioxidant capacity (flavonol content and procyanidin content) of enriched chocolate.	This experimental study analyzed the total antioxidant capacity of the enriched chocolate and compares it with the normal conventional chocolate.	González et al. (2020)
	Flavan-3-ol	To understand the exact mechanisms involved in the prevention of platelet	42 healthy individuals were selected for the experiment. After applying fortified dark	Ostertag et al. (2013)

(continued on next page)

Table 3 (*continued*)

Fortification approach	Fortifying agent	Aim of fortification	Method of fortification	References
		aggregation by applying flavan-3-ol enrich dark chocolate	chocolate several blood and urine parameters were checked for the analysis of platelet aggregation. ADP-induced platelet aggregations and p selectin expression were checked.	
	Flavan-3-ol	To check out the acute effect of flavan-3-ol enriched dark chocolate on cardiovascular disease.	The assessment was done on plasma metabolon before 2 h and after 6 h of taking dark chocolate. A comparative study was done between 3 types of chocolates.	Ostertag et al. (2017)
Rice berry rice	Anthocyanin	The main purpose of this study was to make healthier chocolate by enhancing its anthocyanin content.	Anthocyanin was added in the form of freeze-dried powder. Maltodextrin was used for covering those anthocyanin particles. It was added in the concentration of 5, 10,15 g/g cocoa powder.	Ngamdee et al. (2020)

added in the form of freeze-dried. This freeze-dried lemon balm extract was incorporated to enhance rosmarinic acid content. Rosmarinic acid incorporation was done to increase its antioxidant content. It also plays several vital functions in our body, such as acting as an immunomodulatory, anti-inflammatory, antidiabetic, antimicrobial, hepatic, and renal protectant agent, and anti-allergic. Even when the sensory evaluation was done there was no significant alteration observed after fortification. So, it could be said that lemon balm extract could be used as a fortifying agent for dark chocolate (Sik et al., 2021).

Some researchers have fortified dark chocolate with lyophilized pequi mesocarp (pulp). After fortification, scanning electron microscopy, granulometry, carotenoids and centesimal composition were analyzed. 1.5% lyophilized pequi mesocarp was used for the fortification. Total antioxidant capacity, physiochemical parameters, particle size and rheological properties, and other nutritional properties were significantly altered after fortification. After fortification, the content of oleic, linoleic and palmitic acids content were increased. Lyophilization was chosen for the preservation of existing nutritional qualities. The phenolic content of the fortified chocolate was increased from 235.98 μg GAE mL^{-1} to 813.49 μg GAE mL^{-1}. In this study, lyophilization was chosen for the preservation of existing nutritional qualities (Lorenzo et al., 2022).

According to Polinski et al. dark chocolate was enriched with chokeberries (ChFrE), m elderberries (EFrE) and elderflowers (EFlE). In this experimental study, the dark chocolate which was used for the fortification was already containing zinc lactate. The formulated chocolate was taken for the analysis of total antioxidant capacity By ferric-reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods. Folin–Ciocalteu (F–C) assay was used to check total phenolic content (TPC). The highest TPC and antioxidant capacity were observed in dark chocolate enriched with chokeberries (DCh + ChFrE). Viscosity, total fat content and some physiochemical properties (moisture content) were also checked (Poliński et al., 2021).

The researcher was focused on the fortification of dark chocolate with palm sap (CPS) and date sugar (CDS). Physiochemical and mechanical properties were checked after fortification. The fortified chocolate contains 4.18% (CPS) and 3.96% (CDS) moisture which was 4 times higher than the control (1.24%). CDS produced a higher amount of agglomerated moisture. CPS had the smallest aptitude to hold the load with measured penetration of 180 g/s measured by harness measurement study hence CPS had a much softer texture than other variants. Colour was not affected after fortification. There was a slight increase in the roughness of the chocolate after fortification in both types. After concerning all parameters overall acceptability was increased by the consumer (Ibrahim et al., 2020).

8.2. Dark chocolate fortified with spices and herbs

Indian spices and herbs also could be a good candidate for the fortification of dark chocolate. This review study also includes some types of experimental studies. Here the dark chocolate was fortified with cinnamon essential oil. According to Dwijatmokoa et al. Cinnamon is a very popular type of spice for culinary use. But it also has numerous immunity-enhancing properties due to the presence of cinnamaldehyde. This component is responsible for the antioxidant property by inhibiting oxidative reaction and is also believed to enhance the acceptability of the product due to its excellent flavor-enhancing capacity. It also has anti-inflammatory, and anti-diabetic characteristics. It also reduces serum LDL and triglyceride levels, thus reducing the risk of developing cardiovascular diseases. Certain literature studies showed that cinnamon also has antimutagenic, anticancerous, hypoglycemic, and hypolipidemic properties. Due to the addition of cinnamon oil to the dark chocolate, the sensory property improved more than the control due to its significant aromatic properties. Fortification was done in the concentration of 0.25%, 0.50% and 0.75%. The fortification did not significantly affect the color of the product. In 0.25% addition got the best result in terms of taste because 0.50% and 0.75% were getting much spicy. So from the results of the panelists, it could already be proved that the acceptability of the chocolate was higher when fortified with mild cinnamaldehyde with a concentration of 0.25% due to mild aromatic flavor. The main aromatic compound present in cinnamon is trans-cinnamaldehyde (55.45%) which is responsible for the beneficial properties of chocolate. Cinnamaldehyde was effective in increasing the efficiency of pancreatic β cells when consumed at the concentration of 20 mg/kg body weight. The total antioxidant capacity of cinnamon oil was 0.60 ± 0.05 mg TAE/g (Dwijatmokoa, 2016).

Another experimental study was done by Albak et al. on the fortification of refined dark chocolate with cinnamon powder. This powder was added to the dark chocolate at the time of the conching process. The fortification was done in a laboratory-style conching machine. Aromatic properties were tested both sensorial and analytically. The sensory characteristics were checked by trained panelists, and the olfactometry and solid-phase microextraction (SPME)-gas chromatography (GC)-mass spectroscopy (MS) worked in a combined way to measure the analytical profile of the newly developed product. The innovative product got greater acceptance in the overall inclination test. Conched sample's fruity odor value was lower than the unconched sample. Cinnamon chocolate produced a more specific aroma than normal dark chocolate. This aroma was produced due to the interaction of cocoa mass and cinnamon. The amount of pyrazine was reduced and the amount of aldehyde was increased during conching. But certain textural properties such as hardness and coarseness of the sample were not rich at the satisfactory level. They scored below the reference result. The proximate analysis showed the proportion of macronutrients. The nutritional composition of fortified dark chocolate was Carbohydrate-

57.80%, Protein-8.73%, Fat-30.70%, Ash-2.15%, Moisture-0.54%. After conching 26 aromatic compounds were identified, for example, Butanal, 3-methyl, 1-propen-2-ol-acetate, Pyrazine, 2,5, dimethyl, etc. (Albak and Takin, 2015).

Dark chocolate was also fortified with cinnamon bark to make the chocolate functionally and nutritionally rich. According to Praseptiangga et al. the chocolate fortification was done by the encapsulation method to cover undesirable characteristics of oleoresins. At the time of encapsulation, the cinnamon bark was added in the concentration of 4%, 6%, and 8%. The encapsulation is also used to improve the functional properties of the chocolate and also to improve the bioavailability of the fortified product. After fortification, physical, chemical, and sensory properties were assessed. The fortification at the rate of 8% was the best-accepted product by the panelists. Oleoresin microcapsule addition was markedly affecting the texture, phenolic content, and lightness of the final product. The finished product contains amide, methylxanthines, esters, aldehyde, hydrocarbon, tocopherol, and phytosterol. A higher amount of oleoresin reflected a higher amount of antioxidant capacity. After considering all the parameters, the addition of cinnamon bark in the concentration of 8% was the best product with a higher amount of tocopherol antioxidant content (Praseptiangga et al., 2019).

According to Martini et al. dark chocolate was fortified with sakura green tea leaves and turmeric. After fortification, the polyphenol profile was checked and compared with the original one. Dark chocolate could be used in the treatment of cardiovascular diseases, but the phenolic profiles are not identified properly in previous studies. So, this study was mainly focused on the identification of phenolic compounds present in normal dark chocolate and fortified chocolate. Normal dark chocolate contains catechin and procyanidin that represent more than the 90% phenolic profile of cocoa products. In flavan 3-ols the major monomeric structures are catechin and epicatechin. It has been found that epicatechin has a cardioprotective role in older patients. In the healthy adult male, the elevation of nitric oxide, level occurs after taking flavanol-rich cocoa, which results in enhanced flow-mediated dialated response in arteries. Hydroxycinnamic is another compound that comprises a 20.6 percent phenolic profile of dark chocolate. Another major component is ferulic acid. Whereas sakura green tea leaves fortified dark chocolate (GTDC) contain a higher amount of phenolic compound than normal chocolate. Due to high phenolic content, the antioxidant capability also rises more than normal. The Ferric reducing capacity and ABTS radical scavenging capacity rise to 144% and 40%, respectively. Epigallocatechin, epicatechin (49%), procyanidins, and hydroxycinnamic acid were increased than the normal dark chocolate. On the other hand, turmeric fortification also increased the total polyphenolic content. In addition, that turmeric contains curcuminoids which provide additional health properties such as antimutagenic, anti-inflammatory, anticancer, antioxidant, anticoagulant and antimicrobial, etc. So, it provides better tolerance against several chronic degenerative diseases such as neurological diseases, diabetes, cancer, and obesity (Martini et al., 2018).

8.3. Dark chocolates fortified with prebiotics and probiotics

Another type of fortification was done in dark chocolate, for example, prebiotic fortified dark chocolate. According to Norhayati et al. Inulin is one of the most popular types of prebiotic with multiple health benefits. Prebiotics are one of the essential components of maintaining gut health. They are helpful in maintaining a healthy count of probiotic bacteria in the gut; they protect the human body from an intestinal infection, stimulate mineral absorption, prevent constipation and reduce the incidence of colon cancer. Prebiotics are also responsible for the production of vitamin B. The inulin is added in such a way that the healthy components should be restored. Prebiotic chocolate had a better shelf life of up to 12 months when stored at 18 °C. No fungal growth was observed after processing, and it was also fairly accepted by the sensory panelist due to its good taste. So, after this product development, it could be a better option in terms of sugar-free confectionery items (Norhayati et al., 2013).

Another example of innovative fortification was probiotic fortified dark chocolate. This novel type of chocolate was made with *Bacillus coagulans*. According to Kobus et al. Probiotics are generally used as a medication for the treatment of intestinal problems. It can inhibit the growth of pathogenic bacteria. The concentration of polyphenol and its bioavailability were evaluated in this in-vitro study. *Bacillus* was used for fortification because bacillus can form endospores, so its activity in harsh conditions was not destroyed easily. After enrichment of the chocolate, the polyphenol content, pH and acidity were not affected. The sensory property did not differ sharply from the original dark chocolate. But from this, it was observed that the higher survival rate of *Bacillus coagulans* was observed when it undergoes the GI tract. Thus Dark chocolate provides a suitable matrix to deliver *Bacillus coagulans* to the human body, especially to children so, this novel product could also be an example of functional food (Kobus-Cisowska et al., 2019).

According to Kemsawasd et al. three types of chocolate (milk, white and dark) were fortified with different strains of probiotic bacteria, for example, *Lactobacillus casei* 01 and *Lactobacillus acidophilus* LA5. After fortification stability of those bacteria was checked up to 60days of storage. For both cultures, the viable count of those bacteria was more than 6 logs CFU/g when stored at 4 °C for 60 days. Chocolate provided a suitable transporter to transport probiotic bacteria without affecting its bioavailability in the gastrointestinal pH. The probiotic fortification was not affecting the overall sensory attributes of the chocolate. After 60 days of storage, the overall liking was decreased by the consumers. But within proper storage time, it could be used as the best carrier for delivering the probiotic bacteria to ensure the consumer's overall health status (Kemsawasd et al., 2016).

Another effective study was done on the same fortification by Nebesny et al. Here, and the dark chocolate was supplemented with *lactobacillus casei* and *Lactobacillus paracasei*. In this experimental study, those bacterial strains were lyophilized in milk. The total number used for the supplementation was 7.9×109 cfu/g. Sucrose, Aspartame, and isomaltose were used as bulking agents and sweeteners. The sensory properties of chocolate did not differ from the conventional ones. If sucrose was not used, then the calorie value was decreased by about 11.1–14.6%. Sucrose-free dark chocolate was developed for diabetic patients by using aspartame and isomaltose. This newly developed chocolate was easily stored for 12 months affecting its viable count. Up to 12 months of storage, those probiotics maintain a higher bacterial count, nearly 106–107 cfu/g. The temperature should be maintained at 4–18 °C. The number of volatile acids and textural properties was not altered before and after the addition of probiotic supplements. The sucrose-free chocolate supplemented with lyophilized bacteria contained 3–55% less fat content. So, it's a good choice not only for diabetic patients but also could be used for the treatment of obesity (Nebesny et al., 2007).

Dark chocolate was also fortified with *Lactobacillus plantarum* bacteria in encapsulated form. This study was done by Mirkovic et al. After fortification, volatile properties and sensory profile were evaluated. Two types of strains (*Lactobacillus plantarum* 299v, *Lactobacillus plantarum 564*) were used for the incorporation. Their survival rate was checked after 60days and 180days of preservation. 108 cfu/g was seen after 60days of preservation and 106 cfu/g after 180 days of preservation. No major differences were observed between the control and experimental chocolate in terms of the composition of volatile components. Even there was no effect was observed due to the addition of probiotics to the chocolate. The appearance, aroma, and color were not disturbed even after 60 and 180 days of storage. These strains could be used as a fortifying agent to fortify dark chocolate without affect ting any characteristics of dark chocolate (Mirković et al., 2018).

Some experimental studies have focused on the production of sugar-free dark chocolate. But Fernández et al. have focused on sugar-free chocolate that was fortified with probiotics. This fortified chocolate could be easily used by diabetic individuals. Here L. *acidophilus* La 3 (DSMZ 17742) and *L. plantarum* 299v (L299v) strains were used as probiotic bacteria, whereas; stevia (Stev), polydextrose (Pol), inulin (Inu) and isomalt (Iso) were used as natural low-calorie sweeteners. Low-calorie sweeteners were used in combination with Pol + Inu and Iso + Stev. Sensory acceptability and physiochemical properties were not affected by probiotic addition. The best result was obtained when dark chocolate was fortified with the combination of Iso + Stev + Probiotics. This formulation is successively used by diabetic individuals

According to Waghmodeet al., dark chocolate was fortified with both prebiotics and probiotics. This combined fortification produced symbiotic dark chocolate. The source of the prebiotic was flax seed and the probiotic strain was *Leuconostoc messenteroid*. Flax seed was chosen because it promotes the growth of probiotic bacteria. Biochemical, morphological, and MALDI-TOF tests were done to check several characteristics of the probiotic culture. *Leuconostoc messenteroid* was chosen due to its high tolerance in NaCl, temperature, pH, bile salt, etc. It also has the capability of the production of hydrogen peroxide. Thus it can produce cytotoxic activity against neuroblastoma cells (30%) and MDA, MB 231 (48%). Ultimately the symbiotic chocolate produced more antioxidant activity, for eg. 200 μg torolox/mL when measured by FRAP assay and 90 μ/mL by DPPH method. Ultimately symbiotic chocolate provides excellent nutritional support for maintaining a healthy gut in human beings (Waghmode et al., 2020).

Here also, some researchers are kin interested in the production of symbiotic chocolate. According to Erdem et al., *Bacillus indicus* HU36 and dietary fibers (maltodextrin and lemon fiber) were used as fortifying agents of dark chocolate. *Bacillus indicus* HU36 was used as a probiotic agent after fortification. The viability test was done to check the number of live microorganisms after fortification. For the formulation of this innovative type of dark chocolate, Three-level (1.5, 3.5, 5.5 (g/100 g)), two factorial (maltodextrin, lemon fiber) Central Composite Design (CCD) were adopted. The survival rate of *Bacillus indicus* HU36 after fortification was between 88 and 91%. Sweetness, firmness and adherence were improved after the addition of dietary fibre. No negative effects in color and sensory properties were observed after fortification (Erdem et al., 2014).

8.4. Fortification with leaf

Cvitanovic et al. compared three types of chocolate fortified with concentrated freeze-dried red raspberry leaf extract. In this experimental study, physical, sensory and bioactive properties were measured after fortification. The textural and rheological properties showed that among all variants, semisweet plain chocolate was the hardest variant, then plain chocolate came after that. In the case of milk chocolate, a 3% addition of freeze-dried leaf extract reduced the hardness of the chocolate. 1% addition to semi-sweet chocolate significantly reduced the hardness of the chocolate. On the other side, dark chocolate containing the freeze-dried extract had the lowest hardness. Microstructural examinations showed similar types of structure on the surface image of three types of chocolates. Total polyphenol content was lower in milk chocolate because milk protein interferes with the availability of catechine. In the case of dark chocolate highest amount of Total Polyphenol Content (TPC), procyanidin, flavan-3-ol, and total antioxidant capacity (measured by ABTS and FRAP Assay) were shown. In the case of 1%, the addition of leaf extract gave higher results in terms of TPC content. 1% addition of leaf extract got the sensory acceptance as well as visual and textural characteristics in semisweet and dark chocolate. The antioxidant capacity measured in the FRAP assay showed that 3% concentrated leaf extract fortified dark chocolate had the highest antioxidant capacity 1% fortified dark chocolate came after that. But the semisweet chocolate and milk chocolate showed slightly increased antioxidant capacity than plain chocolate after fortification (Cvitanovic and Bauman, 2012).

8.5. Fortification with polysaccharides

Several researchers focused on the replacement of sugar with some of the popular polysaccharides like polydextrose and inulin. Polydextrose and inulin were replaced with sucrose to make them healthier. The beneficial roles of prebiotics have already been discussed in this review study. After replacement, bulk mixture concentration and the effect of fat content were measured. The effect of inulin and polydextrose on the mechanical and rheological properties of the finished products was checked. Steviol glycosides are used to sweeten this product. Polydextrose and inulin were added with dark chocolate having different fat concentrations (27%, 30%, and 33%). High inulin with low polydextrose concentration gave the best result due to a sharp rise in Casson plastic viscosity and simultaneous reduction in Casson yield stress. This explanation was given by microstructural examination of the sugar replacers that presented large variations in network structure. This finished product would be helpful for the reduction of the total energy load in sugar-free dark chocolate. This application could be done in any type of sugar-free confectionery item (Aidoo et al., 2017).

Some types of differential studies were also included in this review study. According to Syafiq et al. cocoa butter was replaced with xanthan gum or corn starch and glycerine blends. Cocoa butter is one of the important components of dark chocolate. In this experiment, the fat component was replaced with a non-fat substituent. After replacement, the flow parameter was checked with the help of a D-optimal mixture design. The chocolate fortification was done in the concentration of 5%, 10%, and 15%. Xanthan gum had a high water holding capacity and glycerine contributed to high yield stress. So, both components enhanced the stickiness and viscosity of the product. The dual effect of xanthan gum and glycerine gave higher viscosity and positively modified the rheological property of the product. Cocoa butter individually provided only the continuous phase for the transport of solid particles in chocolate, but this combination enhanced the viscosity and shape of the product, which raised the flow behavior of the new variant of chocolate. So, 5%–15% replacement positive modified the rheological characteristics of the chocolate (Syafiq et al., 2014).

Sometimes fortification or replacement was done to improve the physical properties of the dark chocolate. Here, the researchers Lillah et al. replaced cocoa butter with a cocoa butter substitute (CBS: polyglycerol polyricinoleate: water: gum Arabic: invert syrup). This replacement increased the stability of the product in hot weather. The prepared chocolate bar was taken for the evaluation of pH, shape retention index (SRI), color, hardness, water activity, moisture retention, and sensory evaluation. The new variant of chocolate had high moisture and fat content. Up to 60 mL/L CBS, emulsion addition decreased surface whiteness and viscosity of the product, but the sensory attributes improved. Maximum hardness was observed when CBS emulsion was added to the concentration of 80 mL/L. But the appearance of fat bloom decreased the acceptability of the product and sensory property was also below the average value. So, after analyzing 2 variants, it could be concluded that the addition of CBS emulsion in 60 mL/L concentration produced the best result (Lillah et al., 2017).

According to Marazeeq., dark chocolate was fortified with wheat germ to examine the alteration of physical, nutritional, and chemical characteristics after fortification. The fortification improved the total protein and mineral content of dark chocolate. The total fat content was decreased than the control. The calorie content was reduced by about 20.5 kcal/100 g. The amount of carbohydrates and moisture content was similar to the original one. The best result was observed when the fortification was done with 10% wheat germ. But mouth feel, taste, texture, and overall acceptability were better than normal dark chocolate. Colour and sensory properties were not affected by fortification. Two months of controlled storage did not affect the sensory properties of the chocolate. So, it can be claimed fortification provided a healthier option for all age groups people (Marazeeq, 2018).

Replacing sugar in dark chocolate with another healthy option is a common approach tothe production of sugar-free dark chocolate. Here the researchers used D-tagatose and inulin for the production of chocolate that can be easily consumed by diabetics and patients having tooth decay. The glycemic index and overall calorie content were reduced after fortification. The D tagatose and inulin were added in the proportion of 100:0, 75:25, 50:50, 25:75, and 0:100. Moisture contents of the chocolate samples decreased with the reduction of the inulin content and increase of D-tagatose content. D tagatose contributed more hardness to the chocolate. Whereas inulin conferred the least L*,a*,b*,c* and huge value when it was added in 100% concentration. The appearance and plastic viscosity were decreased and linear stress and yield stress were increased when there was a reduction in inulin concentration. The Addition of D-tagatose increased the overall consumer acceptability. The best acceptable dark chocolate was produced when inulin and D-tagatose were replaced by sucrose in the ratios of 25%–75% and 100% (Shourideh et al., 2012).

8.6. Fortification with algae

Petyaev et al. dealt with another type of fortification; for example, dark chocolate was fortified with astaxanthin which is an algal antioxidant. For the prevention of oxidative stress, this innovative product was developed. Here are biologically available polyphenols of lycosome-formulated dark chocolate (DC) which contains a co-crystallized form of astaxanthin (LF-DC-ASTX) to evaluate the oxidative stress parameters on the human body. The study was done in four groups. Each and every group was treated differently. For example, 1st group was taken as control and was treated with 7.5 g DC, and the 2nd group was treated with 4 mg and 7 mg astaxanthin capsule, 3rd group was suggested to take DC bars (7.5 g) and 4 mg ASTX capsules for co-ingestion in the form of two separate formulations. 4th group was treated with LF-DC-ASTX, which contains astaxanthin with different concentrations of 1 mg, 2 mg, 4 mg, or 7 mg co-crystallized with 7.5 g of Dark Chocolate matrix. The treatment was followed for 1 month. After taking, 2–4 weeks, serum malondialdehyde (MDA) level and serum oxidized LDL level was evaluated. No significant changes were observed in the control group. Those people who took ASTX capsules showed a reduction of oxidative marker to some extent, but the best oxidative LDL inhibition result was obtained from the group who was taken astaxanthin combined with the dark chocolate matrix. So it was proved that dark chocolate and ASTX were best effective in combined form and it would be a future alternative for the management of oxidative stress-related disorders (Cancer, CVD, and Diabetes) (Petyaev et al., 2018).

Asti et al. focused on consumer preferences, which were checked after the fortification of dark chocolate with *Spirulina platensis*. Dark chocolate was fortified with beta carotene extracted from *Spirulina platensis* (0.372% dose) to make it energy-dense and vitamin A rich. This study followed the analytical hierarchical method to carry out the survey. In Yogyakarta, 100 respondents were chosen for participation. According to the consumer's response, the taste of fortified chocolate was quite bitter, acidic, and astringent. Caffeine and theobromine were the responsible factors for bitter taste. Apart from these two components, polyphenol and flavonoids were attributed to the bitter taste. During roasting and conching, the acid content of cocoa beans was not evaporated, so those acids contributed to the mild acidic taste of dark chocolate. The astringent taste was given by anthocyanin. Its mouthfeel was good. The texture of the chocolate was smooth, hard, and grainy. The fortified chocolate's price and its health benefit made the product more acceptable to the consumer (Asti and Ekantari, 2020).

Researchers have found one study focused on the consumer presence of dark chocolate fortified with *Spirulina Platensis,* but Ekantari et al. compared the stability of the chocolate when *Spirulina Platensis* was fortified with dark chocolate and milk chocolate. Especially carotenoid used for fortification was isolated from the *Spirulina Platensis*. The fortification was done by forming nano-encapsulate. After enrichment, it was observed that texture, taste, and aroma were not varied with the control. The flat bloom development also lowered fortified chocolate rather than control during storage. But the stability test proved that the shelf life of fortified dark chocolate was 1.5 times higher than milk chocolate (Ekantari et al., 2019).

Several researchers also had done fortification of dark chocolate with *Arthrospira carotenoid*. Ramadhanti et al. used the project mapping method to check consumer acceptance. The researcher wanted to check the *Arthrospira carotenoid* incorporation can influence the buying behavior of customers. The comparison was done between fortified dark chocolate and commercial dark and milk chocolate. *Arthrospira carotenoid* was fortified with dark chocolate in the form of nanocapsule. It was shown that *Arthrospira carotenoid* incorporation would enhance buying interests of customers. The color, texture, and flavor increase the acceptability of the product. The main reason to buy arthrospira product was its good antioxidant capacity. Besides, that arthrospira contains anti-inflammatory phycocyanin pigment; it had polysaccharides with antiviral and antitumor capacity. It's another important component was linoleic acid which was helpful for the reduction of total cholesterol and the effect of immunoglobulin (IgE) (Ramadhanti et al., 2021).

8.7. Fortification with lipid-related components

To reduce the total cholesterol intake by individuals, the next study focused on fortification DC with phytosterol esters. According to Botelho et al. Phytosterol reduces the absorption of cholesterol, so it maintains heart health. Phytosterols could be lost at the time of oxidative reaction or during storage or processing. So, the DC bar containing palm oil or 2.2 g phytosterol was stored at 20 °C and 30 °C for a period of 5 months. The highest value of hydroperoxide was obtained after 60 days of storage at the temperature of 20 °C and 30 days of preservation at 30 °C also gave a better result than the control group. Hydroperoxide could be attributed to the increased amount of alpha-linolenic acid present in phytosterol. After 90 days of preservation, the chocolate bar became softer and lighter. But these changes were not affected the sensory profile of the chocolate. Bioactive components were better reserved during 5months of storage at room temperature. This phytosterol-fortified dark chocolate could be a better option in terms of functional food (Botelho et al., 2014).

Fortification with vegetable oil gives several additional health benefits. Vegetable oil is sometimes called nutritional oil due to the high amount of polyunsaturated fatty acids such as linolenic and linoleic acid content with ample sources of monounsaturated fatty acids. Vegetable oil also provides an adequate amount of natural antioxidant components such as Phytosterol, tocopherols, phenolic compounds, carotenoids, etc. Peanut oil contains tocopherol, palmitic acid, and linoleic acid. Cooper et al. focused on the effect of dark chocolate fortified with peanut oil. In this study, microencapsulation was followed to preserve bioactive compounds. The microencapsulated product had low moisture with great stability. Spray drying was applied to encapsulate the product to make the product at a low cost, easily available with higher flexibility. Arabic gum and maltodextrin are generally used in this encapsulation process. The finished product was thermally stable and the physical characteristics were also sufficient for industrial production. The particle interactions were leading to a decrease in the amount of free fatty acids in the finished products. Ultimately viscosity of the product also increased more than the control. Microencapsulation prevented the fatty acid and antioxidant products from oxidation (Cooper et al., 2007).

Dark chocolate was fortified with DHA (Docosahexaenoic acid) and EPA (Eicosapentaenoic acid). According to Tokeret al., product quality was checked after enrichment. Here EPA was added in the form of microencapsulated powder based on fish gelatin and DHA was added as free-flowing microalgal powder. Melting property and rheological property were not significantly altered. It is also well accepted by consumers due to its satisfactory sensorial properties. EPA and DHA in their triglyceride form reduce the hardness of the finished product. So, this enriched dark chocolate could be a medium for delivering omega-3 fatty acids to the human body (Toker et al., 2018a).

Here the researcher enriched the properties of dark chocolate by fortification with *Santolina chamaecyparissu essential* oil. The fortification was done in the concentration of 0.01 ml/g (w/w), 0.02 ml/g (w/w) and 0.03 ml/g (w/w). Physical, textural, and chemical rheological properties and sensory thermal, and antioxidative properties were analyzed after fortification. The antioxidant property was enhanced after enrichment. Rheological property was not significantly altered after fortification. The processing steps were not modified due to the enrichment. For getting the best possible enriched dark chocolate *Santolina chamaecyparissu* essential oil should be added to 0.02% (Bölek et al., 2022).

8.8. Fortification with protein

Here the researcher enriched the dark chocolate by using whey protein isolate (WPI) and erythritol to make it more suitable for the consumption of athletic and also diabetic individuals. Colour analysis, contact angles and roughness were checked after to get an idea about the surface properties of the dark chocolate. Water activity, melting properties and viscosity was checked. WPI-enriched chocolate produced slight roughness and a more hydrophobic surface. After WPI addition there is an improvement in the shelf life of the chocolate and also the mechanical properties. Protein-enriched chocolate was more resistance to melting. WPI addition produced a denser structure which leads to the firmly packed chocolate. Colour coordinates were not significantly changed by addition. The anti-blooming effect was produced by the addition of WPI and erythritol. Therefore it can be said that highly nutritious dark chocolate was produced ata low cost (Nastaj et al., 2022).

8.9. Fortification with bioactive components

To improve the total flavonol profile of dark chocolate, it was enriched with epicatechin and procyanidin B2. After enrichment, the total flavan 3ol was checked, and this was 4 mg/gm (3 times higher than the conventional chocolate). The amount of procyanidin B1 and B2 was 2.4 times higher in fortified chocolate. The total content of procyanidin B1 and B2 was almost 6 mg/g. The total flavonol content was increased by 39%, which simultaneously increased the total antioxidant capacity by about 56% when compared with the conventional one. Regular consumption of 10 g of enriched chocolate was provided with 200 mg of the flavonol. Cocoa flavonol is believed to maintain the elasticity of the blood vessels that imparts normal blood flow. So this enriched product could be used for the purpose of maintaining healthy cardiovascular health.

Researchers have found that flavonol-rich dark chocolate improves platelet functions. An experimental study was done to understand the exact mechanisms involved in the relation between flavonol-rich chocolate (dark) consumption and the prevention of platelet aggregation. The experiment was carried out on 42 healthy individuals. They were treated with 2 types of chocolate flavan-3-ol-enriched dark chocolate and standard dark and white chocolate. To evaluate the platelet function, blood and urine samples were collected from the individuals after 2 and 6 h of taking enriched chocolate. Flavan-3-ols enriched dark chocolate significantly lowered the adenosine diphosphate-induced platelet aggregation. P-selectin expression in men also decreased simultaneously there is the reduction of platelet aggregation induced by thrombin receptor activating peptide. The new version of dark chocolate is also responsible for the enhancement of thrombin receptor-activated peptide-controlled fibrinogen binding in women and raised the collagen/epinephrine-induced ex vivo bleeding time in women and men. But white chocolate only induced epinephrine/collagen-induced ex vivo bleeding time in men and had a role in decreasing the adenosine diphosphate-controlled platelet P-selectin expression. After considering two types of enriched chocolate, it could be summarised that the two types of chocolates are played a vital role in the reduction of atherogenesis, but Flavan-3-ols in dark chocolate are more effective than in white chocolate (Ostertag et al., 2013).

One comparative study was found in which differentiation was done to check the acute effect of flavan-3-ol-enriched dark chocolate, standard dark chocolate, and white chocolate on the metabolism of humans. Urine and plasma parameters were assessed before 2 h and after 6 h of taking enriched chocolate. Here, 42 healthy volunteers were engaged in this trial. Some significant changes were observed in the level of endogenous metabolites after taking measurements. In plasma, there was no theobromine and catechin was observed. Para-hydroxyphenylacetate (HPA) was increased in both types of dark chocolate after 6 h of intake. The amount of lactate was increased in the case of white chocolate ingestion and concomitantly, it was decreased in flavan-3-ol enriched dark chocolate ingestion. But the amount of urinary pyruvate was reduced because flavan-3-ol enriched dark chocolate increased the rate of gluconeogenesis and glycolysis. Whereas the low level of a glucogenic amino acid (Glycine, arginine, alanine, and valine) indicated an increased rate of gluconeogenesis. Standard dark chocolate and fortified dark chocolate both have the power of oxidative stress-reducing capacity. This statement was well supported by the capacity of creatinine to reduce the power of catechine. The amount of catechine present in dark chocolate can reduce oxidative stress. The reduction of creatinine also plays an indicator of better regulation of ATP usage and PCR pool. Ultimately it was proved that the flavan-3-ol enriched dark chocolate was much more effective in the reduction of oxidative stress by modifying human metabolism (Ostertag et al., 2017).

Another type of unique study was found, where Rice et al. fortified dark chocolate with broken rice berry rice which delivered plenty of anthocyanins to make healthy dark chocolate. Here the researcher partially replaced the cocoa powder with anthocyanin powder. The anthocyanin powder was added in the concentration of 5 g, 10 g, and 15 g. At first, the anthocyanin powder was made from the rice berry by freeze-drying technique and the maltodextrin was taken for coating purposes. The Colour and appearance of the finished product were not affected by the anthocyanin addition. Anthocyanin reduced the hardness of the chocolate. The anthocyanin powder addition increased the total antioxidant capacity measured by the DPPH assay. The DPPH free radical scavenging capacity was increased by about 4–9%. The 10 g anthocyanin fortification produced the best result in terms of sensory evaluation. The amount of anthocyanin concentration was increased with the fortification. This rice berry rice was used to enhance the value of the underutilized Thai crops (Ngamdee et al., 2020). Approaches to dark chocolate fortification are summarised in Fig. 5.

9. Process of addition of bioactive ingredients to the dark chocolate

To produce healthy chocolate, it is necessary to add bioactive ingredients in the proper step, and time of preparation. Bioactive components are very sensitive. They are easily destroyed by light and heat and strong chemical components. So, it should be added after conching, Conching is a crucial step where micro-organisms are inactivated and the flow behavior, color, aroma, and texture are developed (Toker et al., 2019). In this step, the chocolate mass is heated to a specific temperature of >40 °C. This process also includes mixing, shearing, and aeration of cocoa mass. The next step is tempering, and in this process, chocolate transforms liquid mass into a solid mass. Before the tempering step, bioactive components are added to restore their stability. Especially, probiotic bacteria can maintain their viable count if added before the tempering step. High temperatures in conching process can destroy the activity of bioactive components (Fernández et al., 2021; Konar et al., 2018; Toker et al., 2018a).

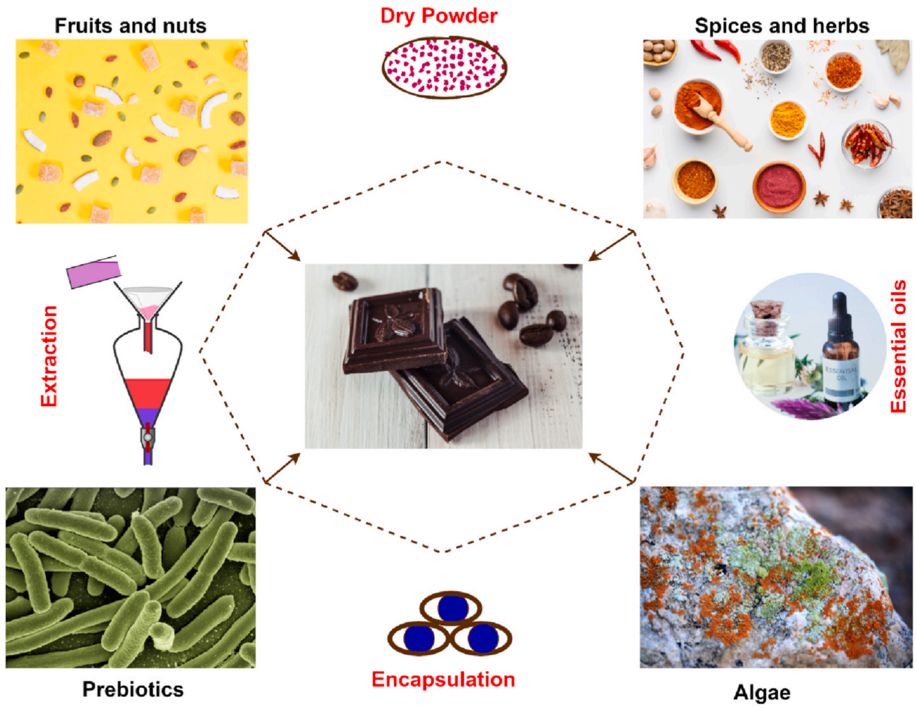

Fig. 5. Approaches to fortify dark chocolate.

10. Effect of dark chocolate fortification on physical, nutritional, antioxidant profile and health immunity

From the above discussion, it has been shown that the dark chocolate was fortified with different categories of ingredients (Including edible parts and waste parts). Thus, this fortification produces several innovative types of effects on the physical properties as well as nutritional properties (Table 4). Those effects are categorized below.

10.1. Effect of fortification on physical and rheological properties

Dark chocolate fortification with different ingredients sometimes imparted alteration of physical properties. Colour, texture, viscosity, flow properties, and Aroma were affected due to fortification. In a maximum number of cases, the fortification or enrichment positively affects the physical properties of dark chocolate. For example, the addition of fruits and nuts will improve the color, texture, and aroma of the product. The sensory properties were not largely affected by the addition of Prebiotic and probiotics. On the other hand, fortification with peanut oil increased the viscosity of the finished product. When cocoa butter was replaced with xanthan gum, the viscosity and shape of the finished product increased. Thus, the new variant of dark chocolate showed better flow behaviour (Didar, 2021; Toker et al., 2018b).

10.2. Effect of fortification on nutritional properties

The main aim of this review study is to focus on the nutritional enrichment of dark chocolate by fortification. After summarising all the experimental studies related to the fortification of dark chocolate, it can be said that fortification would improve the nutrient content and composition of dark chocolate. For example, black mulberry was a rich source of flavonoids, especially anthocyanin. So, fortification with black mulberry would make dark chocolate richer in anthocyanin content. Another study was focused on black mulberry and sea buckthorn enhancing the total polyphenol content in dark chocolate. Lemon balm extract provided rosmarinic acid content to dark chocolate. Prebiotics and probiotics provide a good environment in the GI tract for the synthesis of the vitamin B complex. Fortification with beta carotene increased the total vitamin A content and calorie content of dark chocolate. As well as the fortification enriches the total vitamin D content of dark chocolate (Didar, 2021). Different types of polysaccharides' fortification increase the total fiber content of the novel type of fortified dark chocolate. The amount of monounsaturated and polyunsaturated fatty acids were increased with the fortification of phytosterol and peanut oil. The DHA and EPA fortification enrich the essential fatty acids content in dark chocolate.

10.3. Effect of fortification on antioxidant properties

The overall antioxidant capacity of dark chocolate increased more than the control after adding nutritious ingredients. The bioactive component contains the highest amount of antioxidant capacity. The majority of the studies were focused on the enhancement of the total antioxidant capacity of dark chocolate. Polyphenolic compound and total flavonol content were responsible for the antioxidant capacity. Those components are believed for the best removal of free radicals from the body. This maintains a balance between the total antioxidant status and oxidative stress. Anthocyanin also alters the Nrf2-ARE signaling pathway, which ultimately raises the functioning of several antioxidant enzymes such as Super Oxide Dismutase (SOD), Glutathione Peroxidase, and Catalase (Kropat et al., 2013). Black mulberry, mulberry, buckthorn, lemon balm extract, cinnamon, raspberry leaf extract, astaxanthin, and flavon-3-ol components were added successfully to increase the total antioxidant capacity of dark chocolate. The black mulberry contains a high amount of anthocyanin, and lemon balm extract contains rosmarinic acid, cinnamon contains cinnamaldehyde, etc. are the specific bioactive components present in those fortifying agents. Different properties after fortification are summarised in Fig. 6.

10.4. Effect of fortification on human health

One of the major aims of this review study is to explain the effect on health after fortification. Every study aimed to fortify dark chocolate in such a way that it would provide some extra beneficial role to the human

Table 4
Effect of dark chocolate fortification and their measurement.

Fortifying agent	Effect of fortification	Quantitative results obtained	Instrumental characterization	References
Mulberry	Total anthocyanin content (ACN) and phenolic content (TPC) were increased.	TPC = 69.23 ± 5.1%, and ACN = 56.19 ± 4.8%	Quanta FEG 250 SEM	Ozguven et al. (2016b)
Sea buckthorn and mulberry	Biological activity and antioxidant potential were raised in newly fortified chocolate.	DC + Sea buckthorn = 8.07 mg GAE^-1 DC + Black mulberry = 8.83 mg GAE^-1 DC = 7.32 mg GAE^-1 DPPH value DC + Sea buckthorn-4.10 mgTEACa^-1 Black mulberry-4.39 mgTEACa^-1 DC-3.64 mgTEACa^-1	–	Godočiková et al. (2017)
Lemon balm extract	Total rosmarinic acid content was increased.	Freeze-dried lemon balm extract provided 88.4 ± 1.5 mg/g	HPLC (High-Performance Liquid Chromatography)	Sik et al. (2021)
Pequi mesocarp (pulp)	The amount of essential fatty acids (oleic, palmitic and linoleic acids) increased. TPC content was increased.	The energy value of the fortified dark chocolate was 559.73 kcal.100 g-1. The TPC content was increased by about 235.98 μg GAE.mL-1 to 813.49 μg GAE. mL-1.	SEM, thermobalance (Shimadzu - DTG - 60H), Digimatic Mitutoyo - digital Micrometer Model 293, rheometer	Lorenzo et al. (2022)
elderberries (EFrE), elderflowers (EFlE), and chokeberries (ChFrE)	Chokeberries fortified dark chocolate showed the highest amount of antioxidant capacity and TPC content.	DPPH results showed 90–95% increases in antioxidant capacity in Chokeberries fortified with dark chocolate. The Value of DPPH was (181.07 μmol TE/g).	SEM and Viscometer.	Poliński et al. (2021)
Dates and palm syrup	The overall sugar content was reduced after fortification with some modification of physiochemical properties.	Palm and dates syrup fortified chocolate produced higher moisture content 4.18% and 3.96% respectively. Palm produced the lowest penetration power (180 g/s)	Texture analyzer, moisture analyzer and stereo microscope	Ibrahim et al. (2020)
Cinnamon oil	The main aromatic compound present in cinnamon oil was detected.	An aromatic compound present in cinnamon oil (Transcinnamaldehyde) = 55.45%	GCMS (Gas chromatography-mass spectroscopy)	Dwijatmokoaet al. (2016)
Cinnamon powder	Nutritional composition and flavor were enhanced.	Carbohydrate-57.80% Protein-8.73%, Fat-30.70%, Ash-2.15%, Moisture-0.54%. 26 aromatic compounds were identified after the conching process.	solid-phase microextraction (SPME)-gas chromatography (GC)-mass spectroscopy (MS) and-olfactometry (O)	Albak and Takin (2015)
Cinnamon bark	Total antioxidant and TPC content were increased than the control. The new variant produced a hard texture.	8% fortification produced the best result. Fortified chocolate contained theobromine-11.993%, caffeine-31.671%, cinnamaldehyde-56.336%, Tocopherol content 176.0.43 IU	GCMS	Praseptianggaet al. (2019)
Sakura green tea leaves	Wide varieties of phenolic products had been identified after fortification.	A total of 158 phenolic compounds had been detected in the final product, 67 were freshly identified in dark chocolate, and 38 components were already present in dark chocolate.	LCMS (Liquid chromatography Mass Spectrometry)	Martini et al. (2018)
Inulin	After the replacement of sugar with inulin and polydextrose, Casson plastic viscosity and Casson yield stress changed.	30% and 27% fat-containing chocolate gave increased viscosity among them 30% provided the highest casson viscosity. 27% fat content permitted higher casson yield stress. 25–36% cocoa butter percentage gave the smooth texture of chocolate.	HPLC	Norhayati et al. (2013)
Bacillus coagulans	Several physical properties (pH, moisture content) were altered. Several polyphenolic compounds were recognized.	pH-6.04-6.22 Water activity-0.121–0.134 (after 12 months of storage). Quercetin constitutes about 50% of the total polyphenol compound. The addition of probiotics at the level of 106 CFU $^{g-1}$ gave the best result.	a_w meter, pH meter, HPLC	Kobus-Cisowska et al. (2019)
*Lactobacillus casei*01 *and Lactobacillus acidophilus* 5	Bioactive components were altered after fortification.	Total phenolic content = 196–285 mg GAE/g, flavonoids = 155–274 mg CE/g, antioxidant activity = 4.2–7.7 μg/ml *L. casei* 01 and L. acidophilus LA5 8.4–45.9 and 11.6–69.7 μm ~2 log CFU/ml.	SEM	Kemsawasd et al. (2016)
Lactobacillus casei and Lactobacillus paracasei	The total calorie content was reduced than the control. After fortification casson yield stress was reduced without affecting the taste and flavor of chocolate.	Sucrose-free chocolate content 11.1–14.6% fewer calories than normal chocolate. 10^6–10^7 cfu/g after 12 months of preservation. Casson yield stress was decreased by about 3–55%.	Instron 5544 apparatus, Hygropalm water activity meter	Nebesny et al. (2007)
Lactobacillus plantarum	The sensory quality and nutritional quality were better than control. The viable count of bacteria was not changed after preservation.	Cell count of spray-dried probiotic bacteria = 10.36 log CFU mL−1. After 6 months of preservation, the viable count of probiotic bacteria was 10^6 cfu/g 36 volatile compounds have been identified aldehyde, alcohol; pyrazines, and acids are major examples.	Static headspace solid-phase microextraction (HS-SPME) gas chromatography-mass spectrometry (GCMS)	Mirković et al. (2018)
L. plantarum 299v (L299v) and L. acidophilus La 3 (DSMZ 17742) with		The viable count for L. plantarum 299v (L299v) and L. acidophilus La were 33 ×	Texture analyzer	Fernández et al. (2021)

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Table 4 (*continued*)

Fortifying agent	Effect of fortification	Quantitative results obtained	Instrumental characterization	References
polidextrose (Pol), inulin (Inu), isomalt (Iso) stevia (Stev)	The calorie content is decreased due to the addition of low-calorie sweetener.	1014 CFU/g and 5 × 1010 CFU/g respectively. Iso + Stev + Probiotic, this combination produced less rigid dark chocolate.		
*Leuconostocmesenteroids*and flax seeds	Overall antioxidant properties were increased. Cytolytic properties were increased.	*Leuconostocmesenteroids* can tolerate 1–7% NaCl concentration, pH 3–7 and bile salt 0.5–2%. Total peroxide production was 26.75 mg. DPPH-90μ/mL.	–	Waghmode et al. (2020)
Bacillus indicus HU36, maltodextrin and lemon fibe	The smoothness, firmness, sweetness and adherence were improved.	Survival rate of Bacillus indicus HU36 is 88–91% that was 5 log cfu/g.	–	Erdem et al. (2014)
Raspberry leaf	Rheological properties (plastic viscosity, sheer stress), and microstructural properties were changed. Polyphenolic content was also enhanced.	Microstructural examination showed a 3% addition gave the uneven heterogeneous microstructure. 3% raspberry leaf extract addition increases the TPC content of 1.23 mg GAE/g. Procyanidin content increased by about 0.28–0.88 mg CyE/g. 3% addition gave the most effective antioxidant potential in the case of ABTS free radical scavenging properties. 1% extract addition gave a better result in terms of sensory analysis.	Texture analyzer, DIN Viscometer VT 550 Digital Rheometer	Cvitanovic and Bauman (2012)
Polydextrose and inulin	Rheological properties changed. Microstructural properties and melting properties were also altered.	Casson viscosity increased after the replacement of sugar with inulin and polydextrose. The highest casson yield viscosity (15 Pa s) was obtained in chocolate with 27% fat. Casson yield stress was increased in fortified chocolate. 25–36% cocoa butter containing chocolate produced a smooth texture.	Rheometer	Aidoo et al. (2017)
Xanthan gum, corn starch, and glycerine blends	Rheological properties differed when xanthan gum, corn starch, and glycerine were added in blended form.	Rheological parameters were enhanced in 5–15% cocoa butter replacement. On the other hand xanthan gum and glycerine also uphill the yield stresses. Xanthan gum is the single component in the blend which produced the best result in terms of rheological parameters.	ThermoHaake RS600 Rheometer	Syafiq et al. (2014)
polyglycerol polyricinoleate: water: gum Arabic: invert syrup	The addition of polyglycerol polyricinoleate: water: gum Arabic: invert syrup (CBS) in place of cocoa butter improved the storage properties of dark chocolate.	The addition of CBS to the chocolate bar gave increased the specific gravity of the chocolate. The addition of 80 mL/L CBS produced maximum hardness at 36°c and 40°c. 60 mL/L addition produced good quality dark chocolate with heat-resistant properties.	Texture Analyzer (TA-XT Plus), moisture analyzer (AND MX-50)	Lillah et al. (2017)
Wheat germ	Nutritional quality was enriched after fortification.	Total protein and mineral content were increased. Total fat and calorie (20.5 cal/ 100 g) content were reduced. Carbohydrate and moisture content was remaining the same for both. 10% wheat germ addition gave the best result.	–	Marazeeq (2018)
D- tagatose and inulin	Nutritional quality and physiochemical properties were altered	Fortified chocolate had average 1.2 g moisture, water activity = 0.3184, hardness = 24.48 N, reducing sugar-26.44%, energy-387.36 kcal/100 g, calorie reduction = 25.98%.	Texture analyzer	Shourideh et al. (2012)
Astaxanthin	Significant decrease in the level of MDA (Malonic dialdehyde) and oxidative LDL when ingesting dark chocolate fortified with Astaxanthin.	After 2 weeks of ingestion, the decrease in MDA level was 25.1%, 48.2%, 54.6%, and 63.4% when taken in the concentration of 1,2,4,7 mg ASTX with dark chocolate. The effect was more significant when intake was done for 4 weeks (41.4, 71.2, 71.6, and 85.5%).	–	Petyaev et al. (2018)
Spirulina platensis	After the addition of Spirulina platensis with dark chocolate, consumer acceptance was altered.	The addition of *Spirulina platensis* influences consumer preferences for example 47.8% of people claimed for its bitter taste, 53.3% supported it for its cheap price, 64.2% preferred its sweet flavor, 57.7% liked its smooth texture, 72.8% preferred its aroma. The overall preference value was 55.8%.	–	Asti and Ekantari (2020)
β Carotene	*Spirulina platensis* was used to make dark chocolate beta carotene enriched.	79 aromatic compounds have been identified. Beta carotene addition at a dose of 0.37% produced the best result. The new variant was low in acid and aldehyde content and high in pyrazine and alcohol	GCMS	Ekantari et al. (2019)

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Table 4 (*continued*)

Fortifying agent	Effect of fortification	Quantitative results obtained	Instrumental characterization	References
		content. For example, acetic acid content decreased from 38.79% to 36.07%.		
Arthrospira carotenoid	After addition of Arthrospira carotenoid with milk and dark chocolate improved the consumer acceptability.	The appearance attribute of milk chocolate was 24.31%, the attribute of milk chocolate in terms of texture was 21.42% and in terms of price, the attribute was 16.64%. On the other hand, the attribute of dark chocolate in terms of appearance was 16.53%, 13.08% (taste attribute), and 13.03% (price attribute). So, from these data, it can be said that Arthrospira fortification improved overall consumer acceptance.	–	Ramadhanti et al. (2021)
Phytosterol	Several changes occurred during the 5months of preservation.	After 60days of storage hydrogen peroxide peak was 1.39 mmol/kg. During the shelf-life study amount of saturated fatty acid was 50 g/100 g monounsaturated fatty acid was 40 g/100 g. The amount of linoleic acid was 1 g/100 g.	Texture analyzer, GCMS	Botelho et al. (2014)
Peanut oil	Physical properties were measured after fortification.	Particle size ranges from 5 to 150 µm. The melting point was 21 °C, the peak melting point was 32 °C melting end was 41 °C. Caramelisation peak 183 °C. Flow limit was 11.09 ± 1.73 Pa. PH 6.74 ± 0.14, brittleness = 18.61 ± 3.74 N, water activity = 0.358 ± 0.023, maximum particle size = 0.019 ± 0.001 mm. Peanut oil addition increased Casson's plastic viscosity, whiteness index, and thixotropy.	Rheometer	Cooper et al. (2007)
DHA, EPA	Rheological, sensory, and textural properties were altered after fortification,	The L* and C* values ranged from 32.16 to 33.37 and 7.45–8.09, respectively. Hardness values varied among 6422 and 8367 N.	Rheometer, texture analyzer.	Toker et al. (2018a)
*Santolina chamaecyparissus*essential oil	Antioxidant property was enhanced. No changes in rheological property were observed.	The best possible amount of essential oil is 0.02% for the production ofthe best acceptable enriched dark chocolate.	Colorimeter, texture analyzer, Differential Scanning Calorimetry.	Bölek et al. (2022)
Whey Protein Isolate and erythritol	Overall protein content increased as well as viscosity and shelf life was raised after fortification.	Viscosity = 19.52 Pa s (WPI fortified dark chocolate), Higher WPI means a higher amount of hydrophobic surface. Highest hardness (260.43 N) was produced by WPI fortified chocolate. Water activity was below 0.5. Aw of WPI fortified chocolate = 0.254.	Water activity meter, rheometer, GT Contour Surface Metrology optical profilometer, texture analyzer and confocal microscope.	Nastaj et al. (2022)
Flavonol	Flavonol enrichment increased the antioxidant capacity of the chocolate.	After enrichment flavonol-3-ol content was 4 mg/g, which was 3 times higher than conventional chocolate. Procyanidin content was 2.4 times higher. The total content of procyanidin was 6 mg/g. Total flavonol and antioxidant capacity were increased by 39% and 56% respectively.	HPLC	González et al. (2020)
Flavan 3-ol	Platelet aggregation was reduced after the ingestion of flavan-3-ol fortified chocolate.	Platelet aggregation was reduced from 20 µmol/L to a significant level and the total amount of epicatechin was enhanced by 1.20 mol/L after 2hrs consumption of flavan-3-ol rich dark chocolate.	HPLC	Ostertag et al. (2013)
Flavan-3-ol	The percentage of nutritionally enriched bioactive components was increased.	Flavan-3-ol dark chocolate contained 1.5% flavonoid compared to 0.64% in standard chocolate. Theobromine and caffeine are present in combined forms. The percentage was 1.12 for enriched chocolate and 1.23 for normal standard chocolate.	HPLC, High-Performance Liquid Chromatography and Fluorescence Detection (HPLC-FL)	Ostertag et al. (2017)
Broken rice berry rice	After anthocyanine (extracted from Broken rice berry rice) fortification Physical properties and antioxidants are altered after fortification.	The water activity of the chocolate ranged from 0.42 to 0.45 for all types of chocolate. The hardness of the chocolate was decreased (27.2 N). The percentage of DPPH free radical scavenging capacity was high (22.1%) in anthocyanin-fortified dark chocolate. The anthocyanine of the fortified dark chocolate was 1.01 mg anthocyanins per 100 g.	XT plus texture analyzer, Rotavapor, BÜCHI Rotavapor R-100.	Rice et al. (2020)

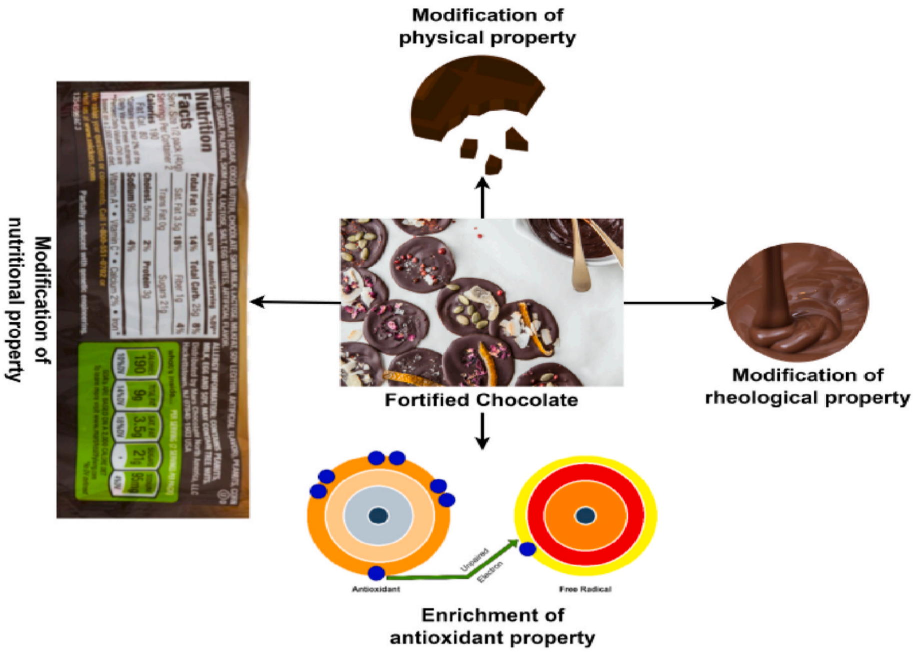

Fig. 6. Alteration of different properties after fortification.

body. For example-when dark chocolate was fortified with black mulberry, it became anthocyanin-rich. Anthocyanin plays multiple roles in our bodies. The most important function was to reduce copper-induced LDL oxidation, which is a very crucial function for the prevention of atherosclerosis. Anthocyanin is responsible for the reduction of total cholesterol, LDL and triglyceride level (Habanova et al., 2016). Sea buckthorn and mulberry fortification imparted several extra beneficial roles to the human body. But the most specific function was to reduce oxidative stress due to its high polyphenol and flavonoid content. When the fortification was done with lemon balm extract, it became rich in rosmarinic acid content as well as it was provided an immune-modulatory, anti-inflammatory, anti-diabetic, anti-microbial, hepatic and renal protectant agent, anti-allergic, etc. (Munoz et al., 2013; Nunes et al., 2017; Rahman et al., 2016). Cinnamon fortified dark chocolate would provide several medicinal properties to the consumers. The main bioactive component cinnamaldehyde was the main responsible factor for the reduction of oxidative reaction. Besides this, cinnamaldehyde plays several roles, such as preventing CVD and diabetes, reducing inflammation in our body, and protecting against cancer and neurodegenerative diseases (Gruenwald et al., 2010; Thorsen, 2009).

Numerous studies were focused on fortification with probiotic bacteria. Probiotic and dark chocolate is a unique combination. Those probiotics are very advantageous for maintaining healthy microflora in the intestine. It also reduces the growth of pathogenic microorganisms and is also helpful for the production of vitamin B complex in the human body. Probiotics can prevent the occurrence of certain types of cancers. Probiotics are used for a therapeutic purpose to resist *Helicobacter pylori* infections. Dark chocolate could be a good vehicle for delivering live microorganisms to the human body (Goldin, 1998; Roobab et al., 2020). On the other hand, prebiotics was also combined with dark chocolate in previous experimental studies. Prebiotics are helpful for the growth of probiotic bacteria. These also increase the bulk of the faeces. Thus regular use of prebiotics could avoid the occurrence of constipation (Slavin, 2013; Verbeke et al., 2015). In some studies, sucrose was replaced with artificial sweeteners with the addition of polysaccharides. We all know that sucrose is an important component of dark chocolate. But the replacement of sugar made the chocolate suitable for diabetic patients. These newly developed finished products contain fewer calories also. So, it could be taken by obese individuals also (Delgado and Tamashiro, 2018).

Another study was found in which the fortification was done with the addition of raspberry leaves. These were also rich sources of polyphenols and flavonoids. It was added due to its high antioxidant capacities. When dark chocolate was fortified with astaxanthin, it was proved that it could be applied as an antioxidant supplement. The consumer would be benefited from a combined effect of dark chocolate and astaxanthin. Astaxanthin can inhibit LDL oxidation. It is capable of neutralizing the amount of singlet oxygen. It also modulates the gene function and immune function (Gentili et al., 2013; Rao et al., 2013). So, it could be helpful for the reduction of several oxidative markers in the human body. It also delays the progression of breast cancer, prostate cancer, and also cancer in fibrosarcoma, embryonic fibroblasts, melanoma, etc. (Chen et al., 2017; Nagaraj et al., 2012). Besides this role of astaxanthin, is very helpful in the treatment of central and peripheral nervous diseases (Fakhri et al., 2018).

Fewer studies had focused on the addition of vitamins with dark chocolate. Vitamin D and beta carotene were added to dark chocolate to increase the vitamin content in dark chocolate. When dark chocolate is fortified with vitamin D, it not only provides good bone health but also protects the endothelial membrane stability and thus maintains the innate and adaptive immune response (Charoenngam and Holick, 2020). The sources of beta carotene were *Spirulina platensis* and arthospira. *Spirulina platensis* is a rich source of essential fatty acids, protein, vitamins, minerals, and essential amino acids. That impended inflammation, hypertension, cancer, etc., and is also responsible for the reduction of the glycemic load of the blood (Matsui et al., 2003). Besides their good antioxidant properties, arthrospira contains anti-inflammatory phycocyanin pigment and also contains antiviral and anti-tumor capabilities. Thus, the fortified product could be easily used for the prevention of vitamin deficiency syndrome and also the prevention of numerous diseases (Jara et al., 2018).

The addition of phytosterol and peanut oil could lead to an increase in the concentration of PUFA and several essential fatty acids in dark chocolate when fortified with these substances. The notable effect of phytosterol is the reduction of blood cholesterol by making the cholesterol insoluble in intestinal miscelle (Quílez et al., 2003; Trautwein and Demonty, 2007). Thus, this product could be used for the maintenance of heart health. On the other side, DHA and EPA fortification lead to better brain development and brain functioning (Menesi et al., 2009; Zhang et al., 2019).

Dark chocolate fortification with flavonol improves platelet functioning. Besides anti-inflammatory, anti-diabetic and anti-obesity effects, it prevents the ADP-induced platelet aggregation in the arteries and decreases P selectin expression, simultaneously stopping the occurrence of atherosclerosis (Ballard and Junior, 2018; Vita, 2005). Flavonol-enriched dark chocolate also provided better utilization of ADP. It was responsible for lowering the creatinine level, which was the indicator of PCR pool and better ADP regulation (Montagnana et al., 2018). The effect of dark chocolate fortification is summarised in Fig. 7.

11. Consumer acceptance of fortified dark chocolate

The success of any food product development resides in its Consumer acceptance. The product's popularity is entirely dependent on the consumer's satisfaction. From the very fast day of dark chocolate production, it was getting popularity due to its sensory properties. Consumers play an important role in the innovation of new product. Recently consumption rate is increased due to its remarkable health benefits (Flammer et al., 2007; Mattia et al., 2017; Moreno et al., 2012). One of the main components in dark chocolate is a polyphenol which is responsible for the anticipation of numerous diseases like high blood pressure, atherosclerosis, and inflammatory diseases (Oracz and Nebesny, 2016). But, due to its ample amount of energy content, it is not permitted for higher intake. To get the best result, dark chocolate intake should be the proper amount (Bartkiene et al., 2021; Zugravu and Otelea, 2019). One of the major causes of dark chocolate's popularity is its flavor and texture. Fatty acids confer texture to the finished products, and volatile compounds give texture to dark chocolate. Polymorphism of cocoa butter could be prevented by the tempering process. Tempering gives a better appearance to chocolate. For example, makes it glossier and increases its shelf life. This is also a good sign of consumer acceptance (Ligęza and Czarkowska, 2019). The melting properties of dark chocolate are dependent on the emulsifier used, fatty acid profile, storage time, particle size, etc. (Afoakwa et al., 2008a, 2009).

In earlier days, the consumption of chocolate was only limited to children, but now it has become more popular among all age groups due to its functional properties mainly developed by fortification (Fernández et al., 2021). The nutritional approach always gives a positive effect on the consumers. Dark chocolate is rich in healthy fatty acids and also several bioactive compounds such as catechine, epicatechin, caffeine, flavonoids, total polyphenolic compounds, etc. (Virgens et al., 2021). Those components provide multiple health benefits, which have already been discussed before. Sometimes these components are responsible for the generation of bitterness and astringency. This could be minimized by fortification. Fortified dark chocolate can support the nutritional status of the population in a better way. The consumer acceptance of dark chocolate is increased after adding micronutrients to it. Researchers have already established the positive relationship between dark chocolate fortification and health promotion. Dark chocolate is already fortified with anthocyanin which imparts modulation of different functions in the human body. It can promote anti-inflammatory and antioxidant effects with a protective effect against DNA damage. LDL, triglyceride, and total cholesterol are reduced by regular intake of anthocyanins (Kuntz et al., 2014; Triebel et al., 2012; Weisel et al., 2006). After the addition of spices to the dark chocolate for example cinnamon consumers get to benefit from its anti-microbial properties (Keith, 2019). Chocolate was fortified with prebiotics and probiotics to promote better gut health (Roberfroid, 2000). Polysaccharide fortification makes dark chocolate much healthy to attract the obese population (Mišurcová et al., 2012). Vitamin fortification increases the vitamin content of dark chocolate thus it can be used as a supplementary product for the prevention of vitamin deficiency disorders. Essential fatty acid fortification could improve skin-related problems (Ruxton et al., 2004). Flavonol fortification gives added antioxidant properties to the consumers which

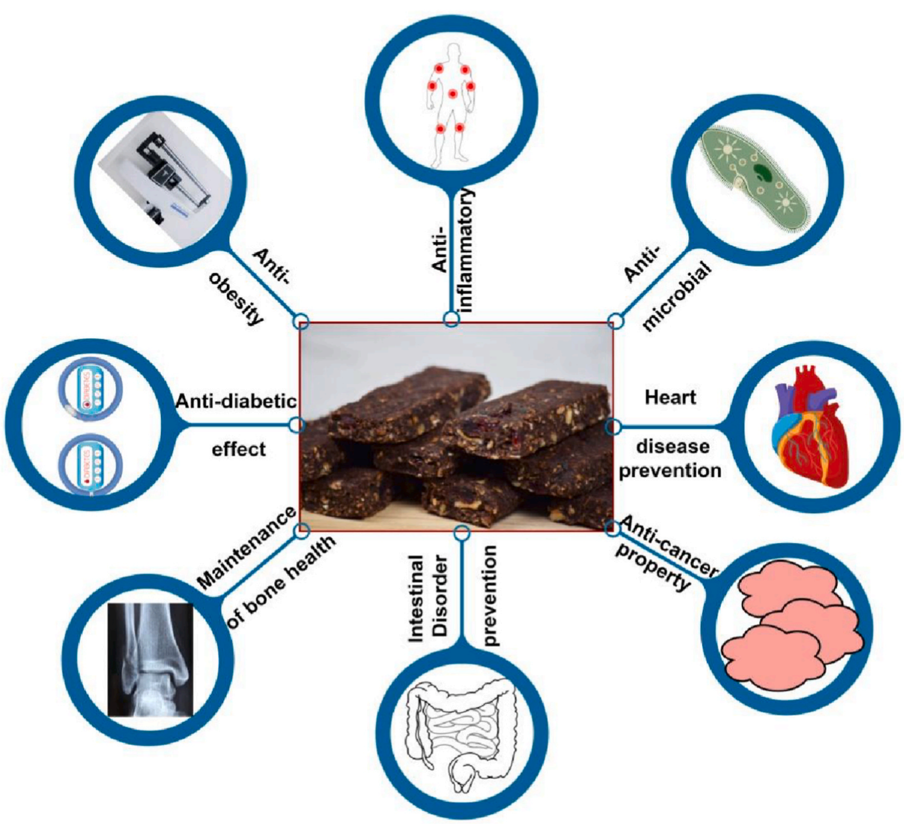

Fig. 7. Health benefits of fortified dark chocolate.

reduce the possibility of degenerative diseases.

In Columbia, cocoa products were taken for getting the remedy of fatigue, gastrointestinal problems, indigestion, etc. (Dillinger et al., 2000). Consumers can use fortified dark chocolate for therapeutic purposes for the prevention of various types of diseases. They can also use for getting instant energy to overcome physical and mental stress. It can be a good nutritional supplement for children as it is dense in valuable nutrients. An aged person also can take this as a dessert item by replacing calorie-dense sweet items. So, fortified dark chocolate can use on multiple grounds for solving various problems.

12. Conclusion and future perspective

Nutritional aspects of dark chocolate were improved after fortification. The fortification was done with numerous ingredients like fruits (mulberry) and spices and herbs (cinnamon), probiotics (*Lactobacillus*), prebiotics (inulin, Xanthan gum), etc. Those fortifications are aimed not only to improve the nutritional qualities of the dark chocolate but also can be used for a therapeutic purpose to avoid the early occurrence of degenerative diseases. Researchers have found that fortification upgrades platelet functioning and enhances cardiovascular health. Even some replacement studies focused on the calorie reduction of chocolate for the weight management of an obese person. The most prominent health benefit is the prevention of cancer due to its excellent antioxidant property. Dark chocolate is believed to scavenge free radicals. Fortification also modulates the physical properties of the chocolate, for example, rheological properties and melting properties, and also amplifies the consumer acceptability of the chocolate. Though a wide range of fortification studies has already been discussed in this review, it can still be modified in several aspects to contribute some unique advantages to societies. So, some future studies can be done to focus on these areas. Dark chocolate fortification can be done with some waste materials thus, the nutrient loss could be minimized. Dark chocolate also can be fortified by applying some inexpensive technologies to decrease the overall cost of the end product. Although all age groups consume dark chocolate, future research can be dealt to enhance the physical performance of individuals such as sportspeople. Fortification could be done in such a way that it can reduce the oxidative stress of sports people and improve their overall performance.

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CRediT authorship contribution statement

Sharmistha Samanta: Conceptualization, Methodology, Writing – original draft, All authors read the final version of the manuscript and approved for final submission. **Tanmay Sarkar:** Conceptualization, Methodology, Writing – original draft, Supervision, All authors read the final version of the manuscript and approved for final submission. **Runu Chakraborty:** Conceptualization, Methodology, Data curation, Writing – original draft, Supervision, Writing – review & editing, Reviewing and Editing, All authors read the final version of the manuscript and approved for final submission. **Maksim Rebezov:** Image preparation, All authors read the final version of the manuscript and approved for final submission. **Mohammad Ali Shariati:** Image preparation, All authors read the final version of the manuscript and approved for final submission. **Muthu Thiruvengadam:** Conceptualization, Methodology, Data curation, Supervision, Writing – review & editing, Reviewing and Editing, All authors read the final version of the manuscript and approved for final submission. **Kannan R.R. Rengasamy:** Conceptualization, Methodology, Data curation, Writing – review & editingEditing, Reviewing and Editing, All authors read the final version of the manuscript and approved for final submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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