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Symbiotic Chocolate: A Review

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ABSTRACT

Prebiotics and probiotics are crucial components of human diets. Research on the possible health advantages of using probiotics and prebiotics has significantly increased in the past few years, with an emphasis on their characterization and validation. Certain probiotics and prebiotic supplements have main effects that have been demonstrated by clinical trials, while other claims have been made based on in vitro testing that need to be validated *in vivo*. One intriguing way to provide health-promoting microorganisms in a well-liked and decadent food matrix is by adding probiotic bacteria to chocolate. The current literature on the encapsulation of probiotic bacteria especially for use in chocolate applications is examined in this study. In addition, the possible health advantages and consumer acceptability of probiotic chocolate products are discussed, emphasizing the prospects and difficulties in this developing industry.

Keywords: Prebiotics, Probiotics, Postbiotic, Chocolate

INTRODUCTION

"Probiotic" refers to the idea that some microorganisms, when given in large enough amounts, directly benefit the host. This phrase refers to "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host," according to a definition provided by an Expert Panel of the United Nations and the World Health Organization (2006). The usage of prebiotics, which are non-digestible food elements that selectively stimulate the growth and/or activity of one or a small number of bacterial species already present in the colon, has increased in recent years (Gibson and Roberfroid, 1995). Prebiotics have several advantageous effects on the host. As of right now, it is believed that neither human nor animal digestive enzymes can break down any of the prebiotics that have been reported. These prebiotics are all short-chain carbohydrates with a polymerization degree of two to about sixty. Prebiotic proteins or lipids are unlikely to exist due to the nature of the metabolism of lactobacilli and bifidobacteria (Cummings and Macfarlane, 2002). When combined with probiotics, chocolate—which is the food that people of all ages love the most—may have a large market potential and health advantages. Probiotics are live microorganisms that, when given in sufficient quantities, enhance the host's health by preserving or enhancing the microbial balance of their stomach, providing defense against gut pathogens. Probiotics use prebiotics as their substrate. Prebiotics and probiotics have widely recognized health benefits, and numerous clinical trials have demonstrated these benefits. The use of probiotics and prebiotics in novel food products is gaining traction in the industry. Most people find chocolates to be quite enticing, and they could be a good vehicle for delivering probiotics to the human gut. The encapsulation of probiotic bacteria involves protecting these beneficial bacteria from adverse environmental conditions.

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Encapsulation methods aim to shield probiotic during industrial processing, ensuring their viability and stability. Various techniques like extrusion, emulsion, and drying are employed for microencapsulation, with each method offering distinct advantages and disadvantages in terms of moisture content, microcapsule size, encapsulation efficiency, and release during digestion. Encapsulation technology plays a crucial role in the food industry by safe guarding probiotics from exposure to external factors, which could compromise their nutritional properties and cell integrity. The primary objective of encapsulation is to maintain the viability of probiotics, ensuring their effectiveness in delivering health benefits when consumed. Encapsulation technology involves enclosing probiotic cells within protective matrices to enhance their stability and survival. Techniques like spray drying, lyophilization, are commonly used for industrial production of encapsulated probiotics, offering different levels of protection and viability maintenance.The selection of appropriate encapsulation is vital to prevent the exposure of sensitive probiotics to external environments, which could lead to cell wall breakage and loss of nutritional properties. Encapsulation technologies are continuously evolving to address challenges such as ensuring non toxicity, resistance to gastric acidity, and compatibility with probiotic cells.

Review of Literature:

Prebiotics:

Gibson and Roberfroid (1995) stated that Prebiotics are mostly a class of substances that benefit their hosts inadvertently. They only aid in the growth of good bacteria in the colon, such as *Lactobacillus* and *Bifidobacterium*, whose metabolic byproducts benefit the hosts. A prebiotic is defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon".

Gibson *et al.* (2004), stated that prebiotics are now described as "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health." Prebiotics, however, mostly have an indirect effect since they preferentially feed one or a small number of bacteria, resulting in a selective alteration of the host's intestinal (particularly colonic) microbiota.

Teitelbaum and Walker (2002) opined that Prebiotics are fermented in the large intestine by advantageous microorganisms. The probiotic organisms mentioned above comprise the microflora in question. The microbiota gets its energy from prebiotics. Prebiotics, however, mostly have an indirect effect since they preferentially feed one or a small number of bacteria, resulting in a selective change of the intestinal (particularly colonic) microflora of the host. In fact, the native lactobacilli and bifidobacteria are the most significant bacterial genera that are the focus of selective stimulation.

Gibson and Roberfroid (1995), opined that *Bifidobacteria* may boost the immune system, generate B vitamins, prevent the growth of pathogens, lower blood ammonia and cholesterol, and aid in the restoration of the natural flora following antibiotic therapy. According to Manning and Gibson (2004), lactobacilli can help those who are lactose intolerant digest lactose, lessen infantile diarrhea and constipation, help the body fight off infections like salmonellae, and ease the symptoms of irritable bowel syndrome. Studies have examined the effects of prebiotics on increases in *lactobacilli* and *bifidobacteria* (Langlands *et al.*, 2004).

Gibson and Roberfroid (1995) stated that: A prebiotic possesses three essential qualities:

- It cannot be broken down by the body's natural enzymes.
- It is fermented by particular genera and species of the gut microbiota that live there.
- The fermentation process leads to a targeted increase in beneficial bacteria that benefit the host's health.

Dietary carbohydrates are often prebiotics. Galacto-oligosaccharides (GOS) and inulin-type fructans are the two main carbohydrates that meet the requirements for prebiotics, however numerous other classes are being studied as well. Prebiotics are believed to enhance health status by decreasing the risk of disease because they positively interact with a variety of physiological processes via the big intestine surface. Prebiotics such as fructo-oligosaccharides and galacto-oligosaccharides mainly promote *Lactobacillus* and *Bifidobacterium* cell growth.

Crittenden and Playne (1996) concluded that Nondigestible oligosaccharides make up the majority of prebiotics and prebiotic candidates that have been found to date. They are obtained either by synthesis (by trans-glycosylation reactions) from mono- or disaccharides such as sucrose (fructooligosaccharides) or lactose (transgalactosylated oligosaccharides or galactooligosaccharides) or by extraction from plants (e.g., chicory inulin), possibly followed by an enzymatic hydrolysis (e.g., oligofructose from inulin).

Moshfegh *et al.* (1999) stated that the most researched prebiotics among them are oligosaccharides and inulin, which are also regarded as dietary fibers in most nations.

Franck (2002) reported that Prebiotics are commonly utilized to provide a dual benefit: an enhanced organoleptic quality and a more nutritionally balanced composition. However, they can be used for their technological or nutritional benefits.

Nelson (2001) reported that Nondigestible oligosaccharides and inulin are simple fiber additives that frequently result in better texture and flavor. These dietary fibers are easily fermented by certain species of lactobacilli and bifidobacteria in the colon, which increases their cell population and simultaneously produces short-chain fatty acids.

Probiotics:

Lily and Stillwell (1965) coined the term probiotics as microbial-derived factors that stimulate other organisms' growth.

Guarner (2003) stated that Probiotics are the Greek word "Probios," which means life.

Probiotics are defined as "Live microorganisms that, when administered in adequate amount confers the health benefit on the host".

Holzapfel (2002) concluded that these probiotic organisms benefit the gut environment by maintaining gut microbial flora, thereby conferring protection against gut pathogens.

Fuller (1989) concluded that *Oenococcus, Wesiella, Pediococcus, Enterobacter,* and *Bacillus* are further probiotic genera. Live microorganisms, or probiotics, are defined as beneficial bacteria that maintain or enhance a person's gut microbial balance.

Yoon *et al.* (2006) stated that Eating foods supplemented with live cells of lactic acid bacteria (LAB), in particular, and their probiotic strains, is thought to be good for human health because of the well-established benefits they have on the immune system, gastrointestinal tract, and cholesterol levels, as well as their notable anticancer activity.

Wang *et al.* (2017) explained that Probiotics have antioxidant enzymatic systems, just like mammals do.

Khaledabad *et al.* (2020) stated that moreover, probiotics can stimulate the antioxidant system in the host and elevate the activities of antioxidants efficiently. There are several possible functions of probiotics that include the production and secretion of antimicrobial substances, which can cause displacement of pathogen colonization in the gut (O'Shea *et al.*, 2012).

O'Shea *et al.* (2012) opined that Additionally, the secretion of substances such as protein, short chain fatty acid (SCFA), organic acids, cell surface active components and DNA from probiotics can exert the same therapeutic effect as probiotics do on gastrointestinal disease. These therapeutic agents are known as pharmabiotics or probioactive (O'Shea *et al.*, 2012).

Agamennone *et al.* (2018) concluded that for instance, clinical trials revealed that co-administration of particular probiotics decreased the risk for antibiotic-associated diarrhea (AAD), a side-effect that is frequently linked to the use of broad-spectrum antibiotics (Agamennone *et al.*, 2018).

According to several studies (Fooks and Gibson, 2002[25]; O'Hara and Shanahan, 2007), explained that probiotics can function as a "barrier" against pathogens by lowering the luminal pH, producing bacteriocins, competing for scarce nutrients, inducing mucosal immune responses, and adhering to the intestinal mucosa.

Lahtinen *et al.* (2007) concluded that Probiotics are typically incorporated in a range of dairy products or fruit juice.

The strains of bacteria from the genera *Bifidobacterium, Lactobacillus*, and yeast that are most advantageous for the gut microorganisms of the host are used to make probiotics. Microorganisms must meet certain requirements in order to be classified as probiotics:

- They must be able to survive in the gastrointestinal tract at low pH levels and when in contact with bile.
- They must adhere to intestinal epithelial cells.
- They must stabilize the intestinal microflora.
- They must not be pathogenic.
- They must be able to survive in foods and be used to produce pharmacopoeia-lyophilized preparations.
- They must multiply quickly, causing either permanent or temporary colonization of the gastrointestinal tractand
- Probiotics must be generically specific. *Saccharomyces boulardii* is another probiotic

that is utilized in addition to lactic acid bacteria.

– According to McFarland and Bernasconi (1993), this kind of yeast was isolated from litchi fruits in Indonesia.

Gibson *et al.* (1997) stated that Another advantage of using probiotics is that they increase an organism's resilience to intestinal infections by inhibiting the growth of harmful bacteria.

For probiotics to have a favorable impact on the host's health and the activity of the intestinal microbiota, they must make it through the gastrointestinal tract and reach their destination alive. According to a recent study, probiotics may even aid in mercury poisoning victims' detoxification. The competitive exclusion of pathogens has been a primary criterion for probiotic selection. On the mucosal surface of the gastrointestinal tract, probiotics either directly compete with infections or impede their adherence by binding to particular receptors.

Salminen *et al.* (2005) stated that They also have an impact on how an infant's intestinal microbiota develops.

Health Benefits of Probiotics:

– *Digestive Health*: Administration of specific probiotic such as lactobacillus and Bifidobacterium reduce the risk of antibiotic associated diarrhea.

– Probiotics can help maintain a healthy balance of gut bacteria, which aids in digestion and may alleviate symptoms of digestive disorders like irritable bowel syndrome (IBS).

– *Heart Health*: Probiotics may help lower blood pressure and cholesterol levels, reducing the risk of heart disease.

– *Skin Health*: Probiotics may improve certain skin conditions like eczema and acne by modulating the immune response and reducing inflammation.

– *Vaginal Health*: Certain strains of probiotics can help maintain a healthy vaginal microbiota, reducing the risk of infections like bacterial vaginosis and yeast infections.

– *Oral Health Support*: Probiotics may contribute to oral health by inhibiting the growth of harmful bacteria in the mouth, reducing the risk of cavities, gum disease, and bad breath. certain strains of probiotics have been incorporated into oral health products like tooth paste and mouth wash for this purpose.

Encapsulation of Probiotic Bacteria:

Corona-Hernandez' *et al.* (2013); Sarao and Arora (2017) concluded that Probiotic bacteria are typically encapsulated to improve their viability during processing and to facilitate target distribution to the gastrointestinal tract. Probiotics are utilized in conjunction with pharmaceuticals, health supplements, and fermented dairy products. They are essential to preserving human health. There is doubt about these bacteria's ability to survive in the human gastrointestinal tract. Many biopolymers, including chitosan, gelatin, whey protein isolate, and cellulose derivatives, are utilized for encapsulation in order to preserve the viability of the probiotic bacteria. Several encapsulation techniques, including spray drying, extrusion, and emulsion, have also been documented. Probiotic cells in capsule form are currently mostly utilized in the marketing of nutraceutical products. However, it has been extensively argued that novel foods must be developed in order to serve as viable carriers for these microorganisms.

Kwak (2014) reported that the inclusion of probiotics

Table 1 : Microorganisms used as probiotics

Lactobacillus	*Bifidobacterium*	Other lactic acid bacteria	Other
L. acidophilus	*B. adolescentis*	*Enterococcus faecium*	*Escherichia coli strain Nissle*
L. casei	*B. animalis*	*Lactococcus lactis*	*Saccharomyces cerevisae*
L. crispatus	*B. bifidum*	*Leuconstoc mesenteroides*	*Saccharomyces bourlardii*
L. curvatus	*B. breve*	*Pediococcus acidilactici*	
L. delbrueckii	*B. infantis*	*Streptococcus thermophilis*	
L. farciminis	*B. lactis*	*Streptococcus diacetylactis*	
L. fermentum	*B. longum*	*Streptococcus intermedius*	
L. gasseri	*B. thermophilum*		
L. johnsonii			
L. paracasei			
L. plantarum			
L. reuteri			
L. rhamnosus			

Table 2 : Some probiotics health benefits effects

Probiotic strains	Health benefit	Reference
Lactobacillus plantarum 299v	Relief of irritable bowel syndrome Reduction of LDL-cholesterol Reduction of the recurrence of *Clostridium difficile* diarrhoea	Niedzielin *et al.* (2001) Bukowska *et al.*, (1998) Wullt *et al.* (2003)
L. Casei Shirota (LcS) *L. Casei* DN114001	Downregulation of LPS-induced IL-6 and INF-γ Immune modulation	Matsumoto *et al.* (2005) Tien *et al.* (2006)
L. rhamnosus GG	Treatment of acute rotavirus and antibiotic associated diarrhea Immune modulation Relief of inflammatory bowel disease Treatment and prevention of allergy Postsurgical prevention of pouchitis	Basu *et al.* (2008) Zhang *et al.* (2005) Gosselink *et al.* (2004) Kalliomäki *et al.* (2003) Kuisma *et al.* (2003)
L. acidophilus La5 *L. acidophilus* M92	Shortening rotavirus and antibiotic associated diarrhoea Immune system activation on patients with irritable bowel syndrome Lowering of serum cholesterol	Sugita and Togawa (1994) Ohman *et al.* (2009) Kos (2001)
L. salivarius UCC118	Relief symptoms of inflammatory bowel disease and modulation of gut microflora	Mattila-Sandholm *et al.* (1999); Dunne *et al.* (2001)
L. reuteri DSM 12246 *L. reuteri* ATCC PTA 6475	Shortening of rotavirus diarrhoea Immune modulation	Shornikova *et al.* (1997); Rosenfeldt *et al.* (2002) Lin *et al.* (2008)
Bifidobacterium breve	Immune modulation and stimulation Reduced symptoms of irritable bowel disease	Hoarau *et al.* (2006); Latvala *et al.* (2008); Okada *et al*. (2009); Brigidi *et al.* (2001); Matsumoto *et al.* (2001)
B. animalis *B. longum* BB536 *B. lactis* Bb12	Increased IgA secretion Treatment of allergy Shortening the frequency of rotavirus and travellers' diarrhoea Inhibitory effect against *Helicobacter pylori*	Bakker-Zierikzee *et al.* (2006) Takahashi *et al*. (2006); Isolauri and Salminen (2008); Saavedra *et al.* (1994) Wang *et al.* (2005)
Escherichia coli Nissle 1917	Fewer relapses of inflammatory bowel disease Immune modulation Recovery of ulcerative colitis Exclusion of pathogenic *E. coli*	Malchow (1997) Sturm *et al.* (2005) Kruis *et al.* (2004); Sartor (2005); Boudeau *et al.* (2003)
Saccharomyces boulardii	Fewer relapses of inflammatory bowel disease Reduction of antibiotic associated diarrhoea Prevention of recurrent *Clostridium difficile* diarrhoea	Guslandi *et al.* (2000) Kotowska *et al*. (2005) McFarland *et al.* (1994)
Streptococcus thermophilus	Enhance lactose intolerance Prevention of rotavirus diarrhoea	De Vrese *et al.* (2001) Saavedra *et al.* (1994)

into food items presents some problems, including the preservation of living cells during processing and storage and the potential for undesired interactions with the food matrix.

Coghetto *et al.* (2016); Kwak (2014)[45] opined that Thus, probiotics encapsulation aims to increase their stability and viability, in addition to provide a controlled release to effectively adhere and colonize the intestine. In this sense, polymers used for microencapsulation should be able to protect the cells in the stomach acidity and release them under neutral to alkaline conditions of small intestine.

De Prisco and Mauriello (2016); Sarao and Arora, (2017) stated that therefore, to ensure that microcapsules are maintaining probiotics viability, it is essential to take into account certain physical and chemical properties, such as coating material concentration, type of culture, initial cell counts, particle size, and water solubility.

Use of prebiotics such as inulin, polydextrose, fructooligosaccharides (FOS) and galactooligosaccharides (GOS) has been characterized as an intriguing and promising approach to extend the shelf life of probiotic microbes.

According to De Prisco and Mauriello (2016), the so-called symbiotics act as a food functionalization tactic in addition to enhancing the survivability of probiotics throughout the manufacturing and storage of microcapsules.

Yao *et al.* (2020) reported that since bacteria are microscopic in size, nanotechnology is not a practical method for encapsulating probiotics (particles smaller than 1 μm, while microbial cells dimensions are typically in the range of about 1–10 μm). Instead, microencapsulation is the only method that can be used. Probiotics can be delivered into the colon and begin to have their health-promoting effects by means of enhanced administration channels that provide sufficient protection during storage and passage through the gastrointestinal tract.

According to (Mahmoud *et al.*, 2020; Sarao and Arora, 2017), the main polymers used for probiotics encapsulation are alginate, starch, chitosan, xanthan gum, k-carrageenan, cellulose acetate phthalate, gelatin, and milk proteins.

Corona-Hernandez *et al.* (2013); Kwak (2014) opined that the most often used techniques, however, are gelling, spray-drying, spray-cooling, extrusion, and emulsification.

Zhang *et al.* (2015) reported that Probiotic bacteria are frequently encapsulated using biopolymer beads made by extrusion in a water-in-oil emulsion.

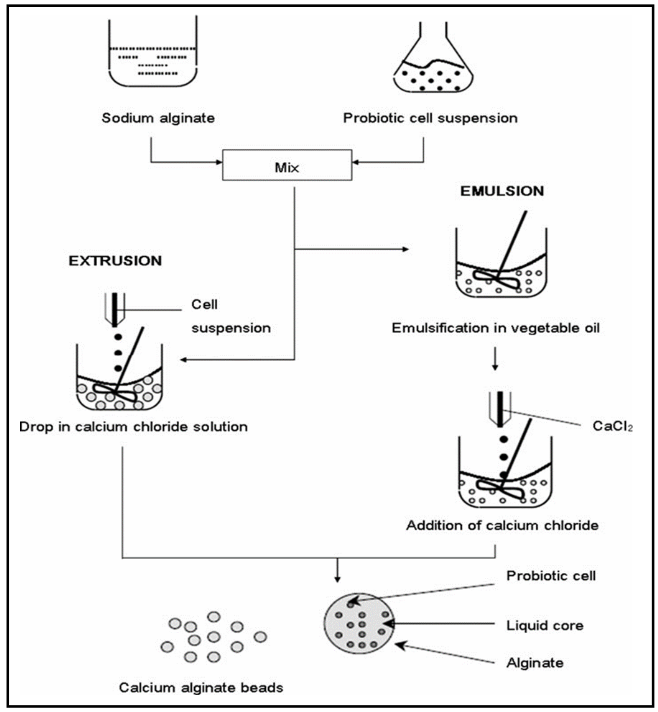
According to Martín *et al.* (2015), extrusion is the most widely used technique for microencapsulating probiotics because of its ease of use, affordability, and formulation conditions that ensure good cell viability.

Coghetto *et al.* (2016); De Prisco and Mauriello (2016) reported that other established methods that show promise include impinging aerosol, vibrating techniques, electrospinning/electrospraying, and coacervation.

According to Marcial-Coba *et al.* (2019), the microencapsulation of probiotics using biopolymers is a potential approach that can enhance their viability and stability throughout upper gastrointestinal transit.

Microencapsulation Technology for Probiotics:

See in Fig.



Protection Needs of Probiotics:

Viernstein *et al.* (2005) stated that during the time from processing to consumption of a food product, probiotic product needs to be protected against the following:

- Processing conditions, like high temperature and shear.
- Desiccation if applied to a dry food product.
- Storage conditions in the food product on shelf and in-home, like food matrix, packaging, and environment (temperature, moisture, oxygen).
- Degradation in the gastrointestinal tract, especially the low pH in stomach (ranging from 2.5 to 3.5) and bile salts in the small intestine.

In the above-mentioned conditions, micro-encapsulation technologies have been developed and effectively deployed to shield probiotic bacterial cells from environmental degradation. Many food applications, including stabilizing food compounds, regulating oxidative reactions, releasing active ingredients (probiotics, minerals, vitamins, phytosterols, enzymes, fatty acids, and antioxidants) gradually or under control, masking off unpleasant flavors and odors, and creating barriers between sensitive bioactive materials and the environment, are made possible by encapsulation technology. The principle behind encapsulation technology is to pack solid, liquid, or gaseous chemicals into milli-, micro-, or nanoscale particles. These particles then release their contents when particular conditions or treatments—such as heating, salvation, diffusion, and pressure—are applied. A thin, robust, semipermeable membrane encircling the solid or liquid core of sealed capsules is spherical in shape. Microcapsules can release active chemicals gradually, and coatings can be made to open at certain locations on the human body. A coating that can resist the stomach's acidity and the pancreatic bile salts after ingestion is typically used when designing probiotic-containing capsules. Hence, during the gastro-duodenal transit, the biological integrity of probiotic products is preserved, which is necessary for the delivery of a high concentration of viable cells to the ileum and jejunum. Before being used in industry or everyday settings, generated particles should undergo testing for swelling, erosion, and disintegration in simulated stomach and intestinal fluids. This is because encapsulates are meant to shield delicate microorganisms from the severe conditions seen in the gut environment. Stabilization of probiotic bacteria, or ensuring their continued viability during storage, is another goal of microencapsulation.

Freeze Drying of Probiotic Bacteria:

Chavez and Ledeboer (2007); Garcia De Castro *et al.* (2000) opined that Probiotics are freeze-dried in the presence of a carrier material at low temperatures, and the water is then sublimated under vacuum. Cryoprotectants aid to stabilize probiotics throughout storage and preserve their activity after freeze-drying. SMP has been the primary drying medium used by many researchers, but other substances such as sorbitol, fructose, lactose, mannose, monosodium glutamate, trehalose, and 30% maltodextrin as well as a mixture of 20% soy protein isolate and 20% maltodextrin have also been used as protective additives in recent research. The resulting dried combination has a low surface area and a wide size variety of final particles that can be ground (Picot and Lacroix, 2003). When stored, freeze-dried probiotics are quite stable, especially at low temperatures and in an inert environment (such as nitrogen or vacuum). Regretfully, the method of freeze-drying is quite costly, costing roughly 4-5 times as much as spray-drying (Chavez and Ledeboer, 2007). But since freeze-drying is one of the safest ways to dry probiotics, it's likely the method most frequently employed, and it serves as a benchmark for other drying procedures as well. The majority of probiotics that are freeze-dried simply offer stability during storage and do not operate locally in the gastrointestinal system.

Postbiotic:

Cicenia *et al.* (2014); Tsilingiri and Rescigno (2013); Kostantinov *et al.* (2013) stated that soluble factors (products or metabolic byproducts) secreted by live bacteria or released following bacterial lysis are referred to as postbiotics. Postbiotics are also known as metabiotics, biogenics, or simply metabolites/CFS (cell-free supernatants). Due to their increased bioactivity, these byproducts boost the host's physiology.

Tsilingiri and Rescigno (2013); Kostantinov *et al.* (2013) reviewed that Various soluble components have been extracted from different strains of bacteria. These factors include peptides, teichoic acids, peptidoglycan-derived muropeptides, endo- and exo-polysaccharides, enzymes, cell surface proteins, vitamins, plasmalogens, and organic acids.

According to Sharma and Shukla (2016), Shenderov (2013), scientific evidence has demonstrated that postbiotics have various functional properties, such as antimicrobial, antioxidant, and immunomodulatory effects, even though the mechanisms underlying their health-promoting effects are not fully understood. These characteristics have the potential to favorably impact the homeostasis of the microbiota and/or the metabolic and signaling pathways of the host, hence influencing particular physiological, immunological, neuro-hormone biological, regulatory, and metabolic processes.

Postbiotics can be classified according to their elemental composition, which includes lipids (like butyrate, propionate, dimethyl acetyl-derived plasmalogen), proteins (like lactocepin, p40 molecule), carbohydrates (like galactose-rich polysaccharides, and teichoic acids), organic acids (like propionic and 3-phenyllactic acid), and complexes molecules (like lipoteichoic acids, peptidoglycan-derived muropeptides) (Kostantinov *et al.*, 2013; Tsilingiri and Rescigno, 2013). Alternatively, postbiotics can be classified according to their physiological functions (Table 1), which include immunomodulation, anti-inflammatory, hypocholesterolemic, anti-inflammatory, anti-hypertensive, anti-proliferative and antioxidant effects (Nakamura *et al.*, 2016).

According to research (Shigwedha *et al.*, 2014; Tomar *et al.*, 2015), postbiotics are generally sought-after due to their many desirable qualities, including their clear chemical structures, safety dose parameters, and longer shelf lives (up to five years when used as nutritional supplements or as an ingredient in foods and beverages).

Furthermore, Shenderov's (2013) study found that postbiotics have favorable capacities for absorption, metabolism, distribution, and excretion. These findings may point to a high potential for signaling various host organs and tissues and triggering a variety of biological reactions. Additionally, Tsilingiri *et al.* (2012) found on an ex vivo assay, that some probiotics can cause a local inflammatory response that is similar to the response caused by *Salmonella.* This shows that postbiotics can mimic the health effects of probiotics without requiring the administration of live microorganisms, which may not always be harmless.Furthermore, theoretical concern associated with live probiotic bacteria administration (e.g., bloating and flatulence, probiotic-related translocation and bacteremia and fungemia, and possible transfer of antibiotic resistance gene) have been described in case reports, clinical trials and experimental models, in patients with major (e.g., immunosuppression, premature infants) and minor (e.g., impairment of the intestinal epithelial barrier, concurrent administration with broad spectrum antibiotics to which the probiotic is resistant) risk factors for adverse events (Williams, 2010; Doron and Snydman, 2015).

Therefore, using postbiotics could be a legitimate and safer way to avoid the risks associated with using live probiotic bacteria. This gives postbiotics some useful functionality and practical applicability, making them a popular treatment option for a variety of diseases (Tsilingiri and Rescigno, 2013; Haileselassie *et al.*, 2016; Vieira *et al.*, 2016).

Kostantinov *et al.,* 2013; Tsilingiri and Rescigno, 2013 reported that, although strains of *Lactobacillus* and *Bifidobacterium* are the most common source of postbiotics, species of *Faecalibacterium* and *Streptococcus* have also been identified to be postbiotic sources. The antihypertensive potential of postbiotic supplements has been demonstrated by their ability to lower blood pressure. The exact mechanism behind this protective effect on endothelial function is unknown, but potential causes include modifications to the gut microbiota and its metabolic byproducts, the restoration of the gut barrier, and effects on inflammation, endotoxemia, and renal sympathetic nerve activity (Robles-Vera *et al.*, 2017).

Li (2013) stated that, the three fundamental processes that propel the effectiveness of postbiotics are pathogen-protective modulation, epithelial barrier augmentation, and immunological and inflammatory response regulation, in that order. Postbiotics are being used in the fermented food sector as well as a potentially effective therapy option for many sub-health issues, including gastrointestinal diseases like diarrhea and bloating. As a result, the use of postbiotics would effectively supplement probiotics and serve as a catalyst for the growth of the whole health sector (Wang *et al.,* 2022 and Fan *et al.,* 2017).

Food potential applications of postbiotics:

According to Venema (2013), postbiotics are meant to be more stable than the live bacteria from which they originate. According to Phister *et al.*(2004), *Bacillus* sp. strain CS93 produces peptides with antimicrobial qualities called bacilysin and chlorotetaine, which are water soluble and active across a broad pH range. This suggests that a wide range of food products may be able to use them.

Thorakkattu *et al.* (2022) opined that, the most popular method involving the use of postbiotics is fermentation, and producer strains of *Lactobacillus* and *Bifidobacterium* are frequently employed.

Rather *et al.* (2013) reported that Additionally, *Lactobacillus plantarum* postbiotics can effectively function as a bio-preservative to increase the shelf life of soybeans.

Makhal *et al.* (2015) Combining the two types of applications mentioned above, Danisco's commercial product Micro GARD is approved by the FDA and used as a top biopreservative in large dairy and food matrices. Propionibacterium freudenreichii subsp. Shermanii, which is present in skim milk, has undergone fermentation. Another innovative method entails augmenting vitamin B levels while diminishing harmful elements via fermentation triggered by probiotics.

Chocolate:

Langner *et al.* (2008) stated that Chocolate came to Europe in the 16th century. Different processes are now used to process cocoa seeds because the contemporary chocolate industry has grown since then. The most frequently craved food worldwide is chocolate. It is now regarded as a medication, while at first it was seen as a luxury good.

Andarea-Nightingale *et al.* (2009) concluded that essentially, chocolate is made up of sugar and cocoa mass suspended in a matrix of cocoa butter. The three main types of chocolate are milk, white, and dark, with variations in the amounts of cocoa butter, milk fat, and cocoa solid in each.

According to Afaokwa (2010), chocolates are semisolid suspensions of fine solid particles of sugar, cocoa, and, depending on the variety, milk; these ingredients make up roughly 70% of the total in a continuous fat phase. People of all ages and from all walks of life consume chocolate across the world.

El-Kalyoubi *et al.* (2011) explained that This food's popularity seems to be mostly related to its ability to elicit pleasurable sensations and happy feelings.

Moramarco (2016) explained that *Theobroma cacao* L., the Latin name for the cacao tree, translates to "Food of Gods." Chocolate is high in polyphenols such catechins, anthocyanidins, and pro-anthocyanidins and primarily composed of fat (cacao butter).

Miller *et al.* (2009) explained that the process of fermenting the seeds from the cacao tree's pods yields cocoa. The end product is high fat "unsweetened chocolate," which is made from dried, roasted, and crushed beans. This intermediary is crushed into cakes and alkalized to create cocoa powder. After homogenizing with sugar, butter, and occasionally milk, chocolate is the result.

Although many different types of cocoa beans grow throughout the world, 3 varieties of cocoa beans are mainly used to make chocolate products.

- Criollo (meaning "native"), distributed to the north and west of the Andes.
- Forastero (meaning "foreign"), found mainly in the Amazon basin; and
- Trinitario (meaning "sent from heaven").

These types of cocoa are separated by their distinct flavors and colors, which arise during manufacturing.

Chocolate contains a high amount of saturated fats, the two major fatty acids are palmitic and stearic acid, which appear to have fewer implications for progression of coronary artery disease than other saturated fatty acids.

Nutritional and Health Aspect of Chocolate:

Macronutrient and Energy Content of the Major Kinds of Solid Chocolate

Nutrient content per (100g)

Macronutrients:

Depending on the type and quantity of cocoa solids present, chocolate can include different levels of macronutrients that provide energy. Table 1 lists the energy content and amounts of fat, protein, and carbs found in the main varieties of solid chocolate.

Fat:

Lopez Huertas (2010) stated that Cocoa butter accounts for the majority of the fat in chocolate. Cocoa butter consists largely of stearic acid (C18:0, 34%), oleic acid (C18:1, 34%), and palmitic acid (C16:0, 27%). Stearic acid has been observed to have minimal effect on serum cholesterol levels, however an accumulating body of data suggests that oleic acid may enhance serum cholesterol levels and other cardiovascular risk factors. It has been demonstrated that palmitic acid slightly raises serum cholesterol levels. Stearic acid is also abundant in tropical oils, such as shea butter, which are commonly utilized as alternatives to cocoa butter.

Carbohydrates:

The main source of carbohydrates in the finished chocolate is the sucrose that is added during the manufacturing process. When making candy products, additional carbohydrates such as glucose, dextrins, flours, and starches may be added. Furthermore, there is a substantial quantity of fiber in cocoa powder (37% by dry weight). The percentage of non-fat cocoa solids in the product determines the final fiber content of solid chocolate; dark chocolate typically has the maximum amount (7g/100g), while white chocolate has the lowest amount (0.2g/100g).

Proteins:

Since cocoa contains poorly digested protein, it is not a substantial source of protein. Because milk proteins are added, milk chocolate has the highest protein content of all chocolate varieties and is also the most digestible.

Vitamins and Minerals:

A variety of minerals are present in cocoa and chocolate (Table 2). The minerals that are most prevalent in cocoa powder are potassium, magnesium, and calcium. Magnesium, selenium, and iron concentrations are also lower. The type of soil used to grow cacao affects the amount of minerals it contains. Reduced levels of thiamine, riboflavin, and vitamin E are among the other vitamins found in cocoa powder. The ultimate vitamin and mineral concentrations in chocolate products are influenced by

Table 1 : USDA National nutrient database for standard reference

Type of chocolate	Protein (g)	Fat (g)	Carbohydrate (g)	Energy (Kcal)
Milk	7.65	29.7	59.4	535
White	5.87	32.1	59.2	539
Dark	4.88	31.3	61.2	546

Table 2 : Variety of minerals present in cocoa and chocolate

Ingredient	Weight of ingredient	Cocoa powder	Chocolate		
			Dark	Milk	White
Iron	mg	13.8	8	2.35	0.24
Calcium	mg	128	56	189	199
Zinc	mg	6.8	2	2.3	0.45
Magnesium	mg	499	146	63	12
Manganese	mg	3.84	1.42	0.47	0.008
Selenium	mcg	14.0	3.0	4.5	4.5
Potassium	mg	1524	559	372	286
Phosphorus	mg	734	206	208	176
Thiamine	mcg	78.0	25.0	112	63.0
Riboflavin	mcg	241	50	298	282
Niacin	mg	2.19	0.725	0.386	0.745
Vitamin B12	mcg	0	0	0.75	0.75
Vitamin E	mg	0.1	0.54	0.51	0.96

the quantity of non-fat cocoa solids, the quantity of cocoa butter, and the inclusion of additional ingredients like milk and nuts, which can raise the concentrations of specific vitamins and minerals (like calcium and vitamin E).

Proanthocyanidins and flavanols:

Miller *et al.* (2009); Hurst *et al.* (2011) explained that Polyphenol contents in cocoa beans are considerable. Studies have generally recorded values of 12–18% by dry weight, however the exact amount varies depending on the type of cocoa bean, growth conditions, and processing procedures. About 10% of the total polyphenols are monomeric polyphenols, primarily (–) epicatechin (EC) and (+) catechin, whereas almost 90% are polymeric and oligomeric proanthocyanidins (PaC). It has been demonstrated that processing affects the composition and amount of polyphenols in cocoa products. Roasting, fermentation, and Table 2 lists the vitamins and minerals that each 100g of chocolate and cocoa contain. Ingredient Weight of ingredient Cocoa powder Chocolate Dark (45–59%) Milk White Iron Milligram 13.8 8 2.35 0.24 Calcium Milligram 128 56 189 199 Zinc Milligram 6.8 2 2.3 0.45 Magnesium Milligram 499 146 63 12 Manganese Milligram 3.84 1.42 0.47 0.008 Selenium Microgram 14.0 3.0 4.5 4.5 Potassium Milligram 1524 559 372 286 Phosphorus Milligram 734 206 208 176 Thiamine Microgram 78.0 25.0 112 63.0 Riboflavin Microgram 241 50 298 282 Niacin Milligram 2.19 0.725 0.386 0.745 Vitamin B12 Microgram 0 0 0.75 0.75 Vitamin E Milligram 0.1 0.54 0.51 0.96 a USDA National Nutrient Database for Standard Reference, Release 27. 524 alkalizations have generally been shown to reduce the levels of total polyphenols in the finished cocoa powder (Miller *et al.*, 2009; Hurst *et al.*, 2011).

It has been demonstrated that roasting and alkalization of specific polyphenols can cause EC to epimerize into catechin. PaCs appear to undergo polymerization upon roasting, and research conducted in our lab has demonstrated that while higher dp PaCs increase with roasting time, lower dp PaCs (*i.e.*, dimers and trimers) decrease (Stanley, personal communication). PaC levels are lowered as a function of time by alkali treatment; however, it is unknown what the final products of this process are.

Methylxanthines:

Franco *et al.* (2013) reported that Theobromine, caffeine, and methylxanthines are all present in chocolate and cocoa in large amounts, with the former having a higher concentration. It has been proposed that theobromine and caffeine, which are present in chocolate, contribute to its health effects on humans, primarily via opposing the adenosine receptor. These substances have the ability to dilate the bladder, relax smooth muscle, cause bronchodilation, and stimulate the heart and central nervous system. Studies have generally indicated that theobromine is not as effective as caffeine at producing these effects. The final cocoa product's composition (*i.e.*, amounts of non-fat cocoa solids, cocoa butter, etc.), processing methods, and source of cocoa beans all affect how much of these compounds are present. According to Franco *et al.* (2013), non-fat cocoa bean solids normally contain 2.5% and 0.24% of the dry weight in theobromine and caffeine, respectively. A typical 50g bar of milk chocolate has 10 mg of caffeine; however, this varies depending on the product. In contrast, a bar of dark chocolate of a comparable size may have 50 mg, depending on the amount of non-fat cocoa solids.

Chocolate Production:

Franco *et al.* (2013) stated that Chocolate is a product widely consumed by all generations. It has a lot of lipids, proteins, carbs, polyphenols, and other healthy ingredients.

Arunkumar and Jegadeeswari (2019) stated that the primary component used to make chocolate is cocoa beans. The steps involved in making chocolate include fermenting the cocoa beans, drying, roasting them, grinding them, combining all the ingredients (cocoa mass, sugar, cocoa butter, emulsifiers, scent, and milk components if necessary), conching, and tempering. The processes of fermenting, drying, roasting cocoa beans, and conching chocolate mass involve significant chemical reactions. The production of flavor and scent is mostly dependent on these interactions.

The process of fermentation, which is carried out on cocoa farms as part of the manufacture of cocoa beans, causes yeasts and bacteria to proliferate in the pulp. In this stage, breakdown of sugars and mucilage occurs.

It consists of three phases:

– The first phase lasts for 24–36 hours and is dominated by anaerobic yeasts. This phase is characterized by low pH (less than 4) and low oxygen concentration.
– Lactic acid bacteria are the main organisms in the second phase. They exist from the start and become active between 48 and 96 hours.
– Third phase: when aeration rises, acetic acid bacteria take over. The temperature rises to 50 °C or more during this final phase due to an exothermic reaction that turns alcohol into acetic acid.

Gutiérrez *et al.* (2017) concluded that Fermentation is the key stage for the production of precursors for development of proper chocolate aroma. After fermentation, the cocoa beans are dried to remove moisture and finish oxidative processes that were started during the fermentation process. Cocoa beans retain 6–8% moisture after drying. Reduced moisture content helps to keep mold at bay and improves the stability of the beans during storage and transportation.

The first step in making chocolate is often roasting cocoa beans in a chocolate factory. It's a high-temperature procedure that's crucial for the occurrence of Maillard reactions; it's often carried out at 120 to 140° C. Cocoa beans are cleaned and given a distinct flavor and aroma by roasting, which also lowers the amount of unwanted ingredients. During this stage, every precursor created in the earlier stages reacts to create a variety of chemicals.

All chocolate ingredients must be ground and well mixed to ensure that each ingredient has the proper particle size. When making chocolate, the primary components are sugar, milk (for milk chocolate), cocoa liquor (which is made by grinding cocoa beans), and cocoa butter (which is made by pressing cocoa liquor).

Conching is a process that involves mixing and heating ingredients to create liquid chocolate (fat coats all solid particles), evaporate volatile acids, reach the right viscosity, eliminate surplus moisture, and generate a desired color. One method of producing a stable product is tempering. Cocoa butter is tempered by conducting heat, which produces stable, uniformly sized crystals that influence the formation of a stable crystalline network during cooling. This study provides an overview of the main chemical events that take place during the manufacturing of chocolate and provides an analysis of the most significant components of chocolate and cocoa beans as they are now understood.

Health Benefits of Chocolate:

– *Reduces Stress*: Chocolate affects stress levels by prompting serotonin production which is a calming neurotransmitter.
– *Improved Cholesterol Level*: Dark chocolate may raise HDL cholesterol level and lower LDL cholesterol levels, which can contribute to better cardiovascular health.
– *Brain Function*: Dark chocolate contains compounds like caffeine and theobromine, which can enhance cognitive functions and alertness.

Conclusion:

Chocolate served as a probiotic source for microbiota proliferation, chocolate nutritional value can be enhanced via fortification with probiotic. Probiotic chocolate could be an excellent source of nutrients for the gut microbiota. Cocoa products are rich in flavonoids, which, as antioxidants and modulators of metabolic and enzymatic processes, may provide some protection against cardiovascular disease and cancer. Although chocolate is often thought of as a luxury item, cocoa products are plant-derived foods that can contribute numerous essential nutrients to the diet. In addition, cocoa products can be rich in polyphenolic compounds, which have been postulated to have health benefits. Although chocolate is often viewed as an indulgence food by virtue of its high fat and sugar content, chocolate and cocoa contain a number of essential nutrients in significant quantities, as well as several classes of chemicals with putative non nutritive bioactivity, including polyphenols, monounsaturated fatty acids and methylxanthine. Chocolate is demanded and loved by everyone. So, in this study we have included probiotics to maintain a healthy gut flora.Probiotic chocolate bars, in summary, offer a promising combination of flavor and health advantages. Manufacturers have discovered a fresh way to provide consumers with these helpful bacteria by adding probiotics into a cherished food like chocolate. Probiotics appear to have many potential health benefits, including better immune system performance, better digestion, and even mood modulation, according to research. Nevertheless, more research is required to properly comprehend the effectiveness and long-term consequences of probiotic chocolate bars. Furthermore, elements including appropriate storage, production methods, and customer preferences play a major role in the success of these products. Probiotic chocolate bars, in spite of these drawbacks, offer a novel way to boost wellbeing and sate desires, which could change the confectionery industry's view on functional foods. At this juncture, we recommend instead of consuming plain chocolate we can consume symbiotic chocolate.

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