

A review of the physicochemical characteristics of dark chocolate and milk enriched with probiotics

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Abstract

Chocolate is widely recognized as a popular food product, with its consumption experiencing significant growth in recent decades, largely attributable to the expanding variety of chocolate offerings available in the market. In response to evolving consumer preferences and health trends, food industry engineers are actively exploring innovative methods to enhance chocolate products, including the incorporation of probiotics. This approach aims to not only diversify the functional properties of chocolate but also to align with the increasing demand for health-oriented food options.

Probiotic-enriched chocolate represents a novel intersection of food science and health promotion, capitalizing on the growing consumer demand for functional foods that offer health benefits beyond basic nutrition. The incorporation of probiotics, live microorganisms that confer health benefits when consumed in adequate amounts, into chocolate products is an innovative approach aimed at enhancing the functional properties of this widely consumed treat. In this study, the physicochemical and sensory characteristics of dark and milk chocolate enriched with probiotics are investigated.

Keywords: Dark chocolate , Milk chocolate , Probiotic , Fortification

۱. Introduction

Chocolate, originating from the seeds of the cacao tree (*Theobroma cacao*), has played a pivotal role in human culture and dietary practices for centuries. Its extensive historical significance, varied applications, and intricate chemical composition render it an intriguing topic for scholarly investigation. The primary constituents of chocolate encompass cocoa solids, cocoa butter, sugar, milk powder, emulsifiers, and flavoring agents, with their proportions varying according to specific formulations. This complexity not only influences the sensory attributes of chocolate but also its nutritional and health-related properties.[^۱, ^۲]. In general, chocolate is classified into two categories: dark and milk chocolate. Dark chocolate composed primarily of cocoa solids, cocoa butter, and sugar, dark chocolate typically contains no milk solids. It is often regarded as the healthiest option due to its high flavonoid content and lower sugar levels compared to other types. Studies have shown that dark chocolate can reduce cardiovascular risk factors due to its antioxidant properties. Milk Chocolate includes milk powder along with cocoa solids and sugar, resulting in a sweeter taste and creamier texture. Milk chocolate generally contains less cocoa than dark chocolate, which may reduce its health benefits [^۳, ^۴].

Probiotics are defined as live microorganisms that confer health benefits when administered in adequate amounts, and they have attracted considerable interest in recent years. Their potential to enhance gut health, bolster the immune system, and mitigate various diseases has established them as essential components of functional foods and dietary supplements [^۵]. The growing body of research underscores the role of probiotics in modulating the gut microbiome, which is increasingly recognized for its influence on overall health. Furthermore, studies have demonstrated that probiotics can play a significant role in preventing gastrointestinal disorders and may have broader implications for chronic disease management. As such, probiotics represent a promising area of investigation within nutritional science and public health, warranting further exploration of their mechanisms and efficacy in promoting health benefits across diverse populations [^۶, ^۷].

The beneficial effects of probiotics are primarily attributed to their capacity to modulate the intestinal microbiota. Probiotics exert their influence by inhibiting the growth of pathogenic bacteria through competitive mechanisms, such as competing for essential nutrients and binding sites within the intestinal epithelium. This competitive exclusion is crucial for maintaining microbiota balance, which is integral to gastrointestinal health. Furthermore, probiotics enhance the immune response by stimulating the production of immunoglobulins and augmenting the activity of immune cells, including macrophages and T lymphocytes. Numerous studies have demonstrated that specific probiotic strains can produce short-chain fatty acids (SCFAs) via the fermentation of dietary fibers. SCFAs play a pivotal role in preserving the integrity of the intestinal barrier, mitigating inflammation, and providing energy to colonocytes. Collectively, these mechanisms underscore the importance of probiotics in promoting gut health and their potential therapeutic

applications in various gastrointestinal disorders [۸-۱۰]. The innovative fusion of probiotics with chocolate has emerged as a promising avenue in functional food development, leveraging the popularity of chocolate to enhance health benefits. Probiotic chocolates not only cater to consumer preferences for tasty treats but also provide a vehicle for delivering beneficial microorganisms that can improve gut health and overall well-being. The innovative combination of probiotics with chocolate has emerged as a promising avenue in functional food development, capitalizing on the popularity of chocolate to enhance health benefits. Probiotic chocolates not only satisfy consumers' preferences for tasty treats, but are also a means of delivering beneficial microorganisms that can improve gut health and overall well-being. In this article, we investigate the physicochemical and sensory effects of chocolate enriched with probiotics and the challenge of using probiotics in chocolate.

۲. Probiotics on milk chocolate

The milk chocolate was invented in ۱۸۷۵ by Daniel Peter in Switzerland. This invention created a new product that quickly became popular because it combined cocoa with milk. The main categories of commercial chocolate are dark, milk, and white chocolates, which differ in their content of cocoa solids, milk fat, and cocoa butter [۱۱].

Jahurul et al. stated that milk chocolate appears to be preferred over any other type of chocolate across the United States. Milk chocolate is a complex rheological system that has solid particles (cocoa, milk powder, and sugar) dispersed in cocoa butter, representing the fat phase. This is further explored in [۱۲], which discusses the effect of the manufacturing process on the microstructural and rheological properties of milk chocolate, as well as comparing quality characteristics between compound and pure milk chocolate [۱۲].

Recently published articles demonstrate that the quality and quantity of antioxidants in cocoa and chocolate are very high, and their flavonoids are believed to reduce the number of free radicals in the body that contribute to medical problems such as cardiovascular disease and cancer, while also being believed to offer some anti-aging health benefits [۱۳].

Chocolate is becoming increasingly popular as a carrier for delivering probiotics to the gut. It is composed of cocoa mass and sugar suspended in a cocoa butter matrix. The popularity of chocolate around the world, combined with the high level of health-related awareness among contemporary consumers, is increasing rapidly. Consequently, the idea of enriching different kinds of chocolate with probiotics has a high market demand [۱۴]. Probiotics are beneficial to the host when consumed in appropriate quantities every day. A number of health benefits of probiotic bacteria include anti-mutagenic effects, anti-carcinogenic properties, improvement in lactose metabolism, reduction in serum cholesterol levels, and stimulation of the immune system [۱۵]. This is further explored in the study titled 'Physicochemical Properties of Probiotic Soymilk Chocolate Mousse During Refrigerated Storage'. The enormous amount of research currently being conducted on the beneficial impact of the gut microbiome on humans has led to the development of novel food products

that directly support gut health. The formulation of probiotics and prebiotics with chocolate is a relatively new area that has not been fully explored, and extensive research is needed to verify the therapeutic effects of probiotic chocolate products. Additionally, novel prebiotics and their effects on the gut microbiota, as well as the rheological, textural, sensory, and nutritional profiles of probiotic chocolate products, need to be investigated to attract health-conscious consumers to these products [۴].

The growing trend of probiotic-enriched milk chocolate not only caters to health-conscious consumers but also appeals to those looking for delicious ways to enhance their diet. Table ۱ shows the physicochemical and sensory characteristics of probiotic milk chocolate

Table ۱. The physicochemical and sensory characteristics of probiotic milk chocolate

Microorganism	Probiotic form	Concentration	Storage	Physicochemical	Sensory	References
Lactobacillus plantarum HM ^{۴۷}	microencapsulated	10^8 log CFU/g	۶ month	Microencapsulated L. plantarum HM ^{۴۷} does not affect Ph. There was no significant difference in the recovery of probiotic bacteria in milk chocolate	addition of probiotic had no significant effect ($p > 0.05$) on the sensory attributes of the milk chocolate.	[۱۶]
Bifidobacteria, lactobacilli and streptococci	Microencapsulated	10^8-10^9 log cfu/g	۱۲ month	The values of plasticity and apparent viscosity in the chocolate samples were completely uniform. The determination of viability revealed that bifidobacteria were less durable than lactobacilli	The overall sensory quality of all chocolate samples analyzed with probiotics remained superior even after the expiration of ۳۶۰ days of storage.	[۱۷]

and streptococci , but the total cell number was high (10^8 – 10^9 log cfu/g) during 360 days, at both tested tem-peratures.

Lactobacillus acidophilus NCFM ,Lactobacillus rhamnosus HN001 and Bifidobacterium lactis HN019	lyophilized	10^8 to 10^9 log (CFU/g)	6 month	Chocolate samples with probiotics have lower yield stress and higher viscosity compared to chocolates without probiotics. Based on the observed results, it could be concluded that chocolate cultured with L. rhamnosus and L. acidophilus strains at 40°C , exhibits high functional quality, and these strains express a	The overall sensory quality of all chocolate samples containing probiotics after 360 days of storage at $20 \pm 2^\circ\text{C}$ is excellent or very good.	[11]
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				great potential for use in production of probiotic chocolate.		
Lactobacillus acidophilus NCFM and Bifidobacterium lactis HN۰۱۹	lyophilized	10^6 log cfu/g	۳ and ۶ month	Viscosity and plasticity did not experience major changes. The probiotic strain L. acidophilus NCFM® had a higher survival in each milk chocolate compared to the strain B. lactis HN۰۱۹	The results of the [۱۸] sensory evaluation of chocolate types with probiotics during the storage period of ۱۸۰ days, it can be concluded that all the analyzed samples were classified in the high sensory quality category in all three test periods.	
Lactobacillus casei ۴۳۱			۶ months	Probiotic sucrose-free chocolate had a higher viscosity than the control chocolate.	Probiotic sucrose-free chocolate was more viscous than control chocolate, although	[۱۹]

			Probiotic milk chocolates could be stored at ۲۰ °C for ۶ months. However, Probiotic viability was better in chocolate that was stored at ۴ °C than ۲۰ °C	displayed satisfactory sensory attributes.	
Lactobacillus plantarum and Lactobacillus acidophilus	Microencapsulation and by spray-drying	۲×۱۰^۷ colony forming unit (CFU) per serving size (۱۲ g)	Sw and Sw + Prob gave values closest to the control in hardness, while Sw without FO increased breakability. Prob + FO increased the resistance of chocolate to shear stress, while Sw + FO showed similar flow behavior. Showed with control. For all chocolate formulations, the addition	Consumers' acceptability of probiotic chocolate and sugar substitutes (steavia + isomalt) was sufficient, while sugar substitutes (steavia + isomalt), fish oil and probiotics were more acceptable than fish oil and probiotics.	[۲۰]

of microencapsulated
probiotics resulted in a
product with $\geq 2 \times 10^7$
CFU per portion (12 g).

Sw: isomalt + stevia,
probiotics (Prob), fish oil
(FO)

Lactobacillus
acidophilus and L.
paracasei

	$9,00 \log \text{ cfu}/20\text{g}$	3 month	Incorporation of probiotics had no significant effect on particle size, hardness and melting characteristics and limited effects on water activity, moisture content. After 90 days, the viability of probiotics was higher than $5,90 \log$ $\text{cfu}/20\text{g}$, L. acidophilus	Incorporation of probiotics had no significant effect on chocolate color and sensory properties.	[21]
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				showed higher levels of viability than <i>L. paracasei</i> .		
Lyophilized <i>Lactobacillus casei</i> and <i>Lactobacillus paracasei</i> . Alternative sweetener (isomaltaspartame)	lyophilised	$6-8 \log \text{CFU/g}$	۱۲ months	The addition of lyophilized <i>Lactobacillus</i> cells in chocolate did not change either the total or the volatile acidity, but increased its Casson viscosity, yield value and increased the hardness of the milk chocolate. The number of live cells of <i>Lactobacillus</i> was kept at a relatively stable level for ۱۲ months at ۱۸ °C (the survival above ۸۵%).	The supplementation did not change the [۲۲] sensorial attributes of chocolate.	

۳. Probiotic effect on dark chocolate

In recent decades, dark chocolate has emerged as a highly sought-after product, gaining significant popularity among consumers worldwide. This surge in interest can be attributed to various factors, including its unique flavor profile and the growing awareness of its health benefits. Dark chocolate is primarily composed of cocoa solids, cocoa butter, and sugar, with a higher concentration of cocoa compared to other types of chocolate. Typically, dark chocolate contains between ۵۰٪ and ۹۰٪ cocoa solids, which contributes to its rich taste and numerous health advantages [۲۳, ۲۴].

Research indicates that dark chocolate is rich in phenolic and flavonoid compounds, which are powerful antioxidants. These compounds play a crucial role in combating oxidative stress in the body, which is linked to various chronic diseases. Studies have shown that the levels of polyphenols and flavonoids in dark chocolate can be up to five times higher than those found in milk or white chocolate. The presence of these antioxidants not only enhances the flavor but also provides significant health benefits, such as reducing blood pressure and lowering the risk of heart disease [۲۵]. Incorporating probiotics into dark chocolate has emerged as a new approach to enhance its health benefits, merging the indulgence of chocolate with the advantages of gut health. This innovative combination leverages the natural properties of dark chocolate, which is already recognized for its rich content of antioxidants and prebiotic fibers, making it an ideal candidate for probiotic enhancement. Probiotics are live microorganisms, often referred to as "good bacteria," that can provide various health benefits when consumed in adequate amounts. They are well-known for their ability to support digestive health by fostering a balanced gut microbiome and inhibiting the growth of harmful bacteria [۲۶-۲۸].

Research has shown that dark chocolate, particularly varieties with high cocoa content, can act as a prebiotic. This means it serves as food for beneficial gut bacteria, promoting their growth and activity. When probiotics are introduced into dark chocolate, they work synergistically with these prebiotics to create a more robust environment for gut health. For instance, studies indicate that dark chocolate enriched with probiotics can enhance the production of beneficial digestive by-products known as postbiotics in the colon. These compounds not only support gut health but also contribute to overall well-being by reducing inflammation and improving metabolic functions [۲۹, ۳۰].

The growing trend of probiotic-enriched dark chocolate not only caters to health-conscious consumers but also appeals to those looking for delicious ways to enhance their diet. Table ۲ shows the physicochemical and sensory characteristics of probiotic dark chocolate



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Table ۲. The physicochemical and sensory characteristics of probiotic dark chocolate

Microorganism	Probiotic form	Concentration	Storage	Physicochemical	Sensory	Reference
<i>Lactobacillus plantarum</i> ۵۶۴	encapsulation	10^8 cfu/g	۲۱ month	No significant difference in chemical composition.	showed excellent sensory quality after ۶۰ and ۱۸۰ days of storage	[۳۱]
<i>Lactobacillus plantarum</i> ۲۹۹۷				good survival of probiotic bacteria after production and during storage		
<i>Bifidobacterium breve</i>	powder	10^9 cfu/g	۳ month	No significant difference in Physicochemical parameters	No significant in sensory parameters	[۳۲]

<i>Bacillus coagulans</i>	lyophilizate	10^6 10^7 10^8 cfu/g	۱۲ month	With the increase of time, pH increased while the amount of aw decreased. No significant change was observed in the amount of phenolic and flavonoid compounds.	No significant change was observed in sensory parameters.	[۳۳]
<i>L. plantarum</i>	Powder	10^8 cfu/g	۳ month	aw, ph and viscosity did not change significantly.	Color and texture (hardness) parameters did not change significantly	[۳۴]
Lactobacillus caseii and Lactobacillus paracasei	lyophilizate	7.9×10^9 cfu/g	۱۲ months	No significant difference in aw, while the amount	—	[۳۵]

				of calories was higher and volatile acidity was lower than the control sample.		
<i>L. plantarum</i>	microencapsulation	$1,1 \times 10^{12}$	۲ month	There was no	No significant	[۳۶]
<i>L. acidophilus</i>		$2,0 \times 10^{12}$		significant	difference in	
		CFU/mL		difference	sensory	
				between dark	parameters(flavor,	
				chocolate	appearance,	
				enriched with	texture,color,	
				probiotics and	overall	
				dark chocolate	acceptability)	
				without		
				probiotics in aw		
				parameter		
<i>Lactococcus lactis sub</i> <i>sp. lactis</i>	Powder	$8,20 \log \text{ cfu/g.}$	۳ month	The pH and	No significant	[۳۷]
				viscosity of	change was	
				probiotic	observed in Color	

				chocolate were definitely higher than the control sample, while the amount of aw was lower.	Appearance Flavor Texture Overall acceptability parameters	
<i>L. helveticus</i> MTCC ۵۴۶۳	Freeze dried	۳,۷۲×۱۰ ^۸ cfu/g	۲ month	No significant change was observed in the compounds.	There was no significant change in sensory parameters such as taste, texture, color between the probiotic dark chocolate sample and the control sample.	[۳۸]
<i>Lactobacillus acidophilus</i> NCFM <i>Bifidobacterium lactis</i> HN019	Freeze-dried	۳,۵ g/kg for <i>Lactobacillus acidophilus</i> NCFM	۲ month	The yield value of probiotic chocolate was higher than the	sensory properties of chocolates were not significantly	[۱۸]

		۱,۷۵ g/ kg for <i>Bifidobacterium</i> <i>lactis HN019</i>		control sample, while there was no significant change in the Viscosity Plastic and Apparent Viscosity parameters.	changed during storage.
<i>Lactobacillus</i> <i>ramnosus</i>	Microcapsules free cells	۹.۱۵ cfu/g	۲ month	-	[۳۹] The effect of microencapsulated probiotic was higher than that of cell wall. No significant difference was observed between probiotic chocolate and the control sample in

organoleptic and
textural properties

4. Challenges of using probiotics in the chocolate industry

Probiotics are live microorganisms that confer health benefits to the host when administered in adequate amounts. Their incorporation into food products, particularly functional foods like dark chocolate, has gained attention due to the potential health benefits associated with both probiotics and dark chocolate. However, the integration of probiotics into dark chocolate presents several challenges that must be addressed to ensure the viability and efficacy of these beneficial microorganisms [۳۷]. One of the primary challenges in using probiotics in dark chocolate is ensuring their survival during the manufacturing process. The high temperatures involved in chocolate processing can adversely affect probiotic viability. For instance, during roasting and conching, temperatures can exceed ۵۰°C, which may lead to significant reductions in probiotic counts. Research indicates that many probiotic strains exhibit sensitivity to heat, which raises concerns about their survival through thermal processing stages [۴۰, ۴۱]. The interaction between probiotics and the chocolate matrix can also pose challenges. The composition of dark chocolate, which includes cocoa solids, fats and sugars, may affect the survival and activity of probiotics. For example, high fat content can create an environment that is less favorable for some probiotic strains. In addition, the presence of polyphenols in dark chocolate may have antimicrobial properties that can inhibit probiotic growth [۴۰, ۴۲, ۴۳]. Regulatory considerations regarding health claims associated with probiotic-infused products should be another important challenge. The classification of probiotics as food supplements or food additives varies by region and can influence marketing strategies and consumer perception [۴۴, ۴۵].

Conclusion

this study has yielded significant insights into the physicochemical and sensory characteristics of dark and milk chocolate enriched with probiotics. The integration of probiotics not only enhances the functional properties of chocolate but also preserves its appealing sensory qualities, positioning it as a viable option for health-conscious consumers. The results demonstrate that both dark and milk chocolate can effectively retain their texture, flavor, and overall acceptability while providing the health benefits linked to probiotic consumption. Consequently, probiotic-enriched chocolate signifies a notable advancement in the realm of functional foods, addressing the increasing consumer demand for products that harmonize indulgence with health benefits. Future research endeavors should focus on optimizing formulations and processing techniques to maximize probiotic viability and further improve the overall quality of these innovative chocolate offerings.

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