Revisiting the Development of Probiotic-based Functional Chocolates

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ABSTRACT

The status of chocolate as functional food is still questionable for some other people as chocolate can also give some adverse effect due to its high fat and sugar content. To overcome this issue, some attempts have made by food scientist to produce chocolate with high potential health benefits and minimum adverse effects such as by fat reduction, sugar replacement and probiotic supplementation. Some of sugar replacer are providentially identified as prebiotic substances. This review, therefore, deeply discuss the potential use of probiotics and sugar replacer in chocolate. The health benefit as well as the effect on the consumer acceptance are also covered as chocolate reformulation may result in the alteration on the chocolate characteristic. Moreover, the regulation in chocolate manufacturing and functional food from different regulatory boards are covered in this review as the guidelines to answer challenges and opportunities in developing functional chocolate. This review also clearly shows a possible direction of designing probiotic chocolate in the future that has not been fully explored until to date.

Keywords

chocolate, probiotic, sensory, sugar replacement

1. Introduction

The development of functional foods is continuously carried out by food scientists to answer global health problems and to respond consumers' needs towards healthier food products [1]. A functional food can be defined as food that has beneficial effects on health beyond basic nutritional values [2]. However, it is worth to note that the definition of functional food is still diverse among countries and regulatory boards in the world [3]. Therefore, regulations, policies and health claims of functional food vary among the countries. For instance, some countries (e.g., Canada, United States, European Union, Japan and China) permit a disease risk reduction claim of a certain food in addition to health function claim. On the contrary, a disease risk reduction claim is not allowed in Russia, India, Taiwan, Thailand, and Singapore [4]. Despite its variation, the similar points among the regulations are that functional food is that taken as part of the usual diet and has a specific health claim.

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Probiotic is widely used in the functional food formulation as it provides health benefits for human [5]. Probiotics are known as ‘living microorganisms’ that confer beneficial health effects on the host by improving its intestinal microbial balance if administered in an adequate amount in daily diet [6]. Probiotics may also provide other health benefits such as reducing cholesterol level, reducing blood pressure, amelioration of arthritis, facilitation of mineral absorption, protection against gastrointestinal pathogens, and enhancement of the immune system [7]. In the last few years, some studies attempted to incorporate probiotics in chocolate [8, 9]. These studies are reasonable as chocolate can be an effective delivery matrix for probiotics. This is based on the fact that nowadays, chocolate becomes the most popular confectionery product throughout the world due to its good taste, unique texture, pleasant physiological effects, and association with an increase in happiness [10]. It was reported that the total consumption of chocolate reached 7.7 million metric tons in 2019 and experienced a 3.2 percent expansion of the market over the last five years [11]. This review, therefore, highlight current studies on the probiotic chocolate. Furthermore, the potential development of synbiotic chocolate by combining probiotic microorganism and prebiotic substances is also covered in this review.

2. Brief history of chocolate

Cocoa beans, the main material of chocolate making, are developed inside the fruit pods of the cocoa tree (*Theobroma cacao* L.). Cocoa is native to Central America. By the Aztecs and the Mayas, cocoa was traditionally consumed as spiced beverage and called as ‘the food from the Gods’ since they believed that cacao seeds were the gift from the god of wisdom and thus cocoa beverage was a source of wisdom and energy [12]. The Spanish introduced cocoa drink to Europe, successively, cocoa drink formulated with sugar and spices was being widely consumed by people from England to Italy by the mid-18th century [13].

In the Industrial Revolution era, Joseph Fry of England used a steam engine to grind cocoa beans; and then in 1828, the Dutch chemist, Van Houten, successfully separated cocoa butter from the dry cocoa solids using a hydraulic press. The cocoa solid could be ground into cocoa powder to make a better chocolate beverage. In the same period, Fry and Sons developed a process to make solid chocolate by the addition of the cocoa butter and sugar [14]. In 1847, the production of solid chocolate started in Great Britain. It was only in the early 20th century that solid chocolate became more popular than chocolate beverage. Due to the increasing demand for cocoa beans, the Portuguese, the British, and the Dutch established cocoa plantation in Africa and Asia in the areas about 20 degrees latitude north and south of the equator which are warm and have moist climates [13]. Recently, Woongnaa [15] reported that the four leading cocoa producing countries in the world were Cote d'Ivoire, Ghana, Nigeria, and Cameroon contributing about 70% of global cocoa production.

Innovative processes were found in Switzerland to improve the quality of chocolate bar. In 1897, Daniel Peter used milk powder to eliminate the perishability issue in chocolate. Nearby, the Lindt Company invented a process known as conching to create a smoother product [13]. Nowadays, the common procedure of making chocolate consists of several consecutive steps, including mixing (to mix the ingredients such as cocoa mass, cocoa butter, sugar, milk and emulsifier), refining (to reduce the particle size), conching (to fine-tune the chocolate flavour), tempering (to form the cocoa butter crystal) and moulding (to shape the chocolate) [16]. Depending on the formulation, there are three well-known basic types of chocolate in the market namely milk, dark and white chocolate. In short, milk chocolate normally contains all of the above-mentioned chocolate ingredients. Dark chocolate is formulated without milk, while white chocolate is made without cocoa mass.

Chocolates is continuously developed by the food scientists and industries, particularly in order to fulfil the demand of consumers. Some product developments focus on the incorporation of functional materials such as herbs,

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spices as well as probiotics [17, 18, 19, 20]. In the effort of innovative chocolate formula, it is important to note that the food industry must still give serious attention on the regulation issued by regulatory. Knowing regulation released by the regulatory bodies of certain area where the chocolates are produced and marketed is substantial for the food industry to start reformulating chocolate recipe. Chocolate regulation regarding dark, milk and white chocolate has been stated in Codex Alimentarius (CODEX STAN 87-1981), European Parliament and the Council (Directive 2000/36/EC) and Food and Drug Administration (Code of Federal Regulations Title 21 Part 163) as shown in Table 1.

3. Incorporation of probiotics in chocolate

Probiotics have been successfully incorporated in various cocoa-derived products, including chocolate bar, mousse and dessert [21, 22, 23]. Chocolate can be a good probiotic carrier for intestinal delivery due to its ability to protect the bacteria from environmental stress condition (gastric acidity and bile salts) [24]. Even though phenols could act as antimicrobials, phenolic compounds in chocolate did not influence the survival of the probiotic bacteria [25].

Table 1: Chocolate regulation

Type	Codex Alimentarius (CODEX STAN 87-1981)	European Parliament and the Council (Directive 2000/36/EC)	Food and Drug Administration (Code of Federal Regulations Title 21 Part 163)
White chocolate	a. Contains not less than 20% by weight of cocoa butter; b. Contains not less than 14% by weight of total milk solids, including 2.5–3.5% by weight of milk fat.	a. Contains not less than 20% cocoa butter; b. Contains not less than 14% dry milk solids obtained by partly or wholly dehydrating whole milk, semi- or full-skimmed milk, cream, or from partly or wholly dehydrated cream, butter or milk fat, of which not less than 3.5% is milk fat.	a. Contains not less than 20% by weight of cocoa butter; b. Contains not less than 3.5% by weight of milk fat and not less than 14% by weight of total milk solids; c. Contains not more than 55% by weight nutritive carbohydrate sweetener; d. Emulsifying agents (if applied) used singly or in combination, the total amount of which does not exceed 1.5% by weight.
Milk chocolate	a. Contains at least 25% cocoa solids, including a minimum of 2.5% fat-free solids; b. Contains minimum 14% non-fat milk solids, including 2.5–3.5% milk fat.	a. Contains not less than 25% total dry cocoa solids; b. Contains not less than 14% dry milk solids obtained by partly or wholly dehydrating whole milk, semi- or full-skimmed milk, cream, or from partly or wholly dehydrated cream, butter or milk fat; c. Contains not less than 2.5% dry non-fat cocoa solids; d. Contains not less than 3.5% milk fat; e. Contains not less than 25% total fat (cocoa butter and milk fat).	a. Contains not less than 10% by weight of cocoa liquor; b. Contains not less than 3.39% by weight of milk fat and not less than 12% by weight of total milk solids; c. Emulsifying agents (if applied) used singly or in combination, the total amount of which does not exceed 1.0% by weight.
Dark chocolate	Contains not less than 35% total dry cocoa solids, including not less than 18% cocoa butter and not less than 14% of dry non-fat cocoa solids.	Contains not less than 35% total dry cocoa solids, including not less than 18% cocoa butter and not less than 14% of dry non-fat cocoa solids.	-

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Previous studies found some interesting findings in the development of probiotic chocolates (Table 2). For instance, *Bacillus coagulans* GBI-30, 6086 strain (10⁶ to 10⁸ CFU/g) were successfully added into dark chocolate at 70 °C using a mixer. This strain has been reported to have a high survival rate after digestion [8]. Konar and his research group [26] reported that *Lactobacillus acidophilus* and *Lactobacillus paracasei* at the level of 9.00 log cfu per 25 g chocolate were successfully incorporated into white chocolate after conching. After 90 days, probiotic viability was above 6.61 log cfu/25 g which *L. acidophilus* showed higher levels of viability than *L. paracasei*. Another study within a similar group research showed that in milk chocolate, the probiotics viability was above 5.90 log cfu/25 g which *L. acidophilus* showed higher levels of viability than *L. paracasei* after 90 days storage [27]. Interestingly, Rad *et al.* [9] reported that *L. casei* 431-incorporated milk chocolates could be stored at 20 °C for 6 months. However, Probiotic viability was better in chocolate that was stored at 4 °C than 20 °C. Encapsulated *L. plantarum* (10 g of encapsulated probiotics/kg of chocolate) were also incorporated into the chocolate at an additional mixing step after tempering. After 60 days and 180 days, the probiotics viability was 8.00 log cfu/g and over 6.00 log cfu/g up, respectively [28].

Different bacteria, such as *L. rhamnosus*, *L. paracasei* F19, *L. casei* DG and *L. reuteri* DSM17938: were incorporated into molten dark chocolate. After 90 days, the probiotics viability was above 6.00 log cfu/15 g except *L. reuteri* DSM17938 which was below 6.00 log cfu/15 g [25]. Almost in the same period, Silva *et al.* [29] reported that semi-sweet chocolate increased the survival of *L. acidophilus* LA3 and *Bifidobacterium animalis* subsp. Lactis BLC1 under gastrointestinal conditions with high viability. Kemsawasd *et al.* [30] also showed that chocolate can protect *L. casei* 01 and *L. acidophilus* LA5 in the stomach and small intestine conditions. According to Yonejima [31], chocolate is an effective matrix to deliver probiotics in a viable form to the intestine because acid tolerance of probiotic bacteria was higher by coating in with the chocolate. Probiotics that were incorporated in chocolate were more stable against gastric acid condition than probiotics powder.

Probiotics has been successfully added into chocolates. Nevertheless, it is noteworthy that until to date the living microorganism used in probiotic chocolate or other food products is bacteria. The species of *Bacillus* Sp., *Lactobacillus* Sp. and *Bifidobacterium* Sp. are the most dominant probiotic bacteria studied in chocolate formulation [32, 33, 34]. Probiotic could be incorporated in chocolate in the freeze dried or microencapsulated form to obtain optimal viability in the gastrointestinal system (Table 2). The probiotic substance may be added to chocolate in the additional mixing process either before or after tempering [29, 31]. Recently, a yeast species *Saccharomyces boulardii* has been reported to have specific probiotic properties [35, 36]. To the best of our knowledge, no study on the application of the yeast in chocolate has been initiated. It might be an interesting topic for the future study.

4. Reducing adverse effect of chocolate: fat reduction and sugar replacement

There are many studies showing the potential health benefit of probiotic chocolate, and thus the prospect of probiotic chocolate as functional food product. Yet, the status of probiotic chocolate as functional food may be questioned by some people. According to Raymond and his co-worker [37], the controversy of chocolate status as functional food is strongly related to its fat content. Even though chocolate with probiotics is proven to have beneficial effect on health, high intake of fat is still strongly associated with some negative implications, such as cardiovascular diseases, high cholesterol and high blood pressure [38]. In addition to the fat content, sugar (sucrose) content may be one of the major concerns of the scientists. On one hand, sugar has a significant contribution in chocolate properties mainly as sweetener and bulking agent [39]. Moreover, sugar has been reported to be able to enhance flavanols absorption in the gastrointestinal track [40]. However, on the other hand, high consumption of sugar triggers some health problems such as obesity, diabetes and dental health [41]. Some efforts need to be

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Table 2: Probiotic enrichment in chocolate

Probiotic enrichment	Type of chocolate	Important findings	Reference
<i>Lactobacillus casei</i> 431	milk chocolate	a. Probiotic milk chocolates could be stored at 20 °C for 6 months. However, Probiotic viability was better in chocolate that was stored at 4 °C than 20 °C. b. Probiotic sucrose-free chocolate had satisfactory sensory attributes and higher viscosity than control chocolate.	[79]
<i>Bacillus coagulans</i> GBI-30, 6086 strains	dark chocolate	Probiotic microorganisms (10^{-6} to 10^{-8} CFU/g) were successfully added into dark chocolate at 70 °C using a mixer. <i>Bacillus coagulans</i> GBI-30, 6086 strains had a high survival rate after digestion. Probiotic dark chocolates had a similar sensory characteristic to chocolate control.	[8]
Lyophilised <i>L. paracasei</i> and <i>L. acidophilus</i>	white chocolate	a. Probiotic microorganisms (9.00 log cfu/25g) were successfully incorporated into white chocolate after conching. After 90 days, probiotic viability was above 6.61 log cfu/25g which <i>L. acidophilus</i> showed higher levels of viability than <i>L. paracasei</i> . b. The incorporation of the probiotics had no significant effect on the water activity, textural properties and melting profile and resulted in a tolerable change on the rheological properties and colour of the chocolate.	[26]
Encapsulated <i>Lactobacillus plantarum</i>	dark chocolate	a. Probiotic microorganisms (10 g of encapsulated probiotics/kg of chocolate) were incorporated into the chocolate at an additional mixing step after tempering. After 60 days and 180 days, the probiotics viability was 8.00 log cfu/g and over 6.00 log cfu/g up, respectively. b. Probiotics incorporation had no significant effect on volatile profiles, aroma, texture and appearance of chocolate of the chocolate after 180 days of storage.	[28]
<i>Lactobacillus acidophilus</i> and <i>L. paracasei</i>	milk chocolate	a. Probiotic microorganisms (9.00 log cfu/25g) were incorporated into milk chocolate after conching. After 90 day, the probiotics viability was above 5.90 log cfu/25g which <i>L. acidophilus</i> showed higher levels of viability than <i>L. paracasei</i> . b. The incorporation of the probiotics had no significant effect on the particle size, hardness, and melting profile, and limited effects on water activity, moisture content, rheological properties and colour of the chocolate.	[27]
<i>Lactobacillus rhamnosus</i> , <i>L. paracasei</i> F19, <i>L. casei</i> DG and <i>L. reuteri</i> DSM17938	dark chocolate	a. Probiotic microorganisms were incorporated into molten dark chocolate. After 90 days, the probiotics viability was above 6.00 log cfu/15g except <i>L. reuteri</i> DSM17938 which was below 6.00 log cfu/15g. b. The incorporation of the probiotics had no significant effect on the sensory profile and water activity of the chocolate	[25]
<i>Lactobacillus acidophilus</i> LA3 and <i>Bifidobacterium animalis</i> subsp. <i>Lactis</i> BLC1	semi-sweet chocolate	a. Semi-sweet chocolate increased bacterial survival under gastrointestinal conditions with high viability. b. After 120 days of storage, fat bloom occurred in the probiotic-enriched chocolate and the control. c. Probiotic chocolates had a high acceptability by panellists.	[29]
<i>Lactobacillus acidophilus</i> NCFM, <i>Lactobacillus rhamnosus</i> HN001, and <i>Bifidobacterium lactis</i> HN019	milk chocolate	a. Inoculation of the probiotics bacteria in milk chocolate at 40 °C resulted in higher scores of overall sensory qualities, lower viscosity and less increase in volume than that at 35 °C. b. After 6 months of storage, the survival of <i>L. acidophilus</i> NCFM and <i>L. rhamnosus</i> HN001 strains was above 90%, with a viable cell count of about 8.1 log CFU/g).	[63]
Immobilized <i>Lactobacillus casei</i> 01 and <i>L. acidophilus</i> LA5 powder	white, milk and dark chocolates	a. Chocolate protected <i>L. casei</i> 01 and <i>L. acidophilus</i> LA5 in the stomach and small intestine conditions. b. Probiotic powders had no significant effect on sensory attributes. However, after 60 days storage, overall liking scores significantly decreased in all the chocolate samples.	[30]

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Table 2: Probiotic enrichment in chocolate (continued)

Probiotic enrichment	Type of chocolate	Important findings	Reference
Freeze-dried <i>Lactobacillus acidophilus</i> NCFM® and <i>Bifidobacterium lactis</i> HN019 powders	milk and dark chocolate	a. <i>Lb. acidophilus</i> NCFM® and <i>B. lactis</i> HN019 were added after tempering process and were able to survive during manufacturing process and storage period (14 months). b. <i>Lb. acidophilus</i> NCFM® and <i>B. lactis</i> HN019 incorporated in chocolate had high numbers of viable cells in the intestinal system. c. To create a sensorial acceptable chocolate, the incorporation of <i>L. acidophilus</i> NCFM® and <i>B. lactis</i> HN019 should be between 2×10^8 – 2×10^9 CFU/g in total.	[80]
Freeze-dried <i>Lactobacillus acidophilus</i> NCFM® and <i>Bifidobacterium lactis</i> HN019	milk and dark chocolate	a. <i>L. acidophilus</i> NCFM® and <i>B. lactis</i> HN019 had good viability in milk and dark chocolate. b. The chocolate was effective to preserve a high level of cell activity during 180 days of storage. The probiotic-enriched chocolates could be stored at room temperature without a significant decline in product functionality. c. The incorporation of freeze-dried <i>L. acidophilus</i> NCFM® and <i>B. lactis</i> HN019 did not lead to a substantial disruption of the sensory properties.	[65]
<i>Lactobacillus brevis</i> subsp. <i>Coagulans</i> (Labre) FERM BP-4693 and <i>L. brevis</i> NTM003 (NTM003) NITE BP-1634	milk chocolate	a. Chocolate was an effective matrix to deliver probiotics in a viable form to the intestine. b. Probiotics that were incorporated in chocolate were more stable against gastric acid condition than probiotics powder.	[31]
Freeze-dried <i>Lactobacillus rhamnosus</i> R0011	dark chocolate	The incorporation of freeze-dried <i>L. rhamnosus</i> R0011 in dark chocolate at 40 °C resulted in a loss of viable cells of approximately 0.2 log per g.	[37]
Synbiotic, consisting of lyophilised <i>Bacillus indicus</i> HU36 and dietary fibre	dark chocolate couverture	a. The addition of carboxymethylcellulose, inulin, wheat fibre, and apple fibre showed a negative effect on taste, mouthfeel, texture and overall acceptability. Maltodextrin and lemon fibre were chosen as the most suitable dietary fibre types for further studies. b. To obtain the best organoleptic properties of the product, the lemon fibre concentration should be kept constant at 1.5 g/100 g chocolate, while maltodextrin concentration should be maintained at 3.20–3.91 g/100g chocolate c. <i>B. indicus</i> HU36 can be used efficiently for probiotic bitter chocolate production. Microbiological analysis proved the <i>Bacillus indicus</i> HU36 had a high survival rate in dark chocolate, and all inoculated samples showed desired probiotic bacteria load (over 5 logs CFU/g product).	[32]
<i>Lactobacillus plantarum</i> (isolated from fermented cocoa beans)	dark chocolate	a. After 3 months of storage, <i>L. plantarum</i> viability decreased from 8 to 6 log CFU/g b. The addition of potential probiotic bacteria did not impair pH, colour, hardness and water activity of the dark chocolate compared to the control. c. Dark chocolate containing probiotic bacteria had a higher viscosity than the control and decreased during storage.	[64]
Free and encapsulated <i>Lactobacillus casei</i> NCDC 298 and inulin	milk chocolate	a. Encapsulation had no significant impact on the survival of probiotic bacteria in milk chocolate during storage at refrigeration conditions, in contrast to at gastrointestinal environment. b. The sensory properties of the chocolates were unchanged due to the minimum metabolic activity of the bacteria during storage.	[81]

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Table 2: Probiotic enrichment in chocolate (continued)

Probiotic enrichment	Type of chocolate	Important findings	Reference
Lyophilized <i>Lactobacillus casei</i> and <i>Lactobacillus paracasei</i> . Alternative sweetener (isomalt-aspartame)	milk chocolate	a. The addition of lyophilised <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. b. The supplementation did not change the sensorial attributes of chocolate. c. The number of live cells of <i>Lactobacillus</i> was kept at a relatively stable level for 12 months at 18 °C (the survival above 85%). However, more bacteria cells survived in chocolate stored in a refrigerated condition.	[82]
A mixture of microencapsulated <i>Lactobacillus helveticus</i> CNCM I-1722 and <i>Bifidobacterium longum</i> CNCM I-3470	milk and dark chocolate	Chocolate protected <i>L. helveticus</i> and <i>B. longum</i> from environmental stress condition. Milk chocolate was more effective than the dark one (respectively 91% and 80% of survival for <i>L. helveticus</i> and <i>B. longum</i>) when evaluated in Simulator of Human Intestinal Microbial Ecosystem (SHIME).	[33]
Powdered yoghurt containing <i>Streptococcus thermophilus</i> MK-10 and <i>Lactobacillus delbrueckii subsp. bulgaricus</i> 151	milk and dark chocolate	a. The incorporation of the bacteria did not lead to a substantial disruption of the sensory properties. b. After 6 months of storage (at the temperature of 4 °C and 18 °C), the viability of the bacteria significantly decreased which <i>S. thermophilus</i> MK-10 had a better viability than <i>L. delbrueckii subsp. bulgaricus</i> 151. Dark chocolate provided a better environment for the survival of the bacteria.	[34]

undertaken by food technologists to produce high-quality chocolate with reduced fat and calories [42]. In order to develop innovative chocolate with an improved nutritional profile fat reduction and sugar replacement techniques can be carried out.

Fat reduction or fat replacement in chocolate have been conducted by different research groups. Fat reduction in chocolate was initially conducted for economical purpose since the price of the cocoa fat is relatively expensive in the market. In nutritional perspective, reducing fat content in a chocolate formula is beneficial to lessen the adverse effects of the chocolate. Reducing fat content is beneficial as it can significantly reduce the total calorie content of the chocolate. The fat-reduced chocolate can be prepared using the protocol of Do *et al.* [43]. Cocoa particles (defatted cocoa powder containing fat below than 1% w/w) were incorporated into the chocolate formula containing molten cocoa butter, milk powder, sugar and lecithin at the level of 8.1% (w/w). In the other studies, fat-reduced chocolate can be prepared by replacing the fat content using other materials such as hydrogenated oils, inulin, β -glucan, xanthan gum and guar gum [44, 45, 46]. However, it is notable that up till now, most of the studies discussed the impact of fat reduction or fat replacement on the quality attributes of the chocolates. As such, the fat reduction in the study of Do *et al.* [43] focused only on the flow properties of the modified chocolate, but did not discuss the nutritional aspect. Thus, investigation on the effect of fat-reduced chocolate on the health are still fairly absent and thus there is still a great opportunity to study this particular topic.

Various studies have also been conducted aiming to investigate the applicability of alternative sweeteners and bulking agents in chocolate. According to Saputro *et al.* [16], one of the main purposes of the use of sugar replacer is targeting to produce chocolate with lower glycemic index than standard chocolate, accordingly having a minimal negative health effect. The combination of high-intensity sweeteners such as sucralose, stevioside, thaumatin and sugar alcohols with a bulking agent such as inulin, maltodextrin, and polydextrose has been widely used to replace sucrose in chocolate [39, 47]. It was reported that sugar replacement has significant impact on the quality of chocolate including particle size, flow behaviour, appearance, texture and melting profile as well as moisture content

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Table 3: Impact of sugar replacement on the quality attributes of chocolate

Sugar replacer	Chocolate type	Results (compared to chocolate control)							Ref.
		Particle size	Viscosity	Yield stress	Lightness	Hardness	Moisture content	Other	
Inulin									
Inulin			↑	↑		↑		Sensory profile †	[68, 69]
Inulin and maltodextrin						↑		Sensory profile †	[70]
Inulin	White chocolate		‡		↔	↓		Water activity ↓ Melting point ↔	[20]
Inulin and stevia with different rebaudioside A	Bittersweet chocolate							Sensory profile †	[71]
Inulin with steviol glycosides	Dark chocolate		↑	↓					[16]
Inulin with polydextrose and thaumatin	Dark chocolate		↑	↓	↓	↓	↑	Melting point ↔ Thixotropy ↓	[72]
Inulin with polydextrose and stevia	Dark chocolate		↑	↓	↓	↓	↑	Melting point ↔ Thixotropy ↓	[72]
Inulin with fine sugar	Milk chocolate	↑	↓	↓	↔	↔		Water activity ↓	[42]
Inulin	Dark chocolate	↑	↑	↓	↓	↑	↑		[73]
Inulin with isomalt	Milk chocolate	↔	↑	↓	↔	↔		Water activity ↔	[47]
Inulin with malitol	Milk chocolate	↔	↑	↓	↑	↔		Water activity ↔	[47]
Inulin with sucralose	Milk chocolate					↓	↑		[74]
Polydextrose									
Polydextrose with steviol glycosides	Dark chocolate		↑	↓					[75]
Polydextrose	Dark chocolate	↔	↔	↑	↓	↑	↑		[73]
Polydextrose with inulin, maltodextrin, stevia, and whey protein	Milk chocolate	↑	‡	↓	↓	‡	↑	• Energy value ↓ • Consumer acceptance ↓	[61]
Polydextrose with sucralose	Milk chocolate					↓	↑		[74]
Polydextrose, sucralose, stevioside, and whey protein concentrate	Milk chocolate							Consumer acceptance ↓	[60]

↑=increase; ↓=decrease; ↔ = remain relatively stable; ‡ = change, depending on the type and the concentration of the material and the process used

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Table 3: Impact of sugar replacement on the quality attributes of chocolate (continued)

Sugar replacer	Chocolate type	Results (compared to chocolate control)							Ref.
		Particle size	Viscosity	Yield stress	Lightness	Hardness	Moisture content	Other	
Others									
Xylitol		↑	↑	↑		↑	↑		[76]
Maltitol		↑	↑	↑		↑	↑		[76]
Galactooligosaccharide		↑	↑	↑		↑	↑		[76]
Palm sugar	Dark chocolate							Aroma †	[59]
Malitol and xylitol	Milk chocolate		‡	‡					[77]
Sucralose and stevia	White chocolate				↔			Melting point ↑	[78]
Palm sugar	Dark chocolate	↑	↑	‡	↔		↑	<ul style="list-style-type: none">• Melting point ↔• Agglomeration ↑• Aroma †	[16]
Coarse palm sugar	Dark chocolate	↑	‡	‡	↑	↑	↑	<ul style="list-style-type: none">• Melting point ↔• Agglomeration ↑• Thixotropy ↑	[16]
Coarse coconut sugar	Dark chocolate	↑	‡	↓	↑	↑	↑	<ul style="list-style-type: none">• Melting point ↔• Agglomeration ↑• Thixotropy ↑	[16]
Fructooligosaccharide and malitol with rebaudioside A	White chocolate							Sweetness ↔	[49]
Fructooligosaccharide and malitol with sucralose	White chocolate							Sweetness ↔	[49]
Malitol	Milk chocolate							Consumer acceptance ↓	[62]
Fructose with various combinations of highly intensive sweetener and bulking agent	Semi-sweet chocolate	↑				↑		Antioxidant activity ↓	[66]
Maltodextrin with sucralose	Milk chocolate					↓	↑		[74]

↑=increase; ↓=decrease; ↔ = remain relatively stable; ↓ = change, depending on the type and the concentration of the material and the process used

sensory profile (Table 3). Research by Akyol *et al.* [48] investigated the replacement of sugar in dark and milk chocolate using β -glucan. However, their study only focused on short-term satiety and energy intake of respondents. With increasing demand for chocolate with energy load reduction, more investigation in this particular area is essential. Thus, until to date most of the previous studies focus on the technological aspect and scarcely on the effect of sugar replacement on the nutritional aspect.

As afore-mentioned, the combination of high-intensity sweeteners and a bulking agent has been widely used to replace sucrose in chocolate [39, 47]. Interestingly, some of those sugar replacers are widely recognised as prebiotic

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substances that can induce the growth or the activity of probiotic microorganisms [49, 50]. The chocolate that contains the combination of prebiotic substances and probiotic microorganism is termed as synbiotic chocolate. In addition to the fact that cocoa fibre has a role as prebiotic substance [51], the current studies on sugar replacement in chocolate lead a bigger opportunity for scientists to develop a more purposeful prebiotic or synbiotic chocolate. This is indeed one of the advantages of carrying out sugar replacement in chocolate that can be further explored in future studies. Also, it indicates that there is still a great opportunity for food scientist to develop synbiotic chocolate.

5. Impact of formula modification on the quality attributes and the consumer acceptance of chocolate

It has been broadly acknowledged that a modification of a recipe of food can have significant impacts on its quality and accordingly its consumer acceptance level. This is true as well in the case of chocolate. As well-stressed by Saputro *et al.* [16], there are at least six important quality attributes in chocolate, including flavour, texture, appearance, flow behaviour, melting profile and particle size distribution. In addition, the presence of moisture significantly affects the quality of the chocolate.

Some cases in chocolate production with fat reduction, sugar replacement or functional ingredients enrichment have been found by different research groups. In technological viewpoint, it has been reported that a fat-reduced chocolate (containing 24.5% of fat) had satisfactory flow properties [43]. However, fat reduction can give some drawbacks in the quality attributes of the chocolate since the cocoa fat can play significant role on the flavour release due to its ability to construct lipophilic flavour interaction, the chocolate's texture due to its functionality to form fat crystal, the chocolate's flow behaviour due to its ability to aid the cocoa particles when they flow past each other and even the chocolate's appearance (lightness) since the crystalline network of cocoa fat scatters light reducing luminance and saturation [52, 53, 54].

Several research groups replaced fat in chocolate using other materials Rodriguez Furlán *et al.* [46] demonstrated a substitution of fat using hydrogenated oils at a concentration of 20% (w/w). However, the effect of the substitution is not clear because they did not use conventional chocolate as the control. In the other reports, a change in the textural properties of the chocolate was observed as a result of a fat replacement. The presence of inulin and β -glucan concentrate decreased the hardness of the chocolates and undeniably, it changed the 'snap' characteristic of the chocolate [44]. In general, a lower level of the lipid phase in chocolate formulation resulted in a less hard chocolate due to its less crystalline network. Moreover, in the case of fat replacement using inulin or β -glucan, the crystalline network could not perfectly coat all the solid particles in the chocolate matrix resulting in rupture structure. On the contrary, fat replacement by using xanthan gum and guar gum blend exhibited a harder texture [45]. They hypothesised that the incorporation of hydrocolloids can induce a network of protein in the chocolate matrix contributing to the increase of the chocolate hardness.

The impact of fat reduction or replacement is not only on the quality attribute of the chocolate, but also the consumer acceptance. Rezende *et al.* [44], for example, reported that a low-fat chocolate with inulin and β -glucan had a lower hedonic score on the panellists' acceptance. Replacing the cocoa fat using another vegetable fat (at the equal total content) has also significant impacts on the physical, rheological and sensorial properties as well as the appearance of the chocolate during storage due to the difference of fat bloom occurrence [55]. The changing in consumer acceptance might be a serious problem, particularly in the context of the product's marketing. Nonetheless, according to Stubenitsky *et al.* [56], giving information to the consumers about the correlation between lower fat content and health could increase consumer acceptance to the product. This can be an alternative strategy for the food industry to promote fat-reduced chocolate.

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Similarly, sugar replacement using high intensity sweeteners in combination with bulking agent causes alteration on the characteristic of chocolate to some extent (Table 3). Even though some studies mentioned in Table 3 are not in the topic of probiotic chocolate, the results clearly indicate that reformulation of chocolate can really give significant impact when applied further in probiotic chocolates. According to Konar *et al.* [42], for instance, some types of sugar replacer, such as inulin, has a water binding activity resulting in a chocolate with a higher moisture content. A similar phenomenon was found when palm sugar was used as sugar replacer in dark chocolate [57, 58]. The increase of moisture content in chocolate influences further on the other quality attributes of the chocolate, such as rheological behaviour and textural properties. Moisture on sugar particle surface increases friction among particles and promotes aggregation to form sugar network; and at the same time the moisture reduces the availability of “free” fat which is beneficial for a swift flow since more fat is used to coat the increased size of the sugar particles resulting in a more viscous chocolate [52, 58]. The sugar network can strengthen the particle-to-particle network system in the chocolate matrix and therefore resulting in a harder chocolate. In addition to the rheology and the hardness, it has been reported that the use of sugar replacement using palm sap sugar also affected the aroma profile and the appearance of the dark chocolate [59]. Change in the quality attributes due to sugar replacement can result in the decrease of the consumer acceptance to the chocolate. As reported in the previous studies, consumers prefer the sensory properties of conventional chocolate to low sugar chocolate [60, 61]. Interestingly, in the report of Markey *et al.* [62], sugar-reduced chocolate obtained a higher consumer acceptance score than the conventional chocolate suggesting that there is an opportunity of the success of the low sugar chocolate in the market.

The impacts of probiotic enrichment in chocolate have been revealed in previous researches. For instance, Succi *et al.* [25] reported that the incorporation of *L. acidophilus* and *L. paracasei* has limited effects on water activity, moisture content, rheological properties and colour of the chocolate. Another study showed that inoculation of *L. acidophilus* NCFM®, *L. rhamnosus* HN001, and *Bifidobacterium lactis* HN019 in milk chocolate resulted in a lower viscosity [63]. Also, dark chocolate containing probiotic bacteria has been reported to have a higher viscosity than the control [64]. In the study of Nebesny *et al.* [34], it has been reported that the addition of lyophilised *Lactobacillus* cells in chocolate increased its Casson viscosity, yield value and increased the hardness of the milk chocolate.

Nevertheless, recently scientists also found that chocolate with probiotics is acceptable. As such, sucrose-free chocolate enriched with *L. casei* 431 have been reported to have satisfactory sensory attributes and higher viscosity than control chocolate [9]. Probiotic dark chocolates formulated with *B. coagulans* GBI-30, 6086 strains had a similar sensory characteristic to chocolate control [8]. Also, the incorporation of the probiotics has no significant effect on the water activity, textural properties and melting profile and results in a tolerable change on the rheological properties and colour of the chocolate [26]. Incorporation of encapsulated *L. plantarum* also has no significant effect on volatile profiles, aroma, texture and appearance of chocolate of the chocolate after 180 days of storage [28]. Kemsawasd *et al.* [30], who worked with probiotic powders, reported that the addition of probiotic powders had no significant effect on sensory attributes of chocolate. Also as reported by Lalicic-Petronijevic *et al.* [65], the incorporation of freeze-dried *L. acidophilus* NCFM® and *B. lactis* HN019 did not lead to a substantial disruption of the sensory properties.

6. Concluding remarks and future perspectives

Innovations in the chocolate industry generally aim to improve the product quality and to fulfil the consumer demands for healthy food. It was shown that chocolate is an excellent carrier for oral delivery of some functional ingredients, including probiotics and prebiotics. Adding probiotics and prebiotics in a chocolate formula are the

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practical way to produce functional chocolate. Even though some people still question the claim of health benefits of chocolate, the combination of the technological application of fat reduction, sugar replacement, and functional material enrichment can be an alternative approach to produce chocolate with high potential health benefits and minimal adverse effects.

The chocolate reformulation may result in the alteration on characteristics and consumer acceptance of the chocolate. Those impacts must be taken into consideration by the food technologists when developing an innovative chocolate. Sensory properties are the primary factor that plays a key role in consumer acceptance and widely recognised as a critical success factor for the market development of food product. Nevertheless, this does not rule out the possibility to make an acceptable functional chocolate as there are already some examples of successful healthier chocolate in the market. Chocolates enriched with glucomannan, insoluble cocoa fibres, multivitamin and probiotic as well as whey chocolate sweetened with non-sucrose sweetener are the examples of former commercial functional chocolates [22, 66]. In the case of low sugar chocolate, food industry can conduct a gradual sugar reduction in the marketed low sugar product to adjust acceptable consumer sweetness levels [67]. This approach may help the food industry to maintain the consumer acceptance and to market the low sugar chocolate.

This paper demonstrates future opportunities for new research focusing on formulating a healthier chocolate product using various potential probiotic bacteria and prebiotic substances. The modification of chocolate formula has clear advantages regarding the functional properties of the product. However, the effects on the quality attributes and the sensory properties must be taken into account. Also, serious attention should be paid on the regulation issued by regulatory bodies in the development of innovative formula of functional chocolates.

Conflict of Interest

The authors declare that they have no conflict of interest.

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