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Review

Improving functionality of chocolate: A review on probiotic, prebiotic, and/or synbiotic characteristics

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ABSTRACT

Background: Chocolate is consumed by people of all ages in all segments of society throughout the world. The popularity of this food is mainly associated with its potential to arouse sensory pleasure and positive emotions. Increasing awareness of the link between healthy eating and well-being is reflected in the current views of the general consumers. Consumers perceive functional foods as a member of the specific food category to which they belong. Also, in developed economies, a key trend at the moment is confectionery products that deliver functional benefits for health and well-being, such as functional chocolate.
Scope and approach: In this review, studies related with production of prebiotic, probiotic and synbiotic chocolates as a functional food were investigated and positive and negative aspects of these functional products when compared with standard one were stated, which could shape the following related studies in food area and the production of prebiotic, probiotic and synbiotic chocolates in the food industry.
Key findings and conclusions: When the studies related with this topic were investigated it could be concluded that the studies associated with chocolate which could play a role in transportation of probiotics and prebiotics might be supported by studies in which bioavailability and bioaccessibility characteristics of them *in vivo* and *in vitro* media will be determined. Moreover, in order to improve bioavailability and bioaccessibility properties product quality optimization studies might be required in the future.

1. Introduction

Chocolate is, in essence, composed of cocoa mass and sugar suspended in a cocoa butter matrix (Andarea-Nightingale, Lee, & Engeseth, 2009). Primary chocolate categories are known as dark, milk and white that differs in content of cocoa solid, milk fat and cocoa butter in the formulation. Chocolates are semisolid suspensions of fine solid particles of sugar and cocoa (and milk, depending on type); making about 70% in total, in a continuous fat phase (Afaokwa, 2010). Chocolate is consumed all over the world, in all segments of society and by people of all ages. The popularity of this food appears to mainly associate with its potential to arouse sensory pleasure and positive emotions (El-Kalyoubi, Khallaf, Abdelrashid, & Mostafa, 2011).

Increasing awareness of the link between diet and health is reflected in the current views of the consumers all over the world (Harwood, 2013). The rise in cardiovascular disease and obesity and in other diet-related illnesses has led to consumers taking a greater interest in the ingredients of food products and valuing those with functional foods. Consumers prefer to consume functional foods as a member of the specific food category to which they belong (Ares, Besio, Gimenez, & Deliza, 2010). The preference for healthier and convenience products has led to a growing demand for functional and ready-to-eat foods that present a suitable sensory acceptance (De Morais, Lima, De Morais, & Blini, 2015).

Motivation elements of consumers in preference of foods have changed especially in the last 20 years. Healthfulness is the main driver of food purchasing behind taste and price and the presence of added beneficial components and fortification have at least

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positive impact on purchasing decisions (Harwood, 2013). Moreover, consumers prefer natural and organic foods or additives, which also be taken into consideration during production. The preference in consumer behavior and choice has directed scientific researches as well as industrial product development activities.

Food companies are constantly searching for ways to innovate and develop novel or improved products to stay competitive (De Pelsmaeker, Gellynck, Delbaere, Declerq, & Dewettinck, 2015). Functional foods have gained prominence in the market, with a large number of products being developed (Morato et al., 2015) and confectionery industry, especially cocoa and chocolate industries are undergoing dynamic changes in recent years, influenced by increased demands for healthy (Belscak-Cvitanovic et al., 2015) or functional chocolates.

Foods with wide acceptance and consumption have been enriched with ingredients that potentially improve the consumers' health. For instance, reducing the fat content in the diet decrease the energy intake and therefore, contribute to the prevention of obesity. There is an opportunity using indulgent foods, such as chocolate to achieve this aim (Rezende, Benassi, Vissotto, Augusto, & Grossman, 2015). Those expectations and inconsistencies warrant the high interest of continuing researches on this area (Fernandez-Murga, Tarin, Garcia-Perez, & Cano, 2011). In developed economies, a key trend at the moment is confectionery products that deliver functional benefits for health and well being, such as sugarless sweets and functional chocolate (Belscak-Cvitanovic et al., 2012).

Within the last several years functional chocolate has gained popularity in America. 35% of Americans have favoured the consumption of chocolate that strengthens the immune system (Saka, 2011). Also, they are longing for chocolate that helps them relax (41%) and 38% for “feel good” (38%) (Callebaut, 2008). Meeting these expectations of consumers could be provided by reducing concentration of functional properties. An increasing number of studies in recent years point towards this trend.

It is required to describe the specific functional term for chocolate and confectionery products. Functional confectionery has been defined as ‘a confectionery item that has undergone the addition, removal or replacement of standard confectionery ingredients with an ingredient that fulfills a specific physiological function or offers a potential health benefit’ by Pickford and Jardine (2000). An European Union directive simplified previous legislation opening up new possibilities for chocolate makes to try new ingredients, which can be used to create new products beneficial to consumers and industry (Bolenz, Amtsberg, & Schape, 2006). This regulation led up the production or improvement of chocolate with functional properties, which can be labeled as a standard chocolate. It also mentions that the new freedom chocolate producers have regarding the ingredients of their chocolate, which opens up interesting possibilities for reducing percentages of expensive cocoa and milk ingredients. Soluble and insoluble fibers, prebiotics, vitamins and minerals, herbal extracts and other phytochemicals are the main ingredients, which are used as substitutes or enrichment agents.

Consumers' sensory and hedonic perceptions could be greatly influenced by the messages highlighted on the front of the packaging, particularly nutrition and health claims for reduced-calorie or functional foods (Miraballes, Fiszman, Gambero, & Varela, 2014). Chocolate lovers want functional chocolate that offers clinically proven physical or emotional health benefits (Callebaut, 2008). However, consumers' attitudes towards functional foods do not depend only on their perceived healthiness, but also on the sensory quality, price and convenience, as any conventional product (Ares et al., 2010). Also, the bioactive compound added to the functional food matrix must maintain its original chemical structure and consequent functionality during the entire shelf-life period (Botelho et al., 2014).

In this review, chocolate-based products, agreed with functional food concept, development studies were investigated. For this purpose, considering the classification of general functional food, the effects of process parameters on bioactive substances and product development studies on the main quality characteristics of chocolate, which could provide important information to the industry and literature could be researched.

2. Chocolate and health

Prominent substances are known as cacao based phenolic compounds, minerals, sugar, and proteins and carbohydrates found in the powdered milk when considering beneficial effects of conventional chocolate on health. However, it is difficult to market chocolate for health benefits since many of the original healthy components inherent in cocoa are lost during processing. The flavanol contents in cocoa products and chocolate vary greatly depending on the bean variety and origin, agricultural and processing practices. In part, the variability of flavanol contents in cocoa and chocolate may be responsible for the mixed outcomes presently observed in research on the effects of cocoa flavanols on neurocognitive and affective functions, executive control, and behavior (Sokolov, Pavlova, Klosterhalfen, & Enck, 2013). Also the sugar added in large amounts to chocolate is harmful for people consuming chocolate. Moreover, consumers' anxiety and perception in calorie reduction, dental health and obesity transforms advantageous of sugar to disadvantages.

Interest on chocolate health advantages is short since its association with protective mechanisms only has initiated in the nineties. Despite so, these less than two decades have witnessed the materialization of a huge amount of literature detailing the flavanols content of chocolate as well as an array of evidences linking them with different protective pathways (Fernandez-Murga et al., 2011).

Most studies so far have been conducted on the effects of chocolate intake on the cardiovascular system (Hooper et al., 2012; Kay, Kris-Etherton, & West, 2006), reducing urinary excretion of the stress hormone cortisol and catecholamines (Martin et al., 2010), skin, cholesterol concentrations, and the release of neurotransmitters anandamide and serotonin, and on the health-related properties of high-quality dark chocolate, containing the stimulants theobromine and caffeine (Katz, Doughty, & Ali, 2011; Lamuela-Raventos, Romero-Perez, Andres-Lacueva, & Tornero, 2005; Sokolov et al., 2013).

However, the case of chocolate is particularly unsettling, given the modifications in composition imposed by processing and the proper lack of evidence that the benefits are, or are not, due to flavanols (Fernandez-Murga et al., 2011). Potential effectiveness of these ingredients depends on bioavailability characteristics, which could be especially influenced by food matrix (Smith, 2011). The final concentration of flavanols depends on the processing treatment applied to reduce their characteristic bitterness and to gain consistency.

As a conclusion, one of the most prominent compounds added to improve functional properties are cacao based phenolic substances for chocolates produced according to conventional methods. However, increasing level of these functional compounds has risk considering sensory properties of chocolate. Astringency resulted from the addition of these compounds could negatively affect the acceptability of the products based on especially age groups, therefore, masking of these negative effects is necessary in the industry. In addition, degradation of those bioactive compounds throughout cacao based raw material production could be reduced by improving novel alternative techniques.

3. Prebiotic chocolate

Recently, dietary fibers have become popular as functional food additives. These fibers are added to food formulations because they have low digestive energy content, a high capacity to absorb water and are said to support the digestive system (Bolenz et al., 2006). American legislations allow the use of functional and health claims on the labels of food products that are sources of dietary fibers. A refined definition of a prebiotic substance has been noted as 'a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits' by Roberfroid (2008a).

Prebiotics can be applied to a variety of foods (Morris & Morris, 2012; Volpini-Rapina, Sokei, & Conti-Silva, 2012). The consumption of foods such as probiotics and prebiotics that promote wellness, health, and a reduced risk of diseases have grown worldwide. During the past decade, more than 500 new products enriched with prebiotics have been introduced to the market (Da Silveria et al., 2015). Prebiotics have attracted the interest of researches and the food industry due to their nutritional and economic benefits (Scheid, Moreno, Marostica-Júnior, & Pastore, 2013). The nutritional value and the possibility of improving some sensory properties of food formulations make the use of prebiotic ingredients advantageous (De Morais et al., 2015).

Among the most widely studied and commercially used prebiotics are inulin, fructooligosaccharide (FOS), and galactooligosaccharides (GOS) (Davis, Martinez, Walter, & Hutkins, 2010) and also polydextrose (Fig. 1). Usage of these beneficial ingredients for enrichment of the corresponding material is also valid for chocolate. Inulin responds to a variety of consumer demands: it is fiber-enriched, prebiotic and low calory. As a dietary fiber, it passes through the digestive tract largely undigested (Roberfroid, Van Loo, & Gibson, 1998). Fructooligosaccharide products containing mainly short-chain molecules enhance flavor and sweetness and are used to partially substitute of sucrose (Aidoo, Afaokwa, & Dewettinck, 2015).

Major prebiotic substances used in the chocolate production can be stated as inulin and polydextrose. There are limited number of studies in the literature related with GOS and FOS. Polydextrose, a functional food ingredient, is also potentially a prebiotic substance. The rise in diet-related illness has led consumers to take a greater interest in the ingredients of food products. Polydextrose is a functional food additive due to its prebiotic properties (Srisuvor, Chinprahast, Prakitchaiwattana, & Subhimaros, 2013). Polydextrose is a non-digestible, odourless, white-to-cream amorphous powder with virtually no sweetness, low molecular weight, randomly bonded polysaccharides of glucose and a calorie content of 1 kcal/g. Polydextrose comprises mainly glucose in its highly branched polymer, with small quantities of randomly distributed sorbitol and citric acid. All possible glycosidic bonds with anomeric carbons of glucose are present, α and β 1–2, 1–3, 1–4 and 1–6, among which the 1–6 bond predominates. This compound has an average degree of polymerisation (DP) of 12 and an average molecular weight of 200 g/mol (Konar, Ozhan, Artik, & Poyrazoglu, 2014). Polydextrose is a well tolerated, and a mean laxative threshold of 90 g/day (1.3 g/kg bw) or 50 g as a single dose has been given (JFECFA., 1985). Polydextrose consumption resulted in dose-dependent decrease in *Bacteroides*, as well as an increase in lactobacilli and bifidobacteria (Slavin, 2013). Inulin is a plant-derived carbohydrate and a polydisperse β (2 $\rightarrow$ 1) fructan which is a bifidogenic, prebiotic and fermentable dietary fiber (Konar, Ozhan, Artik, Dalabasmaz, & Poyrazoglu, 2014). It presents 10% of the sweetness power of sucrose, allowing it to partially replace sucrose in certain formulations (De Castro, Cunha, Barreto, Amboni, & Prudencio, 2009; Villegas, Tarrega, Carbonell, & Costell, 2010).

Interestingly, instead of prebiotic characteristics of inulin and polydextrose as the mostly studied prebiotics their bulking and sweetener properties have been considered for the production of sugar-free chocolate and their fat substitute characteristics have been considered for the production of calorie- or fat-reduced chocolates. However this situation provides an advantage since they could play a role in reducing fat and sugar contents as well as prebiotic characteristics depending on their concentrations in the formula. These multiple functional properties of inulin and polydextrose provide advantageous in using of them in chocolate formulations.

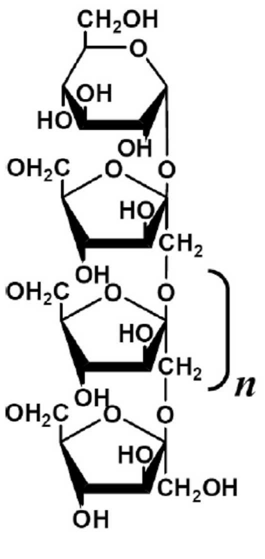
The extensive use of inulin (Aidoo, Afoakwa, & Dewettinck, 2014; Rezende et al., 2015) and polydextrose in the food industry is based on its nutritional and technological properties. Its use as a body agent in sucrose-free chocolates was studied by some researchers, which reported its effects on rheological, physical (Aidoo et al., 2014; Gomes, Vissotto, Fadini, Faria, & Luiz, 2007; Shah, Jones, & Vasiljevic, 2010), and sensory properties (Gomes et al., 2007; Shah et al., 2010) of chocolates (Rezende et al., 2015).

One of the factors taken into consideration during production of functional foods is health claims mentioned on the label of the product and used in the description of the products. In this respect, national regulations, global structure of food market, international legislative regulations and rules should be taken into consideration in order to keep standards of the corresponding material.

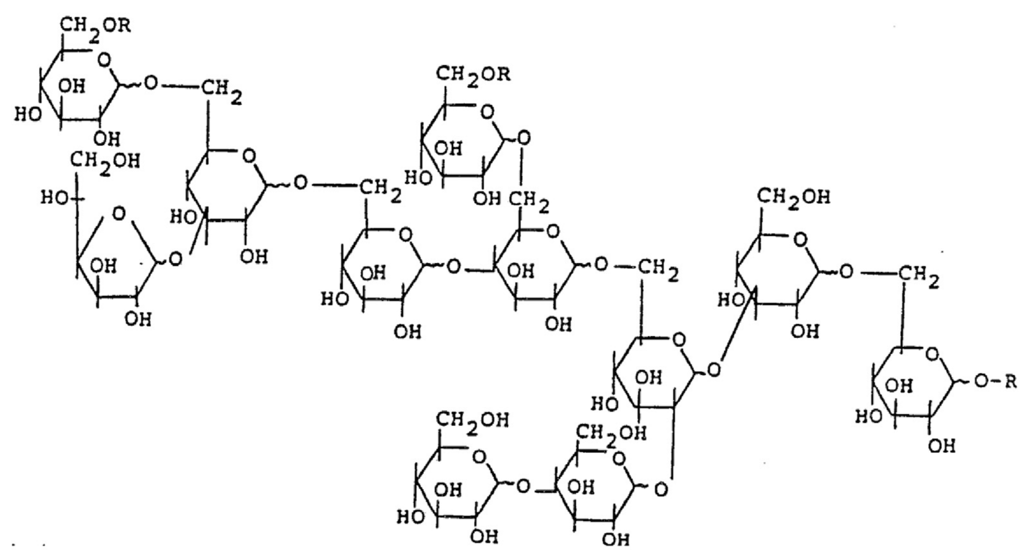
Although inulin is recognized as efficient fat replacer for use in chocolate, In Europe, the minimum reduction is 30% (EC, 2006). The American regulations do not set a minimum percent reduction for food to be considered light, as long as the nutrient percent reduction in relation to the reference food is informed (FDA, 2009) fiber sources (content $\geq$3.0 g fiber/100 g of solid food), as high fiber (content $\geq$ 6.0 g fiber/100 g solid food), (Aidoo et al., 2015; EC, 2006; FDA 2009). Therefore, threshold value for different prebiotics may be accepted as 6.0 g/100 g for chocolates as well as other food products. However, higher amount of prebiotics were added to the chocolate as fat-replacer or sugar-replacer. Shah et al. (2010) and Aidoo et al. (2015) added prebiotics up to 48%, Bolenz et al. (2006) 20%, Konar, Ozhan, Artik & Poyrazoglu (2014, Konar, Ozhan, Artik, Dalabasmaz, et al., 2014) 12% and they examined the influence of prebiotics on some quality parameters of the chocolates. However, these bioactive compounds negatively influenced some physical properties of the chocolates when compared with conventional ones.

Shourideh, Taslimi, Azizi, and Mohammadifar (2012) reported increase in moisture content with increase in inulin concentrations in their dark chocolate formulations containing different mixtures of D-tagatose and inulin. The authors attributed this to hydrophilic groups present in inulin, which causes increase and preservation of moisture in samples with high content of inulin. The low concentrations of inulin (25%) in chocolate formulations of Rezende et al. (2015) could have thus resulted in the chocolates falling within acceptable moisture limits. Therefore, prebiotic concentration added to the chocolates should be determined considering quality characteristics of the end product.

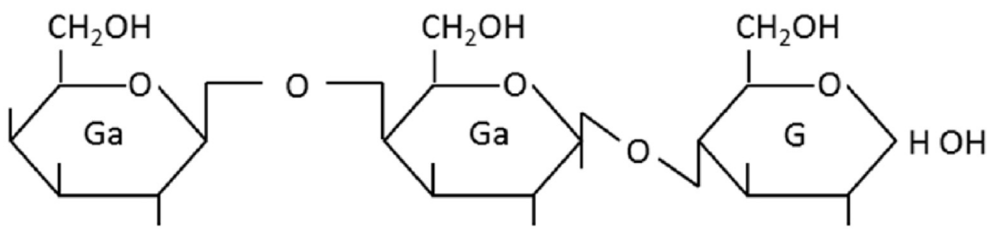
As mentioned above, inulin and polydextrose could be used as a sucrose replacer; however, it is necessary to add high-intensity sweeteners to eliminate deficiency resulted from low relative sweetness value of these ingredients. These sweeteners' amount should be adjusted regarding ADI values due to the legislative regulations. In addition dissolution heat of these compounds should also be considered. Low or high intensity sweetener taking part in formulation should have similar dissolution heat with sucrose. Otherwise, coolness sense observed during consumption of



Inulin



Polydextrose



Galactooligosaccaride

Fig. 1. Chemical structures of polydextrose, inulin and galactooligosaccharide (Duar, 2011; Putaala, 2013).

the product cannot meet a desired expectation of consumers from the chocolate. The other choice and usage criteria are perception of consumers towards food additives. The usage of sweeteners being have negative perception in the eye of consumers could damage the healthy-functional food term gained with addition of prebiotics and result in reduction of commercialization potential of the chocolates. From this reason, usage of stevia (Aidoo et al., 2015; Shah et al., 2010) and thaumatin extracts (Aidoo et al., 2015) having positive effects according to the previous studies should be suggested in terms of addition to prebiotic chocolates.

Demand for use of natural sweeteners and prebiotic compounds for manufacture of sugar-free chocolates has dramatically increased over the past decennium. However, their applicability in product formulation and how these will affect the flow (rheological) and physical quality characteristics still remains a big challenge (Aidoo et al., 2015). The composition of sucrose in chocolate is about 40–50% (depending on type) and this confers multiple functional properties on chocolate including sweetness, particle size distribution (PSD) and mouthfeel (texture). Its impact on rheological properties is also important for the end product quality (Aidoo et al., 2015). Polyols originating from traditional corn syrups are modified by reducing the reactive sites (aldehyde or ketone) through catalytic hydrogenation, enzymatic conversion or fermentation. Only the reactive groups are changed so the polyol retains much of the sugar's structure, bulk and function, making them ideal for 1:1 bulk sugar replacement (Jamieson, 2008). These compounds could be used as a bulk sweetener in prebiotic chocolates and did not result in undesired sensory and physical properties. Ingredients used in low calorie products bringing non-cariogenic properties to products in which polyols are used are known as maltitol (Beards, Tuohy, & Gibson, 2010; Belscak-Cvitanovic et al., 2015; Gomes et al., 2007; Konar, 2013; Konar, Poyrazoglu, & Artik, 2015), isomalt (Belscak-Cvitanovic et al., 2015; Gomes et al., 2007; Konar, 2013) and lactitol (Belscak-Cvitanovic et al., 2015; Gomes et al., 2007). Because of prebiotics are used as a replacer of sucrose in chocolate, quality parameters of the manufactured prebiotic chocolate influenced by sucrose should be determined and they should be compared with those of conventional ones. The researchers conducted about the production of prebiotic chocolate were in accordance with this situation and the main quality parameters taken in consideration in researches could be sorted as texture (especially hardness), rheology, melting profile, color and sensory properties.

Textural characteristics of chocolate are very important quality parameters strongly correlated with sensory properties. A chocolate with desired quality should have a smooth, soft, velvety texture while poor-quality chocolate feels hard, grainy or waxy (Konar, 2013). Farzanmehr and Abbasi (2009) studied milk chocolates containing inulin. These researchers obtained different hardness values (10.1–15.0 N), depending on the composition of the samples. Shourideh et al. (2012) stated that, inulin absorbs moisture, which lead to increase in hardness of chocolates. However in the studies carried out by Konar, Ozhan, Artik & Poyrazoglu (2014 and Konar, Ozhan, Artik, Dalabasmaz, et al., 2014), addition of inulin and polydextrose in concentrations of 6, 9 and 12% did not remarkably influence the texture of the chocolate, which was also supported by the study carried out by the same research group in which the effect of Ca (300–900 mg/100 g) and inulin (9%) on the textural properties of chocolate was also investigated (Konar et al., 2015). Also, there were no significant differences between melting behaviour and texture of the inulin and polydextrose containing chocolates and the reference one (Aidoo et al., 2015). Moreover, addition of GOS as an other prebiotic did not also significantly affect textural properties of the chocolate (Winardi-Liem, 2011). However Belscak-Cvitanovic et al. (2015) reported that addition of inulin and polydextrose increased the hardness value of the chocolates. The findings related with textural properties of prebiotic chocolate highlighted that the addition of inulin, polydextrose and GOS did not have any risk in terms of textural properties; however, it is required to determine usage amount considering their absorption characteristics.

The determination of the rheological properties of chocolate is also important in the manufacturing process for obtaining high-quality products with well-defined texture (Goncalves & Lannes, 2010). Molten chocolate is known as a non-Newtonian fluid with a yield stress, and the flow behaviour can be described using different mathematical models, such as the Bingham, Herschel–Bulkley (Servais, Ranc, & Roberts, 2004) and Casson models (Konar, 2013).

The study of Aidoo et al. (2015) investigated the rheological properties, melting behaviours and other physical quality characteristics of chocolates including inulin (12.0%, w/w) and polydextrose (36%, w/w) mixtures as bulking agents sweetened with stevia and thaumatin extracts (Aidoo et al., 2015). A low level of inulin improved the flow properties but reduced the apparent viscosity. A decrease in the yield stress value of an inulin-containing chocolate was observed by Bolenz et al. (2006). Shourideh et al. (2012) indicated that when a high level of inulin was added, it induced an incremental effect on the plastic and apparent viscosity. In the study conducted by Konar, Ozhan, Artik & Poyrazoglu (2014, Konar, Ozhan, Artik, Dalabasmaz, et al., 2014), addition of inulin and polydextrose in concentrations of 6, 9 and 12% resulted in change of Casson model parameters, namely, plastic viscosity and yield stress. Sucrose replacement with the inulin/polydextrose and stevia/thaumatin extracts resulted in significantly higher plastic viscosity. There were however no significant differences in the melting behaviour and texture of the chocolates and the reference sample (Aidoo et al., 2015). In a study related with comparison of usage of inulin, polydextrose, and maltodextrin as sucrose replacement was found as statistically larger differences for all but one tested formulation in regards to apparent viscosity (Abbasi & Farzanmehr, 2009). A study by Winardi-Liem (2011) performed another study to investigate rheology of prebiotic chocolate bar prepared by using 3.75, 15.4 and 30.4% (m/m) galactooligosaccaharide (GOS). Overall, the result of this study has shown a positive result regarding incorporation of GOS in the chocolate system. Although increases in the yield stress and apparent viscosity were observed upon GOS addition, it was noted that these increases did not contribute to a noticeable quality/textural change.

The increase in the inulin content led to a significant increase in the plastic viscosity (Rezende et al., 2015). However, in the study related with the effect of maltitol and isomalt as a bulk sweetener on the rheological properties of chocolate including 9% inulin, Herschel-Bulkley model was found as the best model and their parameters (yield stress and plastic viscosity) significantly changed based of substitution level of sucrose with maltitol and isomalt and conching temperature (Konar, 2013). In previous studies, researchers reported that chocolate formulations which contain 100% polydextrose show large crystals with dense smaller particles in between the larger crystals and minimal inter-particle spaces in comparison to formulations containing 100% inulin which revealed large crystals with more void spaces between the crystals indicating limited particle–particle interaction strength (Aidoo et al., 2014). A combination of these ingredients will result in chocolates having large crystals with the dense smaller particles of polydextrose filling the void spaces in the crystal network structure of chocolate formulations with inulin (Aidoo et al., 2015).

One need to take into account that the different volume fraction dependence of the side stream materials compared to sucrose leads to higher viscosities at elevated volume fractions and high shear rates. These effects need to be considered when sucrose is replaced with fiber-rich side stream materials in chocolate. However, complete replacement of sugar seems difficult considering quality of the end product. The quantity of sucrose in chocolate that can be replaced with side stream materials without obtaining unfavourable high viscosities and resulting change of sensory perception might be limited as a result of the differences in behaviour of the sugar and fibers in a hydrophobic matrix, like chocolate (Bonarius, Vieria, van der Goot, & Bodnar, 2014). It is possible to attain desired quality of chocolates including prebiotics and bulk or high-intensity sweetener as a substitution of sucrose by optimization of prebiotic concentration and modification of refining and conching process conditions. Moreover, surface-active agent type and concentration might also be another factor for improving the quality of such functional chocolate products.

One of the most important parameters influencing sensory perception of chocolate is colour; therefore, it should be considered during production of novel chocolate products. Konar et al. (Konar, Ozhan, Artik & Poyrazoglu, 2014, Konar, Ozhan, Artik, Dalabasmaz, et al., 2014 and 2015) studied the influence of prebiotic type and concentration on colour parameters (L^*, chroma and hue angle) and they reported that addition of prebiotics in different concentrations did not significantly affect the colour properties. However in a previous study, replacing sucrose with inulin and polydextrose as bulking agents and stevia or thaumatin as sweeteners resulted in darker chocolates (Aidoo et al., 2015). Also in some other studies it was reported that, regardless of the levels of the sugar substitutes used, replacing sucrose with inulin and polydextrose results in darker chocolates (Aidoo et al., 2014). Shourideh et al. (2012) reported darker chocolates for dark chocolate formulations containing 100% inulin. Inulin absorbs moisture, light scattering and lightness decreases, making the chocolate looking darker (Shourideh et al., 2012). Bolenz et al. (2006) also reported chocolate samples with 20% inulin has been the most brownish, and had the lowest L^* (lightness or brightness) value among other texturizing agents when used in milk chocolate. It could be concluded from the findings that the effects of using of prebiotics in chocolate formulation on color properties might be associated with the concentration of prebiotics and the other ingredients used in the formulation.

Another advantage of prebiotic substance is related with their effects on the sensory properties. Gomes et al. (2007) and Shah et al. (2010) observed that the addition of inulin results in good acceptance of sucrose-free chocolates. Shah et al. (2010) studied for the development of a chocolate formulation sweetened with *Stevia rebaudiana* extract and containing polydextrose as a bulking agent. Gomes et al. (2007) produced a diet chocolate using various bulking agents as sucrose substitutes. The bulking agents used in that study were polydextrose (24.14–48.27%), inulin, fructooligosaccharides, lactitol and maltitol. The formulations containing polydextrose, polydextrose/lactitol and polydextrose/maltitol were evaluated for a sensory analysis due to their good technological performance.

In a study performed by Belscak-Cvitanovic et al. (2015), sugar alcohols, dietary fibers, syrups and natural sweeteners were used as sucrose alternatives in the production of sugar-reduced chocolates (50% of cocoa parts) with enhanced bioactive profile. Formulated chocolates were evaluated with respect to sensory properties and also other parameters. Combinations of sugar alcohols (xylitol, isomalt, lactitol, maltitol), dietary fibers (inulin, oligofructose), syrups (rice syrup, agave syrup), and natural sweeteners (lucuma, yacon, dried carrot, acacia flowers, liquorice powder, and stevia leaves and stevioside) were used as ingredients for the production of functional chocolates. Formulated chocolates containing stevia leaves and peppermint exhibited the best sensory characteristics (especially with regard to mouthfeel, sweetness and herbal aroma).

The production process greatly influences the quality of chocolate as the final product (Cidell & Alberts, 2006). The refining process must result in the required particle size (Bolenz, Thiessenhusen, & Schape, 2003). Processing technology is just as important as using the right ingredients for achieving this desired quality. Refining chocolate using a three- or five-roll refiner leads to a reduction of particle size, which is an important step toward obtaining a smooth texture (Torres-Moreno, Tarrega, Costell, & Blanch, 2012). Final particle size critically influences the rheological and sensory properties (Torres-Moreno et al., 2012). In the studies about production of functional foods, another critical point taken into consideration is changing minimally the production technique used in conventional production and minimizing new equipment requirement whenever possible. In this respect, prebiotic substances involving inulin, polydextrose and GOS usage could provide important advantages, because these substances could be taken part in the formulation without any alteration. Prebiotic substances are added to formulation in the first stage of the production and the production occurs in the same way.

However, we have not encountered any study about stability of inulin and polydextrose in the chocolate production. This case could be resulted from physical and chemical properties of these prebiotic substances and production processes performed during chocolate production. However, process stability of GOS was investigated although it has been used in the studies less than the other prebiotics. The results of the study performed by Suter (2010) demonstrated that a chocolate system can serve as an excellent delivery system for GOS and also the GOS maintained concentration and initial profile throughout processing demonstrating stability of these compounds during chocolate manufacturing.

Also, even though inulin, GOS and polydextrose are stated as model prebiotic substances, there is deficiency about investigation of their bio-accessibility and bio-availability potentials as they are found in the chocolate matrix. Because the matrix influence on these parameters is one of the factor should be considered. The only study to which we have encountered related this topic was carried out by Beards et al. (2010). They tested the effects of maltitol, polydextrose and resistant starch addition to chocolate. Forty volunteers consumed reformulated chocolate samples for over a six week period. Their results showed that, consumption of samples containing polydextrose/maltitol blend increased the level of lactobacilli and bifidobacteria levels in faeces after 6 weeks. Increase in the levels of short chain fatty acids i.e. propionate and butyrate was also observed. Formula development of chocolate provided prebiotic effects to consumers in addition to the decrease in energy values. However, different studies are required to support this study.

Moreover, it is necessary to investigate usage possibilities of different forms of prebiotics especially inulin in the chocolate formulation. For instance milk chocolate containing inulin with a higher degree of polymerisation (DP) had higher melting points, greater plastic viscosity and an increased flow behaviour index in the study of Shah et al. (2010). Investigation of degree of polymerisation effect on different chocolates and formulations could be beneficial to produce chocolate product with higher quality.

4. Probiotic chocolate

The concept of probiotics was introduced long before that of prebiotics (Roberfroid, 2008b). A probiotic has been recently defined as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host”. The microbiota of the human gastrointestinal tract plays a key role in nutrition and health (Rastall, Fuller, Gaskins, & Gibson, 2000). Like prebiotics, probiotics do modify the composition of the gut microflora and, as a consequence, they have been shown to influence both intestinal and body functions. However, it is because it is introduced into host intestinal microflora that it causes a selective modification of its composition. Thus the effect of a probiotic is, essentially, direct (Roberfroid, 2008b). It can be stated that probiotic foods are more functional than prebiotic ones. When considering prevalence of chocolate consumption, its importance has become known in terms of carrying of probiotics. Therefore, improvement of chocolate formulations suitable for probiotic definition is of capital importance. The popularity of functional foods continues to increase as consumers desire flavorful foods that will fulfill their health needs. Among these foods, probiotics may exert positive effects on the composition of gut microbiota and overall health. However, in order to be beneficial, the bacterial cultures have to remain live and active at the time of consumption (Coman et al., 2012).

An international expert group of the International Life Sciences Institute (ILSI) has evaluated the categorized and published evidence of functionality of different probiotics in four areas of human application, namely, (i) metabolism, (ii) chronic intestinal inflammatory and functional disorders, (iii) infections, and (iv) allergy. The ILSI report gives concrete examples demonstrating benefits and gaps, and guidelines and recommendations on the design of next generation of probiotic studies, with the aim to substantiate the current body of information on probiotic benefits (Rijkers et al., 2010).

Probiotic bacteria have been incorporated into a wide range of foods, including dairy products (such as yogurt, cheese, ice cream, dairy desserts) but also in non-dairy dairy products (such as cereals, juices) (Anal & Singh, 2007). However, number of researches related with the production of probiotic chocolate and fabricated commercial chocolate is quite a little, which could not be explained by inappropriateness of chocolate matrix for probiotic microorganisms. Because, the incorporation of probiotic cells encapsulated by spray-coating technology has been carried out in chocolate and according to this research, probiotic viability in the small intestine was three times higher when incorporated in chocolate than in dairy product (Maillard & Landuyt, 2008). Also, Possemiers, Marzorati, Verstraete, and Van de Wiele (2010) incorporated encapsulated probiotic cells in chocolate. Results have shown that the introduction of encapsulated probiotic strains into chocolate can be an excellent media to protect them from environmental stress conditions. In chocolate, the lipid fraction of cocoa butter was shown to be protective for bifidobacteria (Burgain, Gaiani, Linder, & Scher, 2011; Lahtinen, Ouwehand, Salminen, Forssell, & Myllärinen, 2007). Yonejima et al. (2015) evaluated *in vivo* method to improve acid tolerance of *Lactobacillus brevis* subsp. *couagulans* by coating with milk chocolate. They noted that probiotics in chocolate was found to be more stable against gastric acid treatment than probiotics powder and those in beverages or in yoghurt.

The term “probiotic” includes a large range of microorganisms, mainly bacteria but also yeasts. Because they can stay alive until the intestine and provide beneficial effects on the host health, lactic acid bacteria (LAB), non-lactic acid bacteria and yeasts can be considered as probiotics (Anal & Singh, 2007; Burgain et al., 2011; Holzapfel, Haberer, Geisen, Björkroth, & Schillinger, 2001). In the previous studies, chocolate was used as a carrier for *Lactobacillus helveticus* (Maillard & Landuyt, 2008), *Lactobacillus rhamnosus* (Coman et al., 2012; Raymond & Champagne, 2015; Saarela, Virkajarvi, Nohynek, Vaari, & Matto, 2006), *Lactobacillus paracasei* (Aragon-Alegro, Alarcon Alegro, Cardarelli, Chiu, & Saad, 2007; Coman et al., 2012), *Lactobacillus acidophilus* (Lalicic-Petronijevic et al., 2015), *L. brevis* (Yonejima et al., 2015), *Bifidobacterium longum* (Champagne, Raymond, Guertin, & Belanger, 2015; Maillard & Landuyt, 2008), *Bifidobacterium lactis* (Lalici-Petronijevic et al., 2015) and *Bacillus indicus* (Erdem et al., 2014) (Table 1). However, possible probiotics used in the chocolate could be specified as *Lactobacillus* and *Bifidobacterium*. Canada and Italy authorities made a requirement thet the use of the term probiotic on food labels require a minimum 9 log cfu/serving or day (Hill et al., 2014). Also, serving size recommendation could be defined as 25 g for chocolate (Versluis, Papies, & Marchiori, 2015; Watson et al., 2016). So that, in studies related with improvement of probiotic chocolate formulations, target probiotic load may be minimum 9 log cfu/25 g in the end product.

In the probiotic product development studies, the activity of the initial inoculum, the storage time and the interaction of other ingredients present in the formulations should be taken into consideration. These important factors markedly affect the survival rate of the probiotics. Advantages of probiotics for health can only be realized if proper probiotic strain or product selections, and dose guidelines of commercial production, are applied in human food or dietary supplement (Coman et al., 2012; Douglas & Sanders, 2008; Kalliomaki et al., 2010).

Probiotic viability in the food matrix depends on factors, such as pH, storage temperature, oxygen levels, and the presence of competing microorganisms and inhibitors. It is important that the formulation maintains the activity and viability of the probiotic for extended periods of time (Shah, 2007). During product development, shelf life stability under various storage conditions and durations should be considered. Lalici-Petronijevic et al. (2015) studied viability of *L. acidophilus* and *B. lactis*, which were inoculated to both milk and dark chocolates, during 180 days at two different storage temperatures as 4 °C and 20 °C. According to the results, they determined that, *L. acidophilus* exerted higher viability compared to *B. lactis* strain in both food matrices while a greater number of cells of both strains were determined at 4 °C. In addition, process step at which probiotics are incorporated and processes and their intensity used in the production of the product up to end product are crucial for their bioavailability and bioaccessibility. In addition to survival of probiotics at the exact dose, it needs to be established that a different food matrix does not influence the functionality of the probiotic bacteria (Coman et al., 2012). It was stated that cacao butter found in the chocolate matrix has the advantage for probiotics (Burgain et al., 2011; Lahtinen et al., 2007). Besides, formulations including milk powder have also advantage for probiotic microorganisms. Possemiers et al. (2010) claimed that chocolate ensured probiotic survival up to 4 times higher than milk-containing products. The other advantage of chocolate matrix for carrying probiotics could be associated with the high phenolic content of cacao. In addition to exhibiting antioxidant activity, chocolate might serve as a better probiotic carrier than dairy products for intestinal delivery (Erdem et al., 2014).

Probiotics could be incorporated to chocolates as lyophlised powdered form (Coman et al., 2012; Saarela et al., 2006) or encapsulated form (Champagne et al., 2015; Possemiers et al., 2010). However, the processing step of the incorporation is also very important for increasing efficiency. We have not encountered any study about this scope. When considering chocolate production process, it might be a good choice for the incorporation of probiotics after conching or prior to pre-crystallization. Thereby, probiotics could be suffered from mechanical forces (refining, shearing) and heat applied in the conching process. However, chocolate type and form of probiotics are among the factors should also be taken into consideration in order to increase efficiency. As known, conching is applied at high temperature levels during dark chocolate production, in milk chocolate conching temperature is lower than that of dark ones. In a recent study, different chocolate types, processed cocoa-based ingredients, manufacturing environment, and workers' hand surface were analyzed to investigate Enterobacteriaceae, coliforms and *Salmonella* detection (Nascimento, Reolon, Santos, Moreira, & Silva, 2015). According to the findings of that study, pre-tempering processes achieved during chocolate production may have negative effects on viability of inoculated probiotics. Nevertheless, particle size of the encapsulated probiotics be incorporated also plays an important role in the determination of primary step of process. In this case, probiotic chocolate process was transposed to a larger scale but the challenge here was to obtain a process, which is compatible with probiotic survival because high temperatures are required in the usual process (Burgain et al., 2011). Large capsules could be mechanically damaged during refining process. Moreoever, independently from process steps, encapsulated probiotics larger than 100 μm could affect the flow properties, textural, sensory and melting profiles.

Maillard and Landuyt (2008) produced probiotic chocolate by incorporating *L. helveticus* and *B. longum* encapsulated with fatty acids using spray drying technique. Possemiers et al. (2010) also carried on a product development study by using same

Table 1
Using probiotics in chocolate matrix.

Probiotic strain(s)	Probiotic form	Inoculation dose	Inoculation conditions	Sample	References
Lactobacillus rhamnosus	Freeze dried and micro-encapsulated	7 log cfu/g	40 °C	Dark chocolate	Raymond and Champagne (2015)
L. acidophilus *B. lactis*	Freeze-dried	8 log cfu/g*	30–32 °C	Milk and dark chocolate	Lalicic-Petronijevic et al., (2015)
L. brevis subsp. *coagulans*	Freeze-dried	6 log cfu/g	32 °C	Milk chocolate	Yonejima et al., (2015)
L. rhamnosus	Freeze-dried	8 log cfu/g	nd	Chocolate coated breakfast cereals	Saarela et al., (2006)
L. paracasei subsp. *paracasei*	nd	7 log cfu/g	40 °C	Chocolate mousse	Aragon-Allegro et al., (2007)
L. rhamnosus *L. paracasei*	Freeze-dried	9 log cfu/g	37–40 °C	Milk and dark chocolate	Coman et al., (2012)
L. helveticus *B. longum*	Microencapsulated	nd	nd	Dark, milk and white chocolate	Maillard and Landuyt (2008)
L. rhamnosus *B. longum*	Freeze dried and micro-encapsulated	8–10 log cfu/g	40 °C	Dark chocolate	Champagne et al., (2015)
Bacillus indicus	Freeze-dried	6.08 log cfu/g	45 °C	Dark chocolate	Erdem et al. (2014)

nd: not defined, *: approximately.

microorganisms and technique. Probiocap™ manufactured with encapsulated forms produced by Institut Rossell & Lal'food and Attune produced by DMS Food Specialties could be given as industrial examples for these types of products. Bifidobacterium and lactobacillus strains are generally used in these products.

Studies related with probiotic chocolates bring to a successful conclusion. In 2007, Barry Callebaut developed a process to produce chocolate containing encapsulated probiotic cells manufactured with the Probiocap technology in partnership with Lal'food. According to Barry Callebaut, the addition of encapsulated probiotic cells has no influence on chocolate taste, texture and mouth feel (Callebaut, 2008).

Also, in the study conducted by Coman et al. (2012) SYNBIO trademarked probiotic bacteria combination composed of *L. rhamnosus* and *L. paracasei* (1:1) was used to produce chocolate as well as ice cream, salami and cheese. Probiotic combination number was adjusted as 10^9 cfu to satisfy the need of daily dose. The products included in the research scope contained 10^7–10^9 CFU/g probiotic living at the end of the shelf life and it was reported that bitter and milk chocolates were good tool in terms of carrying of probiotics. The form of the probiotics used in this study was lyophilized and the incorporation step for chocolate was not mentioned; however, for the other products, probiotics were incorporated during cooling step at 37–40 °C. In addition to survival level of the probiotics, sensory properties of the products were also investigated. Besides, probiotic chocolates were commercialized by some organizations. Institut Rosell has incorporated encapsulated probiotic cells into yogurt-covered raisins, nutrient bars, chocolate bars and tablets (Burgain et al., 2011).

Another important issue is enumerating probiotics in a chocolate matrix. Traditional methods are direct microscopic count (DCM) and colony forming unit counts (cfu). Also, flow cytometry (FC) method was studied by Raymond and Champagne (2015) to evaluate precision and accuracy by using dark chocolate samples containing *L. rhamnosus* as probiotic. They advised using FC and CFU for total and viable counts, respectively.

5. Synbiotic chocolate

Synbiotics are combinations of an exogenous probiotic and a prebiotic, the idea that the probiotic would reach the target site and proliferate in situ using the prebiotic (Bullock, Booth, & Gibson, 2004). The prebiotic should be a specific substrate for the probiotic, being able to stimulate its growth and/or activity while at the same time enhancing indigenous beneficial bacteria. The term synbiotic refers indirectly to a synergy and that is why some authors have suggested that this term should refer exclusively to products in which the prebiotic compounds selectively favor the growth of probiotics (Roberfroid, 2008b).

Roberfroid (2000) suggested that synbiotic products can improve the survival of bacteria when they pass into the upper part of the gastrointestinal tract, and produce greater effects in the large bowel. The results of the previous studies showed that chocolate matrix and production process is suitable for the probiotic and prebiotic substances, indicating that synbiotic functional products could be produced. Results of the limited number of available studies also confirmed this situation (Erdem et al., 2014). In addition to water soluble prebiotic fibers such as lactose derivatives, GOS, FOS, inulin and polydextrose, largely water insoluble fibers could have the potential as probiotic protectants (Charalampopoulos, Pandiella, & Webb, 2002). Therefore, these substances could be used as a carrier agent in chocolate to incorporate probiotics to the corresponding product.

Erdem et al. (2014) performed a study for developing synbiotic dark chocolate formulation. They investigated the effects of probiotic *Bacillus indicus* HU36 and dietary fibers (maltodextrin and lemon fiber) addition on color and organoleptic quality properties of dark chocolate. The viability of *B. indicus* HU36 in dark chocolate was examined as well in the study. After couvertures of chocolate were melted at 45 °C in water bath, lyophilized *B. indicus* HU36 spores were added to have 6.08 log cfu/g of chocolate. And then samples were mixed and tempered manually. According to their results, *B. indicus* HU36 showed survival rate between 88 and 91% in the samples. While bacteria and dietary fiber addition did not show any negative effects on sensory and color properties of the products; dietary fiber addition improved some sensorial features significantly i.e. sweetness, firmness and adherence. However, in this study, conventional production technique was not used and quality parameters of the chocolates such as flow behavior and melting properties were not investigated.

Except for chocolate, during investigation of synbiotic characteristics of some food products chocolate usage was also be discussed. For instance, in the study of Saarela et al. (2006), the capability of different fiber preparations to protect the viability and stability of *L. rhamnosus* during freeze-drying, storage in freeze-dried form and after formulation into apple juice and chocolate-coated breakfast cereals was studied. Moreover, in order to improve probiotic ice cream and to determine storage condition, chocolate used as one of the material which was added to the ice cream as carrier of probiotics. For this aim, *B. longum* R0175 encapsulated with spray dryer was used. The viability of probiotics was further improved when the microencapsulated cells were incorporated into chocolate particles (Champagne et al., 2015). Aragon-Alegro et al. (2007) designed a study to develop a chocolate mousse to which probiotic and prebiotic ingredients were added, and verify the perspectives of the product with regard to potential for consumer health benefits and sensorial acceptance. *L. paracasei* subsp. *paracasei* was added in trials, in order to obtain concentrations of approximately 7 log cfu/g in the final product, and inulin (5.00 g/100 g) was also added in the trial. Chocolate mousse was shown to be an excellent vehicle for the incorporation of *L. paracasei* subsp. *paracasei* LBC 82 and the prebiotic ingredient did not interfere in this viability. Borges, Ferreira, and Costa (2004) evaluated the survival of *L. acidophilus*, microencapsulated in a calcium alginate matrix, in chocolate mousse. Differently to what was observed in the study of Aragon-Alegro et al. (2007), the authors verified that there was a 3 log decrease in counts of the probiotic microorganism, after 20 days of storage of the product when free cells of the microorganism were employed, and a 2 log decrease when microencapsulated cells were employed. In a recent study, Oliveria et al. (2015) studied the influence of sugar reduction on the dynamic sensory profile and consumers' liking probiotic chocolate-flavored milks. A probiotic strain, freeze-dried *L. acidophilus* was used in this study.

Generally, although chocolate is a suitable product for usage of both probiotic and prebiotic at the same time, there are very limited study about this subject. More research is needed in this area. Particularly, researches about the influence of process on these bioactive substances and studies providing data in this scope are required for both researchers and food industry in order to produce the end product with desired functional characteristics. Moreover, the studies about bioavailability and accessibility should be conducted to observe effectiveness and to design production processes and to determine product formulations.

6. Conclusion

In recent years, awareness of people between health and diet has increased, forcing people to consume foods with functional properties. Therefore, increasing functionality of the product without damaging quality characteristics is very crucial to meet people requirement. When considering the fact that chocolate is widely consumed by people of all ages throughout the world, it could be concluded that it is promising bioactive compound carrier. In this study, the researches conducted about production of prebiotic, probiotic and synbiotic chocolate products were reviewed. According to the results it could be concluded that chocolate is suitable for carrying of both probiotic strains and prebiotics. However, deeply researches are necessary to observe the effect of processing conditions on the functionality of these bioactive ingredients and their bioavailability and bioaccessibility.

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