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Current Trend and Future Prospective of Functional Probiotic Milk Chocolates and Related Products – a Review

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Abstract

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The world market of functional dairy products including ice-cream, cheese, sour cream, yoghurt, dahi, butter milk, powdered milk, and frozen desserts has been rapidly growing. The incorporation of probiotics into chocolate could offer a good alternative to common dairy products as it is liked by all age groups people. Chocolate is rich in natural antioxidants and the nutritional quality of it can be further enhanced by the incorporation of probiotics and/or prebiotics or dietary fibers. Current article reviews recent advances in the technologies aimed at incorporating probiotics into chocolate and related products, the ways to enhance or sustain their viability in the presence of stressed surroundings throughout the manufacturing process, and their market potential with future prospects.

Keywords: antioxidants; dietary fibers; functional foods; *L. helveticus* MTCC 5463; microencapsulation

The demand by consumers for value added products with health benefits has been increasing. The beneficial role of food grade organisms such as lactic acid bacteria (LAB) and their association with dairy products is well known. Probiotics are live microorganisms that contribute health beneficial effect to humans and are generally regarded as safe (GRAS). More and more research is undertaken to validate their efficacy; so far, their effectiveness has been demonstrated in gastric disturbances, antibiotic associated diarrhea, allergy, urogenital infections, irritable bowel syndrome, lactose intolerance, oral health, etc. (Reid *et al.* 2003; Patel *et al.* 2013). Probiotic dairy and food products have gained prime position in the market. Most of the foods containing probiotic bacteria are dairy products, although there is a rapidly growing demand for incorporating probiotics in other segments of the food industry because of the above mentioned health benefits without any side effects. Probiotic dairy products such as dahi, yoghurt, ice cream, cheese, and kefir are appropriate vehicles to deliver beneficial bacteria to human host in addition to the available medical health supplements either in the form of pills or capsules (Prajapati & Nair 2003; Shah & Prajapati 2013). The probiotics market has been one of the prime beneficiaries in the recent fad for functional foods. The global probiotic products market was estimated at $26 125.9 million in 2012. According to one survey probiotic market have risen up to worth $1732.8 million by 2019, incorporating probiotics in different kinds of food products (functional foods, dietary supplements, specialty nutrients, animal feed); in medicinal relevance (regular, therapeutic, preventive health care); or by any other convenient mode of application (Anonymous 2014).

The incorporation of probiotics into chocolate could offer a good alternative to common dairy products and allow to broaden the health claims of chocolate based food products. Indeed, recent market research into functional food has shown that, in relation to chocolate, digestive health was one of the most important

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drivers of consumer acceptance (Callebaut 2009). In spite of the high fat and sugar contents, chocolate consumption provides an encouraging role to human nutrition through the provision of antioxidants, predominantly polyphenols including flavonoids such as epicatechin, catechin, and procyanidins (Hii *et al.* 2009). Chocolates also contain minerals, especially potassium, magnesium, copper, and iron. The presence of biologically active phenolic compounds in cocoa has stimulated researches into its effects in ageing, oxidative stress, blood pressure regulation, atherosclerosis, and reduction of the heart disease risk and stroke (Buitrago-Lopez *et al.* 2011). According to Maillard and Landuyt (2008), chocolate has been identified as an ideal carrier for probiotics and has also been found to absorb more probiotics than yoghurt (Deanna 2009).

One of the drawbacks of the probiotics incorporation into food products is connected with the very definition of the probiotics: they are live micro-organisms which have to reach the intestine, their site of action, alive, and in sufficient numbers. Their survival is linked to several factors: first the endogenous properties of the chosen bacteria strain, their environment (other ingredients properties, humidity, temperature, pH, oxygen etc.), the digestive process (gastric acidity, bile salts), as well as various mechanical stresses linked to food processing (Callebaut 2009). Chocolate lipids help to protect probiotics and enhance their survival within the food matrix in various stressed conditions in addition to their fundamental role associated with mouth feel and melting properties of the product. To overcome these limitations, novel technologies have been developed, such as specific microencapsulation methods (Durand *et al.* 2003; Mandal *et al.* 2009), in order to increase the bacterial resistance and broaden their applications from fresh dairy products to long shelf life processed food products. The development of probiotics containing chocolate involves a good understanding of the selected probiotic strains, the chocolate manufacturing process, and various critical points of the process for probiotics survival, as well as the application of specific protective technology. Current article reviews recent advances in the technologies of the probiotics incorporation into chocolate and related products (freeze dried or microencapsulated cell preparations), influence of various processing parameters on the viability of the probiotics, and ways to increase their survivability in probiotic milk chocolate along with their market potential and future prospects.

Probiotic chocolate

Incorporation of probiotics into chocolate. One of the major challenges to incorporating probiotics into liquid chocolate and chocolate related products on an industrial scale is that these products need to be maintained under specific conditions (like temperature, pH, etc.) that do not kill the probiotics. This requires a temperature range narrower than that normally used in chocolate manufacturing, making it difficult to achieve effective mixing and proper probiotic dispersion (Gadhiya 2011). To overcome of these difficulties, a patented process has been reported where two selected probiotic strains were protected with a specific microencapsulation technology (Durand *et al.* 2003).

Mandal *et al.* (2009) reported incorporation of microencapsulated *Lactobacillus casei* NCDC 298 and inulin into milk chocolate and the efficacy of the milk chocolate in delivering the live lactobacilli to modulate the intestinal microenvironment of mice. It is observed that, after 30 days of storage at room temperature, the lactobacilli counts decreased by approximately 3 and 2 log cycles from the initial level of ~8 log CFU/g in milk chocolate with free and encapsulated lactobacilli, respectively; however, at the refrigeration temperature, the viability of the free as well as encapsulated lactobacilli was unchanged in chocolate up to 60 days. Total bacterial counts were decreased by 2 log cycles, while yeast, moulds, and coliforms were found to be absent in chocolate during storage. Sensory panelists liked the chocolate with the encapsulated lactobacilli. The supplementation of milk chocolate with inulin (5%) and free or encapsulated lactobacilli (~8 log CFU/g) increased the faecal lactobacilli, decreased coliforms and β-glucuronidase activity. Khanafari *et al.* (2012) investigated the antimicrobial activity of *L. rhamnosus*, *L. acidophilus*, and *L. plantarum* against *Streptococcus mutans*, an organism responsible for dental plaques and carries, on incorporating these lactic acid bacteria into probiotic chocolate by disc diffusion method.

All these processes make the product more convoluted and more expensive. Hence, a study has been contemplated and undertaken in our laboratory on the incorporation of freeze dried probiotic *L. helveticus* MTCC 5463 (3% w/w) in concentrated form into chocolate, and its survival and product acceptability were studied. A formulation containing lecithin, cocoa butter, sugar, milk powder, cocoa powder, and butter in proper proportions was chosen for making probiotic chocolate, based on sensory and acceptability

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attributes. All these ingredients were mixed together, warmed at 65°C for 60 min, cooled to 40°C, inoculated with probiotics and finally wrapped in aluminium foil. The chocolate had acceptable organoleptic quality up to day 30 of storage but the viability of the bacteria (2.42 × 10^8) remained good only up to day 15 of storage at 10 ± 2°C (Gadhiya 2011).

Microbiology of chocolate based products with probiotics. Recently, Ramakrishna *et al.* (2013) prepared milk chocolates by replacing skim milk powder in the formulation with yoghurt powder at 50% and 100% levels. Microbiological analysis of chocolates showed the presence of *Lactobacillus* species to the extent of 3.37 log CFU/g. Possemiers *et al.* (2010) confirmed chocolate as a potential protective carrier for oral delivery of a microencapsulated mixture of *Lactobacillus helveticus* CNCM I-1722 and *Bifidobacterium longum* CNCM I-3470. A sequential *in vitro* setup was used to evaluate the protection of the probiotics during the passage through the stomach and small intestine, when embedded in dark and milk chocolate or liquid milk. The authors reported that both the chocolates offered superior protection with about 91 and 80% survival for *L. helveticus* and *B. longum* in milk chocolate compared to 20 and 31% found in milk. To simulate the long-term administration, the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) was used. Plate counts, denaturing gradient gel electrophoresis (DGGE) and quantitative PCR showed that the two probiotics successfully reached the simulated colon compartments. This led to an increase in lactobacilli and bifidobacteria counts and the appearance of additional species in the fingerprints. The result indicates that the coating of probiotics in chocolate is an excellent solution to protect them from environmental stresses, and for optimal delivery.

Aragon-Alegro *et al.* (2007) prepared chocolate mousse with *Lactobacillus paracasei* subsp. *paracasei* LBC 82 (probiotic mousse, P) or with *L. paracasei* subsp. *paracasei* plus added inulin as a prebiotic (synbiotic mousse, S). The count of *L. paracasei* remained almost constant (always above 7 log CFU/g) during the whole refrigerated storage of the chocolate mousses P and S, but a significantly ($P < 0.05$) higher population was observed in the probiotic product only on day 21 of storage as compared to the symbiotic one (S). These results show fine maintenance of viability in both products (P and S) and also good conditions for the growth of the probiotic microorganism during the storage of mousse P. The counts in this product increased from 7.36 up to 7.88 log CFU/g, although not significantly, from day 1 up to day 28 of storage.

Nebesny and Zyzelewicz (2006) studied dark chocolate masses and chocolates supplemented with viable cells of 2 bacterial strains, *Lactobacillus casei* and *L. paracasei*, with potential probiotic properties. The two probiotic strains were lyophilised in milk, containing 7.9 × 10^9 CFU/g of the total viable bacteria in the lyophilisate. They determined the number of viable *L. casei* and *L. paracasei* cells at the end of 12 months storage at various temperatures, i.e. 4, 18, 30°C and concluded that the survival was highest at 4°C (89–94%) followed by 18°C (80–87%) and 30°C (60–67%). The total number of probiotic cells remained at the functional level at 4°C (3.6 × 10^7 to 5.8 × 10^7 CFU/g) and 18°C (3.9 × 10^6 to 1.2 × 10^7 CFU/g) while at 30°C the count was found to be below the functional level. However, the disadvantageous phenomenon occurred as soon as, after 4–5 months of keeping at 30°C and from this time of the numbers of the bacteria did not diminish till the end of storage. Thus, the inappropriate conditions during storage of chocolate supplemented with *Lactobacillus* cell can result in a lack of functionality.

In a previous investigation, Nebesny *et al.* (2005) formulated chocolate with isomalt and enriched with lactic acid bacteria *Streptococcus thermophilus* MK-10 and *Lactobacillus delbrueckii* subsp. *bulgaricus* 151, added in the form of powdered yoghurt, prepared by spray-drying as a sucrose-free, low-calorie product with functional properties. The initial numbers of live *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* cells in the powdered yoghurt, accounting for 4% (w/w) in all the chocolates, were 1.2 × 10^9 and 1.3 × 10^7 CFU/g, respectively. The number of live *S. thermophilus* cells added to sucrose-free dark and milk chocolates remained unchanged after 6 months, independently of the temperature of storage. Their survival in dark chocolates exceeded 96% after 6 months, and was higher after storage at 4°C (97.3%), whereas in milk chocolates it was slightly lesser (94%) for both storage temperatures. Lactobacilli were shown to be more sensitive to storage than streptococci. The 2- and 6-months storage of dark and milk chocolates at 4°C reduced the number of lactobacilli to approximately 60 and 33%, respectively. *L. delbrueckii* subsp. *bulgaricus* cells preferred being kept at 18°C. Their survival after 2-months storage was approximately 80% in sucrose-free yoghurt-containing dark and milk chocolates, whereas after

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6 months approximately 33 and 47% survival was observed in dark and milk chocolates, respectively.

Another group of researchers developed a method for the production of milk chocolate, sweetened with either sucrose or isomalt and aspartame, containing 32, 36, or 40 g/100 g fat, and supplemented with viable cells of probiotic bacterial strains: *Lactobacillus casei* and *paracasei* (Zyzelewicz *et al*. 2010). According to the authors, the milk chocolate displayed the same sensory properties as the reference, probiotic-free chocolate, while the total number of viable bacterial cells was maintained at the functional level of 10^6 to 10^8 CFU/g after keeping for 12 months irrespective of the temperature. The highest number of live probiotic bacteria survived in the chocolate kept at 4°C. Similar to this, Ramli *et al*. (2012) observed the viability of *L. plantarum* in dark chocolate over a three month storage period studied. The growth levels of *L. plantarum* in the dark chocolate and mousse decreased until they reached 81.25 ± 0% (6.5 ± 0 log CFU/g) and 76.88 ± 0.88% (6.2 ± 0.07 log CFU/g) in the samples, respectively.

Sensory and physico-chemical properties of chocolate based products with probiotics. Chocolates are semi-solid suspensions of fine solid particles from sugar and cocoa, about 70% total solids being in a continuous fat phase. Central to the sensory character is the lipid composition in the continuous phase, which influences the mouth feel and melting properties. Triglycerides in chocolate are dominated by saturated stearic (34%) and palmitic (27%) acids and monounsaturated oleic acid (34%). Chocolates are solid at ambient temperature (20–25°C) and melt at oral temperature (37°C) giving during consumption a smooth suspension of particulate solids in cocoa butter and milk fat (Beckett 1999; Whitefield 2005). White chocolates differ from milk and dark ones through the absence of cocoa nibs containing antioxidants, which reduces the product shelf-life (Beckett 2000; Whitefield 2005). Chocolates also contain vital minerals, specifically potassium, magnesium, copper, and iron (Holland *et al.* 1991). The differences in the sensory characteristics of chocolate can be attributed to the use of different cocoa types, variations in ingredients proportions, use of milk crumb instead of milk powder, blending techniques, and processing methods. Specifications depend on the chocolate type and its intended use (Jackson 1999).

Rheological properties of chocolate are important in the manufacturing process for obtaining high-quality products with a well-defined texture (Servais *et al*. 2004). Chocolates with a high viscosity have a pasty mouth feel, persisting in the mouth (Beckett 2000). Viscosity relates to the composition, processing strategy, and particle size distribution. The apparent viscosity in aqueous solutions influences flavour 'by-mouth' and taste intensity during consumption (Denker *et al*. 2006), thus the rheological measurements often give information related to the sensory character of chocolate.

Probiotic strain *Bacillus indicus* HU36 in combination with maltodextrin and lemon fiber, demonstrated high survival rate of the probiotic without affecting normal colour, taste, and texture of dark chocolate while dietary fiber addition improved some sensorial features significantly i.e. sweetness, firmness, and adherence of the chocolate (Erdem *et al*. 2014).

Ramakrishna *et al*. (2013) revealed that probiotic chocolates were highly acceptable and similar to control chocolate, while rheological studies showed that milk chocolate prepared using yoghurt powder at 50% showed no significant changes in the yield value compared to that of control, but at 100% addition, a considerable decrease in the yield value was observed. The microstructural properties of chocolate with 50% addition of yoghurt powder showed smaller particles adhering to the cocoa and sugar crystals but, at 100% addition of yoghurt powder, the cocoa particles were completely covered by the smaller yoghurt powder matrix.

Aragon-Alegro *et al*. (2007) studied the sensory characteristic of chocolate mousse prepared with *Lactobacillus paracasei* subsp. *paracasei* LBC 82 (probiotic mousse) or with *L. paracasei* subsp. *paracasei* along with inulin as a prebiotic. Control (C), probiotic (P), and symbiotic (S) samples were stored at 4 ± 1°C for ≤ 28 days. Sensorial results of the chocolate mousse trials did not indicate any significant differences in preference between the samples of mousses C, P, and S evaluated by 42 mousse consumers, even though P was considered the most preferred of the chocolate mousse studied.

In recent years, sucrose-free chocolates have become popular among consumers and manufacturers because of the reduced calorific values, and the fact that these are both noncariogenic and suitable for diabetics (Zumbe & Grosso 1993; Olinger 1994; Olinger & Pepper 2001; Sokmen & Gunes 2006). Sugar alcohols, including xylitol, sorbitol, mannitol, and lactitol are used for the manufacture of lower-calorie or sugar-free products. The replacement of

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sucrose with sugar alcohols conversely affects the rheological properties and thus the processing conditions and chocolates quality (Zumbe & Grosso 1993; Wijers & Strater 2001; Sokmen & Gunes 2006). Sokmen and Gunes (2006) noted that maltitol results in rheological properties of chocolate similar to those with sucrose, and thus may be recommended as a good alternative to sucrose in the chocolate formulations. The authors also observed that chocolate with isomalt resulted in a higher plastic viscosity while xylitol causes a higher flow behaviour index. Polydextrose may be added as an edible carbohydrate and intense sweetener. Such low-calorie sweeteners did not show any adverse effect on the growth of bacteria. Due to the laxative effects, the EU limits the consumption of sugar alcohols to 20 g per day (Kruger 1999). Mandal *et al*. (2005) prepared milk chocolate using the prebiotic inulin and encapsulated *Lactobacillus casei* NCDC-298.

Nebesny *et al*. (2005) established the technique of the production of chocolate sweetened with isomalt with live cells of lactic acid bacteria *Streptococcus thermophilus* MK-10 and *Lactobacillus delbrueckii* subsp. *bulgaricus* 151, added in the form of powdered yoghurt. They determined the physico-chemical and sensory properties as well as the survival of cells during 6-months storage at 4 and 18°C. The yoghurt-containing isomalt-containing milk chocolates gained slightly higher average scores (4.82–4.90 points) than control sucrose-free chocolates (4.83–4.87 points). Sucrose-free yoghurt-containing dark chocolates received lower scores (4.73–4.75) than their yoghurt-free counterparts (4.82–4.86). It can be concluded that the consumers more readily accept the delicate yoghurt taste of milk chocolates than that of dark chocolates.

According to Nebesny and Zyzelewicz (2006), the sensory attributes of dark chocolate masses and chocolates supplemented with viable cells of two bacterial strains *Lactobacillus casei* and *Lactobacillus paracasei*, were not different from those of traditional chocolates. The addition of lyophilised preparations of *L. casei* and *L. paracasei* did not change the sensory attributes of the chocolate. These bacteria do not grow in chocolate and therefore they neither assimilate chocolate constituents nor secrete the products of metabolism. The sensory attributes of the examined batches of lyophilisate containing chocolate, which were sweetened either with sucrose or isomalt and aspartame, received 4.83–4.86 points in the five-point scale. There were no statistically significant differences at the confidence level (α) of 0.05 between the scores received in the evaluation of the sensory properties of all the chocolate batches examined.

The physico-chemical properties of the dark chocolate with *L. plantarum* were almost similar to those of the control dark chocolate based on the measurements of viscosity, texture hardness, pH, colour, and water activity (Ramli *et al*. 2012). However, the physico-chemical properties of the dark chocolate mousse containing *L. plantarum* were significantly different compared to the control and dark chocolates with *L. plantarum* over three months of storage at 4°C.

A dark chocolate containing phytosterols (PS) esters was developed by Botelho *et al*. (2014) to reduce cholesterol in individuals. The phytosterols had kept their potential functionality after 5 months of storage at room temperature in dark chocolates representing an option as a functional food.

Currently, there are several companies that have launched probiotic chocolates in the market viz., Ohso (Belgium), Wysong's chocolate (Wysongs Corporation, USA), Attune chocolates (Attune Foods, USA), Healthy Digestives-ProBiotic dark chocolate bites (Aviva, Canada) and Barryl Callebaut Sdn. Bhd (Malaysia). Wysong's chocolate Therapy^{TM} contain natural whole ingredients with protein, minerals,

Table 1. Probiotic milk chocolates and milk based other products in world market

Product type	Brand	Probiotic species
Attune Probiotic chocolates	Attune Foods, USA	*Lactobacillus acidophilus, Lactobacillus casei*
Ohso Probiotic chocolate	Ohso, Belgium	*Lactobacillus helveticus, Bifidobacterium longum*
Orizins^{TM} bars, Chocolate Therapy^{TM}	Wysongs Corporation, USA	*Lactobacillus acidophilus, Lactobacillus bifidus, Lactobacillus plantarum, Enterococcus faecium*
Healthy Digestives-ProBiotic Dark Chocolate Bites	Aviva Natural Health Solutions, Canada	*Bacillus coagulans* (*Lactobacillus sporogens*)
Healthy Delights^{TM} ProBiotic Bites	FL, USA	*Lactobacillus acidophilus*, *Bifidobacterium lactis*

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antioxidants, and essential fatty acids and probiotics like *Lactobacillus acidophilus, B. bifidus, L. plantarum, Enterococcus faecium* (Hurst *et al.* 2002). In Belgium, Olso developed dark chocolate varieties containing half a billion probiotic *Lactobacillus helveticus* and *Bifidobacterium longum* to obtain the advantage of natural polyphenols and antioxidants from dark chocolates (Table 1). The Canada based company, Aviva Natural Health Solutions has developed Healthy Digestives-ProBiotic dark chocolate bites containing *Bacillus coagulans* (*Lactobacillus sporogenes*), which claims to maintain both a healthy digestive tract and healthy digestive flora. They also produce probiotics chewable bites for children containing billion of *Lactobacillus acidophilus* DDS-1, vitamin D_3 (800 IU), and fructooligosaccharides.

CONCLUSION

Apart from the traditional fermented dairy products, the current trend in the diversification of probiotic foods is aimed towards the non- fermented and heat-treated food product categories. Very few researches have been carried out with the aim to incorporate probiotics into non-fermented foods like chocolate and related desserts. Probiotic milk chocolate is a new addition to the growing list of functional foods. The problems associated with the incorporation of probiotics and/or prebiotics into such foods are their survival and stability during the processing, preservation, storage, and gastrointestinal (GI) transit, and probiotic strains surviving, at a suitable level until the time of consumption. General approach to improve probiotics survivability is the selection of suitable strains for food application. It is very difficult to select a probiotic strain having all required properties by applying classical probabilistic approach. The stress adaptation method is another approach; however, stress responses are strain-specific. A number of techniques have been developed to protect the probiotics from environmental stresses in food matrices, processing, and storage, and GI tract passage. Among these, microencapsulation has been found to be, the most suitable and accessible technology to protect the tiny living organisms.

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