Influence of Probiotics, Prebiotics, Synbiotics and Bioactive Phytochemicals on the Formulation of Functional Yogurt

Nurul Farhana Fazilah[1], Arbakariya B. Ariff[1,3], Mohd Ezuan Khayat[2,3], Leonardo Rios-Solis[4], Murni Halim[1,3*]

[1]Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[2]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[3]Bioprocessing and Biomanufacturing Research Center, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[4]Institute for Bioengineering, School of Engineering, University of Edinburgh, Edinburgh, EH9 3JL, United Kingdom

*** Correspondence:**
Murni Halim (Dr)
Email: murnihalim@upm.edu.my
Phone: 6013-89467477, Fax: 603-89467510

Abstract

The new concept of functional foods has emerged varieties in the production of foods that deliver not only basic nutrition but can also warrant good health and longevity. Yogurt has become one of the prevalent choices and considered as healthy food since it provides excellent sources of essential nutrients. As the popularity of yogurt continues to grow, manufacturers and scientists are continuously investigating on the value adding ingredients such as probiotics, prebiotics and different kinds of plant extracts to produce functional yogurt that consists of extra beneficial properties than the conventional yogurt. This review summarizes the current knowledge on functional yogurt, applications and roles of probiotic, prebiotic and synbiotic in yogurt as well as the effects of phytochemicals that are added in innovative yogurt products. Their important properties are also focused on significance influences on quality and sensory attributes of yogurt products and associated health aspects.

Keywords: yogurt; functional food; probiotic; prebiotic; synbiotic; phytochemical

1. Introduction

Yogurt or yoghurt is a long time known, appreciated dairy food product and is available in a variety of textures (i.e., liquid, set, smooth), fat contents (luxury, low-fat, virtually fat-free) and flavours (natural, fruit, cereal) (Shah, 2003; McKinley, 2005). It is made traditionally from the spontaneous or induced lactic acid fermentation of milk (Widyastuti et al., 2014). Basically, yogurt can be classified into two groups which are standard culture yogurt and bio-yogurt or probiotic yogurt (Pandey et al., 2017). Standard yogurt is typically manufactured from the conventional starter culture strains, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Arena et al., 2015). Meanwhile bio-yogurt or probiotic yogurt is supplemented with probiotic strains such as *Bifidobacterium* and *Lactobacillus acidophilus* that are claimed to have numerous health benefits and should remain live at adequate numbers (Lourens-Hattingh & Viljoen, 2001; Weerathilake et al., 2014; Baltova & Dimitrov, 2014; Chen et al., 2017). For instant, National Yogurt Association (NYA) of the United States specifies bio-yogurt product must contain 10^8 CFU/g lactic acid bacteria (LAB) at the time of manufacture to using “Live and Active Culture” logo while The Australian Food Standards Code regulations require that the LAB used in yogurt fermentation must be present in a viable form in the final product nonetheless numbers of CFU/g are not specified (Pandey et al., 2017). Yogurt is considered as the most popular vehicle for the delivery of probiotics for the consumer (Lourens-Hattingh & Viljoen, 2001). The most commonly consumed yogurts are set type yogurt and strains yogurt but nowadays frozen and drinking yogurts are also part of yogurt’s commercial varieties and become increasingly popular.

Organoleptic, rheological, texture and microstructure properties of yogurt depend on several factors such as fermentation process, type of milk, starter cultures and probiotic strains, packaging and storage conditions. As depicted in Fig 1, the conventional processing for manufacturing of yogurt involved several steps: initial treatment of milk (an optional step for using a higher quality of raw milk (i.e., grade A or grade B milk as defined under the US Pasteurized Milk Ordinance, Food and Drug Administration (FDA) (Murphy et al., 2016) in yogurt production), standardization of milk, homogenization, heat treatment, fermentation process, cooling and ending with the packing of the final yogurt product (Sfakianakis & Tzia, 2014). Yogurt can be manufactured with or without the supplementation of natural derivative of milk (i.e., skim milk powder, caseinates or cream, whey concentrates), the addition of sugars (i.e., sucrose, fructose) and stabilizers (i.e., pectin, starch, gelatine, alginate) and increasing the solids in milk by adding fat and proteins to alter the texture and flavour (Lee & Lucey, 2010). For instant, protein and fat are commonly added to combat the defects in texture, physical properties and mouthfeel of low fat yogurt (Laiho et al., 2017). Meanwhile hydrocolloids stabilizer such as carrageenan, gelatin, xanthan gum and modified starch are often added to milk base in an attempt to improve texture, appearance, viscosity, consistency, mouthfeel as well as to prevent whey separation in yogurt (Nguyen et al., 2017).

In general, the health benefits of fermented food products can be classified into two groups which are nutritional function and physiological function (Bell et al., 2017). The nutritional effect is related to the food function in supplying sufficient nutrients whilst physiological function concerns on the prophylactic and therapeutic benefits (Marco et al., 2017) such as reduction in risk of diabetes (i.e consumption of fermented kimchi decreased insulin resistance and increased insulin sensitivity (An et al., 2013) and reduced muscle soreness from the consumption of fermented milk by *Lactobacillus helveticus* (Iwasa et al., 2013). In response to the consumer awareness of these two imperative benefits, manufacturers are exploiting the demand by producing varieties of fermented food products with additional functional properties (Siro´ et al., 2008). Functional foods are currently part of a new market niche and the industry is kept on expanding with natural ingredients as the most influential driver (Balthazar et al., 2017; da Silva et al., 2016; Granato et al., 2017). In particular, innovative processing of functional yogurt products include the addition of probiotics, prebiotics or their combination which is term as synbiotic and incorporation of various bioactive components from natural sources to improve nutritional values, sensory profile, physiochemical and rheological characteristics as well as to provide therapeutic properties.

2. Varieties and health benefits of yogurt

The microbiology of lactic-producing bacteria and the fermentation biochemistry and technology of yoghurt are well documented (Apostu & Barzoi, 2002). In general, the nutritional composition of yogurt can be varied depending on several aspects including the strains used as the starter culture, type of milk used (whole, semi or skimmed milk), species of that milk is obtained (i.e., cow, goat, sheep, buffalo, ewe, camel, yak, non-dairy milk), type of milk solids, solid non-fat, conditions of fermentation process as well as other components added such as sweeteners and flavour (Weerathilake et al., 2014). Yogurt is considered to have more nutritional benefits than milk as it is nutritionally rich in protein, calcium, riboflavin, vitamin B6 and vitamin B12 (Ashraf & Shah, 2011). Moreover, it can also aid in digestion process, boost immunity, ease diarrhea and protect against cancer (Hassan & Amjad, 2010; Davoodi et al., 2013; Prasanna et al., 2014; McFarland, 2015). Yogurt diet is also favourable towards weight management. A study revealed that high (at least 7 servings per week) consumption of yogurt was associated with lower incidence of obesity as compared to low (1 to 2 servings per week) consumption (Martinez-Gonzalez et al., 2014). Furthermore, yogurt is also associated with reduction of weight gain when consistently in diet for years (i.e., over a 4-years period of consumption) (Winzenberg et al., 2007). The high dairy intake from the yogurt product increased the dairy calcium intake on energy balance that resulted in lower body weight or body fat mass (Zemel et al., 2000).

2.1 Type of yogurt

2.1.1 Yogurt from cow milk

Approximately 85% of the world milk production is derived from cattle (FAO, 2015) and is the most commonly milk for yogurt production (Ranasinghe & Perera, 2016). Yogurts from cow milk composed of ca. 80% caseins (αs1-, αs2-caseins, β-casein and k-casein) and ca. 20% whey protein formed by the four major soluble proteins: β-lactoglobulin (β-LG), α-lactalbumin (α-LA), blood serum protein (BSA) and immunoglobulins (Igs) (Jovanovic et al., 2007; Ruprichová et al., 2012). These proteins represent 50%, 20%, 10% and 10% of the whey proteins fraction, respectively. The whey proteins can bind with many kinds of endogenous and exogenous agents such as dietary polyphenols (Xiao et al., 2011). Whey proteins when exposed to high temperatures (>65°C) irreversibly denature and coagulate, as opposed to caseins, which do not coagulate when subjected to a high heat treatment (Jovanovic et al., 2007). Caseins micelles aggregate through isoelectric precipitation brought about by the action of LAB or organic acids. The casein strands can be broken and the size of the aggregates decreased. The rearrangement and syneresis of the acid induced casein network in yoghurt occur during storage (Everett & McLeod, 2005).

2.1.2 Yogurt from other animal's milk

Apart from cow's milk, yogurts are also being derived from the milk of other animal species. For instant, yogurt derived from goat's, sheep's or buffalo's milk that composed of high fat content often resulted in a more creamy texture than yogurt made of milk with lower fat content (Sfakianakis & Tzia, 2014). While goat milk is not very popular in the Western world, nevertheless it is one of the most widely consumed milk in the rest of the world mainly attributed to its nutrition properties and associated health benefits. In recent years, the production of goat milk worldwide has increased due to increasing demand for raw goat milk and its value added products such as goat milk yogurt (Ribeiro & Ribeiro, 2010). Furthermore, goat milk and its derived product is a good alternative for people suffer from lactose intolerance as the milk has better digestibility and lower allergenicity (Yangilar, 2013). Sumarmono et al., (2015) reported that the predominant saturated fatty acids in goat milk yogurt was comparable to the components found in most traditional Greek yogurt (Serafeimidou et al., 2012) which were myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0). Yogurt from goat milk was reported to compose of higher CLA (0.47 – 0.76 g CLA/ 100g fat) than that in cow milk (0.24 – 0.45 gCLA/100g fat) (Serafeimidou et al., 2013). Free fatty acids were also found to significantly increase during the goat milk yogurt fermentation process as compared to fresh goat milk (Güler, 2007). Frequent consumer complaint on the rancid, goaty off flavour and odor has stimulated into novel formulations of goat milk yogurts that are supplemented with various fruit juices to add a pleasant taste and aroma. For instant, Damunupola et al., (2014) evaluated the quality characteristics of goat milk yogurt fortified with beetroot juice. The inclusion of beetroot juice increased the moisture content and lowered the total solid content as observed during 21 days storage. Sensory evaluation revealed that 98% of the panellists preferred beetroot-goat milk yogurt as compared to plain goat milk yogurt. Beetroot juice managed to mask the goaty flavour and goaty odor of the goat milk yogurt and thus enhanced the consumer preference.

Although sheep milk is rarely consumed in nature, but the milk is quite common in the yogurt making (Balthazar et al., 2017). Sheep milk yogurt possesses high gel strength with minimal syneresis yogurts and tends to have a slightly grainy body and texture due to higher titratable acidity and calcium content as compared to cow's and goat's milk yogurts (Wendorff, 2005). Oleic acid (C18: 1n9) is the most predominant fatty acids in sheep milk yogurt followed by palmitic acid (C16: 0) and myristic acid (C14: 0) (Balthazar et al., 2016). Hence the consumption of sheep milk yogurt may be health beneficial as studies showed that diets high in oleic acid could decrease the level of low-density lipoprotein (LDL) cholesterol without affecting the levels of high-density lipoprotein (HDL) cholestrol (Molkentin, 2000). Sheep milk Greek yogurt was also reported to have high content of conjugated linoleic acid (CLA) (between 0.405 to 1.250 g CLA/100 g fat) that may exhibit immunoregulatory effect and activity as anti-obesity, anticarconogenic, antioxidant as well as anti-diabetic (Wang & Lee, 2015, Yuan et al., 2014). Greek sheep milk yogurt is described as a good source for angiotensin-converting enzyme (ACE) inhibitory peptides that advantages for those with hypertension and congentive heart failure (Politis & Theodoru, 2016).

Buffalo milk has higher concentration of protein, fat, calcium, phosphorus and total solid that other animal's milks (Nguyen et al., 2013; Bilgin & Kaptan, 2016). Consequently, buffalo milk yogurt tends to have contents of higher fat and non-fat dry matters that provide unique texture and sensorial properties. In addition, the high total solid content and high viscosity of buffalo milk lead to an increase of gel firmness and decrease of whey production. From the textural and sensory property perspectives, yogurt made from buffalo milk alone presented distinct characteristics and higher values than mixed milks of cow and ewe's yogurt (Yilmaz-Erzan et al., 2017).

2.1.3 Non-dairy probiotic products

Nowadays, production of yogurt from non-animal based milk such as soy milk, coconut milk, rice milk, sunflower silk milk and cashew milk are also increasing influenced by several factors especially health awareness and changing in consumer demands (Masamba & Ali, 2013). For instant, soy yogurt is becoming popular due to its beneficial advantages in term of nutrition and health as the product contains high protein and absence of cholesterol or lactose and only a small amount of saturated fatty acids (Kolapo & Olubamiwa, 2012). Furthermore, soy milk yogurt is considerably cheap as the soy raw material can be obtained at much cheaper cost than the cow's milk. Makanjuola, (2012) previously reported on the formulation of soy-corn yoghurt as a substitute for milk based yogurt with high content in protein and well balance amino acid composition. Soy milk used for yogurt preparation has low acidification rate and slow growth of probiotic bacteria and prolongs fermentation time due to the low concentration of soluble carbohydrates in soy milk (Donker et al., 2007). Bioyogurt formulated with mixtures of 25% of soy milk and 75% of cow's or buffalo's milk received high scores for sensory evaluation and the optimum combination of milks helped to enhance the viable cells of probiotic bacteria (Ghoneem et al., 2017). Bernat et al., (2015) formulated a non-dairy yogurt-like product from the fermentation of almond milk by a combination of probiotic strains, *Lactabacillus reuteri* and *S. thermophilus*. The viability of both probiotic strains in almond milk yogurt was found to be decreasing throughout the 28 days of cold storage. Nevertheless, the cell count of probiotic *L. reuteri* was above the minimum level recommended for probiotic products which was retained at ~10^7 CFU/mL. Meanwhile, corn milk is another alternative for vegetable based yogurt products bearing balance nutritional content with sweet taste and nice aroma (Yasni & Maulidya, 2013).

Sensory analysis showed that the yogurt formulated with corn extract from corn kernels mixed with 5 % full cream milk powder and 10% sugar obtained the highest score. During 4 weeks of cold storage, the cells counts of probiotics (*L. delbruekii, Streptococcus salivarius* and *Lactobacillus casei*) in the yogurt sample retained at 1.5 x 10^9 CFU/mL which was above the number for probiotics critical threshold.

2.1.4 Fruit yogurt

Besides potential health benefits, consumers are tends to choose flavours as the key factor for food criterion for acceptance and thus addition of different fruits in yogurts to improve its flavour has been attempted progressively (Ndabikunze et al., 2017). Various studies demonstrated that adding some materials particularly fruits can increase the appealing taste of yogurt and improved the quality of yogurt particularly its nutritional properties (Hossain et al., 2012; Çakmakçı et al., 2012; Mahmood et al., 2008). Organoleptic evaluation has shown a marked preference for fruity yogurt as fruit yogurt has more taste and pleasing flavour (Amal et al., 2016). In the meantime, the utilization of persimmon marmalade in yogurt production has improved the taste, odour, appearance, perceived sweetness and fruits taste, acidic taste, structure and overall acceptability scores (Arshlan & Bayrakci, 2016). Common fruits that are frequently used in formulating a functional yogurt production are peaches, orange, strawberry, pineapple, cherries, apricots, and blueberries (Arslan & Özel, 2012; Chandan et al., 1993). In general, fruits may be added to yoghurt formulae as single or blends in the form of refrigerated, frozen, canned fruit, juice or syrup (Cinbas & Yazici, 2008).

3. Roles of Probiotic organisms in yogurt

3.1 Probiotic

Probiotic can be defined as a live microbial food supplement that gives benefit to health through its effects in the intestinal tract (Corliss et al., 2013; Aurelia et al., 2011; FAO/WHO, 2002). Most probiotics fall into the group of organisms' known as lactic acid-producing bacteria and are normally consumed in the form of yogurt, fermented milks or other fermented foods (Handa et al., 2016). Various species of *Lactobacilli* and *Bifidobacteria* are formulated in more than 90 % of probiotic products and popular among health conscious consumers (Shah, 2000; Ranadheera et al., 2014). These bacteria also are

Generally Recognized as Safe (GRAS) (Goldin, 1992; Oakey, 1995). Table 1 shows the genera of bacteria that are commonly used as a probiotics in fermented dairy product.

In dairy fermentation, probiotic taking a role to assist in the preservation of the milk by the generation of lactic acid (Ming et al., 2016; Othman et al., 2017a; Othman et al., 2017b) and possibly antimicrobial compounds (Goudarzi et al., 2017; Halder et al., 2017), production of desirable flavour compounds (i.e., acetaldehyde, diacetyl in yogurt) (Ott et al., 2000; Pinto et al., 2009) and other metabolites. These properties will give a product with organoleptic properties desired by the costumer, improve nutritional value of food and provision of special therapeutic or prophylactic properties as cancer (Davoodi et al., 2013) and control of serum cholesterol levels (Ngongang et al., 2016). For example, *Lactobacillus* isolated from a fermented vegetable called Makdoos, was demonstrated to be able to inhibit the growth of several pathogens and highly effective against *Bacillus cereus*, *Salmonella typhimurium* and methicillin-resistant *Staphylococcus aureus* (MRSA) isolate (Adel et al., 2017). Moreover, the strains also comprise of antibiotic resistance that was pronounced against tetracycline, streptomycin kanamycin, and trimethoprim. In the meantime, *Lactobacillus animalis* LMEM6, *Lactobacillus plantarum* LMEM7, *L. acidophilus* LMEM8 and *Lactobacillus rhamnosus* LMEM9 that were isolated from curd also showed antibiotic like activity against bacterial infection to humans (Halder et al., 2017). The potential benefits may result from the growth and action of the bacteria during the manufacture of cultured foods (Chen et al., 2017).

Additionally, foods that contain viable probiotic microorganisms show several health benefits, such as reduction and prevention of diarrhea, improving the intestinal microbiota balance through antimicrobial effects, decreasing lactose intolerance symptoms and food allergy, improving immune potency, anti-tumorigenic activities and reduction of the risk of colon cancers (McFarland, 2006; Vasudha & Mishra, 2013; Prasanna et al., 2014; Granato, Nazzaro et al., 2018). Probiotics also play roles as immune modulators, anti-hypertensive agent, hypocholesterolemics and perimenopausal treatments (Liong, 2007). The mechanisms by which probiotics exert their effects are largely unknown, but may involve modifying gut pH, antagonizing pathogens through production of antimicrobial compounds, competing for pathogen binding and receptor sites as well as for available nutrients and growth factors, stimulating immunomodulatory cells, and producing lactase (Bengmark 2000; Benchimol & Mack 2004). As depicted in Fig. 2, there may be four difference mechanisms in which probiotic may defend against pathogen (Bermudez-Brito et al., 2012).

3.2 Probiotic yogurt

Probiotic products must contain an adequate numbers of viable cells, at least 10^6 to 10^7 CFU/mL at the time of consumption to certify the beneficial effects (Sohail et al., 2013). Conventional yogurt starter culture strains, *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are lack in the ability to survive passage through the intestinal tract (Mater et al., 2005). These starter culture strains may not play a significant role as probiotics in the human gut due to their incapability of colonizing the human intestine (McFarland, 2015). Therefore, the current trend is to add other probiotic strains during yogurt fermentation along with the starter culture bacteria to induce the probiotic effect. Basically, the manufacture of probiotic yogurt involves several steps starting from milk supplementation with dairy ingredients to increase protein concentration, homogenization of the fortified milk, heated at 90°C for 10 min, cooling down to 42°C prior to inoculation with yogurt starter culture and selected probiotic bacteria (Marafon et al., 2010). In general, probiotic strains are selected on the basis of their safety, nutritive value and health promoting properties besides other valuable properties that may influence the shelf life, texture and appearance of the probiotic yogurt. Furthermore, selection criteria of probiotic strains must also take into account the possible interactions among the strains and dairy product and starter culture bacteria to optimize their performance and survival during storage (Casarotti et al., 2014). It is quite a common practise to combine these probiotic strains with the yogurt starter culture bacteria to reduce fermentation time (Damin et al., 2008). Nevertheless some probiotic bacteria grow slowly in milk due to lack of essential proteolytic activity and their acidifying characteristic may affect the product texture (Lucas et al., 2004). In comparison to yogurt starter culture, probiotic bacteria are often having a poor acidification performance in milk (Almeida et al., 2008). Addition of probiotic culture will reduce the acid accumulation during storage period (Kailasapathy, 2006). Furthermore, post exopolysaccharides was observed in yogurts supplemented with probiotic cultures compared to yogurt without probiotics. High exopolysaccharides may provide a better texture for yogurt (Han et al., 2016). It is known that microbial exopolysaccharides may improve body and texture of fermented products as they serve as emulsifying or gelling agents, thickening and also stabilizing agents. Among various LAB, *Bifidobacteriu*m and *Lactobacillus* are the commonly selected genera to be added in the probiotic yogurt product (Chen et al., 2017). Generally, the efficiency of added probiotic bacteria in yogurt is dependent on dose level and their viability must be maintained throughout storage, and they must survive the gut environment (Aryana et al., 2007). The combination of probiotic bacterium *Bifidobacterium animalis* spp. *lactis* BL 04 with *S.*

thermophilus produces rheological characteristics similar to yogurt and hence suitable to be used in the production of probiotic fermented milk (Damin et al., 2008). *Lactobacillus gasseri* 4/13 was successfully applied as an adjunct culture to yogurt starters (*L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* in combination with a commercial Direct Vat Set (DVS) yogurt starter cultures (LBB 41-8 or LBB 5-54V or LBB 435)) producing yogurt with well-accepted taste and concentration of viable *L. gasseri* 4/13 that remained above the critical threshold of 10^6 CFU/mL during 21 days storage period (Baltova & Dimitrov, 2014). Human origin probiotic strain, *L. gasseri* 4/13 is an attractive adjunct monoculture in the production of functional foods as the strains was demonstrated to have high rate of adhesion to Caco-2 human epithelial cells, good ability in reducing cholesterol and also capable to induce the production of interferon gamma. *L. rhamnosus* GR-1 and RC-14 are other probiotic strain that have the ability to be delivered in a yogurt form with good survival rate and resulted in palatable taste and texture (Hekmat & Reid, 2006). A study on the effect of short term (1 month) consumption of yogurt supplemented with probiotic strains, *L. rhamnosus* GR-1 and RC-14 demonstrated the product promote the formation of a desirable anti-inflammatory environment in the peripheral blood of inflammatory bowel disease patients without any harmful side effects (Baroja et al., 2007).

3.3 Application of encapsulated probiotic bacteria in yogurt

Despite the benefits offered by the incorporation of probiotic bacteria in dairy product especially yogurt, the main challenge is to maintain the viability rate of the bacteria to above the critical threshold of 10^6 CFU/mL throughout the product shelf life (Lourens-Hattingh & Vilijoen, 2001; Shah, 2000). Furthermore, upon consumption, the probiotic bacteria must be resistance to low pH, bile acids and digestive enzymes to remain viable during their passage through the gastrointestinal tract (Halim et al., 2017). Several brands of probiotic yogurt available in the market were analyzed to have inadequate presence of viable cells of probiotic strains such as *L. acidophilus* and *Bificobacteria* (Shah, 2000; Iwana et al., 1993). This inspection has led to a new trend of application of encapsulated bacterial cells in functional food products such as yogurt aiming to increase viability of probiotic bacteria during shelf life. Several commonly used methods for encapsulation of probiotic strains include extrusion (Halim et al., 2017), emulsion (Kumar & Kumar, 2016), spray drying (Hernandez-Carranza et al., 2014) and phase separation (Borza et al., 2010). Alginate (Kumar and Kumar, 2016), gelatine (Mathews, 2017), gellan gum (Totosaus et al., 2013), carrageenan (Cheow & Hadinoto, 2013) and starch (Donthidi et al., 2010), are among the widely used materials for coating probiotic cells for the encapsulation process. Coating materials must be selected based on their attributes in preventing cell release and increases mechanical and chemical stability of the bead produced. Microencapsulated probiotic strains may be added either before or after yogurt fermentation (Krasaekoopt et al., 2004). It was reported that the addition of spray dried-microencapsulated *Bifidobacterium breve* R070 and *Bifidobacterium longum* R023 in whey protein polymers have increased the survival and viability of the probiotic strains in yogurt during 28 days storage at 4°C (Picot and Lacroix, 2004). The advantage of supplementation of encapsulated probiotic cells in yogurt was also presented by Iyer & Kailasapathy (2005). In the study, probiotic strains *L. acidophilus* CSCC 2400 and *L. acidophilus* CSCC 2409 were coated with different coating polymers (alginate, chitosan and poly-L-lysine) by immersion technique. During a 6 weeks storage period, it was observed that the viable cell counts of yogurts in the presence of encapsulated and co-encapsulated (chitosan coated) of probiotic beads were only 2-log and 1-log cycle decrease, respectively, compared to yogurt with non-coated probiotic cells that recorded a 4-log drop in cell numbers. Meanwhile, yogurt supplemented with alginate micoencapsulated *L. rhamnosus* was more stable in term of viability in comparison to carrageenan microencapsulated and free culture probiotic yogurts (Kumar & Kumar, 2016). In a food product application, besides the number of probiotic viable cells that mostly influenced by the encapsulation method and coating materials, the size of probiotic bead produced must also be considered. The presence of microencapsulated probiotics should not affect the sensory attributes of the products. An assessment study conducted by Krasaekoopt & Tandhandskul (2008), found that the consumer acceptances for plain and fruit yogurt containing probiotic beads were as high as 82.3% and 94.9%, respectively. Probiotic cells can also be incorporated in yogurt via immobilization in natural supports such as fruits and grains. For instant, yogurt supplemented with immobilized *L. casei* on fresh apple pieces, wheat grains or dried raisins showed improved cells viability (7 log CFU/g) after 60 days of storage at 4°C than that obtained in yogurt with free probiotic cells (Bosnea et al., 2017). In particular, raisins and wheat grains were the most promising supports for *L. casei* as their matrix seem to protect the cells from acidic environment and also presented less syneresis (appearance of liquid on the milk gel surfaces and gel shrinkage) due to their water holding capacity.

4. Roles of Prebiotics in Yogurt

Prebiotics fall into a category of functional food and can be defined as the non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon thus improve host health (Csutak, 2010; Tomasik, Tomasik, 2006, Corliss et al., 2013). The most prevalent forms of prebiotics are classed as soluble fibre and traditional dietary sources of prebiotics include soybeans, inulin sources (such as Jerusalem artichoke, jicama, and chicory root), raw oats, unripe wheat, unripe barley, onion, banana, asparagus and yacon (Ozcan & Kurtuldu, 2014; Manning & Gibson, 2004; Oliveira et al., 2009). Nevertheless, the levels of prebiotics in these food sources are generally too low to exhibit any significant effect on the composition of intestinal microflora. Thus, prebiotics are commercially extracted and concentrated from fruits and vegetables through the hydrolysis of polysaccharides from dietary fibres or starch, or through enzymatic generation. Prebiotics are mixtures of indigestible oligosaccharides, except for inulin which is a mixture of fructooligo- and polysaccharides (Manning & Gibson, 2004; Gibson et al., 2000). Nowadays, prebiotic oligosaccharides are increasingly added to foods because of their health benefits. Some oligosaccharides that are used in this manner are fructooligosaccharides (FOS), xylooligosaccharides (XOS), polydextrose and galactooligosaccharides (GOS) (Csutak, 2010). Table 2 shows several studies that had been conducted to explore the prebiotic potential of foods and their influences on LAB.

In yogurt, prebiotic act as a substrate for the growth of probiotic bacteria and consequently enhance the gastrointestinal functions and immune system. Prebiotics can also increase the absorption of calcium and magnesium, influence blood glucose levels and improve plasma lipids (Csutak, 2010). Prebiotics may also provide a positive influence on probiotic bacteria multiplication (Younis et al., 2015). Kumari (2015) observed the increase of cell count of *Bifidobacterium* in yogurt that was incorporated with rice as compared to the plain yogurt due to the prebiotic effect. Likewise, Amarakoon et al., (2013) also demonstrated that cooked rice can facilitate the growth and survival of probiotic bacteria such as *Bifidobacteria*. A natural polymer, guar gum that is obtained from the seeds of *Cyamopsis tetragonolobus* is another prebiotic compound that may help to stimulate the growth of probiotic bacteria or native gut microflora (Mudgil et al., 2018). Previously, Mudgil et al., (2016) studied on the supplementation of partially hydrolyzed guar gum to act as soluble fiber enrichment while formulating a functional yogurt. Prior to application in yogurt, guar gum was first subjected to enzymatic hydrolysis by cellulase from *Aspergilus niger* and freeze dried to powder form. Guar gum was observed to have prominent effects on several characteristics of yogurt. In comparison to control yogurt, guar gum fortified yogurt showing an increase in pH, viscosity, water holding capacity but lower in titratable acidity and in general was well acceptable in term of functional and sensory quality. In contrast, Hassan et al., (2015) reported that the addition of 2.5% guar gum or 0.5 % cress seed mucilage did not affect the pH, fermentation time and proteolysis extent of set-yogurt throughout the 15 days storage period at 5±2°C. Nevertheless, the present of these potential prebiotic compounds improved the quality of set-yogurt as compared to the polysaccharide free yogurt. Yogurts with good organoleptic (in term of flavour, appearance, body and texture) acceptance were previously formulated with several polysaccharides extracted from taro corm *(Arum colocasia*), mature okra fruit (*Hibiscus esculents*), whole plant Jew's-mallow (*Corchorus olitorius*) (Hussein et al., 2011). A further study on the effect of these plant polysaccharides on the growth of probiotic bacteria in yogurt will determine their potential as prebiotic compounds.

5. The importance of synbiotic concept in yogurt

Synbiotics is a combination of probiotic and prebiotics that will affect host beneficially by improving the survival and implantation of selected live microbial strains in gastrointestinal tract (Khurana & Kanawjia., 2007). Synbiotics have great benefits to health such as antimicrobial, anticancer, anti-allergic and immune-stimulating properties (Buterikis et al., 2008). The combination of probiotic bacteria with prebiotic compound can cause the release of antibacterial substances such as bacteriocin which can retard the growth of pathogenic bacteria. A study by Kleniewska et al., (2016) has proven that the administration of synbiotics containing $4x10^8$ CFU/mL *L. casei* and 400 mg of inulin have positive influences on human plasma antioxidant capacity and antioxidant enzymes activities. In addition, synbiotics can improve the absorption of minerals, prevent diarrhoea and optimize assimilation of nutrients (Buterikis et al., 2008).

Among the commonly used probiotic strains for synbiotic product formulations are *Lactobacilli*, *Bifidobacteria* spp, *Saccharomyces boulardii* and *Bacillus coagulans* whilst the major prebiotics used include oligosaccharides such as fructooligosaccharide (FOS), galactooligosaccharides (GOS), xyloseoligosaccharides (XOS), inulin and prebiotic from natural sources like yacon roots and chicory (Kavita et al., 2015). The formulation of synbiotic soy yogurt using probiotic strains of *L. acidophilus* NCDC11, *S. salivarius* subsp. *thermophilus* NCDC118 as well as fructooligosaccharide as prebiotic was previously optimized using response surface methodology (RSM) (Pandey & Mishra, 2015). The mathematical modelling and optimization tool were employed to evaluate several parameters (combined effects of FOS, fermentation temperature and time, inoculum level of probiotic strain, whey separation, yogurt texture and sensory attributes) that aimed for improved product characteristics and consumer acceptability. In particular, the synbiotic soy yogurt produced was satisfactory in term of textual and sensory characteristics with good nutritional properties. Mishra & Mishra (2013), also demonstrated an attempt to reduce the after-taste of soymilk yogurt, and improve acidification rates and growth of probiotics via the addition of FOS. The presence of 2% (w/v) FOS as recommended in dairy products provides sweetness that improves the sensory profile of soy yogurt. In the meantime, the supplementation of total dietary fibres from apple and banana in probiotic yogurt also increased the shelf life of probiotic strains, *L. acidophilus* and *B. animalis* subsp. *lactis* (do Espirito Santo et al., 2012). The effects can be associated to the high contents of pectin and fructooligosaccharides in both fruits (Emaga et al., 2008). The addition of passion fruit rinds that are rich in pectin in yogurt containing the similar probiotic strains (*L. acidophilus* and *B. animalis* subsp. *lactis*) exhibited a higher viscosity in comparison to control yogurt (Espirito-Santo et al., 2013). In term of sensory analysis, the probiotic yogurt that enriched with passion fruit fibre received a good score for appearance, colour and odour but the intensity of the flavour was considered weak. A few other studies on synbiotic yogurts and their important findings are summarized in Table 3.

6. Role of Phytochemicals in yogurt

6.1 Bioactive phytochemicals

Phytochemical comes from Greek word phyto, which means plant. It is biologically active, naturally occurring chemical compounds found in plants that impart health benefits for humans that beyond their use as macronutrients and micronutrients (Bloch, 2003). Generally, it is the plant chemicals that help to protect plant cells from environmental hazards or threats such as drought, UV exposure, pollution, stress and pathogenic attack (Gibson, 1998; Mathai, 2000). Phytochemicals that are recognized for their health potentials include phenolic compounds (i.e., flavonoids, phenolic, phytoestrogens), carotenoids, phytosterols and phytostanols, organosulfur and nondigestable carbohydrate compounds (Rodriguez et al., 2006; Saxena et al., 2013). The health related properties of bioactive phytochemicals such as carotenoids and phenolic are believed to be due to their antioxidant activity (Prior & Cao, 2000). Antioxidant activity inhibits the oxidation of molecules caused by free radicals and hence important for dairy food for the shelf life of the product and to provide protection for human body from oxidative damage upon consumption (Alenisan et al., 2017). Phytochemicals can be isolated and characterized from fruits, vegetables, grains, legumes, spices, beverages such as green tea and red wine and numerous other sources (Doughari & Obidah, 2008; Doughari et al., 2009).

6.2 Applications of bioactive phytochemicals in yogurt

Owing to consumer's preferences and demands for functional foods, bioactive phytochemicals from various sources are progressively being applied as an ingredient to improve quality traits, nutritional and therapeutic properties (Alenisan et al., 2017; Granato, Santos et al., 2018). Phytochemicals can be introduced in yogurt in the form of essential oil or plant extract. The present findings by Azizkhani and Tooryan (2016), suggested that adding zataria, basil, or peppermint essential oil into probiotic yogurt formulation could improve the potential functionality of the product and also provide an inhibitory effect against *Listeria monocytogenes* and *Escherichia coli*. The additions of lemongrass leaves and stem into yogurt have improved the physicochemical properties as well as sensory characteristics of the yogurt (Shaaban, 2010). Apart from that, they also play role for decontamination from mycotoxigenic fungi and mycotoxins formation in yogurt. Some *in vitro* studies also showed that phytochemicals in spices significantly enhanced the growth of probiotics while inhibiting pathogens in yogurt (Be et al., 2009; Sutherland et al., 2009). Guava (*Psidium guajava*) leaf extract was supplemented in functional yogurt made from a skimmed buffalo's milk as a source of phenolic compounds and natural antioxidant (Ziena & Abd-Elhamid, 2009). The water extract of guava leaf showed changes in titratable acidity and pH during 5 days of cold storage but did not influence any deterioration effect in the organoleptic properties and the storage ability. Sun-Waterhouse et al., (2013) developed a drinking yogurt with supplementation of blackcurrant berry as a source for polyphenols (i.e., flavonols, flavanols, anthocyanins, proanthocyanidins, hydroxybenzoic acids and hudroxycinnamic acids). Polyphenols hold potential heath promoting properties such as antioxidant, reducing muscle fatigue and increasing peripheral blood flow. Blackcurrant berry can be incorporated into drinking yogurt to add flavour and provide antioxidant properties in form of juice or an extract (higher polyphenol content) during pre- or post-fermentation. Blackcurrant polyphenols added during pre-fermentation of yogurt resulted in polyphenolic metabolism to small phenolic molecules and 3.5-9.5 times the total extractable polyphenol content value of drinking yogurt was obtained when blackcurrant polyphenols was added during post-fermentation. The presence of polyphenols also influenced the appearance, growth and survival rate of *Streptococcus* and *Lactobacillus* yogurt starter cultures. Meanwhile, yogurt fortified with *Azadirachta indica* (neem) showed higher antioxidant effect with higher total titratable acid and lower pH than that observed for the plain yogurt during 28 days of cold storage period (Shori & Baba, 2013). *A. indica* yogurt also showed considerably high inhibitions for α-amylase, α-glucosidase and angiotensin-1 converting enzyme and hence has a great potential to be further developed as a functional yogurt targeted for consumers with diabetes and hypertension. Table 4 summarized several other plants that were applied for formulation of functional yogurt rich of various phytochemical components.

6.3 Bioactive phytocemicals form fruit waste and its application in yogurt

Formulation of functional foods is also directed towards the use of fruit processing wastes as they are rich in bioactive compounds and dietary fibres besides could serve as practical and economic sources of antioxidants (Reddy et al., 2007). High antioxidant activity in yogurt may be favourable in term of reducing lipid oxidation process that might be responsible for unwanted chemical compounds and formation of undesired flavour (Berset et al., 1994). Pomegranate peel extracts was used in the formulation of functional stirred yogurt owing to its therapeutic properties for treating of various illness such as fever, diarrhea, malaria, bronchitis, urinary tract infection and vaginitis (El-Said et al., 2014). The addition of pomegranate peel extract in the yogurt prior to inoculation with yogurt starter cultures resulted in higher antioxidant activity than that measured in yogurt added with pomegranate peel extracts after the inoculation step of starter cultures. Pomegranate peel extracts had no significant effects on the flavour, appearance, body and texture but decreased the viscosity of yogurt when added at concentration of above 25%. Recently, pineapple waste was formulated into a functional yogurt aimed to establish prebiotic potential, antioxidant as well as antimutagenic properties (Sah et al., 2016). The inclusion of oven and freeze dried peel and pomace of pineapple powder increased the cell counts of three probiotic strains (*L. acidophilus*, *L. casei* and *Lactobacillus* spp. *paracasei*) by 0.3 to 1.4 log cycle. The soluble peptide extracts of yogurt samples showed high antioxidant activity via *in-vitro* assays and exhibited antimutagenic activity when tested against mutagenicity effect of sodium azide on *S. typhimurium*. Meanwhile, Marchiani et al., (2016) utilized grape skin flour from grape pomace as a source for polyphenolic compounds in yogurt that prepared by UHT whole milk and YO-MIX 401 starter culture (a mixture of *S. thermophiles* and *L. delbruckii* subsp. *bulgaricus*). Yogurt fortified with grape skin contained higher phenolic content (+55%), antioxidant acitivity (+80%), acidity (+25%) but lower pH, syneresis (-10%) and fat (-20%) than that obtained in the control yogurt. Sensory analysis revealed that the yogurt fortified with grape skin showed a loss of textural quality.

7. Conclusions and future aspects

Yogurt has always been one of the vital players in the spectrum of fermented food products that transform science and technology into health and wellness through diet. The science to develop functional yogurt with specific quality and potential benefits must recognize the complex biology underlying four main features which are milk, bacteria, functional components and consumers. As probiotics and prebiotic industries are flourishing, consumers are more likely to invest on products with the highest quality and benefits. The growing interest and undeniable roles played by both probiotic and prebiotic in improving functionality of the products, enhancing sensory characteristics and extending the shelf life by inhibiting pathogens have nourished their combination as synbiotic yogurt. Moreover, the relationship of food and well-being is further enhanced with the incorporation of bioactive phytochemicals in yogurt varieties to act as functional components for health maintenance. Taking into consideration of the fast evolution of functional yogurts either at research stage or marketplace, further development would demand an accurate measure of the quality, safety and efficacy to meet consumer's expectations on quality and claimable health benefits. The confirmation of health promoting properties and efficacy would involve a broader range of research from *in vitro* experiments to *in vivo* and clinical studies.