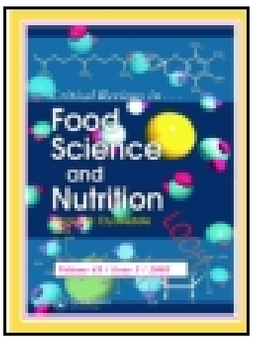

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The underexposed role of food matrices in probiotic products: reviewing the relationship between carrier matrices and product parameters

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Abstract

Probiotic microorganisms are increasingly incorporated into food matrices in order to confer proposed health benefits on the consumer. It is important that the health benefits, sensory properties, shelf-life and probiotic gastrointestinal tract (GIT) survival of these products are carefully balanced as they determine functionality and drive consumer acceptance. The strain-specific effects of probiotic species are imperative in this process but carrier matrices may play a pivotal role as well. This study therefore recapitulates the wealth of knowledge on carrier matrices and their interaction with probiotic strains. The most substantiated carrier matrices, factors that influence probiotic functionality and matrix effects on shelf-life, GIT survival and clinical efficacy are reviewed. Results indicate that carrier matrices have a significant impact on the quality of probiotic products. Matrix components, such as proteins, carbohydrates and flavoring agents, are shown to alter probiotic efficacy and viability. In vivo studies furthermore revealed strain-dependent matrix effects on the GIT survival of probiotic bacteria. However, only a limited number of studies have specifically addressed the effects of carrier matrices on the

aforementioned product-parameters; most studies seem to focus solely on the strain-specific effects of probiotic microorganisms. This hampers the innovation of probiotic products. More human studies, comparing not only different probiotic strains but different carrier matrices as well, are needed to drive the innovation cycle of probiotic products.

Keywords

probiotics; carrier matrix; shelf-life; gastrointestinal survival; efficacy; product quality

ACCEPTED MANUSCRIPT

1. Introduction

Fermented foods have played an indispensable role in ancient cultures and have been widely acknowledged across continents. Fermentation processing generally allowed for longer conservation and enhanced sensory properties (Caplice & Fitzgerald, 1999), which made fermented foods immensely popular. The consumption of fermented milk was later associated with potential health benefits and has been recommended for intestinal problems ever since. But it was not until the early 1900s, that Elie Metchnikoff first theorized that host-friendly bacteria found in yogurt could promote health through manipulation of the intestinal microbiome (Metchnikoff, 1908). These microorganisms are known today as probiotics and fuel a multi-billion-dollar industry (Global Market Insights, 2016).

The World Health Organization (WHO) defines probiotics as "life microorganisms which when administered in adequate amounts confer a health benefit on the host." The vast majority of probiotics are lactic acid bacteria (LAB) such as lactobacilli and bifidobacteria. Some characteristics of probiotics are general such as colonization resistance, competitive exclusion of pathogens, increased turnover of enterocytes and short-chain fatty acids production, whereas immunological, neurological and endocrinological effects are deemed more strain-specific (Hill *et al*., 2014). Probiotic bacteria are being incorporated into numerous carrier matrices and a large variety of probiotic products are available on the commercial market (Champagne *et al*., 2005). Probiotic bacteria are for instance added to frozen yogurts, cheeses, chocolates, cereal bars and juices, but most probiotic products are fermented milks and dairy-based products or lyophilized powders and capsules (Champagne *et al*., 2005).

The safety and potential health benefits of probiotic products have been studied extensively. Probiotic administration is generally recognized as safe (FDA, 2016; Borriello *et al*., 2003; Van den Nieuwboer *et al*., 2014a; Van den Nieuwboer *et al*., 2014b; Van den Nieuwboer *et al*., 2015) and a reduced risk for antibiotic-associated diarrhoea (AAD), necrotizing enterocolitis (NEC) and inflammatory bowel disorders (IBD) is consistently observed in clinical research (Sanders *et al*., 2013; Floch, 2014). However, the European Food Safety Authority (EFSA) deems the scientific evidence to be insufficient to prove a cause and effect relationship between consumption of probiotic products and any health benefit (Agostoni *et al*., 2013). Fueling this problem are the strain-specific effects of probiotics which make it difficult to draw generalizable conclusions on probiotic effectiveness en masse. Current trends in the food industry therefore show a strong focus on strain specificity of probiotics.

Where the benefits of the probiotic matrix once stood central, increased attention for specific strains and their disease targets now leaves the effects of the matrix underexposed (Sanders & Marco, 2010). Sensory properties and ease of use are vitally important aspects of probiotic matrices as they drive consumer acceptance. But it is suggested that matrices may also affect probiotic shelf-life, gastrointestinal tract (GIT) survival and clinical efficacy (Sanders & Marco, 2010). Furthermore, the matrix itself can have a beneficial effect on the host (Sanders & Marco, 2010). All these parameters need to be carefully balanced in probiotic products and carrier matrices may play a vital role in this process. Insufficient attention to the probiotic -- matrix relationship therefore forms a barrier to innovation and calls for new guidelines (Van den Nieuwboer *et al*., 2016).

This paper sets out to recapitulate the wealth of knowledge on probiotic bacteria and their relationship with carrier matrices. It aims to provide a frame of reference for matrices and their interaction with probiotic species and compares matrices with regard to shelf-life, GIT survival and clinical efficacy.

2. Methodology

The present study aims to review two primary aspects of probiotic matrices. The respective benefits and trade-offs of the most substantiated probiotic matrices for both host and microorganism are reviewed first (such as protection through the GIT and impacts on probiotic viability). Matrices are then compared in a systematic way with regard to shelf-life, GIT survival and clinical efficacy. To this end, the online databases PubMed (National library of Medicine, including MEDLINE) and ScienceDirect were used as sources for the search. Eligible articles were retrieved using the search terms ‘probiotics’ in different combinations with ‘matrix’, ‘storage’, ‘viability’, ‘survival’, ‘formulation’, ‘food formats’, ‘carrier’, ‘efficacy’, effectiveness’, ‘shelf-life’ and ‘delivery vehicle’. For the comparison of matrices regarding GIT survival and clinical efficacy, solely human clinical trials were included, thereby excluding all animal studies. Furthermore, all studies in English evaluating a probiotic strain in two or more matrices were deemed eligible for this study.

3. Important product parameters

Two aspects are highly important in any probiotic product. First of all, the characteristics of the probiotic strain itself and secondly the characteristics of the matrix that can be either positive or negative for both strain and host. Although probiotic strains often share common characteristics such as the ability to competitively exclude pathogens or increase the turnover of enterocytes,

their clinical effects are highly diverse and often strain-specific (Hill *et al*., 2014). In order for probiotics to be fully effective, the microorganisms should maintain their viability throughout the GIT transit. First, the microorganisms are exposed to a highly acidic environment in the stomach, where subsequently proteolytic enzymes and bile can significantly impact survival in the small intestines. Proceeding more distally, there is a harsh competitive environment for nutrients with the endogenous microbiota (Fontana *et al*., 2013). Probiotic bacteria are often poor colonizers, and during the transient presence in the GIT, they should exert their potential benefits. Probiotics not only encounter harsh conditions when consumed, but also face various challenges during production, down-stream processing and storage (Figure 1) (Makinen *et al*., 2012). The ability of a strain to cope with stressors such as temperature, oxygen and acid are important in the selection process of appropriate strains (Mills *et al*., 2011). Indeed, different responses to stressors between species are well documented (Corcoran *et al*., 2007; Van de Guchte *et al*, 2002). Other important characteristics that contribute to the potential health benefits of a probiotic product are the concentration of intake, the growth phase of the cells (stationary or logarithmic) and the activity of the probiotic strain in terms of interactions with the host and microbiota (Rivera-Espinoza & Gallardo-Navarro, 2010). It is suggested that the final probiotic product should contain at least 10^6-10^7 colony-forming units (CFU)/g of the probiotic bacteria, or a total of 10^8-10^9 CFU in order for it to exert a therapeutic effect (based on a minimal daily consumption of 100 g or 100 ml food) (Jayamann & Adams, 2006; Talwalker & Kailasapathy, 2004).

There are two ways to incorporate probiotic bacteria into a food product. Via the first route, probiotics are grown into the final product where the product is fermented in the process (e.g. milk to yogurt). Through the second route, dried or encapsulated probiotic microorganisms

are added to the product (e.g. infant formulae and juices; Makinen *et al*., 2012). Fermentation temperature and oxygen exposure have a significant impact on probiotic growth during the processing stage. Intra- and extracellular damage may occur during freezing for example due to ice crystal formation. During thawing, osmotic effects and hazardous components in the melting matrix may cause cellular damage to the probiotics as well. Given that the definition of probiotics dictates the micro-organisms should be alive, one of the key issues concerning the formulation of probiotics is their loss in viability after processing and during storage.

During storage, a range of factors affect the viability of probiotic cells significantly (Figure 1). The most critical factors are (i) food ingredients and additives, (ii) temperature, (iii) pH, (iv) water activity, (v) oxygen contents and redox potentials, (vi) packaging aspects and (vii) competing bacteria (Triphati & Giri, 2014). Food ingredients and additives can play a beneficial, neutral or harmful role in the survival of probiotic cells. Artificial flavors, coloring agents and salting negatively influence probiotic stability, whereas certain carbohydrates promote growth. The presence of carbohydrates that are easily metabolized ensures survival in acidic conditions for example (Corcoran *et al*., 2005) or may increase the viability of probiotics during freezing and thawing (Tripathi & Giri, 2014). The presence of oxygen in the product is negatively correlated with probiotic viability since most probiotics are anaerobic. Peroxides, free radicals and direct oxygen toxicity may result in cell damage, depending on the oxygen sensitivity of the probiotic strain. Water activity (α_w) and storage temperature also significantly impact probiotic survivability. The optimum storage temperature depends highly on the matrix and the probiotic strain. Nevertheless, low temperatures seem to result in lower viability losses, where freeze-dried probiotics are most stable when frozen. High acidity (low pH) significantly hampers the survival

of probiotic cells, although certain probiotic strains are better equipped to deal with acidic environments. Lactobacilli tolerate lower pH levels than bifidobacteria for instance. Their optimum pH for growth is between 5.5-6.0 and 6.0-7.0 respectively (Rivera-Espinoza & Gallardo-Navarro, 2010). Packaging aspects such as light- and oxygen permeability influence microorganism survival as well. Materials with low permeability (e.g. glass bottles) are superior over polymeric containers (Tripathi & Giri, 2014). Various bacteria in the final product (e.g. starter cultures) compete for nutrients and produce bacteriocins. It is important for the probiotic to become the dominant strain, otherwise growth and survival can be significantly affected.

It should be noted that the factors mentioned above might not only affect viability, but can also influence the geno- and phenotype of cells in response to their environment. This could be the reason why for instance heterogeneous strains are found in commercial probiotic products lacking important adhesion molecules (Sybesma *et al*., 2013). The so-called gene decay due to nutrient abundance, as found in dairy products for example, can have a significant impact on functionality (Kort & Sybesma, 2012). Furthermore, addition of compounds such as flavoring to a probiotic yogurt, may alter the gene expression of cells, thereby altering the potential probiotic effect (Reid, 2015; Bisanz *et al.*, 2014).

Probiotic cells can be protected in the production process by the addition of cell protectants such as glycerol, skimmed milk powder and dextran. By reducing the osmotic difference with the environment or stabilizing the cell membrane, cryoprotectants can enhance survival during manufacturing processes (Tripathi & Giri, 2014). Other high potential protectants seem to be gum Arabic, gelatin and pectin. When prebiotics are added, they function as micronutrients and metabolic substrates thereby enhancing survival and activity throughout the

GIT transit. By selecting a suitable prebiotic compound (e.g. *Plantago psyllium* or inulin), viability during storage and protection under gastric conditions can be enhanced (Peredo *et al*., 2016). Another technique to enhance probiotic survival in a matrix is through microencapsulation which entails the enclosing of probiotic cells in polysaccharides such as alginate, gums, chitosan, milk or whey proteins and fats (Chen *et al*., 2015; El-Salam & El-Shibiny, 2015). Other components have been successfully employed for encapsulation and many new substances are being investigated (Champagne & Fustier, 2007). Microencapsulation successfully protects the probiotic cells from the detrimental stressors during the manufacturing process, storage and GIT transit (Chen *et al*., 2015; Tripathi & Giri, 2014).

4. Available probiotic products

Probiotics are available in a wide variety of commercial products but fermented-dairy based products tend to dominate the market. In 2005, probiotic fermented dairy products yielded over 1 billion euro in sales in Europa alone (Saxelin *et al*., 2005). Other natural fermented and non-fermented probiotic foods are considered for marketing due to their pleasurable sensory characteristics and potential as carrier matrix. The rapid growth of this market also lead to the introduction of unsubstantiated and scientifically less credible probiotic products such as mattresses, pizzas and probiotic shampoos (Foligné *et al*., 2013). These so-called “pirate” probiotics harm the reputation of well-substantiated probiotic products and may disrupt the innovation cycle of probiotics and should therefore be strictly regulated by regulatory authorities (Van den Nieuwboer *et al*., 2016). In Table 1, a selection of substantiated probiotic products is portrayed. The most evaluated matrices, including fermented milks/yogurts, cheese, sausages,

ice-creams, fruit and vegetable juices, oats and cereals and freeze-dried probiotics are discussed in further detail.

4.1 Fermented Milk (Yogurt)

Fermented milk and yogurt products have been the most prominent carriers for probiotic strains and possess various favorable characteristics (e.g. the ability to buffer the acidity of the stomach and enhance probiotic viability; Varcoe *et al*., 2002). Furthermore, probiotic milk and yogurt products have been studied extensively, thereby increasing the rational for their use.

Milk contains whey proteins (β-lactoglobulin, α-lactalbumin), caseins (αs1, αs2, β, and κ), immunoglobulins, bovine serum albumin, lactoferrin lactoperoxydase, alkaline phosphatase, catalase and plasmin (Livney, 2010; Burgain *et al*., 2014). In addition, milk also contains fat globules consisting of triacylglycerol and other components (e.g. carotenoids, vitamins A, D, E, and K) and active molecules such as: phospholipids, sphingolipids, cholesterol and milk fat globule membrane proteins. Milk is furthermore rich in minerals such as calcium, inorganic phosphate, citrate and other minerals (e.g. magnesium, sodium, potassium, and chloride). Water-soluble vitamins such as vitamin C, B1 and B2 are also found in dairy products (Burgain *et al*., 2014). Lactose (α-lactose or β-lactose) is the major disaccharide found in dairy products, and serves as a substrate for probiotics, thereby potentially enhancing efficacy (Varcoe *et al*., 2002). It seems that bacteria exhibit affinity to the milk fat globules, which could offer protection through GIT transit (Burgain *et al*., 2014). For instance, in simulated gastric conditions a higher fat and whey protein content results in significantly higher probiotic cell counts (Ziarno & Zareba, 2015). The probiotic strain L. *casei* Zhang furthermore showed higher viability during a stimulated GIT transit in fermented milk than in saline solution (Wang *et al*., 2012). Components

of milk itself can have beneficial clinical effects as well. Exemplifying this phenomenon is a study by De Vrese *et al*. (2011), where milk in combination with probiotics or acidified with lactic acid were both found to significantly reduce *Helicobacter pylori* activity.

L. kefir, L. brevis, L. casei, L. delbrueckii subsp. *Bulgaricus, S. thermophiles, Lc. lactis* subsp. *Lactis, B. bifidum, B. animalis, B. lactis, Ln. mesenteroides* subsp. *Dextranicum and Ln. mesenteroides* subsp. *Cremoris* are common starter cultures for the fermentation of dairy products (Heller, 2001) At 40-45°C the synergistic starter cultures *S. thermophilus* and *L. bulgaricus* produce lactic acid, galactose, free amino acids and fatty acids in the fermentation process and reducing the pH to 3.7-4.3 (Lourens-Hattingh & Viljoen, 2001). During fermentation, folic acid, niacin and riboflavin levels are increased by the lactic acid bacteria (Parvez *et al*., 2006). Probiotics are either incorporated together with the starter cultures, mixed with the starter cultures after fermentation or fermented solely by the probiotic culture (Soccol *et al*., 2010). In the latter, deviating manufacturing procedures might be necessary to prevent undesirable flavors.

Probiotic viability in fermented milks seems stable for up to 4 weeks at 5°C. Viable counts above 10^8 CFU/g have been reported (Rutella *et al*., 2016; Vinderola *et al*., 2011), where the addition of skimmed milk powder (10-15%) may further enhance probiotic survival (Maganha *et al*., 2014). In general, 4 to 6 weeks of storage with sufficient CFU/ml can be achieved in refrigerated conditions (Makinen *et al*., 2012). Signs that milk provides protection through the GIT is demonstrated by Lee *et al*. (2015), since probiotic bacteria supplemented in milk led to higher GIT survival rates in mice than probiotics supplemented in a nutrient-free buffer. Furthermore, milk enhanced the expression of genes involved in metabolism and

membrane biogenesis (Lee *et al*., 2015). The attenuation of colitis in a mouse model by a probiotic strain also seems dependent on the addition of milk. There was a significant lower change in body weight in colitis induced mice after administration of L. *casei* BL23 in milk compared to the strain in a phosphate-buffered saline solution (PBS) (Lee *et al*., 2015). One should however be aware of the use of animal models, as these models often have a poor translation to humans. Clinical efficacy of probiotics in milk and yogurt matrices has been well documented for a large variety of conditions including: constipation, AAD, necrotizing enterocolitis and *Helicobacter Pylori* infection (Barclay *et al,* 2007; Koebnick *et al*, 2003; Sachdeva & Nagpal, 2009., Wenus *et al*, 2008).

Downsides of using fermented milk as a probiotic carrier relate to its low pH, its ability to hamper specific growth of bifidobacteria and the aerobic conditions of production and packaging (Boylston *et al*., 2004). In addition, refrigeration is necessary to maintain viability of bacterial strains and dairy stability.

4.2 Cheese

Cultured dairy products such as cheeses are a popular component in the diets of many cultures and possess several beneficial characteristics. In addition to containing similar nutrients as milk, cheeses contain para-k-casein and other casein hydrolysates as well (produced during rennet activity in the production process). Together with the proteolytic activity of cheese starter cultures, para-k-casein and casein hydrolysates have been suggested as growth-promoting factors for bifidobacteria (Boylston *et al*., 2004). Cheeses are proposedly good carriers for probiotics, in part because they exhibit higher pH levels than yogurt products (4.8-5.6 to 3.7-4.3, respectively). The beneficial fat content (including butyric, palmitic and stearic acids) furthermore offers

protection during the GIT transit. In addition, during ripening cheeses become more anaerobic, favoring persistence of anaerobic probiotic organisms such as bifidobacteria (Boylston *et al*., 2004).

During the development of cheese, rennet and LAB are added to milk, facilitating the coagulation of caseins. Subsequently this curd is heated and pressed, depending on the cheese type. Finally, during the ripening stage, the lipolytic and proteolytic properties of the LAB are responsible for the texture and flavor of the cheese (Boylston *et al*., 2004). Successful incorporation of probiotic strains into cheeses depend on the respective strain, competition with other microorganisms and their ability to respond to high concentrations of salt. Salt content of the cheese is inversely related to the viability of the probiotic bacteria. Furthermore, processing and ripening conditions influence probiotic viability in the final product (Boylston *et al*., 2004). The following probiotic strains have been successfully added into various types of cheeses: *L. acidophilus, L. rhamnosus, Lactobacillus plantarum, L. casei, L. paracasei, L. gasseri, B. bifidum, B. animalis* ssp. *lactis, B. infantis, B. longum and Propionibacterium freudenreichii* ssp. *Shermanii (*Karimi *et al*., 2011). The viability of probiotics in cheese seems promising as $>10^6$/g CFU are retrievable up to 84 days. In a cheddar cheese containing *L. paracasei* NFBC 364 and NFBC 338, probiotic levels around 1×10^8 CFU/g were achieved after 8 months of ripening (Gardiner *et al*., 1998).

Clinical efficacy of probiotics in a cheese matrix has been mainly evaluated in dental care, focusing on the reduction of unwanted oral microorganisms. For instance, probiotic cheeses have been shown to reduce *Streptococcus mutans* and oral *Candida* counts (Ahola *et al*., 2002; Hatakka *et al*., 2007; Jose *et al*., 2013). The cheese matrices allowed survival of probiotic strains

through the GIT, represented by the number of retrievable bacteria from feces (Lahtinen *et al*., 2012; Songisepp *et al*., 2012). However, this was not the case for all participants (Sharafedtinov *et al*., 2013) and it should be noted that this effect is highly strain-dependent. Besides dental health, probiotic cheeses have also been shown to be partly efficacious in the eradication of *Helicopbacter pylori*, reduction of *Clostridium difficile*, reduction of constipation symptoms and lowering blood pressure and body-mass index (Boonyaritichaikij *et al*., 2009; Lahtinen *et al*., 2012; Sharafedtinov *et al*., 2013; Favretto *et al*., 2013; Hütt *et al*., 2015).

4.3 Ice-cream

Ice-cream is a popular product by all age groups due its pleasant sensory and refreshing characteristics. The frozen mixture contains valuable matrix components for probiotic cultures such as milk proteins, fat and lactose, as well as less essential compounds such as sweeteners, stabilizers and emulsifiers (Cruz *et al*., 2009).

Ice-cream is produced by the pasteurization of milk in combination with additional compounds such as: milk powder, sugar and stabilizers. The mixture is cooled to 37-40°C and fermented by starter probiotic cultures. Subsequently the mixture is kept for 24 h at 4°C to mature. By beating the mixture, air is incorporated into the product after which it is frozen to create the final product (Cruz *et al*., 2009).

The following factors of ice-cream significantly influence the viability of probiotic cultures: stress induced by freezing and the formation of ice crystals, high oxygen content and low pH (4.7-4.8). Probiotic cultures can rapidly lose their viability at low temperatures, as formed ice crystals rupture cell membranes. Rapid freezing is correlated with a higher survival rate. In general, the freezing process reduces viability by at least 1 log. Oxygen incorporated by

beating the mixture, although important for sensory characteristics, is toxic for anaerobic probiotic strains, in particular for bifidobacteria. Nevertheless, viability of *L. rhamnosus* GG remains high up to one year (10^8 CFU/g) at low temperatures (-16°C and -28°C; Alamprese *et al*., 2005). Lower counts were found for *L. acidophilus* and *B. bifidum* after 17 weeks of storage (4×10^6 and 1×10^7 CFU/ml, respectively). Ice-cream seems promising with regard to long term storage due to the low temperatures, however, manufacturing, processing and thawing may have a significant impact on the functional efficacy of probiotics (Ranadheera *et al*., 2010).

Whether probiotic cultures still demonstrate therapeutic efficacy after storage in ice-cream remains to be determined. Nevertheless, L. *rhamnosus* GG showed reduced adherence to Caco-2 cells after 7-day storage in an ice-cream matrix (Deepika *et al*., 2011). Consumption of probiotic ice cream is associated with a significant reduction of salivary *Streptococcus mutans* levels, even after a wash-out period compared to the baseline and placebo (Caglar *et al.,* 2008; Singh *et al.,* 2011; Chinnappa *et al.*, 2013; Mahantesha *et al*., 2015; Nagarajappa *et al*., 2015)

4.4 Probiotic meat products

Although the consumption of meat and processed meats has been associated with a higher risk of developing chronic diseases, including cardiovascular disease and cancer, its role as a carrier for probiotic strains is explored by the meat industry (Khan *et al*., 2011). Meat is frequently consumed, has a high diversity of presentations and contains a source of functional ingredients. Meat contains many nutritional components, such as essential proteins and amino acids, conjugated linoleic acid, minerals (e.g. iron, zinc and selenium), l-carnitine, histidyl dipeptides (carnosine and anserine), creatine, taurine, vitamins (B, E), glutathione, ubiquinone and lipoic

acid (Jofré *et al.*, 2015), making them suitable carriers for delivering bioactive compounds (Olmedilla-Alonso *et al*., 2013).

Meat can serve as a good vehicle for probiotic growth and survival through the GIT due to encapsulation by the meat matrix and fat, thereby protecting the bacteria from low pH and bile salts (Khan *et al*., 2011; Rouhi *et al*., 2013). Rubio *et al.* (2014), demonstrated that L. *rhamnosus* CTC1679 in fermented sausage was retrievable in feces at prominent levels (> 7 log CFU/g) up to three days after consumption, demonstrating its ability to survive the GIT passage (Rubio *et al*., 2014). Jahreis *et al*. (2002) demonstrated a significant increase in numbers of L. *paracasei* LTH 2579 after feeding 50 g of probiotic sausage for several weeks, but not in all volunteers. Probiotics are incorporated into meat sausages through several steps including fermentation and drying. Ingredients and probiotic starter cultures are mixed and stuffed into casings and subsequently the sausages are fermented between 22-40°C. This processing step of minimal heating allows survival of probiotic strains. Finally, the sausage is subject to post fermentation steps, including drying and/or smoking, to influence flavor and texture (Rouhi *et al*., 2013).

L.. casei, L. curvatus, L. pentosus, L. plantarum, L. sakei, *Pediococcus acidilactici* and *P. pentosaceus* are commonly used as starter cultures in sausages (Rivera-Espinoza & Gallardo-Navarro, 2010). The viability of probiotic strains in fermented sausages is high, 10^4-10^7 CFU/g for up to 60 days after ripening, storage and drying in salami (Rouhi *et al*., 2013). However, this is heavily dependent on selected strains, fermentation process, ripening and drying procedures. Furthermore, the use of probiotic strains in fermented sausage seems limited due to challenges in the harsh environment of the matrix. Factors such as low pH, water activity, curing salts, temperature, oxygen content, moisture content, salt, sugar, additives and competing

microorganisms present a challenging environment for survival. But as fermented meat products contain a high "natural" microbiota and the product is not heated, probiotic cultures should be able to become dominant in the final product. In addition, the selected strains should exert a certain lipolytic and proteolytic activity to produce aroma components for the desirable sensory quality (Rouhi *et al*., 2013; De Vuyst *et al*., 2008).

Little is known regarding the health promoting effects of probiotic strains in fermented sausages. Moderate changes in immunity, blood cholesterol and triglyceride levels were observed, although not significant (Jahreis *et al*., 2002).

4.5 Fruit and vegetable juices

The use of fruit and vegetable juices for probiotic administration is appealing since they are generally perceived as healthy and have a pleasant sensory profile. Furthermore, fruit and vegetable juices are rich in fibers, vitamins, minerals, flavonoids and antioxidants (Yoon *et al*., 2004). Another benefit of fruit and vegetable juices as a probiotic matrix is the lack of dairy allergens, making it more accessible to a sizeable portion of consumers. Furthermore, fermentation of flavonoids leads to desirable antioxidant activity for the host (do Espírito Santo *et al*., 2011). Fruit and vegetable juices are difficult matrices for probiotics to survive in due to high acidity, oxygen content, natural growth inhibitors and additives. Regardless, some probiotic strains have shown growth potential in juice matrices (Champagne & Gardner, 2008). Lactobacilli and Leuconostocaceae (*Leuc*. *mesenteroides*) are the most common probiotic species found in natural vegetable fermentation (Cleveland *et al*., 2001; Yan *et al*., 2008) but various probiotic bacteria have been added to juice matrices based on their desired clinical effects.

High viability (10^6 CFU/ml) of L. *rhamnosus* (LB11), *L. reuteri* (LB38), *L. fermentum* (LB32), *L. plantarum* (LB42), *L. casei* and *L. paracasei* was seen for up to 80 days in refrigerated storage in orange- and pineapple juice (Champagne & Gardner, 2008; Sheenan, Ross & Fitxgerald, 2007; Rivera-Espinoza & Gallardo-Navarro, 2010). Storage however, did influence probiotic tolerability to simulated GIT stress (Champagne & Gardner, 2008). In some vegetable juices, viability after storage is similar to milk/yogurt products, as 10^6 -- 10^8 CFU/ml of probiotic bacteria were found after 4 weeks of storage in tomato juice at 4°C (Yoon *et al*., 2004). Several bifidobacteria and lactobacilli seem to tolerate fruit juice as a carrier matrix well, whereas others perform poor (do Espírito Santo *et al.*, 2011). Due to the high acidity of fruit juices, one is limited to the selection of probiotic strains that are tolerant of acidic environments. Furthermore, addition of probiotic strains to fruit and vegetable juices can lead to undesirable aromas and flavors (Rivera-Espinoza & Gallardo-Navarro, 2010). It should be noted that there are a lot of different fruit and vegetable juices, all differing in their content making them more or less suitable as a probiotic carrier matrix. For instance, cranberry juice results in poor probiotic survival due to a highly acidic and antimicrobial environment (Shori, 2016). Carrot juice seems promising, as specific probiotic strain were found in high concentrations after 12 weeks of refrigerated storage (Shori, 2016).

The number of clinical trials performed with probiotic fruit and vegetable juices is limited, and usually entails milk-based fruit juices or addition of fruit to another matrix (e.g. Lönnermark *et al.*, 2010; Caglar, 2014; Schrezenmeir *et al*., 2004). However, a human challenge trial with experimentally induced rhinovirus colds demonstrated that L. *rhamnosus* GG in fruit

juice led to lower, but not statistically significant, occurrence and severity of symptoms compared to spray-dried, heat inactivated L. *rhamonsus* GG (Kumpu *et al*., 2015).

4.6 Oats and cereals

Cereals are produced and consumed around the globe, making cereal-based probiotic products easily accessible to a large population. Cereal grains contain proteins, carbohydrates, vitamins, starch, water soluble fibers, oligosaccharides, phytoestrogens, phenolic compounds, antioxidants, phytic acid and sterols. In addition, β-glucan, which has a hypocholesterolemic effect, is found in high concentrations in this matrix (Soccol *et al*., 2010). Furthermore, the concentration of certain vitamins and fibers is higher in cereals than in milk (Gupta & Abu-Ghannam, 2012). Fermentation of cereals may also represent a way to obtain a rich substrate that sustains the growth of beneficial microorganisms, thereby exerting a prebiotic effect (Shori, 2016). Oats and cereals are highly variable in their structure thereby affecting the function of microorganisms. Nevertheless, it seems that many cereals, such as malt, barley and wheat, support the growth and resistance of probiotic bacteria in stressful conditions (Rivera-Espinoza & Gallardo-Navarro, 2010).

Many products come in the form of an oat- or cereal based drink or as cereals with dried probiotic cells sprayed on top. In addition, many traditional products that might exert a probiotic effect are fermented cereal products (e.g. tarhana, togwa and pozol). A large variety of these products exist, all harboring different probiotic species with different characteristics (Rivera-Espinoza & Gallardo-Navarro, 2010). For instance, *L. plantarum, L.. fermentum, Leuc. mesenteroides* and *Sacch. cerevisiae* have been retrieved from fermented maize, sorghum and millet products in West Africa (Ijabadeniyi, 2007). In the Balkan Peninsula, strains of *L.*

fermentum, L. pentosus, L. paracasei and *L. rhamnosus* were isolated from the low-alcoholic beverage Boza (Todorov et al., 2008). Fermented dairy-based products enriched with oats and cereals are also frequently evaluated. However, this makes it difficult to determine whether the beneficial effect can be attributed to the milk compounds or the cereals. There are indications however, that the addition of cereals enhances probiotic growth in dairy matrices (Soccol *et al*., 2010).

Malt, barley, wheat and flours seem to support probiotic growth well above the level of 10^6 CFU/ml (Kedia *et al*., 2008). Furthermore, a fermented oat-based probiotic drink showed high viability of 10^{10} CFU/ml after 24 days of refrigerated storage (Gupta & Abu-Ghannam, 2012). Adding probiotics to a fluid or a dry food matrix poses different challenges. Ouwehand *et al.* (2004) demonstrated that *B. lactis* Bb-12 incorporated into a cereal bar survives the GIT transit. The strain could still be detected 1 week after consumption had seized.

The application of probiotics in cereals seem promising due to good viability and beneficial compounds, but has been underexposed. The variety in cereals complicate the matter. Various health benefits have been associated with cereal-based probiotic products but most clinical studies have been performed with traditional fermented cereal products that are only consumed by specific populations (Nyanzi & Jooste, 2012).

4.7 Drying techniques

Common technologies for the long-term preservation of probiotic bacteria are drying techniques such as freeze-drying (lyophilization), spray-drying, vacuum-drying and fluidized bed-drying. Although all techniques come with trade-offs, it is assumed that freeze-drying results in superior viability and functionality (Iaconelli *et al*., 2015). Freeze-drying starts with rapid freezing,

followed by removal of water by vacuum sublimation and a secondary resorption step (Broeckx *et al*., 2016). Key in the viability of probiotic bacteria is the freezing rate and temperature, whereby higher freezing rates result in less cellular damage. Vacuum drying is highly similar to freeze-drying, although samples are evaporated instead of sublimated, thereby operating at higher temperatures (Broeckx *et al*., 2016). During spray-drying, fluid samples are injected into a convective chamber with air at high temperatures (150°C). Thermal stress and dehydration are believed to negatively impact the viability of probiotics (Iaconelli *et al*., 2015). The fluidized bed-drying technique uses injection of heated gas into a bed of solid particles, however, it cannot be used as a sole drying technique.

Storage conditions and rehydration processes can significantly affect viability. Important are the temperature, moisture and oxygen content, powder composition and rehydration conditions (Ianconelli *et al*., 2015; Broeckx *et al*., 2016). Depending on these factors, freeze-dried supplements can be viable for up to two years, with an average of one log loss per year (in optimal conditions) (Makinen *et al*., 2012). Rehydration of dried products is necessary for revival of the probiotic cells. Important are the rate of recovery, temperature and volume of the media, where slow rehydration with small volumes at 15 -- 25°C results in the highest yield (Tripathi & Giri, 2014). Other important parameters are the rehydration solution, pH and osmolarity.

Lyophilized probiotic strains have been widely evaluated in clinical trials and seem to benefit treatment and prevention of GIT diseases, atopic dermatitis and necrotizing enterocolitis (Ritchie & Romanuk, 2012; Lin *et al.*, 2005; Rosenfeldt *et al.*, 2003). Lyophilized probiotic bacteria are also increasingly incorporated into infant formulas. Clinical efficacy of probiotic

supplementation in infants has been demonstrated for acute diarrhea, rotaviral gastroenteritis and necrotizing enterocolitis (Szajewska & Mrukowicz., 2001; AlFaleh *et al*, 2012).

A schematic overview of the benefits, limitations, contents and production processes of the previously discussed matrices is available as supplementary material in appendix 1.

5. Comparing probiotic matrices

The amount of clinical studies specifically evaluating two or more matrices in their potential as optimal carrier for probiotic administration is scarce, despite a potentially significant impact on probiotic shelf-life, GIT survival and clinical efficacy.

5.1 Shelf-life

Stability data for individual matrices is widely available for various probiotic strains. However, the comparison of multiple matrices with respect to shelf-life in identical conditions is less frequent (Champagne *et al*., 2005). Data on probiotic shelf-life, from clinical studies evaluating two or more matrices, is presented in Figures 2 and 3. Inter-study comparisons should be made carefully as different probiotic strains and methods were used. Phenotypic variations between species may result in strain-dependent matrix effects (Ranadheera *et al*., 2012).

Klu and colleagues (2012, 2014) evaluated the viability of freeze-dried probiotics (*L. acidophilus* (CUL 60), L. *acidophilus* (CUL21), *Bifidobacterium bifidum* (CUL 20), *Bifidobacterium lactis* (CUL 34) and L. *rhamnosus* GG) in two peanut butter matrices differing in fat content. In refrigerated conditions (4°C), both reduced fat (RF) and full fat (FF) peanut butter showed only minor decreases in probiotic viability (< 0.10 log CFU/g) after a storage period of 48 or 52 weeks (Klu *et al.*, 2012; Klu *et al.*, 2014) (Figure 2). At the same temperature,

Sareela *et al.* (2006) compared the viability of lyophilized *B. animalis* subsp *lactis* E2010 in fruit juice and pasteurized milk. Probiotic cell counts decreased with 1-2 log CFU/ml in fruit juice and with less than 1 log CFU/ml in pasteurized milk after two weeks of storage (Saarela *et al.*,2006). *B. animalis lactis* E2010 furthermore exhibited higher bile and acid resistance after two weeks of storage in milk compared to storage in fruit juice (Saarela *et al*., 2006). Pimentel and colleagues (2012) compared the viability of several cultured probiotic strains in milk and yogurt over a two-week period at 4°C. On average, the number of CFU/ml increased with 1 log in milk and decreased with 1 log in yogurt (Pimentel *et al.*, 2012) (Figure 2).

At room temperature (25°C), L. *rhamnosus* GG decreased with < 2 log CFU/gram in RF peanut butter and with of 2-3 log CFU/g in FF peanut butter after 48 weeks of storage (Figure 3) (Klu *et al*., 2012). But in a follow-up study with multiple probiotic strains, viability in both RF and FF peanut butter decreased with an average of 2-3 log CFU/g after 52 weeks of storage (Klu *et al*., 2014). Endo and colleagues (2014) studied the viability of freeze-dried and freshly cultured L. *rhamnosus* GG in various oils at 20°C (extra virgin olive oil, flaxseed oil, olive pomace oil and canola oil). Freeze-dried probiotics showed only minor decreases in viability (0.3-0.6 log CFU/ml) after 120 days of storage in various oils (Endo *et al*., 2014). When freshly cultured cells were added to olive oil however, the probiotic cells were undetectable after one week of storage. Freshly cultured cells suspended in canola and flaxseed oil were detectable up to two months (Endo *et al.*, 2014) (Figure 3).

At 37°C, viability reduction rates increased rapidly in a peanut butter matrix. However, RF peanut butter showed a significantly smaller decrease in probiotic cell counts after 20 weeks

of storage than FF peanut butter (<7 log vs. <4 log and 2.8-3.75 log vs. 2.5-3 log CFU/g) (Klu *et al*., 2012; Klu *et al*., 2014).

5.2.1 GIT Survival

Since matrices can offer probiotic strains vital nutrients, buffering properties and protection throughout the GIT transit, it is important to determine the GIT survival of probiotic strains in different carrier matrices. In simulated gastric conditions, dairy- and water-based products seem to outperform freeze-dried capsules (apart from VSL#3; Fredua-Agyeman & Gaisford, 2015). Data on the *in vivo* survival of probiotic bacteria is presented in Figure 4.

Saxelin *et al.* (2010) reported a strain-dependent matrix effect of yogurt on GIT survival, compared with cheese or capsule matrices, in a study where the GIT survival of four different probiotic strains was evaluated after 14 days of intervention. Higher fecal recovery rates were observed in the yogurt matrix for *B. animalis* BB-12 and *Propionibacterium freudenreichii shermanii* JS (Figure 4 A & B) but not for L. *rhamnosus* GG and L. *rhamnosus* LC705 (Figure 4 C & D). However, due to sizeable dosing differences between matrices these results should be interpreted with caution. Participants in the yogurt group consumed considerably more CFU of *B. animalis* BB-12 (1.4×10^{10} CFU/day) and *Propionibacterium freudenreichii shermanii* JS (7.5×10^{9} CFU/day) than participants in the cheese- (4.2×10^{7} - 1.2×10^{6} and 1.7×10^{9} CFU/day respectively) or capsule group (1.8×10^{9} and 4.2×10^{9} and CFU/day respectively). These differences in daily dosage could be the reason for the different fecal counts between matrices. Dosing differences were also observed for L. *rhamnosus* GG and L. *rhamnosus* LC705 (Saxelin *et al.*, 2010). Participants in the capsule group consumed more CFU of L. *rhamnosus GG* (5.2×10^{9} CFU/day) and L. *rhamnosus LC705* (7.4×10^{9} CFU/day) than participants in the yogurt- (4.7

$\times 10^9$ and 3.3×10^9 CFU/day, respectively) or cheese group (2.8×10^9 and 4.2×10^8 and CFU/day, respectively). Moreover, baseline values for L. *rhamnosus GG* were substantially higher in the capsule group than in the cheese or yogurt group. Rochet and colleagues (2007) investigated the survival of *B. animalis* DN-173 010 in lyophilized- or fermented format in 12 participants during a one week administration. Although the survival of *B. animalis* DN-173 010 was slightly higher in lyophilized form (22% vs. 20% survival), this difference was not significant (Rochet *et al*., 2007) (Figure 4 E). Doses were again higher in the lyophilized format (2.1×10^{11}CFU) than in the fermented format (6.6×10^{10} CFU). Collins *et al*. (2002) administered L. *salivarius* UCC118 for 21 days (10^{10} CFU/day) in fermented milk (n = 20) or in fresh milk (n = 20). Fecal excretion levels of L. *salivarius* UCC118 were found on average to be 15 times higher in fresh milk than in fermented milk (3.7×10^6 vs. 2.4×10^5 CFU/g feces, significance not determined) (Figure 4 F). In both groups the probiotic strain was still present 21 days after the last intake (4 vs. 1 subjects, favoring fresh milk) and one participant still demonstrated the strain 100 days post feeding. Administration of L. *acidophilus* NCFM (1×10^{10} CFU/day) in water, skimmed milk or a skimmed milk premix did not result in significant differences on fecal lactobacilli counts between matrices after 14 days of intervention (Varcoe *et al*., 2002) (Figure 4 G). However, baseline fecal counts of L. *acidophilus* NCFM were significantly higher in the water group than in the milk or skimmed milk premix group, whereas fecal recovery rates after the intervention were slightly lower (Figure 4 G). Participants in the water group therefore showed a relatively smaller increase in fecal counts of L. *acidophilus* NCFM than participants in the milk or premix group. These results suggest there may be a matrix effect of milk over water, but further investigation is required. Finally, Klingberg &

Budde (2006) found that L. *plantarum* MF 1298 was isolated in more individuals (without specification of fecal CFU counts) after 18 days of consuming probiotic sausage than after consumption of lyophilized probiotic powder (10 vs. 4 individuals).

The previously mentioned difference in daily dosage between matrices (Rochet *et al.,* 2007; Saxelin *et al.,* 2010) could be a confounding factor in the assessment of matrix effects on GIT survival. Figure 4 A, B, C, D and E should therefore be interpreted with caution. In an attempt to account for dosing differences, by dividing fecal recovery counts after the intervention by the initial dose in CFU, we observed a different matrix effect on GIT survival. It appears that a cheese matrix may yield higher fecal recovery rates of *B. animalis* BB-12, L. *rhamnosus* GG and L. *rhamnosus* LC705 than a capsule or yogurt matrix after the adjustment. Fecal recovery rates of *B. animalis* BB-12 furthermore appear to be higher in the yogurt group than in the capsule group. A similar matrix effect is observed for *B. Propionibacterium freudenreichii shermanii* JS both for and after the adjustment, where fecal recovery rates are higher after administration in yogurt compared to cheese or capsule. No apparent differences are observed for the revised results of Rochet *et al.* (2007) on GIT survival of B. *animalis* DN-173 in fermented yogurt or lyophilized powder. However, these results should be confirmed by controlled clinical studies, as the present dose-adjustments assume a linear relationship between the applied dose and probiotic fecal recovery. In any case, with or without adjustment for dosing differences, these data clearly show the importance of the matrix with respect to GIT survival.

5.2.2 Colonization

Whether probiotic bacteria colonize the human gut has been subject of debate for some time (Bezkorovainy, 2001). *In vitro* studies suggest that several probiotic bacteria attach to intestinal

epithelial cells and therefore proposedly colonize the gut. For instance, *L. rhamnosus* LC-705 and *L. acidophilus* were found to adhere to Caco-2 cells *in vitro* (Tuomola & Salminen, 1998). However, most *in vivo* studies suggest otherwise and reveal that probiotic bacteria in human feces are usually no longer detectable within a few days or weeks after feeding cessation. For example, *L. GG* appeared in the feces of participants during a probiotic intervention period, but was no longer detectable in 67% of participants within 7 days after the intervention had ended (Goldin *et al*., 1992). Similar results were observed in a clinical trial with premature infants (Millar *et al.,* 1993). It therefore appears that probiotic bacteria only transiently colonize the gut and that permanent colonization is not a strict requirement for probiotics to be effective. During the transient passage through the GIT, they are able to exert their clinical effects (Bezkorovainy, 2001).

It remains to be determined whether carrier matrices have a significant influence on the attachment of probiotic bacteria to epithelial cells. *In vitro* studies suggest that probiotics administered to a fruit yogurt matrix attach better to Caco-2 cells, when compared to probiotics administered to either plain yogurt or goat-milk ice-cream (Ranadheera *et al*., 2012). However, only a limited number of human trials have studied this phenomenon. Fecal recovery studies (as described in paragraph 5.2.1 "GIT Survival") suggest that there may be a small, strain-dependent effect of carrier matrices on transient probiotic colonization (Collins *et al*., 2002; Rochet *et al*., 2007; Saxelin *et al.,* 2010). In all studies, fecal probiotic counts were undetectable within a few days or weeks after feeding cessation but the excretion times differed between matrices. For instance, *L. salivarius* UCC118 was recovered from fecal samples 21 days post-feeding in 4 participants in the fresh milk group compared to 1 participant in the fermented milk group

(Collins *et al*., 2002). Rochet and colleagues (2007) reported that 10 days after their probiotic intervention had ceased, probiotic bacteria were still retrieved from fecal samples in 2 participants in the fermented milk group and in 1 participant in the lyophilized powder group. In addition, Saxelin *et al* (2010) reported that the median excretion time of *B. animalis* BB-12 in a yogurt matrix was significantly longer (17 days) than the median excretion time in capsule (7 days) or cheese matrices (3 days). But again, participants in the yogurt group consumed considerably more CFU of *B. animalis* BB-12 than participants in the cheese or capsule group. Overall, these results suggest there may be a carrier matrix effect on transient probiotic colonization but results are premature and substantially more future research is warranted.

5.3 Clinical Efficacy

Data evaluating the probiotic matrix effect on clinical efficacy is scarce. Most clinical studies focus on two identical matrices differing in probiotic content, rather than exploring the effect of the matrix itself. An overview of studies comparing the matrix effect on clinical efficacy can be found in Table 2. In a study that determined whether probiotic administration could accelerate recovery from acute diarrhea, children received L. *rhamnosus* GG in fermented milk or as freeze-dried powder for 5 days. Both treatments significantly reduced the duration of the diarrhea by approximately 1 day compared to control; however, no matrix specific effects were found (Isolauri *et al*., 1991). Chiang *et al*. (2002) and Sheih *et al*. (2001) described an increase in immune cell activity after intake of *B. lactis* HN019 in either low-fat milk or lactose hydrolyzed low-fat milk, favoring the latter with a higher natural killer (NK)-cell activity. Hütt *et al*. (2015) demonstrated that systolic- and diastolic blood pressure (BP) values were lowered after a three-week administration of L. *plantarum* TENSIA in both a probiotic cheese (1×10^{10} CFU/day) and

yogurt matrix 6×10^9 CFU (Hütt *et al*., 2015). The diastolic BP reductions from baseline were significant for both matrices ($p < 0.005$). The systolic BP reduction from baseline was only significant for the cheese matrix ($p < 0.005$), although a strong trend was found for the yogurt matrix ($p = 0.055$) (Hütt *et al*., 2015). It should be noted that participants in the cheese group consumed a larger daily dose of L. *plantarum* (1×10^{10} CFU) than participants in the yogurt group (6×10^9), which could account for the difference observed between matrices.

6. Conclusions

Current research on probiotics seems to exhibit a disproportioned distribution of efforts, where the majority of scientific studies have evaluated the strain-specific effects of probiotic species but the effects of carrier matrices remain largely underexposed. This focus hampers the innovation cycle of probiotic products as this review demonstrates that both carrier matrices and probiotic strains, influenced by production and storage conditions, ultimately determine the quality of a probiotic product (Figure 5). Matrix components, such as fats, proteins and additives, are shown to alter probiotic viability and efficacy. *In vivo* studies furthermore revealed strain-dependent matrix effects on the GIT survival of probiotic bacteria. These aspects, together with sensory properties of a probiotic product, need to be carefully balanced as they determine functionality and drive consumer acceptance.

Controlled human studies, evaluating two or more matrices with the same probiotic strain, are required to drive the innovation cycle of probiotic products. Moreover, the conventional view of strain-specific probiotic effects as primary indicators of probiotic product value needs to be accompanied by an appraisal of strain functionality in a particular carrier matrix. Any health claim should hence relate to the probiotic product as a whole as opposed to being based on the clinical effects of probiotic strains by themselves.

Disclosure Statement

M. van den Nieuwboer is a paid consultant to the study. O.F.A. Larsen is also Science Manager at Yakult Nederland B.V.

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Table 1. Overview of common matrices for probiotic administration

Format	**Carrier**
Fermented	
Dairy-based	Yogurt
	Cheese
	Kefir
	Fermented Milk
	Ice-cream
	Butter
	Traditional foods (e.g. tempeh and sauerkraut)
Non-dairy based	Sausages
	Vegetables/fruits juices Cereals
Non-Fermented	Biscuits/cookies
	Bread
	Cereals
	Chewing gums
	Chocolates
	Coffee
	Granola bars
	Honey

	Infant formula
	Oat cereal bars
	Peanut butter
	Soy milk
	Vegetable/fruit Juices
	Spread
Supplements	
Freeze-drying (lyophilized)	Tablets
Spray-drying	Powder
Vacuum drying	Pills
Fluidized bed drying	Capsules
	Infant formula
	Straws (sticks)

Adapted from Foligné *et al.*, 2013; Rivera-Espinoza & Gallardo-Navarro, 2010)

Table 2. Probiotic matrix effect on clinical efficacy.

Objective	Matrix 1 (N)	Matrix 2 (N)	Clinical outcome	Difference matrices	Probiotic strains	Ref.
Acute diarrhea recovery	Lactose-hydrolyzed fermented milk (24)	Freeze-dried powder (23)	Significant reduction diarrhea duration in both groups	No statistical differences between matrices	*L. rhamnosus* GG	Isolauri *et al*., 1991*
Immune enhancement	Low-fat milk (27)	Lactose-hydrolyzed low-fat milk (23)	Significant increase in immunologic cell activity in both groups	Significant higher increase in natural killer-cell activity for lactose-hydrolyzed low-fat milk	*B. lactis HN019*	Chiang *et al*., 2000**
Immune enhancement	Low-fat milk (26)	Lactose-hydrolyzed low-fat milk (26)	Significant increase in immunologic cell activity in both groups	Significant higher increase in natural killer-cell activity for lactose-hydrolyzed low-fat milk (71% vs. 147%)	*B. lactis HN019*	Sheih *et al*., 2001***
Lowering blood pressure	Cheese (82)	Yogurt (43)	Reduced systolic and diastolic blood pressure values were detected in both groups	Systolic BP reductions were only significant for the cheese matrix. Diastolic BP reductions were significant in both matrices.	*Lactobacillus plantarum strain TENSIA® (DSM 21380)*	Hütt *et al*., 2015****

*Lactose-hydrolyzed low-fat milk (10^10-11 CFU/day) vs. Freeze-dried powder (10^10-11 CFU/day) (Isolauri *et al*., 1991).

**Low-fat milk (2.5 × 10^10 CFU/day) vs. Lactose-hydrolyzed low-fat milk (2.5 × 10^10 CFU/day) (Chiang *et al*., 2000).

[***]Low-fat milk (2.5 × 10^10 CFU/day) vs. Lactose-hydrolyzed low-fat milk (2.5 × 10^10 CFU/day) (Sheih *et al*., 2001).

[****]Cheese (1 × 10^10 CFU/day). vs. Yogurt (6 × 10^9 CFU/day) (Hütt *et al*., 2015).

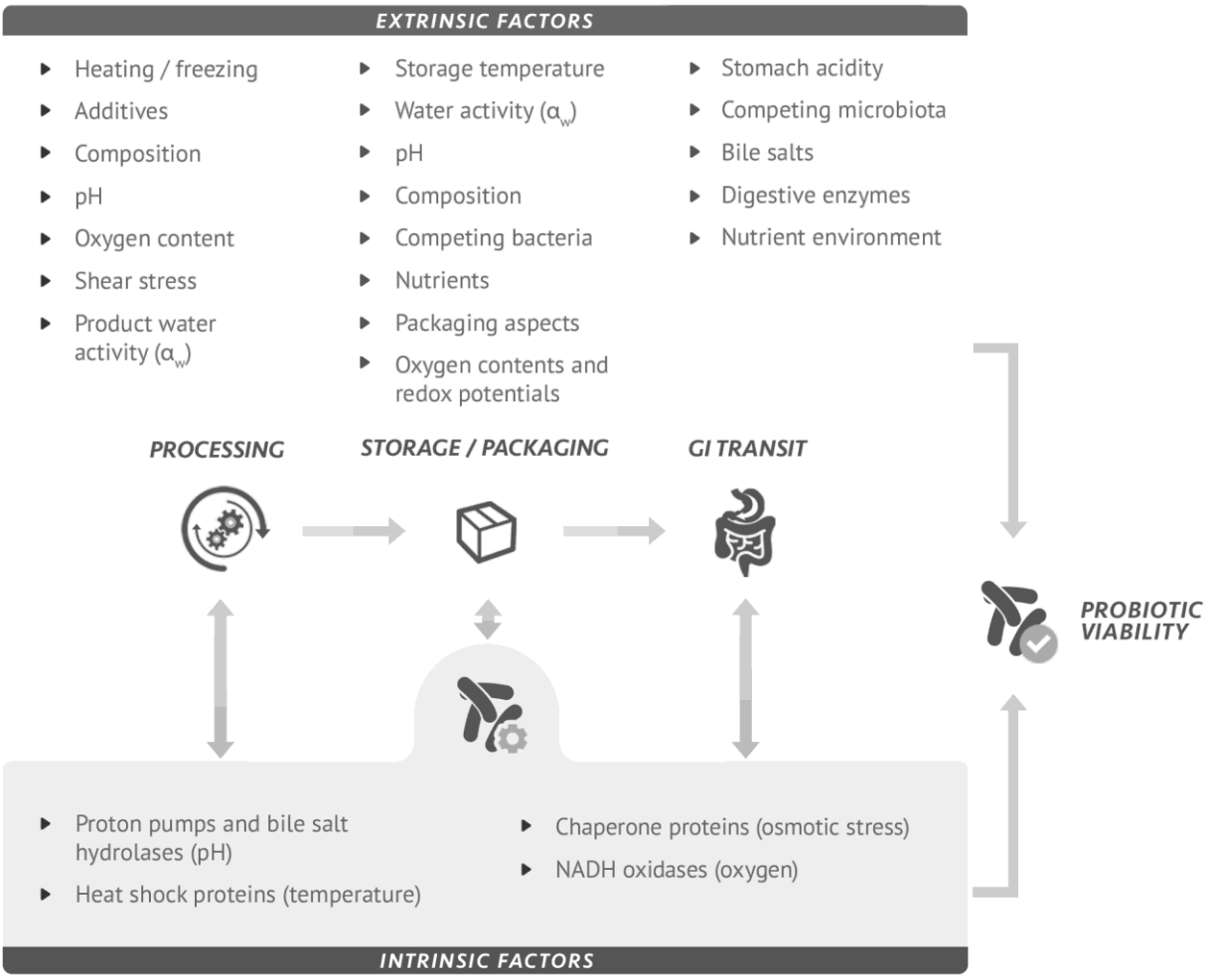

Figure 1. Important intrinsic and extrinsic factors that influence probiotic viability.

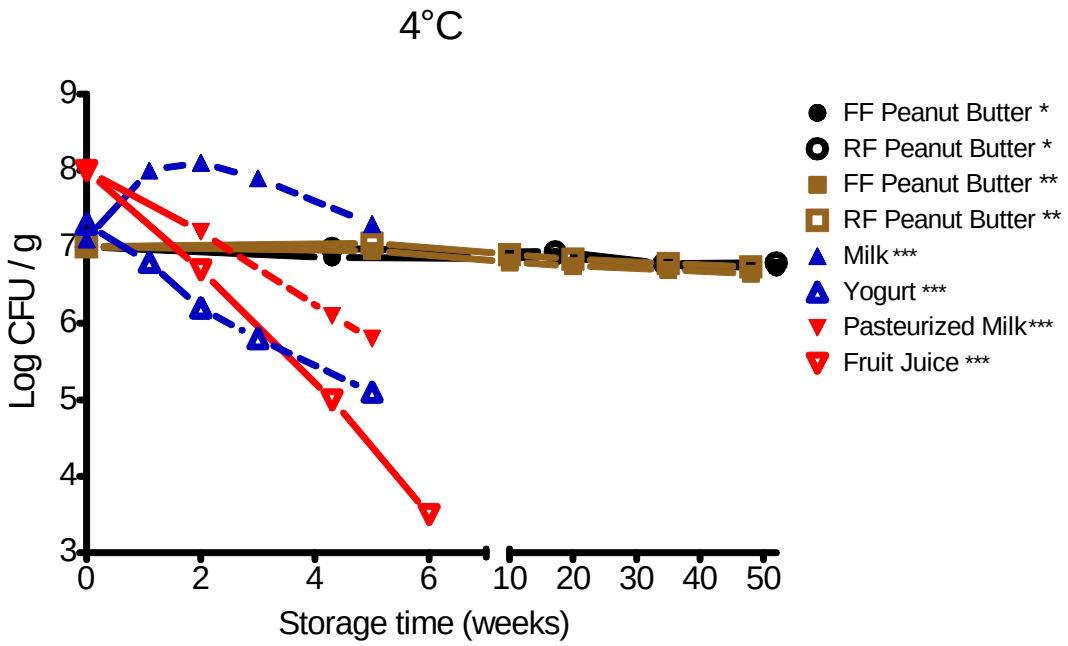

Figure 2. Peanut butter matrix suitable for long-term storage lyophilized probiotics at 4°C. This figure portrays the shell-life of various probiotic products at 4°C, as a function of CFU/gram product over time. Four clinical trials are portrayed in this figure, each represented by a different shape/color. Inter-study comparisons should be made with caution, as different probiotic strains and methods were used. Dotted lines represent projection estimates (Dave & Shah, 1997; Makinen et al., 2010; Mani-López & Mani-López, 2014; Rutela et al., 2016; Sodini et al., 2002). *Klu et al. (2014); Full fat peanut butter vs. reduced fat peanut butter (averaged lyophilized Lactobacillus acidophilus (CUL 60), L. acidophilus (CUL21), Bifidobacterium bifidum (CUL 20) and Bifidobacterium lactis (CUL 34)). **Klu et al. (2012); Full fat peanut butter vs. reduced

fat peanut butter (lyophilized L. rhamnosus GG). ***Pimentel et al. (2012); Milk vs. yogurt (averaged cultured Enterococcus durans 15, E. durans 37, Enterococcus faecium 32 and Enterococcus casseliflavus 40). ****Saarela et al. (2006); Pasteurized milk vs. fruit juice (lyophilized B. lactis BB-12).

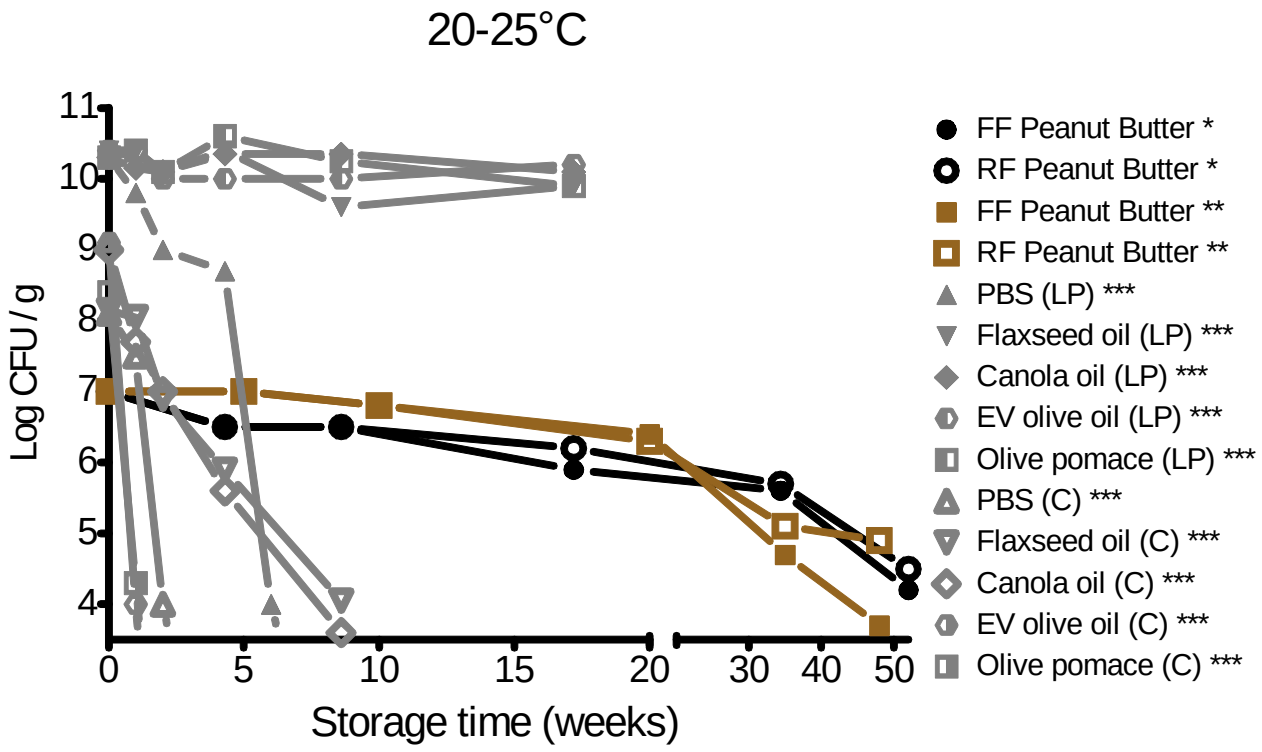

Figure 3. Oil matrices suitable for long-term storage lyophilized probiotics at 20-25°C. This figure portrays the shell-life of various probiotic products at 20-25°C, as a function of CFU/gram product over time. Three clinical trials are portrayed in this figure, each represented by a different shape/color. Inter-study comparisons should be made with caution, as different probiotic strains and methods were used. *Klu et al. (2014); full fat peanut butter vs. reduced fat peanut butter (averaged Lactobacillus acidophilus (CUL 60), L. acidophilus (CUL21), Bifidobacterium bifidum (CUL 20) and Bifidobacterium lactis (CUL 34)). **Klu et al. (2012);

full fat peanut butter vs. reduced fat peanut butter (L. rhamnosus GG). ***Endo et al. (2014); lyophilized (LP) and cultured (C) probiotics in various oils or phosphate buffer solution (PBS) (L. rhamnosus GG).

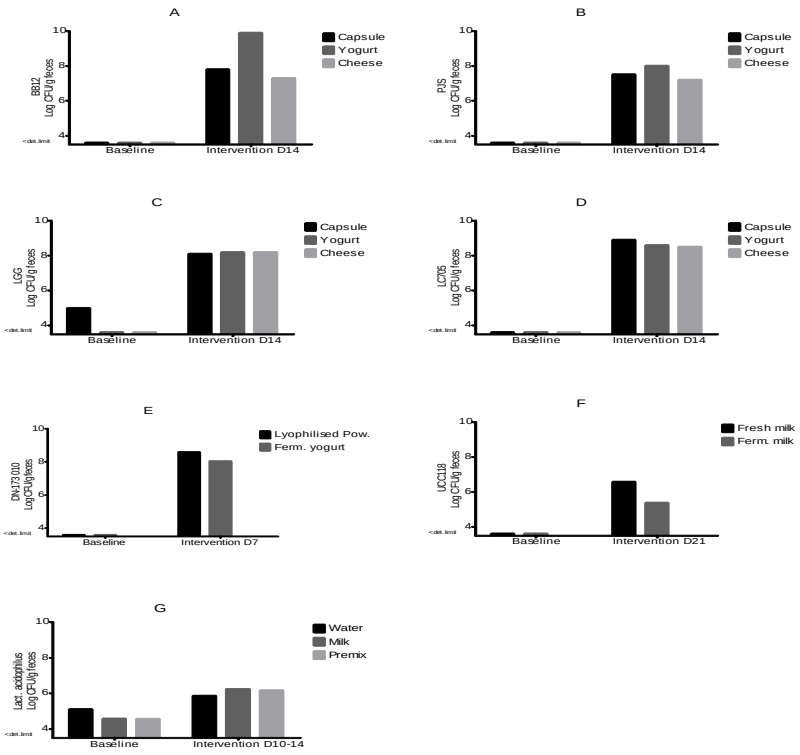

Figure 4. Matrix effects on gastrointestinal survival of probiotic bacteria. These figures portray data the gastrointestinal survival of probiotic bacteria after administration in different carrier matrices. Number of CFU/gram feces are presented at baseline and after 7-21 days of intervention. The seemingly higher GIT survival rates observed for BB12 and PJS (Figure A & B) after administration of probiotic yogurt should be interpreted with caution (see paragraph 5.2). The daily doses for BB12 and PJS were considerably higher in the yogurt matrix than in the cheese or capsule matrix, hence significance asterisks are left out of the figure. A) GIT survival of Bifidobacterium animalis subsp. lactis BB12 in capsule (1.8 × 10^9 CFU/day), yogurt (1.4 × 10^10 CFU/day) and cheese (4.2 × 10^7-1.2 × 10^6 CFU/day) (Saxelin et al., 2010). B) GIT

survival of Propionibacterium freudenreichii subsp. shermanii JS in capsule (4.2 × 10^9 CFU/day), yogurt (7.5 × 10^9 CFU/day) and cheese (1.7 × 10^9 CFU/day) (Saxelin et al., 2010). C) GIT survival of L. rhamnosus GG in capsule (5.2 × 10^9 CFU/day), yogurt (4.7 × 10^9 CFU

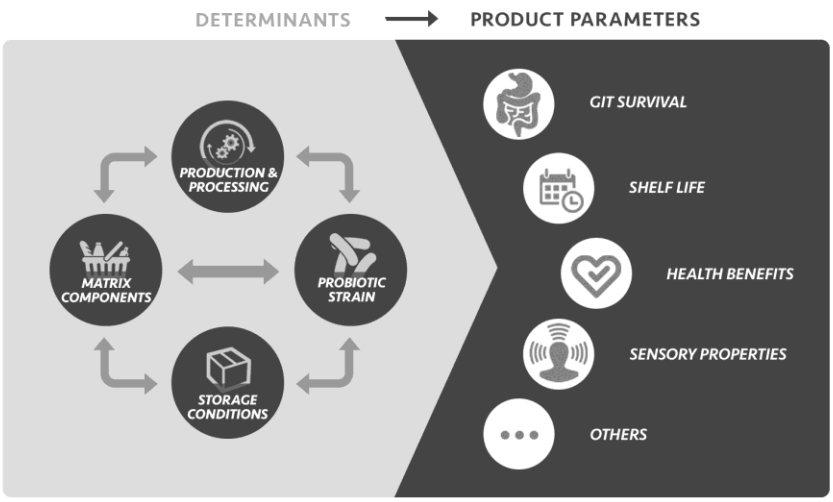

Figure 5. Matrix components and probiotic strains determine the quality of probiotic products. This figure provides an overview of determinants that influence probiotic product parameters. Both carrier matrix components and probiotic strains, influenced by production and storage conditions, determine the gastrointestinal survival, shelf-life, health benefits and sensory properties of a probiotic product.