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Applying probiotics and prebiotics in new delivery formats – is the clinical evidence transferable?

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ABSTRACT

Background: There is substantial demand for gut health products incorporating probiotics and prebiotics. They are being delivered as ingredients in an increasing range of different product formulations. While new delivery matrices are assessed for their potential impact on cell viability and prebiotic degradation, it is unknown whether they should be expected to independently alter the clinical effect of a given probiotic and prebiotic.

Scope and approach: We provide an overview of preclinical and clinical data to examine the degree to which probiotic and prebiotic efficacy may be altered by processing and incorporation into various delivery matrices. We also consider the impact of inter-individual host factors on product efficacy. We further review regulatory positions across the globe on substantiation of prebiotic and probiotic efficacy in the final product format.

Key findings and conclusions: In vitro data suggest that the delivery matrix may interact with prebiotic and probiotic functions via various physicochemical interactions with molecular and cellular structures and changes in cellular expression. However, direct evidence to suggest these changes have a significant *in vivo* impact is very limited. Indeed, meta-analyses suggest a robustness of effect across delivery matrices. Regulatory expectations vary among regions, but scope typically exists for adequate scientific justification to translate probiotic or prebiotic evidence across product formats. Early evidence suggests host factors such as diet, health and microbiome status are likely to play an important role in an individual's response to a given probiotic and prebiotic.

1. Introduction

Gut health is a growing area of interest in the food and nutrition sector, driven in part by the link between the gut microbiome and a diverse range of health outcomes (Integrative Human Microbiome Project Research Network Consortium, 2019). Ingredients with gut and microbiome-associated benefits are increasingly included in a range of foods and supplements. Probiotics and prebiotics (in addition to polyunsaturated fatty acids and antioxidants) are the most widely incorporated ingredients into functional foods and have been dubbed an 'innovation hotspot' with significant levels of current investment into the development of novel technologies and food formats for delivery (Farias et al., 2019; Granato et al., 2020; Terpou et al., 2019).

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill et al., 2014). Care must be taken with probiotic formulations to ensure the product contains concentrations of viable microorganisms, sufficient to deliver the demonstrated health benefit, through the end of shelf life. While the potential impact of product formulation on probiotic viability is well recognised (Shori, 2016), it is not always the case for any potential effects of product formulation on probiotic functionality (Vinderola, Binetti, Burns, & Reinheimer, 2011).

Prebiotics are substrates that are selectively utilised by host microorganisms conferring a health benefit (Gibson et al., 2017). Prebiotics

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targeted for the gut must resist digestion, including the low pH of the stomach, hydrolysis by intestinal enzymes and gastrointestinal absorption. Prebiotics can be consumed as supplements or incorporated as functional ingredients in many types of food products, including biscuits, spreads, cereals, sweeteners, milk, ice cream and yoghurt (Brighenti, 2007). In food processing, they may be subject to temperature, pH and chemical stressors, which could influence their intact delivery and activity in the colon.

There is a solid body of clinical trial research on both probiotics and prebiotics (Sanders, Merenstein, Reid, Gibson, & Rastall, 2019). Study product formulation varies extensively among studies, with trials conducted on capsules, powders, liquids, or conventional food forms, including dairy products, baked goods, confectionary and drinks. Novel formulations continue to proliferate at a rapid rate, with a recent focus on non-dairy matrices (Min, Bunt, Mason, & Hussain, 2019; Valero-Cases, Cerdá-Bernad, Pastor, & Frutos, 2020). However, despite the wide variety of formulations in development and in market, controlled clinical trials directly comparing the efficacy of a given probiotic or prebiotic in different product formulations are rare.

When a particular probiotic strain or specific prebiotic compound has been clinically studied with demonstrated benefits, it is common practice in the food and supplement industry to design novel formulations with this active ingredient. It has been recognised, particularly in the field of probiotics, that product matrix plays a potential role in product efficacy (Gomand et al., 2019; Ranadheera, Baines, & Adams, 2010; Sanders et al., 2014). One question facing regulators and industry alike is the extent to which evidence from studies conducted on specific probiotics or prebiotics delivered in one formulation can be extrapolated to different formulations. For example, will a specific probiotic or prebiotic in a capsule or powder format deliver the same benefit as was demonstrated when it was delivered at the same dose in a yoghurt? What degree of change of delivery matrix should trigger the need to repeat a clinical trial? (see Fig. 1). Repetition of clinical trials for non-substantive changes in product formulation may not be scientifically or ethically justifiable and is likely to be cost-prohibitive.

When considering the nutritional and clinical application of evidence on probiotic and prebiotic benefits, a key question is whether a given individual consumer can reasonably expect to experience the health benefit demonstrated in a clinical trial. While delivery matrix of the probiotic or prebiotic may conceivably play a role, host factors are also undoubtedly important. Study populations are typically selected for health status, and sometimes for age and sex; however it is uncommon for baseline dietary habits, genetics or microbiome composition to be considered in study subject recruitment. There is increasing recognition that such factors may play a role in the physiological response to a given prebiotic (Rodriguez et al., 2020) or probiotic (Szymanski et al., 2020; West et al., 2019). We therefore consider in this article the data comparing variability of effect across product formulations weighed against the potential variability arising from host factors (Fig. 2). The regulatory context in which such evidence must be considered is also examined.

2. Prebiotic functionality and the influence of the food matrix

Most prebiotic development work to date has focused on carbohydrates, particularly oligosaccharides. Considering the published prebiotic literature overall, the most studied prebiotics are the soluble fibres inulin, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and more recently human milk oligosaccharides (HMOs). Emerging prebiotics include isomalto-oligosaccharides (IMO), xylo-oligosaccharides (XOS) and resistant starch. In terms of their production, these prebiotics are obtained by extraction from plants, for example inulin is extracted from chicory (Tripodo & Mandracchia, 2019); by enzymatic or chemical hydrolysis of plant polysaccharides, as is the case for FOS (Martins, Ureta, Tymczyszyn, Castilho, & Gomez-Zavaglia, 2019) and XOS (Poletto et al., 2020); or by enzymatic synthesis using disaccharides or other substrates, as is done for HMOs (Benkoulouche, Fauré, Remaud-Siméon, Moulis, & André, 2019; Chen, 2018), IMO (Goffin et al., 2011), GOS and FOS (Martins et al., 2019; Torres, Goncalves, Teixeira, & Rodrigues, 2010). The mechanism they utilise to affect the host is largely through indirect effects based on their selective utilisation by a group or groups of host bacteria. This modulation of the microbiota can lead to protection against harmful microorganisms, to the strengthening of the epithelial barrier function, and to immune stimulation, although a possible direct immune signalling/immune receptor effect by prebiotics has also been suggested (Jeurink, van Esch, Rijnierse, Garssen, & Knippels, 2013; Wu et al., 2017). Important compositional and structural features that influence prebiotic microbial and immune interactions include the degree of polymerisation, the sugar composition, the degree of branching, and the anomeric configuration and type of glycosidic bonds (Rajendran, Okolie, Udenigwe, & Mason, 2017). Further, the concentration of prebiotics in preparations is an important determinant of activity. Prebiotic concentrations range between 50 and 95%, with main impurities being mono- and disaccharides, depending on the upstream and downstream production processing (Martins et al., 2019).

Prebiotics can be susceptible to structural degradation when exposed to certain stresses during processing and storage. Studies have been performed primarily with GOS, FOS and inulin investigating the effect of pasteurisation (temperature, time) and pH (2–5) and structural integrity, using both simplified model systems and whole foods. GOS has been reported to be very stable to acidic conditions and high temperatures (Playne & Crittenden, 1996), likely due to the presence of β-linkages (Sangwan, Tomar, Singh, Singh, & Ali, 2011; Voragen, 1998). For this reason, they have been added to a variety of acid or heated foods, such as acidified milks and yogurts, pasteurised fruit juices and bakery products (Duar et al., 2015; Klewicki, 2007; Sangwan et al., 2011).

On the other hand, inulin and FOS have been found to be relatively sensitive to combinations of high acid and temperature. Moderate levels of FOS degradation, ranging from 10% to 30%, were observed at temperatures between 60 °C and 70 °C and pH $<$ 3, in model solutions (Wang, Sun, Cao, & Tian, 2009). Moreover, in a study investigating the long-term stability of FOS, it was shown that at pH 2 (relevant to some juices), 38% of FOS was hydrolysed after 18 weeks of storage at 4 °C (Courtin, Swennen, Verjans, & Delcour, 2009). When prebiotics were added to extruded cereals, which are subject to the combination of heat with pressure and shear stress (dependent on extrusion screw speed) during processing, >50% of FOS was degraded at all temperatures and screw speeds tested. Inulin recovery was significantly altered by changes in temperature and screw speed, with near 100% recovery at

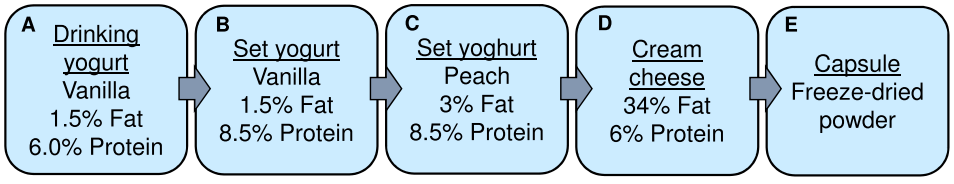

Fig. 1. Hypothetical product formulation evolution in a probiotic or prebiotic product In this example, if clinical validation studies were conducted in product format A, would it be justifiable to use the evidence to support product B (yoghurt format change), C (flavour and fat change), D (new dairy product), or E (change from food to non-food)? Whether an efficacy study is required to be repeated for these translations is a matter of both scientific and regulatory concern.

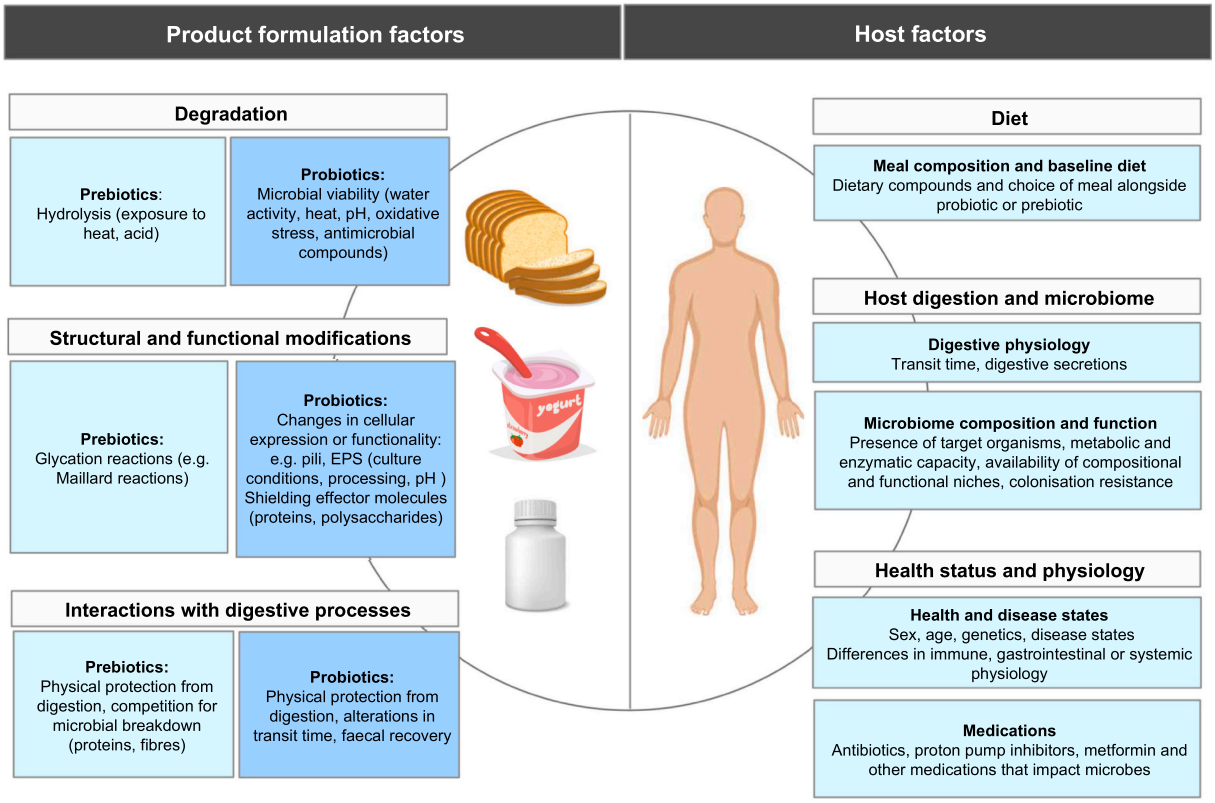

Fig. 2. Potential factors influencing the effect of probiotics and prebiotics
A range of factors, both inherent to the product formulation as well as specific to the individual consuming it, may determine the activity and clinical effectiveness of any given prebiotic or probiotic product. EPS = exopolysaccharide.

120–140 °C and optimal screw speed; dropping to 35% at 170 °C with optimal screw speed, and 25-24% recovery at 140 °C with higher pressure or shear stress. GOS recovery was not significantly affected by any temperatures or screw speeds tested (Duar et al., 2015). Such results suggest that, especially in the case of FOS and inulin, any treatment process involving heat, pressure or acidity needs to be carefully considered and the impact assessed during processing and subsequent storage.

Recent studies have investigated the effect of non-thermal processing technologies, including ultrasound, high pressure processing and atmospheric cold plasma on prebiotic stability (particularly FOS and inulin) in fruit beverages (Fonteles & Rodrigues, 2018), such as cranberry juice (Gomes et al., 2017), apple puree (Keenan, Brunton, Butler, Wouters, & Gormley, 2011) and orange juice (Almeida et al., 2017). The results from these studies indicate an improved prebiotic stability compared to standard thermal treatments and during subsequent chilled storage. Finally, although relatively little work has been conducted investigating the interaction of the food matrix components (e.g. proteins, amino acid, lipids) with prebiotics, it has been shown in model systems as well as food matrices (e.g. infant formulae, fruit puree) that prebiotics with reducing ends, such as GOS and FOS, can participate in Maillard reactions taking place during heat treatment, leading to the formation of prebiotic-protein conjugates. These could potentially increase prebiotic stability during processing and formulation and positively influence prebiotic activity such as the bifidogenic effect, although more studies are needed to investigate the mechanism by which these reactions affect prebiotics (Joubran, Moscovici, & Lesmes, 2015; López-Sanz, Montilla, Moreno, & Villamiel, 2015; Sabater et al., 2018; Seifert, Freilich, Kashi, & Livney, 2019). It is relevant to note that excessive dietary consumption of advanced glycation end products (AGEs) produced through Maillard reactions is generally considered to be detrimental to health (Nowotny, Schröter, Schreiner, & Grune, 2018). Although prebiotic-containing food products are not anticipated to contribute significantly to the dietary load of AGEs, due to comparatively milder processing techniques and the low dose of prebiotics consumed within the dietary context, further research focus on AGE content in these food products and their *in vivo* impact is recommended.

3. The influence of product formulation on prebiotic digestion

After a prebiotic in formulation remains intact through processing, if it is targeted to the gut, it must then withstand gastrointestinal digestion and absorption, including the impact of gastric acids, intestinal brush border and pancreatic enzymes (Ferreira-Lazarte, Moreno, & Villamiel, 2020). Both the delivery matrix as well as the structural characteristics of the prebiotic have the potential to influence the interaction of the prebiotic with the digestive system and its secretions. Assessing the fate of prebiotics during gastrointestinal digestion *in vitro* has been a key feature of prebiotic research, albeit using diverse models and non-standardised approaches. Nevertheless, the indications are that prebiotics are generally able to resist gastric digestion, whereas their resistance to intestinal digestion is influenced considerably by their composition, degree of polymerisation, and structure. In general, oligosaccharides with smaller degrees of polymerisation are hydrolysed first (Hu, Winter, Chen, & Gänzle, 2017; Kaulpiboon, Rudeekulthamrong, Watanasatitarpa, Ito, & Pongsawasdi, 2015), whereas in the case of GOS, the structure influences resistance, e.g. GOS β (1–6) > β (1–4) > β (1–3) (Torres et al., 2010; Playne & Crittenden, 2009).

We were unable to find any clinical studies on the impact of incorporation into food matrices on prebiotic digestion. The food matrix has potential to physically protect a prebiotic from enzymatic digestion, through non-covalent interactions or barrier effects (e.g. for protein and fibre-rich products); however this is a hypothesis that needs to be tested. Multicompartment models which including simulation of oral, gastric

and upper intestinal digestion, as well as lower intestinal fermentation, are needed to explore this hypothesis (Bohn et al., 2018).

There is also the potential for competitive interactions to occur with fibre-rich products in the lower digestive system when prebiotics are delivered with other fermentable microbial substrates. Fibres can modulate the microbiota composition and activity; therefore it would be complex to prove to which extent prebiotics or fibres are being utilised and by which bacterial groups. An interesting approach was tested *in vitro* using 13C labelled prebiotics (Maathuis, van den Heuvel, Schoterman, & Venema, 2012). Prebiotic 13C-GOS was inoculated into TIM-2 colonic model and LC-MS and 16 S-rRNA stable isotope probing coupled to a phylogenetic micro-array were used to detect, respectively, label incorporation in metabolites and bacterial biomass. This is a unique method which could allow the identification of which metabolites are produced from which source and which bacteria ferment such source. The use of stable isotope was also tested in a small exploratory clinical study, to detect the systemic availability of SCFA in healthy subjects (Boets et al., 2017). Such approaches could serve as a starting point to further explore this fibre-prebiotic competition theory.

Further, there have been some recent studies showing that prebiotics can increase bio-accessibility of other food components, such as dairy proteins and plant sterols (Blanco-Morales et al., 2018; Ferreira-Lazarte et al., 2017). This is also an area worth further investigation as it can align prebiotic research with the research in functional foods and nutrition, creating a more complete picture for the role of prebiotics.

4. Evidence from human intervention trials comparing prebiotic matrices

In clinical trials, prebiotics are administered in many different formats, including breakfast cereals, biscuits, chocolate, ice cream, yoghurt, spreads, pasta, syrups, and powders suspended in water or other beverages. Most of these studies compared the prebiotic-containing food to a control version of the same food without prebiotic. We found no clinical trials that directly compared the efficacy of a given prebiotic across multiple food formats. We did, however, find one prebiotic preparation tested as a supplement, a fortified food (bread) and fortified beverage (orange juice) in three independent trials on metabolic parameters. Vulevic, Juric, Tzortzis, and Gibson (2013), administered 2.75 g/day galacto-oligosaccharides (5.5 g/day Bimuno B-GOS) in supplemental form (powder in water) to overweight subjects at-risk for metabolic disease. The prebiotic significantly decreased insulin (12 pmol/L), total cholesterol (0.3 mmol/L), triglycerides (0.1 mmol/l) and C-reactive protein (not quantified) compared to control after 12 weeks. In two further studies (as reviewed in Scott et al., 2020), the administration of the same dose of the same prebiotic incorporated into bread or orange juice failed to result in any clinical or microbiological effects. As the fortified formulations used in these studies had been previously chemically verified for prebiotic content and for impact on microbiota composition using *in vitro* models (Costabile et al., 2015a, 2015b), the lack of *in vivo* equivalence was somewhat surprising, however it must be noted that *in vitro* prebiotic screening models cannot fully predict *in vivo* activity. It is possible that matrix effects such as the food processing techniques or constituents created an *in vivo* impact that was not detected in the chemical or functional *in vitro* analysis. Another potential explanation for these inconsistent results among trials could be found in the different characteristics of the baseline population. In the food matrix studies, not all volunteers were overweight or had dyslipidaemia or hyperinsulinemia, compared to the metabolically unhealthy subjects studied by Vulevic and colleagues in 2013. For this reason, subjects in the juice study were further stratified for triglyceride concentrations and reductions were found in those with baseline triglyceride concentrations of over 1 mmol/L (Scott et al., 2020).

Meta-analyses may provide useful insight on potential elasticity of effect for specific prebiotics across studies and delivery matrices. A meta-analysis by Brighenti (2007) showed a positive effect of inulin-type fructans on triglyceride concentrations across multiple study populations (healthy, hypercholesteraemic or hypertriglyceridaemic, type II diabetic, and subjects with non-alcoholic fatty liver disease), and divergent doses and food formats, including biscuits, spreads, cereals, sweeteners, milk, ice cream and yoghurt. In a 2017 meta-analysis, Liu, Prabhakar, Ju, Long, & Zhou evaluated the efficacy of inulin-type fructans on metabolic syndrome parameters across and between four subgroups - healthy, dyslipidaemic, overweight or obese, and type 2 diabetic subjects. The included clinical trials administered prebiotics across differing food and supplement formats. Results showed that inulin-type fructans significantly reduced low density lipoprotein (LDL) cholesterol (mean 0.15 mmol/L reduction) compared to control in the overall analysis. Further, blood insulin was significantly reduced (4.01 mU/L) and high density lipoprotein (HDL) cholesterol was significantly increased (0.07 mmol/L) by prebiotic administration in a subgroup comprising subjects with type 2 diabetes. In addition, further subgroup analyses found that while inulin administration lowered total cholesterol and LDL cholesterol, FOS was not effective. Matrix was not identified as a factor contributing to heterogeneity in either meta-analysis. While these meta-analyses demonstrate a positive impact of inulin-type fructans across a variety of matrices, study limitations including sample size and heterogeneity between studies suggest that more consistent study designs and subgroup analysis by delivery matrix would enable stronger conclusions in this area.

5. Concluding thoughts and research directions for prebiotics

Overall, the data show that formulation or processing that exposes prebiotics to acid, heat and pressure can lead to degradation and that prebiotic composition and structure play a key role in susceptibility. The majority of data documenting such degradation is derived from *in vitro* assessments; studies of the clinical impact of such changes are lacking. One of the limitations of most clinical trials using prebiotics is that chemical and structural analysis of the prebiotic within the food matrix is rarely evaluated. These analyses are fundamental to assess how the food processing and formulation may have affected the prebiotic concentration, structure and potentially its efficacy. State-of-the-art analytical techniques are available to address this gap, including high-performance anion-exchange chromatography coupled with mass spectrometry (Coulier et al., 2009; Mechelke et al., 2017), hydrophilic interaction liquid chromatography (Mernie, Tolesa, Lee, Tseng, & Chen, 2019), nuclear magnetic resonance spectroscopy (Coulier et al., 2009; Ramakrishnan & Luthria, 2017), matrix-assisted laser desorption/ionisation mass spectrometry (Harvey, 2018; Huang et al., 2019), and collision-induced dissociation tandem mass spectrometry (Hsu, Liew, Huang, Tsai, & Ni, 2018). Embedding such techniques in prebiotic research to characterise the oligosaccharide structures in detail will identify potential degradation and conjugation reactions taking place during food processing and storage that might influence prebiotic activity. Such knowledge will also help to design improved prebiotic-containing foods through food structuring and encapsulation, particularly for oligosaccharides that are more sensitive to gastric and intestinal digestion. Technologies available in this area include multiparticulate dosage forms (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2014; Fayed, Abood, El-Sayed, Hashem, & Mehanna, 2018) and protein-alginate encapsulation (Klemmer, Korber, Low, & Nickerson, 2011; Varankovich et al., 2018) for the delivery of synbiotics. Besides increasing stability, such designs would also provide the opportunity to target prebiotic (and probiotic) release in specific parts of the intestine.

Overall, although a wealth of research has demonstrated the stability of FOS, GOS and inulin in model systems and to an extent in some beverages and foods, further work is needed in evaluating prebiotic stability during production and storage of complex food matrices, such as dairy, bakery and confectionary products. Such work should also consider the relationship between food matrix, structure and stability

for emerging prebiotics (e.g. XOS, IMO). A fundamental understanding of how prebiotics interact with food components, particularly proteins (e.g. casein, whey proteins, gluten proteins) and lipids, is also needed to determine the extent that these reactions take place within a food matrix, and their effect on prebiotic structure, prebiotic activity *in vitro* and *in vivo*, and on the organoleptic properties of the products. More research is also needed to understand the effect of purity of prebiotic substances on their ability to withstand processing, and to assess the potential beneficial effect of non-thermal processing technologies on prebiotic stability, particularly for more sensitive and less robust prebiotic structures.

Finally, it is necessary to conduct further human trials comparing the efficacy among different prebiotic formulations. The comparison of three clinical studies on B-GOS (Vulevic et al., 2013; unpublished data within; Scott et al., 2019) demonstrates that a difference in effect of B-GOS was seen between food matrixes; however, differences in study population limit the conclusions that can be drawn (see 'Host factors impacting the clinical effect of probiotics and prebiotics' - below). Multi-arm studies comparing prebiotics in unmodified supplemental form with those incorporated into food matrices, with relevant placebo controls, would be informative. We further propose that all human trials involving prebiotic-containing foods are accompanied with detailed physicochemical and structural analysis of the prebiotic and by *in vitro* gastrointestinal digestion studies, as this information is important to draw conclusions on prebiotic efficacy and potentially of the mechanisms involved. The above research directions and the consistent publication of such findings will lead to the design of more effective products that maintain their prebiotic health effects during food formulation, processing and storage.

6. The impact of processing and formulation on probiotic functionality

Viability is a requirement to meet the definition of a probiotic and probiotic efficacy is dependent upon delivering an adequate dose throughout product shelf life. Probiotic dosages typically range between 10^7 and 10^{11} colony-forming units (CFU)/day for the majority of human clinical studies (Dronkers, Ouwehand, & Rijkers, 2020). Briefly, the mechanisms of action described for probiotics include modulation of the intestinal microbiota and its metabolites, influencing gut-associated and systemic immune responses, enhancing epithelial barrier function and mucin secretion, metabolism of biliary salts and other luminal compounds, such as lactose, and most recently interaction with the brain-gut axis by regulation of endocrine and neurologic functions (Plaza-Diaz, Ruiz-Ojeda, Gil-Campos, & Gil, 2019).

The influence of the food matrix on the viability of probiotic bacteria in dairy and non-dairy beverages has been previously reviewed (do Espirito Santo, Perego, Converti, & Oliveira, 2011; Shori, 2016). During probiotic storage, a range of factors may affect viability, including temperature, pH, water activity, oxygen content, redox potential, the presence of other cultures, or interactions with ingredients, additives, or packaging materials (Tripathi & Giri, 2014). Due to these factors, numerous consensus and consultation panels have stressed the need for verification of the viable cell count in the finished product at the end of shelf life (FAO & WHO, 2001; Hill et al., 2014; Jackson et al., 2019).

A range of processing effects may impact some aspects of probiotic physiology, without changes in cell viability (Vinderola et al., 2011). One potential influence is probiotic culturing conditions, such as the pH of the growth medium. For example, Sashihara, Sueki, Furuichi, and Ikegami (2007) reported that *Lactobacillus gasseri* OLL2809 induced different levels of the pro-inflammatory cytokine IL-12 in mice splenocytes depending on the pH at which the bacteria were produced (pH constant of 4, 5 or 6), though the same numbers of viable bacteria were obtained under the three pH values assessed. In line with these findings, Deepika, Karunakaran, Hurley, Biggs, and Charalampopoulos (2012) reported that the growth curves of the probiotic strain *Lacticaseibacillus rhamnosus* GG were similar at 37 °C at different pH conditions, but hydrophobicity and adhesion to Caco-2 cells were significantly higher at pH 5. Further, Biagioli and colleagues (Biagioli et al., 2017) reported divergent effects on attenuation of experimental colitis as well as *in vitro* metabolomic expression between the same multistrain VSL#3 probiotic formulation produced at two different manufacturing sites. These differential effects were so large that only one formulation could attenuate inflammation, intestinal permeability and disease activity in the experimental colitis models, while the other batch did not. A metabolomic analysis of the two formulations found a three-fold enrichment in the concentrations of four metabolites, including 1–3 dihydroxyacetone (1-3DHA), in supernatants of the non-effective batch. Feeding mice with 1-3DHA increased intestinal permeability, highlighting that the specific metabolites produced in different formulations have potential importance. Strain identity and viability were not verified beyond the manufacturers' claims, potentially introducing confounders, however the study is interesting because it proposes that metabolomics and screening for 1-3DHA could be included in quality control of fermentation processes.

These experiments suggest that probiotic functionality may be altered by growth conditions without altering cell viability. While there is not yet convincing evidence that culturing conditions can significantly influence clinical efficacy, preclinical evidence suggests caution is warranted with significant changes in probiotic manufacturing techniques, and *in vitro* or *in vivo* tests of equivalence may be warranted in such cases (see 'A path forward – demonstrating essential equivalence' – below).

Further processing of the probiotic culture can also modulate its functionality. Probiotic culture processing steps such as centrifugation (Tripathi et al., 2013) and spray drying (Kiekens et al., 2019) can physically remove key adhesins such as the pili of *L. rhamnosus* GG. Under optimal conditions, these adhesins can be re-expressed *in vivo* after a few hours, although the reliability of this regeneration in the human gut after consumption is unknown. Moreover, it is important to recognise that the well-studied pili of *L. rhamnosus* GG are not the main 'active pharmaceutical ingredient'; they are rather 'facilitators' of efficacy (Segers & Lebeer, 2014). When their expression is altered, the impact on probiotic functionality is generally not known. However, it seems relevant to check for pili presence during quality control of products containing *L. rhamnosus* GG.

In addition to processing steps, when probiotic cultures are incorporated into foods, the food components such as proteins and polysaccharides have the potential to impact probiotic efficacy: for example, by shielding effector molecules such as adhesins and immunomodulatory molecules on the probiotic surface. These effects are probably minor inside the gastrointestinal tract, because of digestion of these food matrix components. For example, Burgain et al. (2015) and Guerin et al. (2016) have shown that the pili of the probiotic *L. rhamnosus* GG bind to components of dairy matrices such as whey proteins and β-lactoglobulin, but not α-lactalbumin and bovine serum albumin. Low pH was also demonstrated to lead to collapse of the exopolysaccharide surface layer of this probiotic. This resulted in reduced binding of the probiotic to whey as shown by atomic force microscopy and single molecule force spectrometry, an interesting analytical tool to study the physical impact of food matrix components on probiotic features (Burgain et al., 2015). Recently, it was also shown that the exopolysaccharides of *L. rhamnosus* GG could protect during tablet production, promoting the survival of this probiotic strain. Since exopolysaccharide can be important for probiotic immune interaction, these findings demonstrate that monitoring of exopolysaccharide and the impact of factors such as pH is important to better understand the impact on probiotic functionality.

7. Evidence from human intervention trials comparing probiotic matrices

The extent to which potential changes in *in vitro* functionality may

affect efficacy in humans remains unknown. However, several meta-analyses point to clinical health benefits of probiotics independent of processing or formulation. For example, although not intended to compare probiotic efficacy across different formats, Ritchie and Romanuk (2012) performed an interesting meta-analysis of probiotic efficacy for various gastrointestinal diseases. They found consistent effects for probiotics over placebo in pouchitis, antibiotic-associated diarrhoea, and *Clostridioides difficile*-associated diarrhoea, independent of the probiotic strain or formulation used, with the strongest and most consistent reduction of risk for well-known probiotics such as *L. rhamnosus* GG and *Saccharomyces boulardii*. Similarly, Szjawenska, Wanke, & Patro (2011) determined that *L. rhamnosus* GG was effective for the prevention of healthcare-associated diarrhoea in children when supplemented as capsule or in fermented milks. Another systematic review with meta-analysis reported that *Limosilactobacillus reuteri* DSM 17938, irrespective of the formulation (calcium milk, powder), reduced the duration of diarrhoea and increased the chance of cure (Urbańska, Gieruszczak-Białek, & Szajewska, 2016). Although meta-analyses can inform on durability of effect across matrices, they are not sufficiently granular to assess possible changes in effect size that might exist due to different matrices. The effects of different probiotic formats on probiotic efficacy would ideally be studied by comparing arms within a double-blind placebo controlled randomised human clinical trial. Such data are available on intestinal survival of the same probiotic delivered in different food and dried supplement matrices, suggesting that large changes in matrix can impact intestinal survival (Sanders et al., 2014; Flach, van der Waal, van den Nieuwboer, Claassen, & Larsen, 2018; Gomand et al., 2019). For example, *in vivo* persistence of a combination of *Lacticaseibacillus*, *Bifidobacterium* and *Propionibacterium* strains in the gastrointestinal tract when administered as capsules, yoghurt, or cheese was studied by Saxelin et al. (2010). Their results showed that the administration matrix did not influence the faecal quantity of *Lacticaseibacillus* spp., but when the probiotic was consumed in cheese, there were lower faecal counts of the *Propionibacterium* and *Bifidobacterium* strains compared to other dosage forms. However, Flach et al. (Flach et al., 2018) highlighted that daily dosages consumed in this study varied between matrixes, which could also have contributed to differences in faecal recovery. It should also be noted that survival during gastrointestinal transit or recovery in faeces are not health benefits.

Isolauri, Juntunen, Rautanen, Sillanaukee, and Koivula (1991) conducted one of the first human clinical trials comparing the efficacy of the same probiotic administered in two different matrices, as a supplement and as a fermented milk. The authors reported that *L. rhamnosus* GG either in the form of fermented milk or as freeze-dried powder was equally effective in shortening the course of acute diarrhoea. In another study, Meng et al. (2016) compared the impact of the consumption of *Bifidobacterium animalis* subsp. *lactis* BB-12 on upper respiratory tract infections and the function of NK and T cells in different delivery forms in a crossover design. Healthy adults consuming BB-12 capsules or yoghurt with BB-12 added during fermentation had elevated IL-2 secretion and NK-cell cytotoxicity, concurrent with fewer days of upper respiratory tract infection. These findings add to those of Isolauri and colleagues in 1991, supporting an elasticity of effect across fermented and freeze-dried formats. However, in two other subgroups, Meng and colleagues found that while plain yogurt (without BB-12 addition) also improved the immune and clinical parameters, a yoghurt with BB-12 added after fermentation failed to elicit these responses. While the authors suggested the potential that BB-12 added after fermentation could blunt the beneficial effects of plain yogurt, they also did not rule out a placebo effect across the arms, and a limitation due to small sample size (n = 30).

A 2020 study by Grom and colleagues compared the impact of three different dairy products containing *Lacticaseibacillus casei* 01 on post-prandial blood glucose concentrations. Healthy subjects consumed bread alone or in combination with one of three *L. casei*-containing dairy products (fresh cheese, ripened cheese or whey beverage) in a repeated measures design. The dose of probiotic consumed per serving was not standardised. There was a significantly lower increase in postprandial blood glucose concentrations at 45 min with the consumption of ripened cheese (13 mg/dl increase) compared to other matrices (average 20 mg/dL for fresh cheese and 30 mg/dL for whey beverage) and control (19 mg/dL increase). These results correlated with the greater inhibitory action of this matrix on α-glucosidase and α-amylase demonstrated *in vitro*. Authors suggested that the greater efficacy of the ripened cheese was due to the higher levels of protein and bioactive peptides created during dairy fermentation and maturation. In this example, the superiority of one format over another should be considered to be a function of the specific food itself, rather than any modulating effect of matrix on probiotic bioactivity.

In their 2018 review, Flach and colleagues identified three additional clinical trials comparing matrix effects (Flach et al., 2018). Two of these studies tracked immune biomarker changes with the administration of *Bifidobacterium lactis* HN109 (Chiang, Sheih, Wang, Liao, & Gill, 2000) and *L. rhamnosus* HN001 (Sheih, Chiang, Wang, Liao, & Gill, 2001)) in low-fat milk compared to lactose-hydrolysed low-fat milk. Both studies found increases in leukocyte activity across both active treatment arms. Chiang et al. observed significantly higher levels of natural killer (NK) cell activity in the lactose-hydrolysed milk group (approximately 240% increase from baseline) compared with non-hydrolysed milk (approximately 100% increase), while Sheih et al. found non-significantly higher NK activity levels in the lactose-hydrolysed milk group (mean 147% increase) compared to non-treated milk (71%). Such findings were attributed to the creation of GOS during the milk treatment. The remaining clinical study (Hutt et al., 2018) was not a single head-to-head comparison trial, instead reporting on two distinct trials delivering *Lactiplantibacillus plantarum* DSM 21380, one in yoghurt and one in cheese. Diastolic blood pressure was significantly reduced by both products, however systolic blood pressure values decreased significantly only due to consumption of probiotic cheese. Baseline blood pressure and BMI were significantly different between the cohorts, as was the daily dose of probiotic (1×10^{10} CFU in cheese and 6×10^{9} CFU in yoghurt), factors which may have influenced different results between the groups.

In the case where there is plausible evidence that a food matrix may negatively influence the efficacy of the probiotic, clinical trials with the new matrix may be needed. In the case of *L. plantarum* 299v, the probiotic was shown to increase the absorption of non-haem iron from phytate-rich oat gruel in healthy women, potentially via the production of organic acids in the intestine (Bering et al., 2006). Whether an iron absorption effect would be seen in non-phytate rich matrices was not clear, and further studies were conducted in new formats. Subsequently, Hoppe, Önning, Berggren, and Hulthén (2015), and Hoppe, Önning, and Hulthén (2017) demonstrated an increase in absorption of more bioavailable iron from low phytate meals, using 299v administered in an iron-supplemented fruit drink and 299v administered in iron-containing capsules, respectively. Therefore, the impact of *L. plantarum* 299v on iron absorption does not appear dependent on the food matrix.

8. Concluding thoughts and research directions for probiotics

To summarise, the available evidence suggests that processing, storage conditions and product formulation may alter probiotic structure or functionality *in vitro*, while culturing conditions have been shown to influence both *in vitro* and *in vivo* functionality in animal models. Variations in product formulation have also been demonstrated to alter faecal recovery counts of administered probiotics in clinical trials. However, when probiotic viability, dose and presence of other bioactive ingredients is controlled for, there is no human evidence that any clinical effect is altered among different matrices in head-to-head comparisons in a single study. Further, meta-analyses of different studies across different probiotic formulations show clinical benefits, suggesting a robustnessof effect on clinical outcomes.

A useful parallel to these conclusions comes from considering food-borne pathogens. Common food pathogens such as *Listeria monocytogenes,* members of the *Salmonella* genus, or certain strains of *Escherichia coli* are regarded as pathogenic microorganisms regardless the food matrix where they are present. While the food context of one pathogen (*Vibrio parahaemolyticus*) has been shown in an animal model to alter the quantitative effect (Wang et al., 2016), core pathogenic qualities remain. Although evidence for matrix-driven quantitative reduction in clinical effects for probiotics is lacking, future research may find that matrix may impact probiotic effect sizes, while not eliminating a clinical effect altogether. Further, as is the case with both pathogens and probiotics, the provision of a minimum infective or efficacious dose (respectively) as well as the host health status are considered more significant considerations for clinical impact than the food in which the microbe is delivered.

A future area of exploration is the development of quality control techniques that go beyond live cell enumeration, to assay structural integrity and functional activity of probiotics. Such assays could monitor metabolite production and surface architecture (such as for the pili of *L. rhamnosus* GG) in line with the available knowledge of modes of action. We also encourage researchers to consider evaluating more than a single matrix in clinical trials of probiotic strains destined to be delivered in different matrices.

9. Host factors impacting the response to probiotics and prebiotics

When considering the ability of probiotics and prebiotics to have a meaningful effect on health, characteristics of the person who uses them are likely important (Fig. 2). Indeed, stratification among study subjects is possible based on response or non-response to a probiotic or prebiotic intervention (Reid et al., 2010). Such individual responses are not unique to probiotics and prebiotics as many drugs are also effective in only a subset of patients. In most cases, what leads to a response or non-response is not known. The question being addressed in this paper is how important is the delivery matrix to probiotic and prebiotic functionality. This question encompasses a broader scope: considering the many factors likely to influence overall function of probiotics and prebiotics, is delivery matrix a major or minor factor? Although we do not have the data to quantitatively answer this question, considering the different factors may provide insight.

A factor that is likely very important in overall probiotic or prebiotic function is the nature of the microbial community in the consumers of these substances. Most efficacy studies do not characterise baseline microbiome status. However, research by Maldonado-Gómez and colleagues in 2016 is informative. They found that the absence of *B. longum* in the microbiota at baseline predicted the ability of the probiotic strain, *B. longum* AH1206, to stably colonise. Although no clinical endpoints were tested, the paper demonstrates that the resident microbiota can dictate probiotic residence time, a factor that would plausibly influence the duration or intensity of *in vivo* effects.

This finding demonstrates the impact of the availability of compositional niches in an individual's microbial community. There is also a potential role for functional niches. Probiotics can produce many bioactive compounds that are also produced by commensal microbes (Suez, Zmora, Segal, & Elinav, 2019), one example being bile salt hydrolase (BSH), which has demonstrated cholesterol reducing properties (Joyce, Shanahan, Hill, & Gahan, 2014). When Jones, Martoni, Parent, and Prakash (2012) administered a BSH-producing probiotic strain, *Limosilactobacillus reuteri* NCIMB 30242, to hypercholesteraemic individuals, a mean reduction in LDL cholesterol of 8.9% was observed. However, there was a large number of non-responders; out of 56 individuals in the treatment group, 21 experienced an increase in cholesterol concentrations. BSH of various forms and activity levels is produced by many commensal microbes, including common genera such as *Enterococcus*, *Clostridium*, and *Bacteroides* (Song et al., 2019). In an individual whose microbiome already possesses this functionality, it seems possible that a probiotic benefit mediated by this mechanism would be less effective. The presence of compositional and functional microbiome niches is a potential explanation for non-responders to probiotic interventions and stratifying for such factors has been suggested as a strategy to better understand probiotic efficacy (Zeilstra, Younes, Brummer, & Kleerebezem, 2018). In recent work, Szymanski et al. (2020) performed a post hoc metabolomic analysis of faecal samples from responders and non-responders to a *L. reuteri* intervention in children with acute gastroenteritis. They found significantly lower baseline levels of several metabolites including lactate in the responder group and hypothesised the existence of a vacant 'metabolic niche' within the responder group which the administered *L. reuteri* strain was able to fill.

In the field of prebiotics, Martínez, Kim, Duffy, Schlegel, and Walter (2010) found significant inter-subject variations in microbiome compositional changes from resistant starch administration. While some of the taxonomic shifts induced by prebiotics (namely resistant starch types 2 and 4) achieved significance across the group as a whole, none of the changes occurred consistently across the 10 subjects. The authors speculated that the inter-subject differences might be due to the presence or absence of certain bacterial groups with the relevant metabolic capacity and binding affinity for the particular substrate. They also suggested a role for other host factors beyond the microbiome, such as variations in digestive enzyme secretions or transit time, which are both factors that can alter the degree and duration of exposure of the prebiotic to the relevant bacterial groups. In a 2020 clinical study with obese subjects consuming inulin, weight-loss responders could be differentiated from non-responders by the baseline presence of certain genera including *Anaerostipes*, *Akkermansia* and *Butyricicoccus* (Rodriguez et al., 2020). Based on these findings teamed with parallel results from humanised mice experiments, authors suggested that improvement in metabolic disorders by inulin depends on the presence of specific bacteria within the microbiome. Such results suggest that interindividual differences have the potential to influence whether or not a given individual will receive a benefit from a probiotic or prebiotic ingredient, however, validated biomarkers are not yet available to guide recommendations to consumers. More research is needed to establish an individual's microbiota pattern or other phenotypic characteristics as key determinants of responsiveness to an administered probiotic or prebiotic.

Probiotics and prebiotics not only interact with the gut microbiome, but their chemical structures and metabolites also produce clinical effects via direct interaction with host cells, including the intestinal epithelial barrier, liver, immune system, and nervous system (Suez et al., 2019). Differential clinical response may not only result from heterogeneity in the microbiome, but also in physiological variations in function or baseline status of relevant host systems. For example, West et al. (2019) found a difference in the baseline immune gene expression between responders and non-responders to probiotic supplementation in allergic rhinitis. Sex, age, and health status also affect physiological response to any intervention, including probiotics and prebiotics.

A further consideration weighs heavily in a pragmatic view of the significance of product matrix effects. An individual's dietary habits and acute meal effects could constitute a larger background 'matrix' effect than the prebiotic or probiotic delivery vehicle itself. When an individual takes a probiotic or prebiotic supplement with food, the meal content will be extremely heterogeneous among individuals and among dosing occasions. The volume of food constituents in a meal is usually much greater than the volume within the matrix of a supplemental food, suggesting that dietary context could provide a much larger 'matrix' influence. This broader food matrix issue (the meal context) is an uncontrolled factor in most clinical studies, as well in everyday consumption - consider the almost infinite number of combinations of foods that could make up a consumer's (or study participant's) meal. Further, diet will independently impact gut microbiota composition and gut

function, providing ways that diet can indirectly influence probiotic or prebiotic function. In addition, other lifestyle factors and ingested substances such as medications (Maier et al., 2018) could also account for inter-individual variation in response.

Clinical studies which document detailed participant characteristics (including dietary intake and pre and post microbiome analysis) and attempt stratification of analyses based on the inter-individual differences described above will continue to grow our understanding of the role of host factors in the efficacy of probiotics and prebiotics. Such data could be examined alongside the earlier recommended comparative studies on product formulation, in order to understand the relative impact of host and product-related factors.

10. The regulatory perspective around the globe

Probiotics and prebiotics are regulated in many different manners across the globe. Regulations as they pertain to probiotics were recently reviewed for Asia (Au et al., 2019), Latin America (Binetti, Burns, Tomei, Reinheimer, & Vinderola, 2019), the United States (Smith, 2019), Canada (Powers, 2019), and the European Union (Von Wright, 2019). Procedures and documentation required for making a health benefit claim for a probiotic or prebiotic product vary among different regions, although there are some commonalities. Regulatory authorities typically require human intervention studies in generally healthy populations that provide suitable evidence that the intervention leads to the health benefit (Salminen & van Loveren, 2012). Further, the probiotic or prebiotic must be adequately characterised (chemical or microbiological description and identity) (Jackson et al., 2019). Such requirements are coupled with relevant quality assurance steps to ensure adequate dose and viability of probiotics and dose of prebiotics in the finished product.

Across different regions, there are variations in the regulatory positions regarding acceptance by regulatory authorities of evidence generated in one food or supplement format being used to substantiate health benefits for a different delivery format. Opinions solicited among experts on probiotic and prebiotic regulation from Asia, Canada, United States of America, European Union, Australia, New Zealand, Brazil and Argentina indicated that overall, specific guidance regarding extrapolation from one matrix to another is not clearly stated in regulations. On the whole, there appears to be significant room for applicants to offer appropriate scientific rationale that evidence can be extrapolated to other product formats. Further, experts stated it is rare that differences in inactive components (such as flavours, sweeteners, fat content, and the like) of the product formulation would preclude use of the evidence from a regulatory perspective.

In the United States, the Food and Drug Administration (FDA) and the Federal Trade Commission (FTC) share responsibility for assuring claims made on products are not misleading. The FDA has enforcement power, which it uses if food or supplement products claim to cure, treat, mitigate or prevent disease (in other words, are labelled as a drug) or if they deem products to be unsafe. The FTC is the U.S. agency responsible for truth in advertising, and this agency will challenge companies if they deem their products are labelled with unsubstantiated claims. In guidance for industry making dietary supplement claims, the FTC states, “If there are significant discrepancies between the research conditions and the real life use being promoted, advertisers need to evaluate whether it is appropriate to extrapolate from the research to the claimed effect” (Federal Trade Commission, 2001). This clearly allows a company to make an argument of substantial equivalence between different formulations of a product.

Health Canada allows even more flexibility by accepting claims for certain probiotics in foods without a requirement for strain-specific (or matrix-specific) human trials, as long as the species used is on Health Canada’s list of acceptable species and 10^9 live CFU/serving is delivered in the product.

In the European Union (EU), all health claims for foods or active food ingredients are submitted to the European Food Safety Authority (EFSA) for pre-approval, and once authorised, the claims for an ingredient may be used in new products according to the published conditions for use (European Commission, 2020). Such conditions have not typically stipulated dependency upon a specific delivery matrix in the finished product, although there are exceptions. For example, the claim for improved lactose digestion from yoghurt cultures specifies delivery in a fermented milk product. In the case of an approved bowel health claim for native chicory inulin, a minimum daily dose and chemical composition (carbohydrate structural and polymerisation limits) are specified for the inulin, but no delivery matrix requirements are included. While there is a paucity of approved claims on probiotics and prebiotics to consult as precedents, we expect that EFSA will continue to assess these on a case-by-case basis, and various conditions (including delivery matrix limitations) may be imposed depending on available evidence (EFSA Panel on Dietetic Products, Nutrition and Allergies et al., 2017). It is relevant to note that where matrix conditions have been prescribed in the approved health claim, it may not be possible to extend this claim to other matrices (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2012).

In some other regions, more definitive guidance is provided. In Argentina, health claims (including ‘with probiotics’) require authorisation after satisfactory evaluation of ‘*in vivo* trials with the food as it is to be consumed’ (Secretary Politicas, 2011). In Australia, while food products have more flexible regulations (Food Standards Australia New Zealand, 2015), dietary supplement forms of prebiotics and probiotics are regulated within the medicine framework, where it is stipulated that the preparation and dosage form in the product should match those used in the evidence substantiating the claim (Therapeutic Goods Administration, 2019).

In summary, with some exceptions, most authorities do not stipulate specific legal requirements for substantiation of health claims using studies with the exact formulated product. However, on the whole, if claims are challenged it is clear that regulatory authorities will require that any extrapolation of data from a study conducted in a product different from what is being marketed must be based on sound scientific evidence, principles and opinion.

11. A path forward - demonstrating essential equivalence

Demonstrating ‘essential equivalence’ may provide a path forward. The concept of ‘essential equivalence’ is reflected in different regulatory frameworks. In the United States, the Federal Trade Commission uses the following definition: “Essentially equivalent” product means a product that contains the identical ingredients, except for inactive ingredients (e.g., binders, colours, fillers, excipients) in the same form and dosage, and with the same route of administration (e.g., orally, sublingually), as the Covered Product; provided that the Covered Product may contain additional ingredients if reliable scientific evidence generally accepted by experts in the field indicates that the amount and combination of additional ingredients is unlikely to impede or inhibit the effectiveness of the ingredients in the Essentially Equivalent Product.’ (United States District Court, 2019).

A path to demonstrate essential equivalence in the case of probiotic products via a scientifically valid rationale was proposed by Sanders et al. (2014), consisting of two key activities: quantification of survival in the finished product and phenotypic performance mapping. Performance mapping was proposed to consist of comparative *in vitro* or *in vivo* tests of relevant readouts of mechanistic functionality (such as lactase activity, antimicrobial activity, organic acid production, immunomodulatory profiling *in vitro* or *ex vivo*, and integrity of probiotic effector molecules such as pili), probiotic culture growth performance, acid resistance, bile resistance, or recovery from target host site (faeces, oral, stomach, or vaginal sites). This approach also has the potential to be applied to prebiotics. For prebiotics, chemical analysis to ensure equivalent prebiotic concentration and composition could replace quantification of probiotic survival. Relevant performance maps for

prebiotics could also include functional assays such as *in vitro* organic acid production, or more detailed structural integrity analysis techniques.

If *in vitro* or *in vivo* performance maps demonstrate differences between the new and original formulations, further clinical investigations may be needed. It is worth noting that a performance mapping approach may identify *in vitro* differences in functionality between matrices that may not translate to significant differences in clinical endpoints, in line with our findings so far. Tolerance limits for differences in *in vitro* effects may be able to be developed in the future, through the combination of performance map data and comparative head-to-head clinical trial data, which may avoid undue focus on *in vitro* matrix effects that do not have a significant impact on clinical efficacy. To enable the creation of pertinent assays, further preclinical and clinical research is needed to further elucidate the mechanism of action and relevant functional markers for probiotics and prebiotics.

Sanders and colleagues suggested that the conduct of such a performance map was indicated when substantive changes in the product format had the likely potential to influence probiotic activity (Sanders et al., 2014). A noted difficulty with this model at the time was that insufficient evidence existed to guide the rational identification of what constituted substantial product change. In our view, a starting place for such evaluation could be review of the clinical trial literature, to determine if integrity of clinical effect has been demonstrated across clinical trials and meta-analyses for similar matrices with similar levels of variation. Novel, previously unstudied product formats as well as significant changes in manufacturing techniques of the probiotic or prebiotic itself would be strongly recommended for a performance mapping approach.

Performance mapping approaches may provide a practical solution to the challenge of providing confidence in new product formulations in a scientifically, ethically and economically justifiable manner, compared to the repetition of clinical trials for each matrix change. Further research to develop robust approaches in this space could support innovation in delivery vehicles for probiotics and prebiotics and enable a more widespread and accessible delivery of health benefits, with potential public health benefits (Lenoir-Wijnkoop, Gerlier, Roy, & Reid, 2016).

12. Conclusion

With recent trends in the development of novel probiotic and prebiotic product formulations, it is of significant importance to ensure that they reliably deliver claimed health benefits. It is clear that product formulation and processing factors such as heat, pH, moisture, mechanical stress, and chemical reactions have the potential to degrade or inactivate probiotics and prebiotics. Therefore, care must be taken to avoid formulations and processing techniques that may cause degradation or inactivation of these active ingredients. Further, finished formulations should be verified to assure they deliver the required composition and concentration of prebiotics and identity, number and viability of probiotic cells.

In addition to adverse effects on viability or degradation, delivery matrices and the processing steps inherent in their production have the potential to affect probiotic and prebiotic functionality, but there is limited convincing evidence to suggest a significant impact of these changes on clinical endpoints. Clinical trials directly comparing delivery formats are rare and provide mixed data with several potential confounders, such as dose variability among arms; while comparing directly across studies introduces variability in study populations. Meta-analyses suggest that probiotics and prebiotics are able to exert their beneficial action across a range of delivery matrices, although such evidence does not enable assurance that the magnitudes of effects are not impacted. Taken together, we conclude that probiotics and prebiotics can be considered as active ingredients, with benefits that may extrapolate to new product formulations, provided formulation characteristics antagonistic to functionality are absent and quality assurance standards are met. However, such extrapolations should be substantiated by a considered rationale based on scientific evidence and principles.

We recommend regulatory authorities place an increased weight on meta-analyses where available to determine the robustness of effect of probiotic and prebiotic ingredients across matrices. Where there is an absence of data and the theoretical potential for negative interactions in a new formulation exists, we recommend the demonstration of "essential equivalence" between products through the application of a performance mapping approach based on sound scientific principles. The development of assays to characterise structural and functional properties of prebiotics and probiotics in more detail will aid robust and standardised performance mapping approaches.

Further, while data are lacking that enable us to quantify their relative importance, the impact of host factors, such as accompanying meal composition, dietary habits, lifestyle factors, medications, resident microbial populations and baseline health status, may be more significant than the delivery matrix on response to a probiotic or prebiotic product. Research exploring host response factors that determine responsiveness to specific prebiotic and probiotic interventions will advance understanding in this area.

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Authorship statement

RG, MC and MES conceptualised and organised the sharing of these viewpoints at the annual meeting of the International Scientific Association of Probiotics and Prebiotics (ISAPP). MC took primary responsibility for manuscript planning, collation and administration. All authors wrote, edited and reviewed the manuscript.

Declarations of competing interest

M.C. and R.G. are involved in research and development projects as employees of companies that produce, sell and distribute healthcare products, including probiotics and prebiotics (M.C -Metagenics, R.G. - GSK Consumer Healthcare).

G.V. serves on the board of ISAPP and has led industry-sponsored research projects on dairy products and probiotics. These projects were independently carried out and had no influence on the content of this manuscript. He is member of the Argentinian board of the Yoghurt in Nutrition Initiative (Danone Argentina).

D.C. has led industry-sponsored on probiotics and prebiotics. These projects were independently carried out and had no influence on the content of this manuscript.

S.L. serves on the board of ISAPP and has led industry sponsored research projects on probiotic supplements. She is a member of the scientific advisory board of Yun focusing on probiotics for skin applications (www.yun.be)

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