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Food for thought! Inulin-type fructans: Does the food matrix matter?

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ABSTRACT

Food matrices can be described as the final composition of a food product which results from complex interactions between compounds found within different ingredients and the processing parameters used in production. These factors, not only impact on the final structure of a product, but also have the potential to alter both the structural integrity and bioavailability of potentially beneficial compounds present, for example, dietary fibres. As a result, there is growing curiosity amongst the scientific community on whether the food matrix may impact on the prebiotic efficacy of inulin-type fructans. Therefore, the purpose of this review is to explore previous food-based inulin-type fructan supplementation studies to determine whether the food matrix directly impacts on their prebiotic efficacy. Our working hypothesis is that other potentially prebiotic ingredients and components present within the food may alter inulin-type fructans prebiotic effect.

1. Introduction

As diet is a key driver of gut fermentation and thus can strongly influence the composition and functionality of the gut microbiota, one way to modify the composition of the gut microbiota and potentially improve health outcomes is via the use of prebiotics, as they provide a safe, affordable and effective dietary approach (Sanders et al., 2019). Under the latest definition prebiotics are categorised as a substrate that is selectively utilized by the host microorganisms conferring a health benefit (Gibson et al., 2017). The most supported of all prebiotics are oligofructose (OF) and inulin, which belong to a class of non-digestible carbohydrates referred to as inulin-type fructans (ITF) (Karimi et al., 2015).

ITF were first discovered over two centuries ago and are a natural component of several plant species including Jerusalem artichokes, bananas, garlic, leeks, dandelion and chicory amongst others (Roberfroid et al., 2010). The amount and DOP of ITF present vary significantly between species. For examples, wheat, bananas and onions possess short-chain ITF (max DOP < 10). Jerusalem artichokes possess medium-chain ITF (max DOP < 40) with globe artichokes and chicory root possessing long-chain ITF (max DOP $\langle 100$) (Roberfroid et al., 2010). In this regard, with exception of chicory root which possesses approximately 70% inulin on a dry weight basis, most of these fruits and vegetables only possess trace amounts of ITF and as a result isolation of supplemental ITF primarily focuses on chicory root (Mensink et al., 2015).

From a chemical standpoint, in general, ITF are linear polydisperse carbohydrates composed of monomers of fructose linked by β-(2–1) glycosidic linkages. A starting α-D-glucose moiety may or may not be present (Roberfroid, 2007). Based on the degree of polymerisation (DOP) ITF can be separated into oligofructose (OF) (DOP 2–9) and inulin (DOP 2–60 +) (van Loo, 2006) See Fig. 1.

The ability of ITF to alter the composition of the gut microbiota and manipulate health parameters has been investigated extensively (Gibson & Roberfroid, 1995b, Kleessen et al., 2007, Marteau et al., 2011, Ramnani et al., 2010, Rao, 2001). These benefits include increasing and decreasing the numbers of beneficial and potentially harmful bacteria, for example stimulating the growth of bifidobacteria as they possess the necessary glycosidases to hydrolyse the β-(2–1) glycosidic (fructosyl-fructose) linkages (Falony et al., 2009, Riviere et al., 2018). This

Abbreviations: BMO, Bovine Milk Oligosaccharides; DOP, Degree of polymerisation; HPAE-PAD, high-performance anion exchange chromatography-pulsed amperometric detection; ITF, Inulin-type fructans; LC, Long chain; OF, Oligofructose; 3′SL, 3′sialyllactose; 6′ SL, 6-sialyllactose; 6′ SLN, 6′-sialyllactosamine; DSL, Disialyllactose; CLA, Conjugated linoleic acid concentration; CFU, Colony forming unit.

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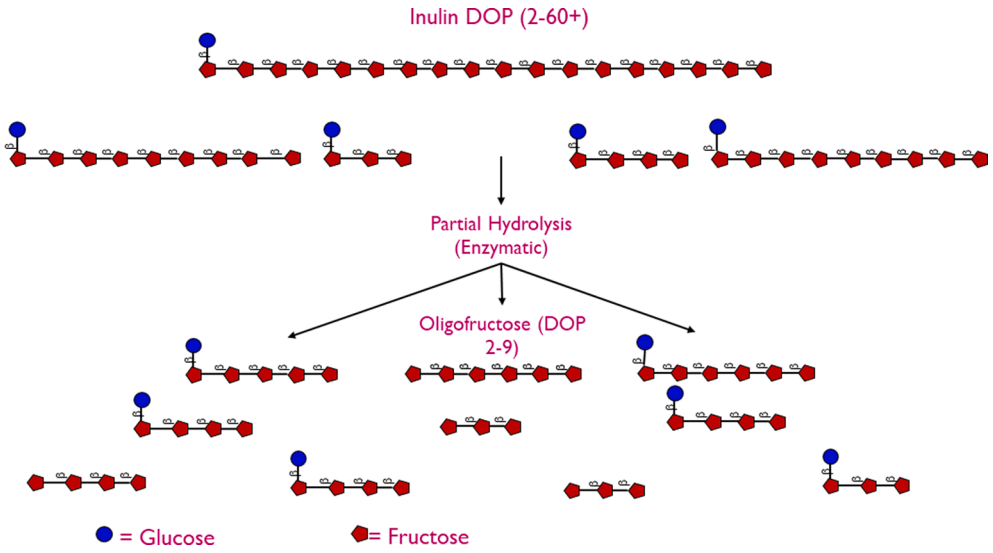

Fig. 1. Structure of inulin type fructans.

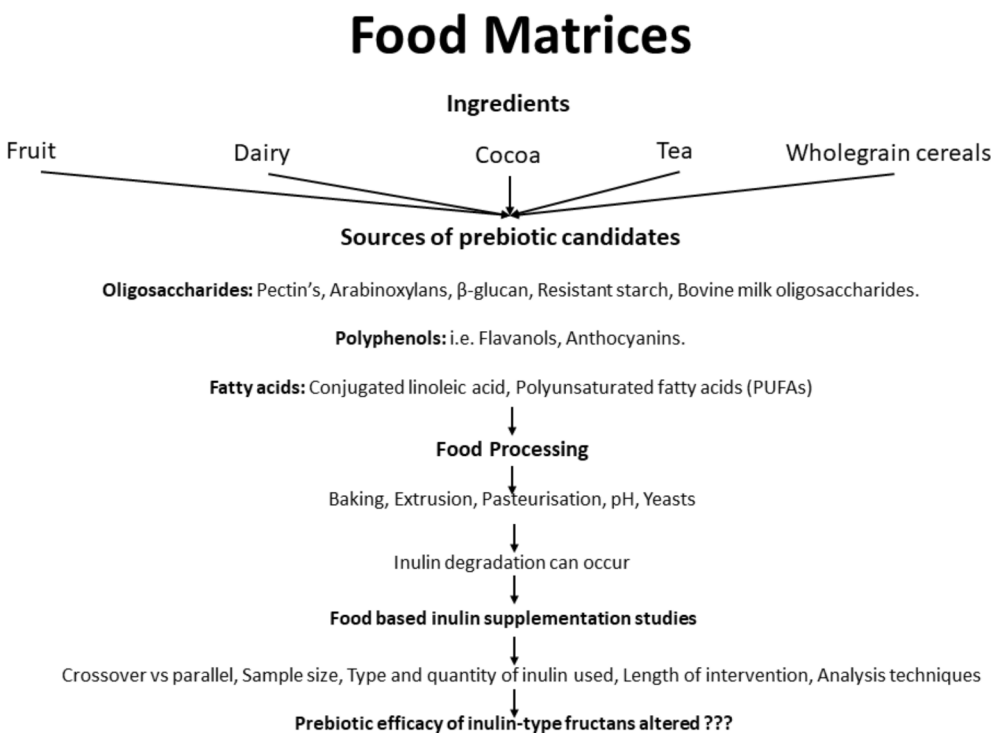

Fig. 2. Overview on how the food matrix may impact on the prebiotic efficacy of inulin-type fructans.

saccharolytic fermentation reduces the fermentation of undigested or endogenous proteins (Wang et al., 2019, Wang et al., 2020) and produces beneficial metabolites such as short-chain fatty acids, associated with increased satiety (Morrison & Preston, 2016), modifications in immune function (Delgado et al., 2012), and improvements in bowel transit function (Buddington et al., 2017) amongst others.

The concept that the food matrices may impact on the prebiotic efficacy of ITF has become of increasing interest amongst the scientific community in recent years. This is due in part to previous research suggesting that food matrices may either hinder or enhance the bioavailability of phenolic compounds, fatty acids and other nutrients (Ribas-Agusti et al., 2018, Thorning et al., 2017).

Yet, while several studies have utilised various food products such as biscuits, yoghurt, stewed apple, cereal bars, cocoa drinks and fruit juices as vehicles for ITF supplementation (Brighenti, Casiraghi, Canzi, & Ferrari, 1999; Gibson, Beatty, Wang, & Cummings, 1995a; Kleessen et al., 2007; Ramnani et al., 2010; Rao, 2001; Slavin & Feirtag, 2011) none of these were specifically designed to determine the effects that the food matrices had on the prebiotic efficacy of ITF. Furthermore, the food products used in these studies can also be sources of several other potential prebiotic candidates including phenolic acids, β-glucan, arabinoxylans and bovine milk oligosaccharides. Many of these possess the potential to alter microbial selectivity and is an aspect often overlooked by researchers when considering study designs regarding food-based prebiotic supplementation studies.

The only study to date to consider whether the matrix altered the prebiotic efficacy of ITF (Kleessen et al., 2007). The results suggested that the prebiotic (bifidogenic) efficacy of ITF was unaltered as a result.

Therefore, this leads to the question of whether food matrices matter in the supplementation of ITF. This question is becoming increasingly important to answer given the interest in the addition of ITF into various foods products which are supposed to be beneficial for health. Thus, the purpose of the remainder of this review is to explore previous food-based ITF intervention studies aiming to conclude whether the matrix likely impacts on the prebiotic efficacy of ITF. Fig. 2 presents a theoretical overview of how the food matrix may potentially impact on the prebiotic efficacy of ITF.

2. The food matrix effect: What are food matrices?

The potential importance of the food matrix has received much attention as the composition of the matrix can directly influence the bioavailability of nutrients due to its effect on the digestion process. For example, during digestion the bioavailability of phenolic compounds, mineral and fats in the gastrointestinal tract can be directly influenced by the presence/absence and combination of carbohydrates, proteins, fats, calcium and minerals (Palafox-Carlos et al., 2011, Thorning et al., 2017, Zeng et al., 2016). Given the strong structure–function relationship existing between dietary fibre and its behaviour during digestion, one must comprehend this relationship in order to recognise the potential effects this may have on physiological outcomes (Capuano, 2017). For example, there is evidence existing that the presence of high levels of dietary fibre present within the matrix can directly influence the absorption of the afore mentioned compounds, via the sequestering of ions and binding of phenolic compounds (Capuano, 2017, D'Archivio et al., 2010, Palafox-Carlos et al., 2011). This concept also applying to the microbial fermentation of unabsorbed compounds and the absorption of resulting metabolites within the colon (Aguilera, 2019).

Food matrices at face value can be crudely classified into several basic types: liquid, emulsions, gels, viscoelastic, dense, and porous (Aguilera, 2019) amongst others, and represent a wide variety of different products including yoghurt, cakes, sauces, soups, biscuits, ready-to-eat breakfast cereals and snacks, orange and other fruit juices with several food products consisting of more than one matrix. Additionally, how these food products are produced can not only modify the structure of the matrix (i.e. viscosity, porosity, density etc) but when considered alongside other processing factors including, milling, grinding, pH, pressure and temperature, have the potential to directly influence the physicochemical (particle size, intactness, exposedness) properties of dietary fibres (Duar et al., 2015, Klewicki, 2007, Poinot et al., 2010). Furthermore, one must consider other biological ingredients present within the food matrices, including phenolic compounds, dietary fats and other fermentable dietary carbohydrates that can also directly influence the composition of the gut microbiota. As a result, it is increasingly difficult to predict the behaviour of dietary fibre and their physiological effects when administered in solutions (beverages) or a solid food matrix, even those of the same class and compositional nature (Capuano, 2017). It is for these reasons that food matrices might have effects on the prebiotic efficacy of ITF.

2.1. Effects of food processing

The physiochemical properties (compositional nature) of ITF are the hallmark of the prebiotic efficacy of ITF. Given that ITF can also function as both fat and sugar replacers as well as texture modifiers and there has been much interest in the addition of ITF into several different food products. in order to help reduce not only the consumption of both saturated fat and sugar (sucrose) but also increase people's dietary fibre intakes (Shoaib et al., 2016). Yet, ITF can be subject to structural degradation when exposed to specific processing conditions.

One examples of this is low pH with the critical cut-off point appearing < 4 (often seen in fruit juice production) where at below this pH hydrolysis begins to occur due to protonation of the glycosidic bond, potentially resulting in a loss of functional properties (Glibowski & Wasko, 2008, Mensink et al., 2015). The sensitivity of ITF to low pH only heightens with increasing time and temperature. For instance, during pasteurisation it has been demonstrated that between 70 and 87% of OF was degraded under two-stage processing of an apple and blackcurrant juice drinks (Klewicki, 2007). Yet, the same authors reported that under less extreme pasteurisation parameters (pH 4.2; 95 °C 30 sec), 80% of OF survived the pasteurisation process. A finding similar to those reported by both Duar et al. (2015) and (Glibowski et al., 2020) who noted that OF, native and HP inulin were stable at pH 4 and pH 3 in a model and apple juice drink respectively implying when both pH and time are considered little-to-no breakdown of ITF occurs.

In addition to pasteurisation, other heating processes including baking also have the potential to alter the structural integrity of ITF. One illustration of this comes from Poinot et al., (2010) who demonstrated that bread containing 5% ITF darkened 3 mins quicker than bread containing no ITF. Several other studies have reported that the addition of ITF in baked products altered the colour of the final product suggesting that structural degradation of ITF may have occurred (Zahn et al, 2010; Rodriguez-Garcia et al., 2012). The potential for ITF to alter the browning of a product is suggested to be down to the ability of ITF to participate in the Maillard and caramelisation reactions due to the presence of reducing groups (Mensink et al., 2015). Under this premise, there are multiple pathways by which ITF can participate in the Maillard caramelisation reactions. Firstly, via direct participation to due to availability of free reducing ends and secondly due to presence of invertase/inulinase which can specifically target and hydrolyse the β-(2–1) glycosidic (fructosyl-fructose) linkages where 80–90% of non-ITF/ITF can be degraded (Morreale et al., 2019; Verspreet et al., 2013) producing more reducing groups. However, to what extent the participation of ITFs in either/both the Maillard and caramelisation reactions has on the bifidogenic properties of ITF is not well understood.

Furthermore, it has been reported that when ITF were added into ready-to-eat breakfast cereals at temperatures including 140 °C at a screw speed of 170 RPM, >50% OF was degraded. LC-ITF appeared to be unaffected by low temperature (120 °C) extrusion, however, when screw speeds were adjusted to 120 and 170 rpm only 25% and 34% of LC-ITF were recovered. Low levels of inulin were recovered (35%) when temperatures and screw speeds were operated at their most extreme conditions: temperature (170 °C) and pressure (170 rpm) respectively (Duar et al., 2015).

Overall, these results imply that the time, temperature, and degree of polymerisation of ITF used during the production process appears to be critical if the potential degradation of ITF is to be avoided, with each of these aspects needing to be carefully considered in order to optimise product quality while maintaining ITF integrity.

2.2. ingredient-ingredient interaction – Food for thought?

One aspect that is frequently overlooked when designing food-based inulin supplementation studies is the effects that other potential compounds present within the food matrix may have in altering the prebiotic efficacy of ITF. For example in the production of food products, including baked goods (cakes, biscuits and bread), dairy products (yoghurt and ice cream) and fruit juices (orange and apple) several ingredients including numerous types of wholegrain flours or cereals (wheat, spelt and barley), dairy ingredients including milk and skimmed milk powder, as well as cocoa powder and fresh and/or dried fruit and vegetables are often combined to produce a final product (Brighenti et al., 1999; Kleessen et al., 2007; Menne et al., 2000) with each of these ingredients being a potential source of microbially active compounds. Examples of this include cranberries, apricots, lemons and oranges which are known sources of fermentable polysaccharides and polyphenols, including pectins and hesperidin (Sadler et al., 2019; Sanchez-Patan et al., 2015). Pectins have been shown to increase the levels of bifidobacteria, *Lactobacillus*, some strains of *Bacteroides*, *Enterococcus*, *Prevotella,* and *F. prausnitzii*. The ability of pectins to stimulate changes

in microbial composition appear to be structure and microbiome composition dependant (Gomez et al., 2016; Larsen et al., 2019). While polyphenols found in black tea have been shown to increase levels of Bacteroidetes (Kemperman et al., 2013), the flavanols present in cocoa have been shown to increase levels of both bifidobacteria and lactobacilli *in vivo* (Tzounis et al., 2011). Yet, while the research on the ability of both pectins and polyphenols to have beneficial effects on the composition of the gut microbiota is building, it is still in its infancy with many of the mechanisms of gut microbiota modulation, particularly by polyphenols, not being well understood (Scott et al., 2019).

Wholegrain cereals, including wheat, rye, spelt and barley, are desirable sources of numerous fermentable carbohydrates namely non-ITF, arabinoxylans and β-glucans (Knudsen, 2015; Maccaferri et al., 2012) for which a body of evidence on prebiotic efficacy is rapidly growing. Bifidobacteria and potentially *Bacteroides* and lactobacilli appear to be main bacteria to benefit from the fermentation of these dietary fibres (Costabile et al., 2008; Knudsen, 2015; Scott et al., 2019; Valeur et al., 2016; Walker et al., 2011).

Another common food ingredient is bovine milk (a common constituent of mousses, ice cream and milk drinks) and is a source of bovine milk oligosaccharides (BMO) with 10 of these BMO being identical to those found in human breast milk (Kirmiz et al., 2018; Zivkovic et al., 2011). However, compared to human breast milk, the oligosaccharide content of bovine milk is nearly 10-times less at approximately 100 mg/L (Robinson, 2019) with the predominant oligosaccharides being 3′sialyllactose (3′ SL), 6-sialyllactose (6′ SL), 6′-sialyllactosamine (6′ SLN), and disialyllactose (DSL). Together these make up the majority at 60–94 μg/mL to 347–460 μg/mL (Fong et al., 2011).

Whey permeates, a by-product of cheese making and skimmed milk powder production, are becoming an increasingly common ingredients used in baked goods, meats, soups and confectionary (Krolczyk et al., 2016; Smith et al., 2016). As a result, the bovine milk oligosaccharide concentration in whey permeate and skimmed milk powder is likely to be higher than in milk. However, what these increases in BMO mean regarding alterations in microbial composition has yet to be determined. Moreover, dairy products including milk, cheese and yoghurt are also a source of conjugated linoleic acid, an acid produced as a result of microbial fermentation in the rumen (Devillard et al., 2007). Similar to BMO, the conjugated linoleic acid concentration (CLA) in bovine milk is low at 0.55 to 1.53 g/CLA 100 g but may be increased as a result of yoghurt and cheese production (Prandini et al., 2007) and has been shown to decrease proportions of Firmicutes ($P = 0{\cdot}003$) and increase proportions of Bacteroidetes ($P = 0{\cdot}027$) in mice respectively (Marques et al., 2015).

However, it can be speculated that several of these potential prebiotic candidates present within various food ingredients may potentially interfere with the prebiotic efficacy of ITF. Due to differences in growing conditions and location, time of the year and the species used in production (Huynh et al., 2008; Marcotuli et al., 2016; Robinson, 2019) there can be significant variations in the concentration of each of these potentially beneficial compounds present within the final product. Thus, unless each ingredient is measured and standardised for levels of potential prebiotic candidates prior to production, the role each of these potentially bioactive compounds play in altering the composition of the gut microbiota may never be fully known.

2.3. Baked goods

2.3.1. Cereal bars

One of the only studies to date to question whether the food matrices and the processing methods used altered the prebiotic efficacy of ITF was undertaken by (Kleessen et al., 2007). In this study commercial inulin from chicory (Fibruline® Instant) and Jerusalem artichoke inulin were fortified into snack bars produced from purely vegetable ingredients (not identified) at 7·7 g/per bar. A non-inulin containing cereal bar was used as a control. The control bar contained several wholegrain cereals, dried fruits and fruit juice concentrates. The study design was a randomized, double-blind, placebo-controlled study with parallel groups that included forty-five healthy volunteers. After a one-week run-in period, subjects were randomly assigned into one of three groups (control; chicory inulin bar; Jerusalem artichoke inulin bar). Bars were consumed for 3-weeks once per day during week 1, and twice per week during week 2 and 3. Faecal samples were collected after the run-in period had been completed and then again at day 14, 21 and 28. Changes in microbial composition were determined by fluorescent *in situ* hybridization combined with selective media techniques to determine changes in some less abundant microbial groups/species. Differences in fructans before and after processing were determined via high-performance anion exchange chromatography-pulsed amperometric detection (HPAE-PAD). The authors reported that after consumption of either the chicory or Jerusalem artichoke inulin bar, total numbers of faecal bacteria remained constant; while there was a steady increase in bifidobacteria by approximately 1·2 $\log_{10}$ cfu/g faeces (wet weight) after 3 weeks and was significantly different from the placebo group ($P < 0{\cdot}05$). Both chicory and artichoke bars also reported lower levels of *Bacteroides/Prevotella* compared to the placebo group ($P < 0{\cdot}05$). Along with an approximately 0·6 $\log_{10}$ cfu/g faeces (wet weight) fewer numbers of *Clostridium coccoides/Eubacterium rectale* group at the end of the intervention period (Day 28). Unfortunately, due to the lack of detail over the ingredients of the intervention bars, it is difficult to put these data into the context of the food matrix. Furthermore, no structural differences were detected in either chicory or Jerusalem artichoke inulin before and after processing, confirming their stability during processing, which is the prerequisite for ITF to exert a prebiotic effect.

The addition of ITF into snacks bars was also undertaken by (Reimer et al., 2020)..

The study design was a single-center, placebo-controlled, double-blind, crossover study with a 4-wk washout period involving fifty healthy adults. Subjects were randomly assigned to one of two trials: Trial 1—Moderate Dose ITF (7 g/d) snack bar and Control 1 snack bar; or Trial 2—Low Dose ITF (3 g/d) snack bar and Control 2 snack bar with the composition of the intervention and control block varying between Trial 1 and Trial 2. Subjects were instructed to consume 1 bar/d for 4 wk in each treatment arm. The total duration of the study including the 4wk washout period was 12 weeks. Each subject provided 10 stool samples: 1 at baseline and 1 for every week of the trial with changes in faecal microbiota composition being determined using 16S ribosomal RNA–based approaches. The results of this study indicated that, compared to the control group, the moderate dose group showed significant differences across multiple microbial taxa with most notable increases being detected in *Bifidobacterium* (mean ± SEM) 5.3% ± 5.9% to 18.7% ± 15.0% over the 4wk period. With the low-dose ITF snack bar significant increases in *Bifidobacterium* were no longer present after correction for multiple comparison ($P = 0.55$). However, targeted analysis with qPCR showed significant increases in relative abundance of *Bifidobacterium* for the low bar dose at week 2 ($P = 0.027$) and a trend toward an increase at week 4 ($P = 0.056$) compared with the Control 2 bar.

2.3.2. Biscuits

One of the first studies to utilise food products as means of ITF supplementation was undertaken by (Gibson et al., 1995a).. Under the premise of this study, eight healthy adults were fed an initial control diet for 15 days consuming 15 g/day sucrose daily, followed by another 15-days in which 15 g sugar was replaced with OF, followed by further 15 days on the sucrose control diet. Additionally, four adults went on to complete a further 25-days study, comprising the same control sucrose diet for 10-days, with sucrose then being substituted for 15 g/day inulin for a further 15 days. 5 g of the OF and inulin were consumed as a supplement with the remaining 10 g being incorporated into biscuits. Stool samples were collected three times during the last three days of each dietary period. Changes in total anaerobes, total aerobes,

coliforms, Gram-positive cocci, bifidobacteria, *Bacteroides*, fusobacteria, lactobacilli, and clostridia were analysed via selective media techniques. The results of this study indicated that while supplementation had little to no effect on the total viable counts of aerobes or anaerobes, both OF and inulin increased bifidobacterial counts by 0.7–0.9 $\log_{10}$/g faeces (wet weight).

(Tuohy et al., 2001) also used biscuits as means of ITF supplementation but with a larger sample size of thirty-one participants in total. OF was not the only dietary fibre used in this study as partially hydrolysed guar gum was utilised alongside OF. Each subject consumed 6.6 and 3.4 g/day of OF and partially hydrolysed guar gum fortified into three biscuits resulting in a total biscuit consumption of up to 37.5 g/day. Volunteers consumed the experimental biscuits for one 21-day period and then the placebo biscuits for a second 21-day period. Changes in faecal microbial composition were analysed by fluorescent *in situ* hybridisation. The authors of this study reported that the consumption of oligofructose and partially hydrolysed guar gum resulted in a 0.487 $\log_{10}$ cfu cells/g faeces increase in *Bifidobacterium* spp., suggesting that the biscuit matrix does not impact on the bifidogenic effect of inulin-type fructans. The authors also noted there were little-to-no changes in numbers of total bacteria, *Bacteroides* spp., *Clostridium* spp. or *Lactobacillus/Enterococcus* spp.

2.3.3. Extruded ready to eat snacks

Ready-to-eat breakfast cereals and snacks represent a class of matrices referred to as porous and are composed of mixtures of wholegrains or slurries of grains, sugar and water, extruded under high pressure and temperature conditions and with varying degrees of shear (Peressini et al., 2015; Sacchetti et al., 2005; Tsokolar-Tsikopoulos et al., 2015). As the extrusion process allows manufacturers to fortify food products with vitamins and minerals, which may be lost in other parts of the production process, it should come as no surprise that the addition of ITF to extruded food products has been extensively studied (Brennan et al., 2008; Capriles et al., 2009; Tsokolar-Tsikopoulos et al., 2015). Yet to date, only one study has aimed to determine the effects of addition of ITF to extruded ready-to-eat breakfast cereals on the composition of the gut microbiota (Brighenti et al., 1999). In this study, inulin (Fibruline® Instant) with an average DOP of 7 with 30% of inulin present possessing a DOP $>$ 30 was, incorporated at 18% (dry weight) into a test cereal, prepared by puffing a dough consisting of rice flour, salt, sucrose, maltodextrin, and water. Participants consumed 50 g of a rice-based ready-to-eat cereal (placebo), then the same cereal containing 18% inulin (test) in substitution of their regular breakfast. After which, they then returned to the regular habitual diet (wash-out). They followed no other dietary restrictions. Stool samples were collected at baseline and on the fourth day of the last week of the intervention period, with changes in microbial composition determined by selective media techniques (total facultative anaerobes on Difco Tryptic Soy agar, bifidobacteria on NPNL-agar, Bacteroidaceae on kanamycin-vancomycin blood, clostridia on sulfite-polymyxin-milk and coliforms on Difco levine-eosine-methylene-blue agar). The authors noted that although not significant compared to the basal numbers, upon consumption of the placebo there was a 0.49 and 1.47 $\log_{10}$ CFU/g faeces dry weight increase in *Bacteroides* and clostridia. As well as a 0.08 $\log_{10}$ CFU/g faeces decrease in bifidobacteria. The increases seen in *Bacteroides* in this study could have occurred due to the formation of novel carbohydrate complexes as a result of the extrusion process. Given *Bacteroides* possess the widest array of loci able to target dozens of highly complex glycans (Flint et al., 2012). This being an area of much needed research if the functional effects of food processing on dietary fibre behaviour during digestion and the gut microbiota are to be fully understood (Capuano, 2017). Yet, upon consumption of the inulin test breakfast cereal numbers of bifidobacteria were significantly higher at the end of the test period compared to the placebo ($P < 0{\cdot}05$). Numbers *of Bacteroides* decreased returning close to basal levels. This suggesting that bifidobacteria in the presence of inulin are able to outcompete *Bacteroides* for substrates becoming the dominant genus. This further indicated the high level of selectivity of ITF toward bifidobacteria. Yet, the increase in bifidobacteria seen in this study was relatively small at just 0.33 $\log_{10}$ CFU/g faeces dry weight. The smaller than expected response likely occurring from the higher levels of bifidobacteria present in baseline stool samples. Although one must also consider that the ITF used in this study possessed an average DOP of 7 with $<$ 30% of the ITF possessing a DOP $>$ 30, it could have undergone a substantial amount of degradation during the extrusion process (Duar et al., 2015; Tsokolar-Tsikopoulos et al., 2015). This may have also contributed towards the low bifidogenic response. However, as the authors did not analyse carbohydrate structure prior to or post extrusion this cannot be determined with any real degree of certainty.

2.4. Dairy

2.4.1. Cheese

To date, only one study has investigated if the addition of ITF to cheese was able to stimulate changes in microbial composition. In this study, the ability of Swiss cheese containing Beneo Orafti® ST and Orafti® P95 to alter the composition of the gut microbiota was investigated using pH-controlled *in vitro* fermentation experiments in which changes to microbial composition were analysed via fluorescent *in situ* hybridisation (Cardarelli et al., 2007).

The results showed petit Swiss cheese containing a mixture of both Orafti® ST and P95 was able to act as substrates for faecal bacteria. However, rates of fermentation appeared to decline after 6 h. Furthermore, rates of substrate breakdown were lower than expected likely due to the concentration of ITF used. This is because the majority of *in vitro* studies involve the inoculation of faecal samples with substrate concentrations in the region of 1% ITF (w/v) (Saman et al., 2017; Wang et al., 2019). In contrast, the cheeses used in this study contained a substrate concentration equating to 0.25 % (w/v) at most, suggesting there was likely not enough substrate present to sustain microbial growth. Yet these results do demonstrate the ability of the addition of ITF to cheese to modify the composition of the gut microbiota suggesting ITF-fortified cheese could be the subject of future human intervention ITF-supplementation studies once product formulation has been optimised.

2.4.2. Yoghurt

While biscuits, cereal bars, ready-to-eats and Swiss cheese represent more solid and porous matrices, other dairy products such as yoghurt and ice cream have also been used in supplementation studies involving ITF and represent that middle ground between liquid and solid. In one study (Kruse et al., 1999) investigated using inulin as a replacement for dietary fat in 11 healthy adults. The quantity of ITF FIBRULINE® (DOP 2–50 (average DOP 9) consumed was based on individual energy requirements and resulted in an inulin intake of up to 34 g/day and was incorporated into commercial yoghurt which was consumed for 64-days. Stool samples were collected at day 8, 27 and 62 of the intervention as well as 34-days following the end of the intervention. Changes in microbial composition were measured by fluorescent *in situ* hybridisation. The authors reported that the consumption of ITF-supplemented yoghurt resulted in an approximate 1 $\log_{10}$/g dry faeces increase in bifidobacteria compared with the control group with numbers returning close to baseline 34 days after ceasing inulin consumption. However, the limitations with this study include that levels of ITF consumed by volunteers were based on each individual's energy needs, thus drawing any conclusion on whether specific dosages of ITF are required to impact on changes in microbial composition cannot be drawn. As well as only analysing changes in bifidobacteria.

The only other study to date to utilise yoghurt as means of ITF supplementation was undertaken (Marteau et al., 2011) with the authors using stewed apple and pear as a means of ITF supplementation. The study design was a double-blind placebo controlled trial in which native

chicory inulin supplemented at 15 g/day (2x7.5 g/sachets) was consumed in either yoghurt or stewed apple or pear. Stool samples were collected the day before Day 0 (V1), Day 14 (V2), and Day 28 (V3) with changes in bifidobacteria being analysed by quantitative polymerase chain reaction (qPCR). The results of this study showed that the consumption of inulin in either yoghurt or stewed apple/pear increased faecal bifidobacteria counts by 0.6 $\log_{10}$/g faeces between Day 0 (V1) and (Day 28) V3 ($P < 0.01$) and were significantly higher compared to the control ($P < 0.001$). Yet, no significant differences were detected in bifidobacteria between the placebo and the inulin intervention at Day 0 (V1) and Day 14 (V2). However, the authors did not monitor changes in any other microbial groups. Along with not stratifying results into participants who consumed yoghurt and those who consumed the stewed apple and pear. Thus, it cannot be determined whether changes in numbers of bifidobacteria or other microbial genera varied between the two different means of supplementation.

2.4.3. Ice cream

To date, only one study has aimed to determine the effects that the addition of chicory inulin to low-fat ice cream had on stool weight, transit time and gut microbiota composition (Slavin & Feirtag, 2011). The study design was a double-blind randomised-controlled crossover trial with the control group consuming a low-fat ice cream (no inulin) while the intervention group consumed low-fat ice cream containing 20 g inulin. Ice creams were consumed for 21-days after which ice creams were swapped and consumed for a further 21-days. Stool samples were collected on day 16 and 37 of the trial.

From the results, the authors noted that despite consuming 20 g chicory inulin/day, *Bifidobacterium* spp. increases in the intervention phase were not statistically significant compared to the control phase (0.44 $\log_{10}$ CFU/g faeces) ($P = 0.33$) with only the number of *Lactobacillus* spp. recording a statistically significant increase throughout the course of the intervention ($P < 0.05$).

Regarding changes in microbial composition, these results are unusual given the known response of bifidobacteria to ITF (Roberfroid et al., 2010). There are several reasons these results may have occurred. Firstly, the sample size was small ($n = 12$) with it being well established that there is a high degree of variability in gut microbial composition from person-to-person (Ames et al., 2017), hence a greater number of participants may have been needed to achieve statistical significance.

Secondly, analysis techniques used to determine changes in microbiota composition may have resulted in the generation of false results likely due to the lower sensitivity of the spread plates being used to determine changes in bacterial counts as well as subjectivity in the scoring of *Bifidobacterium* spp. Thirdly, no baseline stool sample was taken, and finally and critically no washout period was undertaken between treatment periods which likely confounded results due to a lack of a structural re-shift in the composition of the gut microbiota between stage 1 and stage 2 of the intervention (McBurney et al., 2019).

2.5. Juices and drinks

Regarding liquid matrices, ITF supplementation studies have been conducted in a diverse variety of different drinks including fruit juices and cocoa drinks. The addition of ITF from Jerusalem artichokes to fruit and vegetable juice shots and their effects on microbial composition was investigated by (Ramnani et al., 2010). The study design was a double-blind, randomised control trial in which Jerusalem artichoke ITF were incorporated into two different fruit juices: pear-carrot-sea buckthorn and plum-pear-beetroot. Each shot contained 2.5 g of Jerusalem artichoke ITF and was consumed twice a day over 3-weeks, followed by a 3-week washout period. Fruit and vegetable juice shots containing ITF were compared against a water-based control containing blood orange, carrot and raspberry extracts and flavours but no ITF, with changes in faecal bacteria analysed by fluorescent *in situ* hybridisation. The authors noted that after 21 days of ITF juice supplementation, there was a significant increase in bifidobacteria of between 0.5 and 0.7 $\log_{10}$ cells/g faeces ($P < 0{\cdot}0001$) along with a smaller yet still significant increase in *Lactobacillus/Enterococcus* groups of 0.2 $\log_{10}$ cells/g faeces recorded in both ITF/fruit juice interventions ($P < 0{\cdot}042$). This potentially implies that the presence of polyphenols in fruit juices may have aided in the selective stimulation of *Lactobacillus/Enterococcus*. Given that it has been previously demonstrated, both *in vitro* and *in vivo*, that *Lactobacillus* are predominant polyphenol utilisers within the gut (Hidalgo et al., 2012; Tzounis et al., 2011).

(Kolida et al., 2007) also investigated the prebiotic efficacy of ITF this time using a powdered cocoa drink as a means of supplementation. In this study, fifteen men and fifteen women consumed a cocoa drink containing either a placebo (maltodextrin) or 5 or 8 g inulin/day for a two-week period. Each treatment period was followed with a one-week washout before the next treatment commenced. Stool samples were collected at the start of the study (baseline) end of each treatment and washout period, with changes in faecal microbial composition analysed via fluorescent *in situ* hybridization. The results indicated that levels of bifidobacteria compared to the control significantly increased with consumption of both 5 g/day and 8 g/day of inulin ($P < 0.05$) with no significant differences between the low and high inulin dosage. Additionally, the authors noted there was a slight decrease in *C. perfringens – histolyticum* subgroup upon completion of the higher inulin dosage with respect to levels at washout 2 ($P < 0.01$). Along with a significant decline in *C. perfringens – histolyticum* upon consumption of the low inulin dose with respect in washout period 1 ($P < 0.05$).

Beverages were also the preferred method of (Rao, 2001) for the supplementation of ITF. In this small-scale study, eight subjects were recruited: four males and four females. The intervention was split into two distinct 3-week periods. In the first 3-week period, subjects consumed 5 g of sucrose a day, and in the second 3-week period subjects consumed 5 g/day of RAFTILOSE® P95 adjusted to the sweetness and colour of sucrose with aspartame at 2.7 g per kg oligofructose. Both sucrose and RAFTILOSE® P95 were dissolved in the subject's beverage of choice. Stool samples were collected before the start in the intervention and at the end of period 1 (sucrose control) and again at day 11 and finally at end of week 3 of period 2 (RAFTILOSE® P95). Changes in microbial composition were analysed using selective media techniques. The results of the study indicating that the consumption of RAFTILOSE® P95 resulted in nearly a 1 Log_{10} CFU/g wet faeces in bifidobacteria. As well as a 0.66 Log_{10} CFU/g wet faeces increase in *Bacteroides* between the end of the control and day 11 of the oligofructose intervention ($P < 0.001$ and $P < 0.01$). Furthermore, while there was a slight decline in bifidobacteria between days 11 and 21 of the intervention, this was not statically significant ($P > 0.05$). However, as the beverages consumed were the preferred choices of the subject in question and were not documented, determining if the type of beverages had a significant impact on the prebiotic effect of oligofructose cannot be undertaken.

In contrast, Azpiroz et al., (2017) did note the drinks that volunteers consumed as means on ITF supplementation with drinks including water, milk, tea, coffee and juice drinks. The study design was a single-center, placebo-controlled, parallel randomized and double-blind study involving 36 adults. Adults were randomised into one of two groups (intervention and control). The intervention group consumed 8 g/day HSI ITF with the control group consuming 8 g/day maltodextrin. Both ITF and maltodextrin were incorporated into 200 mL of the volunteers' preferred beverage during breakfast and dinner at 4 g per serving. The intervention lasted four weeks where during the first three days of the intervention only half the dose of ITF/maltodextrin was administered for adaptation. Faecal samples were collected on the two days before their scheduled visit at the end of each study period (baseline and intervention). Changes in total bacteria and bifidobacteria were analysed by real-time PCR. The results of the study showing that the effect of inulin on bifidobacteria was significantly greater compared to that of the placebo ($P = 0.011$). The limitations of this study are similar to those of (Rao, 2001) as the authors did not diversify results by drink type.

Table 1
Summary of food-based inulin-type fructan supplementation studies.

Reference	Food matrices	Type of inulin	Quantity of inulin	No. of Volunteers	length of intervention	Analysis technique	Outcome
Kleessen et al. (2007)	Cereal bars	Fibruline® Instant and Jerusalem Artichoke extract	7.7 g/per bar (2 bars per day)	45	7 day run in − 1 bar per day. Then 14 days at 2 bars per day	FISH-FLOW	1.2 log10/ g wet weight increase in bifidobacteria ($P = < 0.05$); Reduction in numbers of *Bacteroides*/Prevotella ($P < 0{\cdot}05$) and fewer numbers of *Clostridium histolyticum*/*C. lituseburense*
Reimer et al. (2020)	Cereal bars	ITF (Type not stated)	Low (3 g) and moderate (7 g)	48	12 weeks (2 × 4 weeks with 4 week washout)	16S rRNA sequencing and QPCR	Moderate group = increase in bifidobacteria; Increase in bifidobacteria in low dose at week 2 ($P = 0.027$) and a trend toward an increase at week 4 ($P = 0.056$) compared with the Control 2 bar
Gibson et al. (1995a)	Biscuits	OF and Inulin	15 g/day	8 and 4	(30 and 25 days total) (15 days on OF or Inulin)	Selective media techniques	Both OF and inulin increased bifidobacteria counts by 0.7–0.9 $\log_{10}$/g faeces. OF but not inulin resulted in decreases in counts of *Bacteroides* ($P < 0.01$), clostridia and fusobacteria ($P < 0.05$ and $P < 0.01$).
Tuohy et al. (2001)	Biscuits	OF + (partially hydrolysed guar gum)	6.6 g (OF) and 3.4 g/day (PHGG)	31	42 days in total (21 on OF)	FISH-FLOW	0.487 $\log_{10}$ cfu cells/g faeces increase in *Bifidobacterium* spp. No other changes were detected.
Brighenti et al. (1999)	Extruded ready to eat cereal	Fibruline® Instant (av DoP < 7, 30% DoP > 30)	Inulin was incorporated into dry cereal at 18% dry weight basis	12	12 weeks total (4 weeks: control, 4 weeks intervention and 4 week washout)	Selective media techniques	Increase in bifidobacteria counts by 0.33 $\log_{10}$ CFU/g faeces dry weight. Small decrease in total facultative anaerobes.
Marteau et al. (2011)	Yoghurt/ Stewed apple and pear	Fibruline® Instant	2 × 7.5 g sachets	50	28 days	RT-qPCR	Increase in bifidobacteria counts by 0.6 log10/ g faeces
Kruse et al. (1999)	Yoghurt	Fibruline® (DOP 2–50 (average DOP 9)	Based on individual's energy requirements	11	64 days	FISH-FLOW	1 $\log_{10}$/g dry faeces increase in bifidobacteria
Slavin and Feirtag, (2011)	Ice cream	chicory inulin (Frutafit)	20 g/day	12	21 day intervention, 21 days control	Selective media techniques	No sig increase in *Bifidobacterium* spp. Small decline in of Clostridium spp. ($P = 0.33$). Significant increase in *Lactobacillus* spp. ($P < 0.05$)
Ramnani et al. (2010)	Juice shots	Jerusalem artichoke ITF	2 × 2.5 g per day	66	22 day intervention, 21 washout period	FISH-FLOW	Increase in bifidobacteria between 0.5 and 0.7 $\log_{10}$ cells/g faeces. Increase in *Lactobacillus* and *Enterococcus* groups of 0.2 log10 cells/g faeces
Kolida et al. (2007)	Cocoa drink	Inulin (av DP 9–10 Frutafit IQ)	Low (3 g) and moderate (7 g)	30 (15 men and 15 women)	14 days followed by a 7 day washout period	FISH	0.12 log log10 cells/g faeces increase in bifidobacteria. No significant increases were seen between the low and high dose groups. Decrease in *C. perfringens – histolyticum* subgroup upon completion of the higher inulin dosage. slight decline in *C. perfringens – histolyticum* subgroup upon consumption of the low inulin dose with respect in washout period 1 ($P < 0.05$)
Rao, (2001)	Beverages - preferred choice of subject	Raftilose® P95	5 g/day	8 (4 males and 4 females)	2 × 3 week period (3 weeks sucrose followed by 3 weeks P95)	Selective media techniques	1 Log_{10} CFU/g wet faeces in bifidobacteria. No differences in total aerobes or coliforms were detected.
Healey et al. (2018)	Hot and cold beverages of participant choice	Orafti® Synergy 1	16 g/day (2 × 8 g doses)	33	2 × 3 week periods (3 weeks Synergy I, 3 weeks placebo)	16S rRNA sequencing and QPCR	Actinobacteria relative abundance significantly increased ($P < 0{\cdot}001$) and Firmicutes relative abundance significantly decreased ($P = 0{\cdot}007$) and a trend towards a reduction in Proteobacteria relative abundance ($P = 0{\cdot}070$) during the prebiotic intervention phase. increase in the relative abundance of *Bifidobacterium* ($P < 0{\cdot}001$) and a reduction in *Coprococcus* ($P = 0{\cdot}016$), *Dorea* ($P = 0{\cdot}029$), *Ruminococcus* (Lachnospiraceae family) ($P = 0{\cdot}007$) and *Oscillospira* relative abundance ($P = 0{\cdot}031$). There was also a trend towards an increase in *Faecalibacterium* relative abundance ($P = 0{\cdot}088$). In low fibre group only increase detected was in *Bifidobacterium* ($P = 0{\cdot}001$). In the high fibre group *Bifidobacterium* ($P < 0{\cdot}001$) and *Faecalibacterium* relative abundance ($P = 0{\cdot}010$), and a significant reduction in *Coprococcus* ($P = 0{\cdot}010$), *Dorea* ($P = 0{\cdot}043$)

(*continued on next page*)

Table 1 (*continued*)

Reference	Food matrices	Type of inulin	Quantity of inulin	No. of Volunteers	length of intervention	Analysis technique	Outcome
							and *Ruminococcus* (Lachnospiraceae family) relative abundance ($P = 0{\cdot}032$
Azpiroz et al. (2017)	Drinks: water, milk, orange juice,	Orafti® HIS	8 g (2 × 4 g;/ day)	36	4 weeks (1st 3 days only half dose of inulin was consumed)	RT-qPCR	Inulin significantly increased the abundance of bifidobacteria ($P = 0.001$)
Menne et al. (2000)	Mixed model concept	Raftilose® L60	8 g/day	8	3 periods: Period one: controlled diet no ITF: Period two: Controlled diet supplemented with ITF. Period three: normal diet supplemented with inulin	Selective media techniques	1 $\log_{10}$ cfu/g faeces increase in bifidobacteria after 2 weeks compared to the control ($P < 0.01$) with numbers in bifidobacteria still being 0.8 $\log_{10}$ cfu/g faeces higher at the completion of treatment II than at baseline ($P < 0.01$). No changes in total bacteria or other microbial genera were detected.
Hiel et al. (2019)	Mixed model	Diet naturally high in inulin rich vegetables	Mean 15 g/day	26	3 Period: T0 baseline. T1-end of 14 day controlled diet high in inulin-rich vegetables T2 End of return to habitual diet	16S rRNA sequencing and qPCR	Significant 3.8-fold increases in relative abundances of *Bifidobacterium* ($P < 0.0001$). Decrease in relative abundance of Clostridiales ($P < 0.0001$) and a trend towards a decrease in *Oxalobacteraceae* ($P < 0.052$). Significant increase in *Bifidobacterium* by qPCR 3-fold ($P < 0.0001$)

Secondly, participants were instructed to consume substantial amounts of dairy (cheese and milk) daily throughout the course of the intervention which may not give a fair reflection of volunteer's typical habitual diet. Finally, the authors only reported changes in bifidobacteria. Thus, it cannot be determined whether changes in other microbial genera occurred. Although the results do suggest that the bifidogenic effect of ITF appears to be unaltered.

2.6. Mixed meals

The only study to utilise multiple foods products incorporated with ITF was undertaken by (Menne et al., 2000). In this small-scale study eight participants were recruited with the intervention being split into three distinct periods. Period I) A control period in which the volunteers were all given a controlled diet without any addition of OF. Period II) Treatment I which lasted two weeks during which the controlled diet was supplemented with 8 g/d of chicory OF. Finally, Period III) intervention treatment II, a second treatment period of 3 wk, during which the volunteers consumed their usual home-cooked diet to which they added 8 g/d of chicory OF. The ITF used in this study was Raftilose® L60 and was incorporated into orange juice, various desserts (puddings, creams and fruit mousses), cakes and biscuits. Additionally, participants undertook dietary restraint of naturally occurring ITF foods i.e. onions, leeks, bananas and artichokes. Stool samples were collected on the last day of week 2 of the control period; the last day of week 4 of treatment period I and finally at the last day of week 7 at the end of treatment period II. Changes in microbial composition were analysed via selective media techniques. The results indicated that consumption of 8 g/day OF resulted in a 1 $\log_{10}$ cfu/g faeces increase in bifidobacteria after 2 weeks compared to the control ($P < 0.01$) with numbers in bifidobacteria still being 0.8 $\log_{10}$ cfu/g faeces higher at the completion of treatment II than at baseline ($P < 0.01$). No changes in total bacteria or other microbial genera were detected. However, as participants consumed a variety of different food products throughout the course of the intervention, often in combination, no conclusions can be drawn on the impact of differing food matrices on changes in the microbiota. Yet, these results do suggest that mixed food models likely have no impact on the prebiotic efficacy of ITF toward bifidobacteria.

A mixed model concept was also employed by (Hiel et al, 2019). This time not involving direct supplementation of IFT into food products, but instead involving a diet rich in ITF-containing vegetables. The study design was single-group involving 26 healthy adults. The trial lasted 33 days. Volunteers were instructed to consume a controlled diet based on ITF-rich vegetables providing an average intake of 15 g ITF/d over 14 days. Test days were organised day 0 (T0 - baseline), Day 14 (T1- end of inulin-rich diet) and Day 33 (T2 - end of return to habitual diet). Stool samples were collected at within 2 days before each test day. Changes in gut microbiota composition were analysed via 16 rRNA sequencing and qPCR. Major increases in microbial composition were reported in *Bifidobacterium* (3.8-fold, $P < 0.0001$). along with a decrease in unclassified *Clostridiales* ($P < 0.0001$), and a trend toward a decrease in Oxalobacteraceae *family* ($P = 0.052$). The 3-fold increase in *Bifidobacterium* was also confirmed by qPCR ($P < 0.0001$). The limitations of this study are a lack of control group, limited number of subjects focusing purely on hydrogen-producing individuals, along with not analysing for presence of other bioactive compounds such polyphenols (flavanols), also naturally high in several of vegetables (artichokes, leeks) (Negro et al., 2012; Ren et al., 2017) used in this study. Nevertheless, the results of the study do still imply that consuming adequate amounts of fructan-rich vegetables are able to beneficially shift gut microbiota composition.

2.7. So does the food matrices matter: Jury out?

Overall, while these results of all of these studies seemingly suggest that the bifidogenic effect of ITF is unaltered as a result of the food matrix, there were several confounding factors including crossover vs parallel study design, number of participants and length of the intervention in the studies conducted thus far. Furthermore, the implementation of controlled vs non controlled and exclusion diets (excluding or not excluding other fructans) the type and amount of ITF supplemented (inulin vs OF) and when stool samples were collected combined with the lack of washout periods, differences in how studies report changes in microbial numbers (dry vs wet weight of faeces) and analytical techniques used (FISH vs selective media vs qPCR). Along with several studies only reporting changes in bifidobacteria mean that drawing definitive conclusions based on these findings should not be undertaken with any real degree of certainty (summarised in Table 1).

3. Future direction and call to action

The popularity of prebiotics and their potentially beneficial effects on microbial composition and health outcomes continues to gain momentum. There is increasing motivation by researchers to design food-based interventions which promote favourable shifts in the gut

microbiota, however, due to differences in the type of ITF, the food product and analytical method used, along with variances in the populations and primary and secondary outcomes studied, it has become increasingly difficult to make direct comparisons between studies, hindering of the ability to draw appropriate conclusions on whether the food matrices alter the prebiotic efficacy of ITF. As a result, if we are to establish the impact of food matrices on the prebiotic efficacy of ITF, interventions must ensure several criteria are met. These criteria include ensuring that all ITF-containing foods products are standardised to contain the same type and amount of ITF, combined with undertaking identical eligibility criteria, intervention periods, primary and secondary measurements and analytical techniques. Thus, will allow for more reliable interpretation of results and generation of a consensus regarding ITF-food based intervention studies.

4. Ethics statement

No animal or human experimentation was conducted in the writing of this review.

Declaration of Competing Interest

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