University of Reading

Effects of food matrix on the prebiotic efficacy of inulin-type fructans: a randomised trial

Article

Accepted Version

Jackson, P. P. J., Wijeyesekera, A. ORCID: https://orcid.org/0000-0001-6151-5065, Theis, S., Van Harsselaar, J. and Rastall, R. A. ORCID: https://orcid.org/0000-0003-1775-5226 (2023) Effects of food matrix on the prebiotic efficacy of inulin-type fructans: a randomised trial. Beneficial Microbes, 14 (4). pp. 317-334. ISSN 1876-2891 doi: https://doi.org/10.1163/18762891-20220120 Available at https://centaur.reading.ac.uk/113491/

It is advisable to refer to the publisher's version if you intend to cite from the work. See Guidance on citing.

To link to this article DOI: http://dx.doi.org/10.1163/18762891-20220120

Publisher: Brill

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the End User Agreement.

www.reading.ac.uk/centaur

University of Reading

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

1 **Research article**

2 **Effects of food matrix on the prebiotic efficacy of inulin-type fructans: a randomised**
3 **trial**

4 P. P. J. Jackson[1], A. Wijeyesekera[1], S. Theis[2] & J. Van Harsselaar[2], R. A. Rastall[1],

5

6 1. p.p.j.jackson@pgr.reading.ac.uk
7 1. r.a.rastall@reading.ac.uk*
8 1. a.wijeyesekera@reading.ac.uk
9 2. stephan.theis@beneo.com
10 2. jessica.vanharsselaar@beneo.com

11

12 **A.** University of Reading, Department of Food and Nutritional Sciences, Harry Nursten
13 Building, Pepper Lane, Whiteknights, Reading RG6 6DZ

14 **B.** BENEO-Institute, BENEO GmbH, Wormser Str. 11, 67283 Obrigheim (Germany)

15

16 # Abstract

17

18 Recently there is much debate in the scientific community over the impact of the food matrix
19 on prebiotic efficacy of inulin-type fructans. Previous studies suggest that prebiotic
20 selectivity of inulin-type fructans towards bifidobacteria is unaffected by the food matrix.
21 Due to differences in study design, definitive conclusions cannot be drawn from these
22 findings with any degree of certainty. In this randomised trial, we aimed to determine the
23 effects that different food matrices had on the prebiotic efficacy of inulin-type fructans
24 following a standardised 10-day, 4-arm, parallel, randomised protocol with inulin either in
25 pure form or incorporated into shortbread biscuits, milk chocolate or a rice drink. Similar
26 increases in *Bifidobacterium* counts were documented across all four interventions using both
27 fluorescence *in situ* hybridisation (pure inulin: +0.63; shortbread: +0.59; milk chocolate:
28 +0.65 and rice drink: +0.71 (log_{10} cells/g wet faeces) and 16S rRNA sequencing quantitative
29 microbiome profiling data (pure inulin: +1.21 x 10^9; shortbread: +1.47 x 10^9; milk chocolate:
30 +8.59 x 10^8 and rice drink: +1.04 x 10^9 (cells/g wet faeces) (all $P \leq 0.05$). From these results,
31 we can confirm that irrespective of the food matrix, the selectivity of inulin-type fructans
32 towards *Bifidobacterium* is unaffected, yet the compositional make-up of the food matrix
33 may have implications regarding wider changes in the microbiota.

34

35 **Trial registration**: clinicaltrials.gov ID: NCT05581615.

36

37 # Key words

38

39 Prebiotics, food matrix, carbohydrates, inulin-type fructans, gut microbiota

1. Introduction

Diet, being one of the key drivers of fermentation in the gut, can strongly influence the composition and thus the functionality of the gut microbiota. One way to modify the composition and activity of the gut microbiota is via prebiotic functional foods as they provide a safe, affordable and effective dietary approach (Sanders *et al.*, 2019). Oligofructose (OF) and inulin are the most widely researched prebiotics belonging to a class of non-digestible carbohydrates referred to as inulin-type fructans (ITF) (Karimi *et al.*, 2015). ITF are linear polydisperse carbohydrates composed of monomers of fructose linked by β-(2-1) glycosidic (fructosyl-fructose) linkages. A non-reducing *α*-D-glucose moiety may or may not be present (Roberfroid, 2007) and based on the degree of polymerisation (DP), ITF can be separated into OF (DP 2-9) and inulin (DP ≥ 10) (van Loo, 2006).

Due to their structure and the absence of brush border β-fructosidases the majority of ITF reach the colon intact functioning as prebiotics by displaying high selectivity towards certain beneficial microbial groups such as *Bifidobacterium.* This is a key feature of the prebiotic concept along with providing a series of health benefits to the host as summarised in these series of reviews (Ahmed and Rashid, 2019; Gibson *et al.*, 2017; Sanders *et al.*, 2019; Wilson and Whelan, 2017). Furthermore, due to their physicochemical properties ITF can also act as fat and sugar replacers as well as texture modifiers while still providing potentially prebiotic dosages. They are becoming an increasingly common ingredient within the food industry (Shoaib *et al.*, 2016).

The concept that the food matrix may impact on the prebiotic efficacy of ITF has become of increasing interest in recent years. This is in part due 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). Furthermore, there is evidence that high levels of dietary fibre present within the matrix can influence the absorption of such compounds via the sequestration of ions and formation of complexes (D'Archivio *et al.*, 2010; Palafox-Carlos *et al.*, 2011). This concept also applies to the microbial fermentation of unabsorbed secondary metabolites in the diet and resulting metabolites within the colon (Aguilera, 2019).

Depending on the processing parameters, ITF may or may not be subject to degradation during the production process. Critical processing parameters include pH, with the critical cut-off appearing to be ≤ 4 (Glibowski and Wasko, 2008; Mensink *et al.*, 2015), pasteurisation (often used during fruit juice production) (Klewicki, 2007), heating such as during baking (Poinot *et al.*, 2010; Rodriguez-Garcia *et al.*, 2012) resulting in participation in caramelisation and Maillard reactions (indicated by the level of browning in bread, cakes, biscuits, etc) (Mensink *et al.*, 2015). Degradation could also be caused by high temperature and pressure extrusion (ready-to-eat cereals and snacks) (Duar *et al.*, 2015) and enzymatic hydrolysis via yeasts and bacteria (bread and beer production) (Struyf *et al.*, 2017). Generally, the processing time, temperature, and the DP of ITF used appear to be critical if the potential degradation of ITF is to be avoided. Each aspect needs to be carefully considered in order to optimise product quality while maintaining ITF integrity (Jackson *et al.*, 2022b).

86 To date, studies have explored the effects of ITF on the gut microbiota in both pure form, as
87 well as several food products such as biscuits, yoghurt, stewed apple, cereal bars, cocoa
88 drinks, and fruit juices as vehicles for ITF supplementation (Azpiroz *et al.*, 2017; Brighenti *et*
89 *al.*, 1999; Gibson and Roberfroid, 1995; Healey *et al.*, 2018; Kleessen *et al.*, 2007; Ramnani
90 *et al.*, 2010; Rao, 2001; Slavin and Feirtag, 2011). The results of these studies all document
91 that the selectivity of ITF towards *Bifidobacterium* is unaltered as result of the food matrix.
92 However, as a subgroup analysis from So *et al.*, (2018) concluded, fibre interventions
93 delivered through supplementation resulted in significantly higher *Bifidobacterium* spp.
94 compared to placebo/lower fibre controls (SMD: 0.75; 95% CI: 0.52, 0.98; $P \leq 0.00001$, I2 =
95 83%). No differences were found between food interventions and comparators (SMD: 0.20;
96 95% CI: −0.36, 0.76; P = 0.49, I2 = 88%), although considerable heterogeneity persisted in
97 both analyses. This emphasizes that definitive conclusions on whether the food matrix
98 matters in the supplementation of ITF cannot be drawn due to differences in study design
99 (crossover vs parallel study design, number of participants, length of the intervention),
100 differences in the implementation of controlled vs non controlled and exclusion diets
101 (excluding or not excluding other fructans), the type and amount of ITF supplemented (inulin
102 vs OF), time point of stool samples collection), combined with the lack of washout periods,
103 differences in reporting changes in microbial numbers (dry vs wet weight of faeces) and
104 analytical techniques used (fluorescence *in situ* hybridization (FISH) vs selective media vs
105 quantitative polymerase chain reaction (qPCR)).

106

107 Many of the food products utilised in the studies mentioned above are sources of other
108 potential prebiotics including phenolic acids, β-glucan, arabinoxylans and bovine milk
109 oligosaccharides. Each possesses the potential to alter the fermentation selectivity and have
110 been shown to influence levels of *Lactobacillus*, *Bacteroides*, *Enterococcus*, *Prevotella,* and
111 *F. prausnitzii* (Gomez *et al*., 2016; Kemperman *et al*., 2013; Scott *et al*., 2019; Valeur *et al*.,
112 2016) amongst others. A critical aspect often overlooked by researchers when considering
113 study designs regarding food-based prebiotic supplementation studies. This leads to the
114 question of whether the food matrix matters in the supplementation of ITF? This question is
115 becoming increasingly important to answer given the interest in the addition of ITF into
116 various food products with several manufacturers marketing these products as beneficial for
117 health (Rolim, 2015). Therefore, this study aims to determine the effects that different food
118 matrices may have on the prebiotic efficacy of ITF following a standardised protocol. The
119 hypothesis to be tested is that the food matrix does not impact on the selectivity of ITF
120 towards *Bifidobacterium.*

121

122 # 2. Materials and methods
123

124 **Subjects and recruitment**
125

126 Healthy adults, both males and females, were recruited from the Reading area via previous
127 email lists and posting on social media. The inclusion criteria were volunteers aged 18-65,
128 BMI ≥ 18.5 and ≤ 30 kg/m^2, no evidence of gastrointestinal diseases and following what
129 could be deemed a typical Western European diet. They were free of food allergies and had a
130 stool frequency of at least 3 bowel movements per week. Exclusion criteria were extreme
131 diets (i.e., ketogenic, vegetarian, vegan, intermittent fasting), antibiotic treatment in the four

months preceding the study, anaemia, chronic or acute diseases i.e., (pre)-diabetic. Potential subjects were also excluded if they had undergone surgical resection of any part of the bowel, were current smokers and/or had a history of alcohol or drug misuse. Potential volunteers were excluded if they were pregnant or lactating. Use of laxatives was not permitted 4 weeks prior to beginning of the intervention.

Study design and interventions

The study design was a prospective, non-placebo controlled, parallel-group, randomised trial lasting ten days. Ten days was the chosen intervention length based on the results of previous research demonstrating that the bifidogenic effect of ITF can be seen after approximately seven days of daily intake (Nagy *et al.*, 2022). Prior to commencing the study, eligible subjects were provided with both verbal and written study information and gave their informed consent. Enrolled subjects were asked to undergo a two-week run-in period in which they were required to restrict the use of any probiotics, prebiotics and prebiotic or probiotic containing foods or supplements. After the run-in phase enrolled subjects were randomised using REDCap (see below) into one of four groups (n = 24 per group) stratified by sex using a ratio of approximately 2:1 (female : male): (Group A (16 : 8) – pure inulin), (Group B (18 : 6) – inulin-enriched shortbread), (Group C (16 : 8) – inulin-enriched milk chocolate), and (Group D (18 : 6) – inulin-enriched rice drink).

The ITF used in the was highly soluble inulin (Orafti® HSI, DP 2-60, min. 88% inulin, maximum of 12% glucose, fructose, and sucrose (DM), BENEO-Orafti, Tienen, Belgium) produced from chicory. The interventions used in this study were provided by BENEO. Interventions were chosen based on the outcomes of our literature review reflecting the most common food products that undergo inulin fortification (Jackson *et al.*, 2022a; Jackson *et al.*, 2022b). This not only reflects a wide degree of matrices (baked, semi-solid and liquid), but also those consumed as part of the population's habitual diet (Murakami and Livingstone, 2016). Each portion of pure inulin or enriched food product contained 5 g of ITF and was consumed twice per day resulting in a total daily ITF intake of 10 g. This dosage was chosen based on the amount of ITF that can be successfully fortified into study products without changes in product characteristics. Pure inulin was used as the comparator to determine if the prebiotic efficacy was altered as a result of different food matrices. Details on composition of each study product per 100 g and per daily portion can be found in Table 1.

	Pure inulin		Shortbread		Milk Chocolate		Rice Drink	
Amounts	**per 100 g**	**per 11.4 g daily portion**	**per 100 g**	**per 58 g daily portion**	**per 100 g**	**per 52 g daily portion**	**per 100 mL**	**per daily 300 mL portion**
Energy kJ/kcals	875/216	87.5/21.6	1766/422	1024.28/244.76	2187/523	568.36/271.96	465/111	1534.5/330
Carbohydrates (g)	11	1.1	54.7	31.73	31	16.12	20.4	61.2
of which is sugars (g)	11	1.1	12	6.96	30.4	15.81	11.8	35.4
Fat (g)	Negligible	Negligible	15.9	9.22	36.3	18.88	2.3	6.9
of which is saturates (g)	Negligible	Negligible	7.2	4.18	21.6	11.23	0.8	2.4
Protein (g)	Negligible	Negligible	5.4	3.13	7.2	3.74	0.5	1.5
Fibre (excluding fructans) (g)	0	0	1.36	0.79	2.46	1.28	0.77	2.3
Fibre (including fructans) (g)	88	10	18.6	10.79	21.7	11.28	4.1	12.3
Salt (g)	Negligible	Negligible	1	0.58	0.2	0.104	0	0

166 Table 1. Compositional breakdown of study products per 100 g and per daily portion.

167 Stool and urine samples were collected at Day 0 and Day 10. Details of sample collection are
168 presented below. No intervention was given until both baseline samples had been provided.
169 Subjects were instructed to consume their assigned pure inulin supplement or food product
170 for the entire 10 days, one portion in the morning and one portion in the evening with no
171 other food or drink and within 15 min of opening. Volunteers were told to not alter their diet
172 or fluid intake during the trial with exception of portion size to make allowances for
173 additional calories consumed as part of the intervention. Volunteers were only considered
174 compliant if consumption for the whole ten-days of the intervention was achieved. In order to
175 assess compliance volunteers were asked to complete an online daily check-in dairy. Changes
176 in habitual dietary intakes at Day 0 and Day 10 were assessed using a modified version of the
177 validated eNutri2019-DE web application specifically designed to capture short-term changes
178 in dietary intake. In-depth details on the eNutri2019-DE web application have been described
179 elsewhere (Franco *et al.*, 2019).

180

181 Data were collected and managed using REDCap electronic data capture tools hosted at the
182 University of Reading (Harris *et al.*, 2009). REDCap (Research Electronic Data Capture) is a
183 secure, web-based application designed to support data capture for research studies,
184 providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data
185 manipulation and export procedures; 3) automated export procedures for seamless data
186 downloads to common statistical packages; and 4) procedures for importing data from
187 external sources.

188

189 **Outcomes**

190

191 Primary outcomes

192

193 The primary outcome was differences in *Bifidobacterium* count as measured by fluorescence
194 *in situ* hybridisation flow cytometry (FISH-FLOW).

195

196 Secondary outcomes

197

198 The secondary outcomes were changes in microbial composition and urinary metabolites as
199 measured 16S rRNA sequencing and ^{1}H-nuclear magnetic resonance (^{1}H-NMR). Details on
200 sample collection, processing and analysis are detailed below.

201 Bowel habit and GI sensation diaries were completed daily throughout the of the ten-day
202 intervention, in order to assess day-to-day changes in flatulence, intestinal bloating,
203 abdominal pressure, abdominal pain and feeling of fullness (all none, mild, moderate and
204 severe) (Costabile *et al.*, 2008; Ramnani *et al.*, 2010; Walton *et al.*, 2012), stool frequency
205 and consistency according to the Bristol Stool Form Scale (Lewis and Heaton, 1997). Any
206 medication use or adverse events were also recorded.

207 # Sample collection
208

209 Faecal samples
210

211 Volunteers were provided written and verbal instruction on how to collect stool samples, and
212 with sterile stool sample pots for Day 0 and Day 10 collections. Freshly collected faecal
213 samples were kept in 2.5L Oxoid™ AnaeroJar™ (Oxoid, Hampshire, United Kingdom) with
214 Oxoid™ AnaeroGen™ 2.5L sachets (O_2 ≤0.1%; CO_2: 7-15%). Faecal samples were collected
215 from the volunteer's place of residence within 2 hours of voiding. Samples (1.5 g) for
216 metabolic profiling were stored at -80 °C until the study had been completed. An additional 3
217 g of the same faecal sample was diluted 1:10 (w:w) in anaerobic phosphate-buffered saline
218 (PBS, 0.1 M; pH 7.4), then homogenised using a stomacher (260 paddle beats/min) for 2 min
219 at room temperature. 20 mL of faecal slurry were then vortexed with 3 mm diameter glass
220 beads for 30 s before being centrifuged at 1,500 x g for 3 min at room temperature. 75 µL
221 were then diluted in 675 µL phosphate buffered saline (PBS mol l^{-1}; pH 7.4) (1:100 dilution),
222 aliquoted in to 1.5 mL Eppendorf tubes and stored at -80 °C until cells could be fixed.
223 Samples were then centrifuged at 11,337 × g for 5 min and the supernatant was decarded.
224 Pellets were then resuspended in 375 µL of 0.1 M PBS and fixed in 4% (w:v)
225 paraformaldehyde (1,125 µL) for 4 h at 4 °C. Fixed cells were centrifuged at 11,337 × g for 5
226 min at room temperature. Samples were then washed with 1 mL PBS, pellets aspirated and
227 centrifuged at 11,337 × g for 5 min. The washing process was repeated twice more. Samples
228 were re-suspended in 150 µL PBS and stored in ethanol (1:1, v:v) at −20 °C until analysis via
229 fluorescence *in situ* hybridisation – flow cytometry (FISH-FLOW).

230

231 Urine samples
232

233 Day 0 and Day 10 mid-stream urine samples were collected as the first urine sample after
234 waking in sterilised specimen pots. Urine samples were collected from volunteers at the same
235 time as faecal samples. Urine samples were stored at – 80 °C until analysis by Proton Nuclear
236 Magnetic Resonance spectroscopy (^{1}H-NMR) could be conducted.

237

238 ## Enumeration of faecal microbial populations by fluorescence *in situ*
239 ## hybridisation flow cytometry (FISH-FLOW)
240

241 FISH by flow cytometry was carried out as described by (Grimaldi *et al.*, 2017). Probes used
242 in this study are listed in Table 2. Fluorescence measures were performed by a BD Accuri™
243 C6 Plus (BD, Erembodegem, Brussels) measuring at 488 nm and 640 nm. Thresholds of 9000
244 in the forward scatter area (FSC-A) and 3000 in the side scatter area (SSC-A) were placed to
245 discard background noise, a gated area was applied in the main density dot to include 90% of
246 the events. Flow rate was 35 uL/min, with limit of collection set for 100,000 events and
247 analysed with Accuri CFlow Sampler software. Bacterial counts were then calculated through
248 consideration of flow cytometry reading and PBS dilution. The number of log_{10} cells is
249 presented as per gram of wet fresh faeces.

250

	Sequence (5' to 3')	Target groups	Reference
Non Eub	ACTCCTACGGGAGGCAGC	Control probe complementary to EUB338	(Wallner *et al.*, 1993)
Eub338	GCTGCCTCCCGTAGGAGT	Most Bacteria	(Amann *et al.*, 1990)
Eub338II	GCAGCCACCCGTAGGTGT	*Planctomycetales*	(Daims *et al.*, 1999)
Eub338III	GCTGCCACCCGTAGGTGT	*Verrucomicrobiales*	(Daims *et al.*, 1999)
Bif164	CATCCGGCATTACCACCC	*Bifidobacterium* spp.	(Langendijk *et al.*, 1995)
Bac303	CCAATGTGGGGGACCTT	Most *Bacteroidaceae* and *Prevotellaceae*, some *Porphyromonadaceae*	(Manz *et al.*, 1996)
Erec482	GCTTCTTAGTCARGTACCG	Most of the *Clostridium* coccoides-*Eubacterium rectale* group (*Clostridium* cluster XIVa and XIVb)	(Franks *et al.*, 1998)
Rrec584	TCAGACTTGCCGYACCGC	*Roseburia* spp.	(Walker *et al.*, 2005)
Prop853	ATTGCGTTAACTCCGGCAC	*Clostridium* cluster IX	(Walker *et al.*, 2005)
Fprau655	CGCCTACCTCTGCACTAC	*Feacalibacterium prausnitzii* and relatives	(Suau *et al.*, 2001)

251 **Table 2:** Name, sequence, and target group of oligonucleotide probes used for bacterial enumeration

252

253

Microbial Profiling

254

255 Bacterial DNA extraction

256

257 Bacterial DNA was extracted from faecal samples using the QIAamp Fast DNA Stool mini
258 kit (QIAGEN) according to the manufacturer's instructions. Faecal samples were
259 homogenised and allocated into 2 mL screwcap tubes containing 0.6 g 0.1 mm glass beads.
260 Bead beating was run on a fastprep24 instrument (MPBiomedicals); 4 cycles of 45s at speed
261 4). 200 mL of raw extract were then used for DNA isolation.

262

263 DNA isolation, library preparation and 16S rRNA gene sequencing

264

265 Extracted bacterial DNA was subjected to PCR amplification of the V4 region of the 16S
266 rRNA bacterial gene using two-stage Nextera PCR libraries using the primer pair 515F (5′-
267 GTG YCA GCM GCC GCG GTA A -3′) and 806R (5′- GGA CTA CNV GGG TWT CTA
268 AT -3′). Raw sample extracts were diluted to 2.5ng/mL, using Tris-Buffer and 5 mL were
269 used in 1st Step PCR, together with 5x HOT FIREPol® MultiPlex Mix (Solis BioDyne,

270 Estonia) and 4uM primer mix (fwd+rev) 515F/806R (Microsynth, Balgach,
271 Switzerland). 1st Step PCR samples were purified with NGS Clean Beads (Labgene,
272 Switzerland). Bead ratio was 1:1:2, Beads were washed with 75% ethanol, airdried and
273 resuspended in Tris buffer. The 2nd step PCR, each sample was individually barcoded, using
274 Nextera XT Index Kit v2 (Illumina, San Diego, California) and 5x HOT FIREPol®
275 MultiPlex Mix (Solis BioDyne, Estonia). 2nd Step PCR samples were purified with NGS
276 Clean Beads (Labgene, Switzerland). The final 2nd Step PCR products were quantified using
277 a Quant-iT™ PicoGreen™ ds DNA Assay Kit (Thermo Fisher Scientific, Waltham, USA).
278 Amplicons were pooled equimolar prior to sequencing. The final pool was quantified using a
279 Quant-iT™ PicoGreen™ ds DNA Assay Kit (Thermo Fisher Scientific, Waltham, USA) and
280 Fragment analyzer (Agilent).

281

282 Subsequent PCR libraries were sequenced on an Illumina MiSeq platform using a v2 500
283 (2*250 bp read length). Pools were diluted to 9.2 pM and loaded together with 15% PhiX
284 (Illumina, FC-110-3001) to increase the diversity of the run resulting in a raw cluster density
285 of 631 and a cluster passed filter rate of 98%. Paired-end reads which passed Illumina's
286 chastity filter were subject to de-multiplexing and trimming of Illumina adaptor residuals
287 using Illumina's bcl2fastq software version v2.20.0.422. Quality of the reads was checked
288 with the software FastQC version 0.11.8 and sequencing reads that fell below an average Q-
289 score of 20 or had any uncalled bases (N) were removed from further analysis. The locus
290 specific V4 primers were trimmed from the sequencing reads with the software cutadapt v3.2.
291 Paired-end reads were discarded if the primer could not be trimmed. Trimmed forward and
292 reverse reads of each paired-end read were merged to reform *in silico* the sequenced molecule
293 considering a minimum overlap of 15 bases using the software USEARCH version 11.0.667.
294 Merged sequences were again quality filtered allowing a maximum of one expected
295 erroneous base per merged read. Reads that contained ambiguous bases or were outliers
296 regarding the amplicon size distribution were also discarded. Samples that resulted in less
297 than 5000 merged reads were discarded, to avoid distortion of the statistical analysis.
298 Remaining reads were denoised using the UNOISE algorithm implemented in USEARCH to
299 form Amplicon Sequencing Variants (ASVs) discarding singletons and chimeras in the
300 process. The resulting ASV abundance table was then filtered for possible barcode bleed-in
301 contaminations using the UNCROSS algorithm. ASV sequences were compared to the
302 reference sequences of the RDP 16S database provided by
303 https://www.drive5.com/usearch/manual/sintax_downloads.html and taxonomies were
304 predicted considering a minimum confidence threshold of 0.5 using the SINTAX algorithm
305 implemented in USEARCH. The resulting library was then corrected by taking into
306 consideration numbers of 16S copies and rarefying to an even sampling intensity to reduce
307 bias in diversity metric calculations and quantified as described by (Vandeputte *et al.*, 2017).

308

309

310

311

312 **Metabolic profiling using ^{1}H-NMR spectroscopy**
313

314 For analysis urine samples were thawed, A phosphate buffer (pH 7·4 sodium phosphate with
315 0.2M disodium phosphate (Na_2HPO_4), 0.04M monosodium phosphate (NaH_2PO_4) in
316 deuterium oxide (99·9 %) was prepared, with 1mM 3-(trimethylsilyl) propionic acid-d_4
317 sodium salt (TSP) and 3mM sodium azide in the solution. 400 µL of each urine sample were
318 mixed with 200 µL buffer. 550 µL aliquots of supernatant were collected and dispensed into 5
319 mm NMR tubes.

320

321 ^{1}H-NMR spectroscopy analysis was carried out using a Bruker Avance DRX 500 MHz NMR
322 spectrometer (Bruker Biospin, Germany). The spectrometer was operated at 500.13 MHz.
323 Urine water spectra were acquired using a standard 1D pulse sequence [recycle delay (RD)-
324 90˚-t1-90˚-Tm-90˚-acquire free induction decay (FID)] with water suppression applied during
325 RD of 2 s, a mixing time Tm of 100ms and a 90° pulse set at 7.70 µs. Per spectrum, a total of
326 128 scans were carried out with a spectral width of 14.0019 ppm. The FIDs were multiplied
327 by an exponential function corresponding to 0.3 Hz line broadening. Acquired spectroscopic
328 data were processed using the TopSpin 3.6.5 software package (Bruker Biospin, Rheinstetten,
329 Germany). Data Processing was undertaken using the nPYc-Toolbox 1.2.7. Further details on
330 the nPYC-Toolbox can be found at https://github.com/phenomecentre/nPYc-Toolbox

331

332 Chemometric analysis
333

334 Processed spectroscopic data were imported to the SIMCA 13.0 software package (Umetrics
335 AB, Umeå, Sweden) to conduct unsupervised multivariate statistical analysis. Principal
336 components analysis was used to evaluate similarities/differences in urinary metabolite
337 composition between groups. The R^2 and Q^2 variables provided an indication of goodness of
338 fit (R^2) as well as goodness of prediction (Q^2) of the models.

339

340 **Ethics**
341

342 The study was given favourable ethical consent by the University of Reading's Research
343 Ethics Committee (36/2020). The trial was registered as a clinical trial (clinicaltrials.gov ID:
344 NCT05581615) and conducted in accordance with the Declaration of Helsinki. All
345 participants gave written informed consent prior to study entry. There were no protocol
346 changes once the trial commenced.

347

348

349

350 ## Sample size and statistical analysis
351

352 The primary outcome measure was bifidobacterial population as log_{10} cells/g wet faecal
353 sample as measured by fluorescence *in situ* hybridisation. It was calculated that to detect a
354 difference in *Bifidobacterium* populations between interventions, a total of 92 volunteers was
355 required. This is based on an 80% probability that the study could detect a 0.5 log_{10} cells/g
356 wet faecal sample difference in colonic bifidobacterial population at a two-sided 0.05
357 significance level based on the assumption of a standard deviation of 0.7 log_{10} cells/g wet
358 faecal sample bifidobacteria.

359

360 Statistical Package for Social Science version 27 (SPSS Inc., Chicago, IL, USA) was used for
361 all statistical analyses. Changes in bacteriology (FISH-FLOW, RMP and QMP), dietary data
362 and bowel habit data were analysed using a linear marginal model (LMM) in order to assess
363 both repeat measures (changes from baseline) and Day 10 group comparisons. Baseline
364 values were included as a covariate to assess differences between groups. Participant metrics
365 were assessed using a one-way ANOVA. All comparisons were corrected for type 1 errors
366 using a Bonferroni adjustment within each LMM and ANOVA. Results are presented as
367 mean and standard error (SE) unless otherwise stated. All tests were two tailed and *P* values
368 ≤ 0.05 were considered statistically significant.
369

370 # 3. Results
371

372 Subject characteristics
373

374 110 subjects expressed interest in the trial with 100 potential subjects completing the
375 screening visit. Of these, 14 did not meet the inclusion criteria, 96 eligible subjects were
376 randomized ($n = 24$ per group) and included in the analysis for all primary and secondary
377 outcomes (Figure 1). Baseline characteristics are reported in Table 3.

378

379 **Figure 1.** CONSORT diagram of participant flow through the trial

380 Table 3 reports the subject data (age, height, weight, and BMI) mean and range segregated by
381 intervention. Average subject age was 37.89 y, weight 68.05 kg, height 169.08 cm and BMI
382 23.70 (kg/m^2). No significant differences were recorded between any of the groups.
383
384

Metric	Pure inulin (*n* =24)	Shortbread (*n* =24)	Milk Chocolate (*n* =24)	Rice Drink (*n* =24)	*P* (b)
Age (y)	39.46 (25-63)	34.46 (20-62)	38.29 (19-64)	39.33 (19-64)	*P* = 0.54
Weight(kg)	69.86 (50-110)	67.76 (51-105)	66.98 (53-86)	67.82 (45-98)	*P* = 0.89
Height (cm)	170.2 (157-193)	168.4 (152.4-189)	170.2 (155-193)	167.5 (147-195)	*P* = 0.73
BMI (kg/m²)	23.89 (18.37-30.37)	23.79 (19.57-30.79)	23.11 (19.71-28.72)	24.03 (18-29.9)	*P* = 0.74

385 **Table 3:** Subject data – age, weight, height, and BMI mean and SE segregated by intervention (*n* = 24 per group). *P* values
386 are the results of using a one-way ANOVA to compare differences in categorical data.
387

388 Dietary intake
389

390 Nutrient data collected at Day 0 and Day 10 of the intervention are presented in Table 4.
391 No significant differences were detected in total energy, protein, carbohydrates, total sugar,
392 starch and PUFAs intakes (Table 4). Analysis of total fat revealed significant differences
393 between interventions at day 10 ($P = 0.026$) with fat intakes in the milk chocolate
394 intervention being significantly different from the rice drink intervention ($P = 0.019$).
395 Repeated measure comparisons showed that total fat intake was significantly greater at Day
396 10 in the milk chocolate group only ($P = 0.042$). Finally, no significant differences in dietary
397 fibre were detected between interventions at Day 10 (Table 4). Follow-up comparisons
398 revealed that dietary fibre intake was significantly greater at Day 10 within each group (all P
399 ≤ 0.001) (Table 4).

	Pure Inulin (*n* =24)			Shortbread (*n* =24)			Milk Chocolate (*n* =24)			Rice Drink (*n* =24)			***P* (b)**
	Day 0	**Day 10**	***P* (a)**	**Day 0**	**Day 10**	***P* (a)**	**Day 0**	**Day 10**	***P* (a)**	**Day 0**	**Day 10**	***P* (a)**	
Total energy (kcals)	2139 (156.60)	2056 (167.90)	0.58	2127 (149.40)	2302 (180.80)	0.25	2429 (168.20)	2570 (172)	0.35	1990 (135.70)	2083 (129.90)	0.53	0.552
Protein (g)	93.51 (6.98)	96.17 (5.9)	0.69	88.4 (8.07)	89.71 (8.40)	0.84	98.22 (6.76)	97.73 (6.4)	0.94	79.99 (6.91)	76.83 (6.82)	0.64	0.293
Fat (g)	88.04 (8.11)	84.31 (6.82)	0.59	87.48 (8.03)	81.98 (8.72)	0.59	98.92 (9.70)	113.2 (9.24)	**0.042**	83.38 (6.12)	79.27 (6.38)	0.55	**0.026**
PUFA (g)	16.44 (1.43)	15.09 (1.27)	0.38	15.86 (1.74)	15.41 (1.85)	0.77	17.98 (1.92)	18.52 (1.71)	0.72	14.50 (1.38)	14.73 (1.31)	0.88	0.499
CHO (g)	250.30 (18.51)	247.80 (25.59)	0.89	248.60 (22.59)	276.50 (19.77)	0.13	280.70 (18.34)	276.90 (17.33)	0.84	228.20 (16.59)	236.40 (17.06)	0.66	0.599
Starch (g)	130.90 (10.71)	127.50 (15.07)	0.74	133.80 (10.36)	143.30 (11.70)	0.37	147.80 (12.79)	138.20 (11.73)	0.33	122.20 (11.23)	132.50 (11.84)	0.33	0.616
Total sugar (g)	116.80 (10.91)	116.90 (15.36)	0.99	112.80 (13.82)	110.60 (11.11)	0.85	129.80 (12.44)	134.10 (9.48)	0.71	104.50 (10.62)	115.40 (10.18)	0.35	0.748
Fibre (g)	31.04 (2.09)	38.64 (2.11)	**≤ 0.001**	27.06 (2.38)	38.04 (2.51)	**≤ 0.001**	30.23 (2.18)	39.01 (39.01)	**≤ 0.001**	21.69 (21.69)	35.14 (1.76)	**≤ 0.001**	0.902

400 **Table 4:** Energy and nutrient intake at baseline (Day 0) and at completion (Day 10) of intervention study in 96 volunteers (*n* = 24 per group). Mean and standard error (SE). (a) *P* values are as a
401 result of planned Day 0 vs Day 10 comparisons (grey columns). **(b)** *P* values are as a result of using Day 0 data as a baseline covariate for between group Day 10 comparisons (orange column).
402 Keyword: CHO = Total carbohydrates; PUFA = Polyunsaturated fatty acids

403 **Bacterial enumeration by FISH**
404

405 96 subjects provided stool samples at baseline and end of the intervention. Figure 2 and
406 Figure 3 report changes in bacterial counts observed in the four intervention groups between
407 Day 0 and Day 10 of the intervention.

408
409 Figure 2A reports the changes seen in total bacteria counts (Eub I-II-III). Analysis revealed
410 no significant differences between interventions at completion (*P* = 0.315). There was an
411 average 0.07 $\log_{10}$ cells/g wet faeces increase in Eub I-II-III counts across all four
412 interventions going from 9.74 to 9.81 (0.07) ± 0.025 (SE) $\log_{10}$ cells/g wet faeces. All values
413 at end of intervention were significantly different compared to respective baseline samples
414 (all $P \leq 0.05$) (Supplemental Data Table 1).

415

416 Similarly, regarding Bif164 (*Bifidobacterium* spp.) counts no significant differences were
417 detected between interventions at Day 10 (*P* = 0.641). Repeated measures analysis revealed
418 significant increases in Bif164 counts at Day 10 across all four interventions: average
419 numbers increasing from 8.36 to 9.00 (mean difference 0.64) ± 0.05 (SE)) Log_{10} cells/g ($P \leq$
420 0.001) (Figure 2B).

421

422 **Figure 2.** Bacterial groups measured by FISH-FLOW (Log_{10} cells/g wet faeces) using probes: (A) total bacteria (Eub338 I-
423 II-III), (B) *Bifidobacterium* spp. (Bif164). Box and whisker plot - min and max with all points. 96 volunteers (*n* = 24 per
424 group). Results that are statistically significant within and between subject (intervention) are displayed by specified *P* values.

425 *Bacteroides* (Bac303) counts are reported in Figure 3A. Increases in Bac303 counts were
426 observed across all four interventions, yet the extent of change varied greatly. Largest
427 increases in numbers of Bac303 were observed in the shortbread intervention increasing from
428 8.06 to 8.31 (mean difference 0.25 ± 0.04 (SE)) log_{10} cells/g wet faeces ($P = 0.002$). Bac303
429 counts at the end of the interventions (Day 10) were not significantly different between
430 interventions ($P = 0.201$) (Supplemental Data Table 1).

431

432 In contrast, significant differences in Rrec584 (*Roseburia/Eubacterium rectale*) counts were
433 observed between interventions at Day 10 ($P = 0.022$). Subsequent analysis identified
434 significantly greater increases in Rrec584 counts in the shortbread intervention compared to
435 milk chocolate ($P = 0.021$). Significant increases from baseline in Rrec584 counts were only
436 detected in the shortbread group going from 8.39-8.61 (mean difference 0.22 ± 0.07 (SE))
437 log_{10} cells/g wet faeces ($P = 0.005$) (Figure 3B).

438

439 Additionally, *Faecalibacterium prausnitzii* (Fprau655) (Figure 3C) counts differed
440 significantly between interventions at Day 10 ($P = 0.029$), with increases in the shortbread
441 intervention being significantly different from milk chocolate ($P = 0.048$). In Day 0 vs Day
442 10 comparisons the most noticeable changes in Fprau655 were recorded in both the
443 shortbread and rice drink interventions with increases from 8.73 to 8.93 (0.20 mean
444 difference ± 0.07 (SE)) log_{10} cells/g wet faeces (shortbread) and 8.77 to 8.84 (0.18 mean
445 difference ± 0.08 (SE)) log_{10} cells/g wet faeces (rice drink). Both changes were statistically
446 significant compared to respective Day 0 samples - shortbread ($P = 0.004$) and rice drink ($P =$
447 0.012) (Figure 3C).

448

449 Finally, no significant differences were observed in changes of numbers of *Clostridium*
450 *coccoides-Eubacterium rectale* group (Erec458) or *Propionibacterium* (Pro853) either within
451 or between intervention at completion (Supplemental Data Table 1).

452

453 **Figure 3.** Bacterial groups measured by FISH-FLOW (Log_{10} cells/g wet faeces) using probes: (A) most *Bacteroidaceae* and
454 *Prevotellaceae* (Bac303), (B) *Roseburia* (Rrec584) and (C) *Faecalibacterium prausnitzii* (Fprau655). Box and whisker plot -
455 min and max with all points. 96 volunteers ($n = 24$ per group). Results that are statistically significant within and between
456 subject (intervention) are displayed by specified *P* values

457 Microbiota Profiling Analysis
458
459 Figure 4 reports 16S rRNA sequencing results for Relative Microbiome Profiling (RMP)
460 along with Quantitative Microbiome Profiling (QMP) for *Bifidobacterium* data across all four
461 interventions.
462
463 **Figure 4.** Relative Microbiome Profiling (RMP) (A) and Quantitative Microbiome Profiling data (QMP) (B) of
464 *Bifidobacterium* 16SrRNA sequencing results. Mean and standard error (SE). 96 volunteers (n = 24 per group). Results that
465 are statistically significant within and between subject (intervention) are displayed by specified P values.
466
467 Relative Microbiome Profiling (RMP)
468
469 There were no significant differences in phylum level abundances detected between
470 interventions at Day 10 (Supplemental data Table 2) (all $P \geq 0.05$). At phylum level largest
471 changes were documented in *Actinomycetota* (*Actinobacteria*), *post hoc* analysis
472 documenting significant increases across all four interventions at Day 10: shortbread (P =
473 0.002), milk chocolate, pure inulin and rice drink (all $P \leq 0.001$) (Supplemental Data Table
474 2). Subsequently, there were also significant decreases detected in *Bacillota* (*Firmicutes*):
475 milk chocolate (P = 0.002), and pure inulin, rice drink and shortbread (all $P \leq 0.001$). These
476 changes coincided with those seen in *Bifidobacterium* at genus level.
477
478 Accordingly, no significant differences were detected at genus level in any bacterial group
479 between interventions (all $P \geq 0.05$) (Supplemental Data Table 2). In line with phylum level,
480 largest changes were recorded in *Bifidobacterium* with significant increases being detected
481 across all four interventions averaging an 92% increase above baseline (all $P \leq 0.001$),
482 (Figure 4A). In addition, while no differences were detected between interventions, several
483 differences in bacterial taxa were documented within intervention including decreases in
484 *Blautia* (pure inulin, shortbread and rice drink), *Clostridium* cluster IVXA + IVXB (pure
485 inulin, milk chocolate and rice drink), *Dorea* (shortbread and rice drink), *Lactococcus*
486 (shortbread), *Ruminococcus2* (milk chocolate), *Lachnospiraceae incertae sedi* (pure inulin
487 and shortbread), *Ruminococcus* (pure inulin, shortbread and rice drink), and increases in
488 *Prevotella* (milk chocolate) (Supplemental Data Table 2).
489
490 There were no significant differences in any measure of α-diversity detected between
491 interventions at Day 10 (all $P \geq 0.05$). Several within group differences were detected with
492 significant decreases in Shannon index in both the pure inulin (P = 0.003) and rice drink (P =
493 0.033) interventions. Trends towards reductions in both shortbread (P = 0.061) and milk
494 chocolate interventions (P = 0.073) were noted. There was also a significant decrease in
495 richness (no. of species) in both the pure inulin (P = 0.011) and rice drink interventions (P =
496 0.026). Simpson index was reduced in the pure inulin intervention (P = 0.011) (Supplemental
497 Data Table 3).
498

499 Quantitative Microbiome Profiling (QMP)
500

501 Upon quantification of RMP data no significant differences were detected between groups at
502 Day 10 (all $P \geq 0.05$) (Supplemental Data Table 4). As per RMP, largest increases at phylum
503 level were documented in *Actinomycetota:* pure inulin and rice drink (both $P = 0.003$), milk
504 chocolate ($P = 0.015$) and shortbread ($P = 0.001$). Significant decreases in *Bacillota*
505 (*Firmicutes*) were documented in both the pure inulin ($P = 0.016$) and shortbread ($P \leq 0.001$)
506 interventions, but not in the milk chocolate (all $P = 0.612$) or rice drink interventions (all $P =$
507 0.514).

508

509 Largest changes in microbial counts at genus level were detected in *Bifidobacterium*, *post hoc*
510 analysis revealing significant increases across all four interventions: shortbread ($P \leq 0.001$),
511 milk chocolate ($P = 0.036$), pure inulin ($P = 0.004$) and rice drink ($P = 0.011$) (Figure 4B).
512 This mirrors the changes observed in RMP. Additionally, as per RMP there were a number,
513 albeit fewer, changes in bacteria groups detected within each intervention. These included
514 decreases in numbers of *Blautia* (pure inulin and shortbread), *Clostridium* cluster IVXA +
515 IVXB (pure inulin), *Lachnospiraceae incertae sedi* (pure inulin and shortbread), *Collinsella*
516 (pure inulin) and *Ruminococcus* (shortbread). Along with increases in *Prevotella* (milk
517 chocolate) and *Roseburia* (shortbread) (Supplemental Data Table 4).

518

519 **^{1}H-NMR spectroscopic profiles**
520

521 Metabolic profiles of urine samples across the four intervention groups were analysed using
522 unsupervised (PCA) methods (first two components), showing separation between the four
523 interventions at completion (R^2Cum = 0.18, Q^2Cum = 0.122) (Figure 5). We did not observe
524 any differences in ^{1}H-NMR metabolic profiles between interventions as points did not show
525 any clustering or patterns in relation to intervention. As a result, no subsequent downstream
526 analysis was carried out.

527

528 **Figure 5.** Urinary ^{1}H magnetic resonance (^{1}H-NMR) profiles across the four intervention groups. Unsupervised principal
529 components analysis (PCA) scores plot of endpoint urine samples. R^2Cum = 0.18, Q^2Cum = 0.122. Key: IN = Pure inulin;
530 MC = Milk chocolate; RD = Rice Drink; ST = Shortbread

531

532 **Bowel habit and function**
533

534 Changes in gastrointestinal symptoms (flatulence, intestinal bloating, abdominal pressure,
535 abdominal pain and feeling of fullness) were self-recorded daily throughout the 10-day
536 intervention and are reported as averages of Days 0-5 and Days 6-10. Scores of 0, 1, 2, and 3
537 corresponded to none, mild, moderate, and severe. Changes in stool consistency were
538 measured as per Bristol Stool Form Scale and stool frequency are reported in Figure 6. There
539 were no differences in flatulence, intestinal bloating, abdominal pressure, abdominal pain or
540 feeling of fullness detected between interventions at completion (D6-10) (Supplemental Data

541 Table 5), although there was a trend towards significant differences in feeling of fullness (*P* =
542 0.058). This reflected the level of significance documented between the rice drink and pure
543 inulin interventions at completion (*P* = 0.058). Repeated measures analysis revealed a
544 significant decrease in feeling of fullness in the pure inulin intervention only (*P* = 0.002).

545

546 Stool consistency was significantly different between interventions (*P* = 0.017), with values
547 documented in pure inulin being higher than in the rice milk intervention (*P* = 0.010). These
548 results are in line with post hoc analysis revealing increases in stool consistency ratings were
549 only detected in the pure inulin group (*P* = 0.009). Finally, there were no changes in stool
550 frequency either within or between interventions although there was a trend towards increases
551 in stool frequency identified in the pure inulin intervention (*P* = 0.080) (Figure 6 and
552 Supplemental Data Table 5).

553

554 **Figure 6**. Stool consistency (Bristol Stool Form Scale, A) and stool frequency (B) at (Day 0-5) and again at Day 6-10 after
555 intervention in 96 volunteers (*n* = 24 per group). Results that are statistically significant within and between subject
556 (intervention) are displayed by specified *P* values.

557

558 # Discussion

559

560 This is the first study to investigate whether the food matrix impacts on the prebiotic efficacy
561 of ITF using a standardised protocol. In total 96 volunteers provided stool samples at baseline
562 and end of the intervention. One of the main pre-requisites of a prebiotic is to stimulate
563 beneficial changes in microbial composition in certain, but not limited number of bacteria
564 (Gibson *et al.*, 2017). ITF prebiotics primarily target bifidobacteria as they possess the
565 necessary glycosidases and transporters needed to degrade fructans and to assimilate low
566 molecular weight carbohydrates (Falony *et al.*, 2009; Riviere *et al.*, 2018). In this study we
567 used both targeted and untargeted analyses to determine the impact of the food matrix on the
568 prebiotic efficacy of ITF.

569

570 In this study, we demonstrate, using both targeted and untargeted analysis, that, irrespective
571 of the food matrix, the selectivity of ITF towards bifidobacteria appears to be unaffected.
572 FISH-FLOW determined similar increases in Bif164 counts across all interventions averaging
573 a 0.64 ± 0.10 Log_{10} Cells/g wet faeces at completion. These findings were further validated
574 using untargeted analysis with an average 92% ± 5.43% (SE) and $1.14 \times 10^9 \pm 1.52 \times 10^8$
575 (SE) *Bifidobacterium* increase in RMP and QMP abundance respectively. This further
576 confirms the selectivity of ITF towards *Bifidobacterium* (Costabile *et al.*, 2010; Gibson and
577 Roberfroid, 1995; Kruse *et al.*, 1999). No significant differences were detected between
578 interventions (all $P \geq 0.05$). These results are in line with those documented by several
579 previous food-based ITF supplementation studies (Gibson *et al.*, 1995; Healey *et al.*, 2018;
580 Marteau *et al.*, 2011; Ramnani *et al.*, 2010; Reimer *et al.*, 2020; Tuohy *et al.*, 2001). This
581 does not, however, match those recorded by (Slavin and Feirtag, 2011) who documented that
582 upon consumption of 20g/day of ITF supplemented into ice cream, no significant differences

583 in *Bifidobacterium* counts were detected. These differences likely result from subjectivity in
584 using plate counts, lack of a washout period and lack of collection of baseline stool samples
585 (Slavin and Feirtag, 2011).

586

587 Upon completion differences between the interventions in microbial load and composition
588 among the differing food matrices were detected. Using targeted FISH-FLOW analysis there
589 were significant increases in Bac303, Rrec584 and Fprau655 detected in the shortbread
590 intervention. In the rice drink intervention significant increases were seen in numbers of
591 FPrau655. The microbial loads (QMP) documented in both *Roseburia* and *Faecalibacterium*
592 *prausnitizii* were similar to those recorded by FLOW-FISH. The levels of *Roseburia* and
593 *Faecalibacterium prausnitizii* at completion of the shortbread intervention using FISH-
594 FLOW were significantly different from milk chocolate at Day 10 (both $P \leq 0.05$), but not
595 from pure inulin or rice milk (both $P \geq 0.05$).

596

597 These results are of interest because several previous food-based supplementation studies by
598 (Gibson *et al.*, 1995; Kleessen *et al.*, 2007; Tuohy *et al.*, 2001) either noted reductions or no
599 changes in numbers of *Bacteroides* upon consumption of ITF-fortified cereal bars and
600 biscuits. In contrast (Brighenti *et al.*, 1999) and (Rao, 2001) recorded 0.49 and 0.69
601 $\log_{10}$ CFU/g faeces dry weight increases in *Bacteroides* upon consumption of ITF containing
602 extruded ready-to-eat cereal and when pure ITF was supplemented into drinks. These
603 discrepancies probably occur due to the higher levels of *Bacteroides* present in the study
604 conducted by (Kleessen *et al.*, 2007; Tuohy *et al.*, 2001). It should be noted that different
605 analytical techniques were used (FISH-FLOW vs selective media) which directly impedes the
606 comparison and evaluation of results across such studies (Jackson *et al.*, 2022b).

607

608 Additionally, it is difficult to compare results of Rrec584 and FPrau655 to previous food-
609 based ITF supplementation studies due to most studies using targeted analysis not reporting
610 changes in both targeted groups. One food-based supplementation study that counted
611 Fprau655 using FISH-FLOW recorded no change in numbers upon consumption of fruit juice
612 drinks containing Jerusalem artichoke inulin (Ramnani *et al.*, 2010). A trend towards an
613 increase in relative abundances of *Faecalibacterium prausnitzii* was detected upon
614 consumption of pure ITF (Healey *et al.*, 2018).

615

616 *Bacteroides* possess a large number of loci responsible for the assimilation of complex
617 carbohydrates including arabinoxylans (Pereira *et al.*, 2021) as well as complex starches
618 (Dobranowski and Stintzi, 2021). Arabinoxylans are components of the wheat flour used in
619 production of the shortbread biscuits in this study. From this, one could speculate that the
620 significantly larger increases seen in *Roseburia* and *Faecalibacterium prausnitzii* in the
621 shortbread intervention resulted from the utilisation of resulting motifs from the breakdown
622 of arabinoxylans by *Bacteroides*. For example, it was previously demonstrated by (Walton *et*
623 *al.*, 2012) that, consumption of *in situ* produced arabinoxylan-oligosaccharides in bread,
624 resulted in significant increases in *Bacteroides*, *Roseburia* and *Faecalibacterium prausnitzii*

625 (all $P \leq 0.05$). However, it has also been demonstrated that upon consumption of 2 x 44 g
626 bowls of wheat bran arabinoxylan-rich ready-to-eat cereal no changes in *Bacteroides*,
627 *Roseburia* and *Faecalibacterium prausnitzii* could be detected (Maki *et al.*, 2012). Taking
628 this into consideration, increases in both *Roseburia* and *Faecalibacterium prausnitzii* often
629 coincide with increases in *Bifidobacterium* in *in vitro* studies likely as a result of cross-
630 feeding on acetate and lactate (Kim *et al.*, 2020; Riviere *et al.*, 2016). From this, it could be
631 hypothesised that increases in both *Roseburia* and *Faecalibacterium prausnitzii* in the
632 shortbread intervention may have also occurred from both the utilisation of resulting
633 breakdown arabinoxylan motifs by *Bacteroides* along with cross-feeding on acetate and
634 lactate produced by *Bifidobacterium*.

635

636 It can be implied that complementary effects may exist from the presence of other bioactive
637 compounds present within the matrices. For example it was demonstrated by (Ramnani *et al.*,
638 2010) that upon consumption of high polyphenol-containing fruit shots containing Jerusalem
639 artichoke ITF, in addition to an increase of bifidobacteria, significant increases in
640 *Lactobacillus/Enterococcus* group were detected ($P = 0.042$). Finding means to increase
641 numbers of *Bacteroides*, *Roseburia* and *Faecalibacterium prausnitzii* alongside
642 *Bifidobacterium* may be of clinical importance via the potential to increase butyrate
643 production, given that butyrate plays a vital role as an energy source for colonocytes, in the
644 regulation of tight cell junction integrity, and in the repair of the intestinal mucosa (Canani *et*
645 *al.*, 2011). *Faecalibacterium prausnitzii* is considered to be a keystone species and has been
646 associated with lowered risks of IBD and ulcerative colitis (Leylabadlo *et al.*, 2020). Overall,
647 from the findings of this study we can conclude that the selectivity of ITF towards
648 bifidobacteria is independent of the food matrix. Yet, the compositional makeup of the matrix
649 may likely have important implications towards stimulating changes in the wider microbiota.

650

651 During the trial volunteers did not alter their diet or lifestyle, with exception of consumption
652 of study product and adjustment of portion sizes to compensate for additional calories
653 consumed. On average, fibre intakes were estimated at 27.5 g/day which is slightly below the
654 current UK recommendations of 30 g/day as laid down by SACN (Scientific Advisory
655 Committee on Nutrition, 2015). They do, however, far exceed those of the average population
656 at just 14.9-18 g/day (Gressier and Frost, 2022; Scientific Advisory Committee on Nutrition,
657 2015).

658

659 Significant increases in dietary fibre intakes were detected across all four interventions (Table
660 4). Between baseline and completion there was an average increase of 10.2g fibre with an
661 average 37.71 g/day of fibre being consumed by completion suggesting that the addition of
662 10 g/day of inulin into food products could help people reach or even exceed the daily
663 minimum recommendation. Increasing fibre intake is the 1st line of treatment to improve
664 bowel function. In order to assess changes in stool consistency the validated Bristol Stool
665 Form Scale was used. However, despite an additional consumption of 10 g/day ITF
666 significant changes in stool consistency were only detected in the pure inulin intervention at
667 Day 10 ($P = 0.023$).

In our cohort no differences in stool frequency were detected and scores were stable throughout the intervention. Given that, in this study, volunteers started with higher daily stool frequency at baseline and that increases in stool frequency are often seen in subjects with low fibre intakes, the higher baseline fibre intakes seen in this study likely contributed towards a lack of change in stool frequency (Buddington *et al.*, 2017; François *et al.*, 2014; Grider and Piland, 2007; Isakov *et al.*, 2013; Micka *et al.*, 2017; Ramnani *et al.*, 2010; Slavin and Feirtag, 2011).

Gastrointestinal sensations including flatulence, intestinal bloating, abdominal pressure and abdominal pain were rated as none to mild and remained unchanged throughout the course of the intervention. No discomfort was reported and no discontinuation of the study by any volunteers was recorded. The only significant difference was a decrease in feeling of fullness in the pure inulin intervention ($P = 0.002$). This indicates that chicory inulin in both pure form and supplemented into differing matrices is well tolerated, but the food matrix may have implications regarding satiety. It has been documented that matrices higher in lipids and other non-digestible carbohydrates content such as the interventions used in this study can induce/sustain satiety by regulating smooth muscle stretch receptors and delaying gastric emptying (Aguilera, 2019).

Conclusion

In conclusion, we can confirm that irrespective of the food application and matrix, prebiotic ITF are selectively utilized and lead to specific changes in the gut microbiota. *Bifidobacterium* was the only genus consistently impacted by inulin-type fructans, yet the compositional make-up of food matrix may have implications regarding changes in the wider microbiota. For example, differences in several bacterial groups including *Roseburia* and *Faecalibacterium prausnitzii* were documented at the completion between the shortbread and milk chocolate interventions.

Supplementary material

Supplemental Data Table 1. Targeted microbial analysis vis fluorescence *in situ* hybridisation at Day 0 and Day 10 of intervention.

Supplemental Data Table 2. 16S rRNA relative microbial profiling data at Day 0 and Day 10 of intervention

Supplemental Data Table 3. Alpha diversity measures of 16S rRNA sequencing at Day 0 and Day 10 of intervention.

Supplemental Data Table 4. 16S rRNA quantitative microbiome profiling data at Day 0 and Day 10 of intervention.

Supplemental Data Table 5. Gastrointestinal sensation and bowel habit diary data displayed by day and intervention.

Acknowledgements

We would like to acknowledge Carlos Poveda for his initial help and expertise in preparation and analysis of faecal samples.

Conflict of Interest

We acknowledge that this work was financed by BENEO. ST and JVH are employees of BENEO.

Data Sharing

The data that support the findings of this study are available from the corresponding author upon reasonable request.

742 # References

743

744 Aguilera, J.M., 2019. The food matrix: implications in processing, nutrition and health. Critical
745 Reviews in Food Science and Nutrition 59: 3612-3629. 10.1080/10408398.2018.1502743
746 Ahmed, W. and Rashid, S., 2019. Functional and therapeutic potential of inulin: A comprehensive
747 review. Critical Reviews in Food Science and Nutrition 59: 1-13.
748 10.1080/10408398.2017.1355775
749 Amann, R.I., Binder, B.J., Olson, R.J., Chisholm, S.W., Devereux, R. and Stahl, D.A., 1990.
750 Combination of 16s rRNA-targeted oligonucleotide probes with flow-cytometry for analyzing
751 mixed microbial-populations. Applied and Environmental Microbiology 56: 1919-1925.
752 10.1128/aem.56.6.1919-1925.1990
753 Azpiroz, F., Molne, L., Mendez, S., Nieto, A., Manichanh, C., Mego, M., Accarino, A., Santos, J.,
754 Sailer, M., Theis, S. and Guarner, F., 2017. Effect of Chicory-derived Inulin on Abdominal
755 Sensations and Bowel Motor Function. Journal of Clinical Gastroenterology 51: 619-625.
756 10.1097/mcg.0000000000000723
757 Brighenti, F., Casiraghi, M.C., Canzi, E. and Ferrari, A., 1999. Effect of consumption of a ready-to-
758 eat breakfast cereal containing inulin on the intestinal milieu and blood lipids in healthy male
759 volunteers. European Journal of Clinical Nutrition 53: 726-733. 10.1038/sj.ejcn.1600841
760 Buddington, R.K., Kapadia, C., Neumer, F. and Theis, S., 2017. Oligofructose Provides Laxation for
761 Irregularity Associated with Low Fiber Intake. Nutrients 9. 10.3390/nu9121372
762 Canani, R.B., Di Costanzo, M., Leone, L., Pedata, M., Meli, R. and Calignano, A., 2011. Potential
763 beneficial effects of butyrate in intestinal and extrainitestinal diseases. World Journal of
764 Gastroenterology 17: 1519-1528. 10.3748/wjg.v17.i12.1519
765 Costabile, A., Klinder, A., Fava, F., Napolitano, A., Fogliano, V., Leonard, C., Gibson, G.R. and
766 Tuohy, K.M., 2008. Whole-grain wheat breakfast cereal has a prebiotic effect on the human
767 gut microbiota: a double-blind, placebo-controlled, crossover study. British Journal of
768 Nutrition 99: 110-120. 10.1017/s0007114507793923
769 Costabile, A., Kolida, S., Klinder, A., Gietl, E., Bauerlein, M., Frohberg, C., Landschutze, V. and
770 Gibson, G.R., 2010. A double-blind, placebo-controlled, cross-over study to establish the
771 bifidogenic effect of a very-long-chain inulin extracted from globe artichoke (Cynara
772 scolymus) in healthy human subjects. British Journal of Nutrition 104: 1007-1017.
773 10.1017/s0007114510001571
774 D'Archivio, M., Filesi, C., Vari, R., Scazzocchio, B. and Masella, R., 2010. Bioavailability of the
775 Polyphenols: Status and Controversies. International Journal of Molecular Sciences 11: 1321-
776 1342. 10.3390/ijms11041321
777 Daims, H., Bruhl, A., Amann, R., Schleifer, K.H. and Wagner, M., 1999. The domain-specific probe
778 EUB338 is insufficient for the detection of all Bacteria: Development and evaluation of a
779 more comprehensive probe set. Systematic and Applied Microbiology 22: 434-444.
780 10.1016/s0723-2020(99)80053-8
781 Dobranowski, P.A. and Stintzi, A., 2021. Resistant starch, microbiome, and precision modulation. Gut
782 Microbes 13. 10.1080/19490976.2021.1926842
783 Duar, R.M., Ang, P.T., Hoffman, M., Wehling, R., Hutkins, R. and Schlegel, V., 2015. Processing
784 effects on four prebiotic carbohydrates supplemented in an extruded cereal and a low pH
785 drink. Cogent Food & Agriculture 1. 10.1080/23311932.2015.1013782
786 Falony, G., Lazidou, K., Verschaeren, A., Weckx, S., Maes, D. and De Vuyst, L., 2009. In Vitro
787 Kinetic Analysis of Fermentation of Prebiotic Inulin-Type Fructans by Bifidobacterium
788 Species Reveals Four Different Phenotypes. Applied and Environmental Microbiology 75:
789 454-461. 10.1128/aem.01488-08
790 Franco, R.Z., Fallaize, R., Hwang, F. and Lovegrove, J.A., 2019. Strategies for online personalised
791 nutrition advice employed in the development of the eNutri web app. Proceedings of the
792 Nutrition Society 78: 407-417. 10.1017/s0029665118002707
793 François, I.E.J.A., Lescroart, O., Veraverbeke, W.S., Windey, K., Verbeke, K. and Broekaert, W.F.,
794 2014. Tolerance and the effect of high doses of wheat bran extract, containing arabinoxylan–

795 oligosaccharides, and oligofructose on faecal output: a double-blind, randomised, placebo-
796 controlled, cross-over trial. Journal of nutritional science (Cambridge) 3: e49-e49.
797 10.1017/jns.2014.52
798 Franks, A.H., Harmsen, H.J.M., Raangs, G.C., Jansen, G.J., Schut, F. and Welling, G.W., 1998.
799 Variations of bacterial populations in human feces measured by fluorescent in situ
800 hybridization with group-specific 16S rRNA-Targeted oligonucleotide probes. Applied and
801 Environmental Microbiology 64: 3336-3345.
802 Gibson, G.R., Beatty, E.R., Wang, X. and Cummings, J.H., 1995. Selective stimulation of
803 bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology 108: 975-
804 982. 10.1016/0016-5085(95)90192-2
805 Gibson, G.R., Hutkins, R., Sanders, M.E., Prescott, S.L., Reimer, R.A., Salminen, S.J., Scott, K.,
806 Stanton, C., Swanson, K.S., Cani, P.D., Verbeke, K. and Reid, G., 2017. Expert consensus
807 document: The International Scientific Association for Probiotics and Prebiotics (ISAPP)
808 consensus statement on the definition and scope of prebiotics. Nature reviews.
809 Gastroenterology & hepatology 14: 491-502. 10.1038/nrgastro.2017.75
810 Gibson, G.R. and Roberfroid, M.B., 1995. Dietary modulation of the human colonic microbiota -
811 introducing the concept of prebiotics. Journal of Nutrition 125: 1401-1412.
812 10.1093/jn/125.6.1401
813 Glibowski, P. and Wasko, A., 2008. Effect of thermochemical treatment on the structure of inulin and
814 its gelling properties. International Journal of Food Science and Technology 43: 2075-2082.
815 10.1111/j.1365-2621.2008.01825.x
816 Gomez, B., Gullon, B., Yanez, R., Schols, H. and Alonso, J.L., 2016. Prebiotic potential of pectins
817 and pectic oligosaccharides derived from lemon peel wastes and sugar beet pulp: A
818 comparative evaluation. Journal of Functional Foods 20: 108-121. 10.1016/j.jff.2015.10.029
819 Gressier, M. and Frost, G., 2022. Minor changes in fibre intake in the UK population between
820 2008/2009 and 2016/2017. European Journal of Clinical Nutrition 76: 322-327.
821 10.1038/s41430-021-00933-2
822 Grider, J.R. and Piland, B.E., 2007. The peristaltic reflex induced by short-chain fatty acids is
823 mediated by sequential release of 5-HT and neuronal CGRP but not BDNF. American Journal
824 of Physiology - Gastrointestinal and Liver Physiology 292: 429-437.
825 10.1152/ajpgi.00376.2006
826 Grimaldi, R., Cela, D., Swann, J.R., Vulevic, J., Gibson, G.R., Tzortzis, G. and Costabile, A., 2017. In
827 vitro fermentation of B-GOS: impact on faecal bacterial populations and metabolic activity in
828 autistic and non-autistic children. Fems Microbiology Ecology 93. 10.1093/femsec/fiw233
829 Harris, P.A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N. and Conde, J.G., 2009. Research
830 electronic data capture (REDCap)-A metadata-driven methodology and workflow process for
831 providing translational research informatics support. Journal of Biomedical Informatics 42:
832 377-381. 10.1016/j.jbi.2008.08.010
833 Healey, G., Murphy, R., Butts, C., Brough, L., Whelan, K. and Coad, J., 2018. Habitual dietary fibre
834 intake influences gut microbiota response to an inulin-type fructan prebiotic: a randomised,
835 double-blind, placebo-controlled, cross-over, human intervention study. British Journal of
836 Nutrition 119: 176-189. 10.1017/s0007114517003440
837 Isakov, V., Pilipenko, V., Shakhovskaya, A. and Tutelyan, V., 2013. Efficacy of inulin enriched
838 yogurt on bowel habits in patients with irritable bowel syndrome with constipation: a pilot
839 study. Faseb Journal 27.
840 Jackson, P.P.J., Wijeyesekera, A. and Rastall, R.A., 2022a. Inulin-type fructans and short-chain
841 fructooligosaccharides-their role within the food industry as fat and sugar replacers and
842 texture modifiers-what needs to be considered! Food Science & Nutrition. 10.1002/fsn3.3040
843 Jackson, P.P.J., Wijeyesekera, A., Theis, S., Van Harsselaar, J. and Rastall, R.A., 2022b. Food for
844 thought! Inulin-type fructans: Does the food matrix matter? Journal of Functional Foods 90:
845 104987. 10.1016/j.jff.2022.104987
846 Karimi, R., Azizi, M.H., Ghasemlou, M. and Vaziri, M., 2015. Application of inulin in cheese as
847 prebiotic, fat replacer and texturizer: A review. Carbohydrate Polymers 119: 85-100.
848 10.1016/j.carbpol.2014.11.029

849 Kemperman, R.A., Gross, G., Mondot, S., Possemiers, S., Marzorati, M., Van de Wiele, T., Dore, J.
850 and Vaughan, E.E., 2013. Impact of polyphenols from black tea and red wine/grape juice on a
851 gut model microbiome. Food Research International 53: 659-669.
852 10.1016/j.foodres.2013.01.034
853 Kim, H., Jeong, Y., Kang, S.N., You, H.J. and Ji, G.E., 2020. Co-Culture with Bifidobacterium
854 catenulatum Improves the Growth, Gut Colonization, and Butyrate Production of
855 Faecalibacterium prausnitzii: In Vitro and In Vivo Studies. Microorganisms 8.
856 10.3390/microorganisms8050788
857 Kleessen, B., Schwarz, S., Boehm, A., Fuhrmann, H., Richter, A., Henle, T. and Krueger, M., 2007.
858 Jerusalem artichoke and chicory inulin in bakery products affect faecal microbiota of healthy
859 volunteers. British Journal of Nutrition 98: 540-549. 10.1017/s0007114507730751
860 Klewicki, R., 2007. The stability of gal-polyols and oligosaccharides during pasteurization at a low
861 pH. Lwt-Food Science and Technology 40: 1259-1265. 10.1016/j.lwt.2006.08.008
862 Kruse, H.P., Kleessen, B. and Blaut, M., 1999. Effects of inulin on faecal bifidobacteria in human
863 subjects. British Journal of Nutrition 82: 375-382. 10.1017/s0007114599001622
864 Langendijk, P.S., Schut, F., Jansen, G.J., Raangs, G.C., Kamphuis, G.R., Wilkinson, M.H.F. and
865 Welling, G.W., 1995. Quantitative fluorescence in situ hybridization of Bifidobacterium spp.
866 with genus-specific 16S rRNA-targeted probes and its application in fecal samples. Applied
867 and Environmental Microbiology 61: 3069-3075. 10.1128/aem.61.8.3069-3075.1995
868 Lewis, S.J. and Heaton, K.W., 1997. Stool form scale as a useful guide to intestinal transit time.
869 Scandinavian Journal of Gastroenterology 32: 920-924. 10.3109/00365529709011203
870 Leylabadlo, H.E., Ghotaslou, R., Feizabadi, M.M., Farajnia, S., Moaddab, S.Y., Ganbarov, K.,
871 Khodadadi, E., Tanomand, A., Sheykhsaran, E., Yousefi, B. and Kafil, H.S., 2020. The
872 critical role of Faecalibacterium prausnitzii in human health: An overview. Microbial
873 Pathogenesis 149. 10.1016/j.micpath.2020.104344
874 Maki, K.C., Gibson, G.R., Dickmann, R.S., Kendall, C.W.C., Chen, C.Y.O., Costabile, A., Comelli,
875 E.M., McKay, D.L., Almeida, N.G., Jenkins, D., Zello, G.A. and Blumberg, J.B., 2012.
876 Digestive and physiologic effects of a wheat bran extract, arabino-xylan-oligosaccharide, in
877 breakfast cereal. Nutrition 28: 1115-1121. 10.1016/j.nut.2012.02.010
878 Manz, W., Amann, R., Ludwig, W., Vancanneyt, M. and Schleifer, K.H., 1996. Application of a suite
879 of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum
880 cytophaga-flavobacter-bacteroides in the natural environment. Microbiology-Sgm 142: 1097-
881 1106. 10.1099/13500872-142-5-1097
882 Marteau, P., Jacobs, H., Cazaubiel, M., Signoret, C., Prevel, J.M. and Housez, B., 2011. Effects of
883 chicory inulin in constipated elderly people: a double-blind controlled trial. International
884 Journal of Food Sciences and Nutrition 62: 164-170. 10.3109/09637486.2010.527323
885 Mensink, M.A., Frijlink, H.W., Maarschalk, K.V. and Hinrichs, W.L.J., 2015. Inulin, a flexible
886 oligosaccharide I: Review of its physicochemical characteristics. Carbohydrate Polymers 130:
887 405-419. 10.1016/j.carbpol.2015.05.026
888 Micka, A., Siepelmeyer, A., Holz, A., Theis, S. and Schon, C., 2017. Effect of consumption of
889 chicory inulin on bowel function in healthy subjects with constipation: a randomized, double-
890 blind, placebo-controlled trial. International Journal of Food Sciences and Nutrition 68: 82-
891 89. 10.1080/09637486.2016.1212819
892 Murakami, K. and Livingstone, M.B.E., 2016. Energy density of meals and snacks in the British diet
893 in relation to overall diet quality, BMI and waist circumference: findings from the National
894 Diet and Nutrition Survey. British Journal of Nutrition 116: 1479-1489.
895 10.1017/s0007114516003573
896 Nagy, D.U., Sandor-Bajusz, K.A., Body, B., Decsi, T., Van Harsselaar, J., Theis, S. and Lohner, S.,
897 2022. Effect of chicory-derived inulin-type fructans on abundance of Bifidobacterium and on
898 bowel function: a systematic review with meta-analyses. Critical Reviews in Food Science
899 and Nutrition. 10.1080/10408398.2022.2098246
900 Palafox-Carlos, H., Ayala-Zavala, J.F. and Gonzalez-Aguilar, G.A., 2011. The Role of Dietary Fiber
901 in the Bioaccessibility and Bioavailability of Fruit and Vegetable Antioxidants. Journal of
902 Food Science 76: R6-R15. 10.1111/j.1750-3841.2010.01957.x

903 Pereira, G.V., Abdel-Hamid, A.M., Dutta, S., D'Alessandro-Gabazza, C.N., Wefers, D., Farris, J.A.,
904 Bajaj, S., Wawrzak, Z., Atomi, H., Mackie, R.I., Gabazza, E.C., Shukla, D., Koropatkin,
905 N.M. and Cann, I., 2021. Degradation of complex arabinoxylans by human colonic
906 Bacteroidetes. Nature Communications 12. 10.1038/s41467-020-20737-5
907 Poinot, P., Arvisenet, G., Grua-Priol, J., Fillonneau, C., Le-Bail, A. and Prost, C., 2010. Influence of
908 inulin on bread: Kinetics and physico-chemical indicators of the formation of volatile
909 compounds during baking. Food Chemistry 119: 1474-1484.
910 10.1016/j.foodchem.2009.09.029
911 Ramnani, P., Gaudier, E., Bingham, M., van Bruggen, P., Tuohy, K.M. and Gibson, G.R., 2010.
912 Prebiotic effect of fruit and vegetable shots containing Jerusalem artichoke inulin: a human
913 intervention study. British Journal of Nutrition 104: 233-240. 10.1017/s000711451000036x
914 Rao, V.A., 2001. The prebiotic properties of oligofructose at low intake levels. Nutrition Research 21:
915 843-848. 10.1016/s0271-5317(01)00284-6
916 Reimer, R.A., Soto-Vaca, A., Nicolucci, A.C., Mayengbam, S., Park, H., Madsen, K.L., Menon, R.
917 and Vaughan, E.E., 2020. Effect of chicory inulin-type fructan-containing snack bars on the
918 human gut microbiota in low dietary fiber consumers in a randomized crossover trial.
919 American Journal of Clinical Nutrition 111: 1286-1296. 10.1093/ajcn/nqaa074
920 Ribas-Agusti, A., Martin-Belloso, O., Soliva-Fortuny, R. and Elez-Martinez, P., 2018. Food
921 processing strategies to enhance phenolic compounds bioaccessibility and bioavailability in
922 plant-based foods. Critical Reviews in Food Science and Nutrition 58: 2531-2548.
923 10.1080/10408398.2017.1331200
924 Riviere, A., Selak, M., Geirnaert, A., Van den Abbeele, P. and De Vuyst, L., 2018. Complementary
925 Mechanisms for Degradation of Inulin-Type Fructans and Arabinoxylan Oligosaccharides
926 among Bifidobacterial Strains Suggest Bacterial Cooperation. Applied and Environmental
927 Microbiology 84. 10.1128/aem.02893-17
928 Riviere, A., Selak, M., Lantin, D., Leroy, F. and De Vuyst, L., 2016. Bifidobacteria and Butyrate-
929 Producing Colon Bacteria: Importance and Strategies for Their Stimulation in the Human
930 Gut. Frontiers in Microbiology 7. 10.3389/fmicb.2016.00979
931 Roberfroid, M.B., 2007. Inulin-type fructans: Functional food ingredients. Journal of Nutrition 137:
932 2493S-2502S.
933 Rodriguez-Garcia, J., Puig, A., Salvador, A. and Hernando, I., 2012. Optimization of a Sponge Cake
934 Formulation with Inulin as Fat Replacer: Structure, Physicochemical, and Sensory Properties.
935 Journal of Food Science 77: C189-C197. 10.1111/j.1750-3841.2011.02546.x
936 Rolim, P.M., 2015. Development of prebiotic food products and health benefits. Food Science and
937 Technology 35: 3-10. 10.1590/1678-457x.6546
938 Sanders, M.E., Merenstein, D.J., Reid, G., Gibson, G.R. and Rastall, R.A., 2019. Probiotics and
939 prebiotics in intestinal health and disease: from biology to the clinic. Nature Reviews
940 Gastroenterology & Hepatology 16: 605-616. 10.1038/s41575-019-0173-3
941 Scientific Advisory Committee on Nutrition, 2015. Carbohydrates and Health. TSO, London.
942 Scott, K.P., Grimaldi, R., Cunningham, M., Sarbini, S.R., Wijeyesekera, A., Tang, M.L.K., Lee,
943 J.C.Y., Yau, Y.F., Ansell, J., Theis, S., Yang, K., Menon, R., Arfsten, J., Manurung, S.,
944 Gourineni, V. and Gibson, G.R., 2019. Developments in understanding and applying
945 prebiotics in research and practice-an ISAPP conference paper. Journal of Applied
946 Microbiology. 10.1111/jam.14424
947 Shoaib, M., Shehzad, A., Omar, M., Rakha, A., Raza, H., Sharif, H.R., Shakeel, A., Ansari, A. and
948 Niazi, S., 2016. Inulin: Properties, health benefits and food applications. Carbohydrate
949 Polymers 147: 444-454. 10.1016/j.carbpol.2016.04.020
950 Slavin, J. and Feirtag, J., 2011. Chicory inulin does not increase stool weight or speed up intestinal
951 transit time in healthy male subjects. Food & Function 2: 72-77. 10.1039/c0fo00101e
952 So, D., Whelan, K., Rossi, M., Morrison, M., Holtmann, G., Kelly, J.T., Shanahan, E.R., Staudacher,
953 H.M. and Campbell, K.L., 2018. Dietary fiber intervention on gut microbiota composition in
954 healthy adults: a systematic review and meta-analysis. American Journal of Clinical Nutrition
955 107: 965-983. 10.1093/ajcn/nqy041

956 Struyf, N., Van der Maelen, E., Hemdane, S., Verspreet, J., Verstrepen, K.J. and Courtin, C.M., 2017.
957 Bread Dough and Baker's Yeast: An Uplifting Synergy. Comprehensive Reviews in Food
958 Science and Food Safety 16: 850-867. 10.1111/1541-4337.12282
959 Suau, A., Rochet, V., Sghir, A., Gramet, G., Brewaeys, S., Sutren, M., Rigottier-Gois, L. and Dore, J.,
960 2001. Fusobacterium prausnitzii and related species represent a dominant group within the
961 human fecal flora. Systematic and Applied Microbiology 24: 139-145. 10.1078/0723-2020-
962 00015
963 Thorning, T.K., Bertram, H.C., Bonjour, J.P., de Groot, L., Dupont, D., Feeney, E., Ipsen, R., Lecerf,
964 J.M., Mackie, A., McKinley, M.C., Michalski, M.C., Remond, D., Riserus, U., Soedamah-
965 Muthu, S.S., Tholstrup, T., Weaver, C., Astrup, A. and Givens, I., 2017. Whole dairy matrix
966 or single nutrients in assessment of health effects: current evidence and knowledge gaps.
967 American Journal of Clinical Nutrition 105: 1033-1045. 10.3945/ajcn.116.151548
968 Tuohy, K.M., Kolida, S., Lustenberger, A.M. and Gibson, G.R., 2001. The prebiotic effects of biscuits
969 containing partially hydrolysed guar gum and fructo-oligosaccharides - a human volunteer
970 study. British Journal of Nutrition 86: 341-348. 10.1079/bjn2001394
971 Valeur, J., Puaschitz, N.G., Midtvedt, T. and Berstad, A., 2016. Oatmeal porridge: impact on
972 microflora-associated characteristics in healthy subjects. British Journal of Nutrition 115: 62-
973 67. 10.1017/s0007114515004213
974 van Loo, J., 2006. Inulin-type Fructans as Prebiotics. In: G.R. Gibson and Rastall. R. A. (Eds.),
975 Prebiotics : development & application. John Wiley & Son, Chichester pp. 57-99.
976 Vandeputte, D., Kathagen, G., D'Hoe, K., Vieira-Silva, S., Valles-Colomer, M., Sabino, J., Wang, J.,
977 Tito, R.Y., De Commer, L., Darzi, Y., Ermeire, S.V., Falony, G. and Raes, J., 2017.
978 Quantitative microbiome profiling links gut community variation to microbial load. Nature
979 551: 507-+. 10.1038/nature24460
980 Walker, A.W., Duncan, S.H., Leitch, E.C.M., Child, M.W. and Flint, H.J., 2005. pH and peptide
981 supply can radically alter bacterial populations and short-chain fatty acid ratios within
982 microbial communities from the human colon. Applied and Environmental Microbiology 71:
983 3692-3700. 10.1128/aem.71.7.3692-3700.2005
984 Wallner, G., Amann, R. and Beisker, W., 1993. Optimizing fluorescent in situ hybridization with
985 rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms.
986 Cytometry 14: 136-143. 10.1002/cyto.990140205
987 Walton, G.E., Lu, C.Y., Trogh, I., Arnaut, F. and Gibson, G.R., 2012. A randomised, double-blind,
988 placebo controlled cross-over study to determine the gastrointestinal effects of consumption
989 of arabinoxylan-oligosaccharides enriched bread in healthy volunteers. Nutrition Journal 11.
990 10.1186/1475-2891-11-36
991 Wilson, B. and Whelan, K., 2017. Prebiotic inulin-type fructans and galacto-oligosaccharides:
992 definition, specificity, function, and application in gastrointestinal disorders. Journal of
993 Gastroenterology and Hepatology 32: 64-68. 10.1111/jgh.13700

994