

Impact of bread making on fructan chain integrity and effect of fructan enriched breads on breath hydrogen, satiety, energy intake, PYY and ghrelin

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1 Impact of bread making on fructan chain integrity and effect of fructan enriched
2 breads on breath hydrogen, satiety, energy intake, PYY and ghrelin

3

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13

14 Abstract:

15 Recently, there has been considerable interest in the satiety inducing properties of
16 inulin type fructans (ITF) as a tool for weight management. As a staple food, breads
17 provide an excellent vehicle for ITF supplementation however the integrity of the ITF
18 chains and properties upon bread making need to be assessed. Breads enriched
19 with 12% fructooligosaccharides (FOS) and 12% inulin were baked and the degree
20 of polymerisation of fructans extracted from the breads were compared to those of
21 pure compounds. An acute feeding study with a single blind cross-over design was
22 conducted with 11 participants to investigate the effect of ITF enriched breads on
23 breath hydrogen, self-reported satiety levels, active ghrelin, total PYY and energy
24 intake. Size exclusion chromatography indicated that little or no depolymerisation of
25 inulin occurred during bread making, however, there was evidence of modest FOS
26 depolymerisation. Additionally, ITF enriched breads resulted in increased
27 concentrations of exhaled hydrogen although statistical significance was reached
28 only for the inulin enriched bread ($p=0.001$). There were no significant differences
29 between bread types in reported satiety ($p=0.129$), plasma active ghrelin ($p=0.684$),
30 plasma PYY ($p=0.793$) and energy intake ($p=0.240$). These preliminary results
31 indicate that inulin enriched bread may be a suitable staple food to increase ITF
32 intake. Longer intervention trials are required to assess the impact of inulin enriched
33 breads on energy intake and body weight.

34 Keywords: inulin, fructooligosaccharides (FOS), bread, fructans, degree of
35 polymerisation (DP), satiety, PYY, ghrelin, breath hydrogen

36 Introduction

37 There has recently been considerable interest in the potential satiety inducing
38 properties of inulin type fructans (ITF) with a view to facilitate weight management¹.
39 Indeed, a number of studies have investigated the impact of ITF
40 (fructooligosaccharides and inulin) on satiety regulating gut hormones²⁻⁵, satiety^{2, 3, 5-}
41 ¹¹, energy intake^{2, 3, 5-8, 10, 11} and weight/BMI^{9, 12} with mixed findings. The discrepancy
42 between reported results may originate from different study designs and/ or the small
43 number of participants. A recent systematic review of published trials concluded that
44 there was limited data to suggest that long-term administration of ITF contributed to
45 weight reduction¹³. Considering that many consumers have been shown to be
46 receptive to nutrition and health claims associated with ITF enriched breads¹⁴, it is
47 not surprising that the incorporation of ITF into staple foods such as bread has been
48 used as a tool to facilitate intake¹⁵⁻²⁴. A review of the textural, rheological and
49 sensory properties of ITF enriched bread concluded that low fortification levels
50 should be feasible²⁵, however possible issues were identified around the integrity of
51 ITF chains during bread making²⁶ as heat^{27, 28} and yeast²⁹ have been shown to
52 impact on the molecular integrity of ITF chains. **In particular, high temperatures (195**
53 **°C) have been shown to alter the structure of dry inulin²⁷ whereas in solutions, the**
54 **effect of temperature has been shown to be pH dependent^{28, 30}. Similarly, the**
55 **percentage of ITF retention has been shown to be both temperature and matrix**
56 **dependant in a study investigating the kinetic rates of loss of ITF chain integrity at**
57 **different temperatures in buffer, tomato juice or orange juice³¹. Despite these well**
58 **documented effects of temperature and matrix, the effect of bread making remains**
59 **unknown.** The aim of this study was therefore to assess whether ITF chains and their
60 properties are affected during the bread making process. Fructooligosaccharides

61 and inulin enriched breads were prepared and the degrees of polymerisation of
62 water-soluble polymers extracted from the breads were measured. Moreover, the
63 effect of ITF on breath hydrogen levels, satiety, active ghrelin concentration, total
64 PYY concentration and energy intake were followed over time after a breakfast of
65 ITF enriched breads or an energy matched control bread.

66

67 Materials and Methods

68 Materials: The FOS (Orafti® P95) and inulin (Orafti® HPX) were provided by Beneo
69 (Tienen, Belgium). The flour (strong white flour, Nelstrops), yeast (Fermipan red
70 instant yeast) and table salt were bought from H N Nuttalls. The fat (Trex vegetable
71 shortening) was bought from a local supermarket.

72 Bread making: all the ingredients (Tables 1 and 2) were mixed for 8 minutes. The
73 dough was then proved for 45 minutes, knocked back and weighed to the required
74 weights (Tables 1 and 2). The samples were then placed in the proofer for an
75 additional 25 minutes before being baked at 240°C for 20 minutes.

76 Degree of polymerisation: to determine the effect of bread making on the degree of
77 polymerisation of ITF, breads were prepared with 0%, 4%, 8% and 12% FOS and
78 inulin. The 12% ITF enriched breads were used in the feeding trial. The recipes for
79 all formulations are presented in Table 1.

80

81

82

83 Table 1 ingredients for breads prepared to estimate the degree of polymerisation

	Control	4% FOS	8% FOS	12% FOS	4% inulin	8% inulin	12% inulin
Flour (g)	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Salt (g)	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Yeast (g)	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Water (g)	71.7	76.7	76.7	71.7	76.7	76.7	76.7
Fat (g)	2.0	2.0	2.0	2.0	2.0	2.0	2.0
FOS (g)	0.0	4.0	8.0	12.0	0.0	0.0	0.0
Inulin (g)	0.0	0.0	0.0	0.0	4.0	8.0	12.0

84

85 The ITF standard solutions were prepared using 70 mg of inulin or FOS suspended
86 in 15 mL of distilled water and heated at ~ 90 °C for 30 minutes to solubilise the
87 fructans. The solutions were then centrifuged (Eppendorf 5702, Eppendorf,
88 Stevenage, UK) at 3000 g for 30 minutes to remove any insoluble material. For each
89 bread a representative sample was taken from both the crust and the crumb and 1.5
90 g was suspended in 15 mL of distilled water and heated at ~ 90 °C for 30 minutes to
91 solubilise the fructans. The bread extract was then centrifuged (Eppendorf 5702,
92 Eppendorf, Stevenage, UK) at 3000 g for 30 minutes to remove any insoluble
93 material. The absolute weight-average molecular weights and degrees of
94 polymerisation (DP) were determined using size exclusion chromatography coupled
95 with multi-angle laser light scattering (SEC-MALLS). Size exclusion chromatography
96 was carried out at ambient room temperature on a PL aquagel guard column
97 (Polymer Labs, Amherst, U.S.A.) which was linked in series with PL aquagel-OH 60,
98 PL aquagel-OH 50 and PL aquagel-OH 40 (Polymer Labs, Amherst, U.S.A.) and was

99 eluted with distilled water at a flow rate of 0.7 mL/min. The eluent was detected on-
100 line by a DAWN EOS light scattering detector (Wyatt Technology, Santa Barbara,
101 U.S.A.) and a rEX differential refractometer (Wyatt Technology, Santa Barbara,
102 U.S.A.). The refractive index increment, dn/dc was taken to be 0.131 mL/g³².

103

104 Feeding study: the breakfast composition with nutrient content and associated
105 energy for the test breakfasts are presented in Table 2. As several studies have
106 reported that an ITF intake of 16 g significantly increased breath hydrogen^{8, 33} or
107 modulated the secretion of gut peptides⁵, this amount was therefore chosen as an
108 appropriate dose to be ingested as part of the enriched breakfast.

109

110 Table 2: composition and energy of test breakfasts (2 baps).

	Control	12% FOS	12% Inulin
Flour (g)	69.7	66.7	66.7
Salt (g)	1.0	0.9	0.9
Yeast (g)	2.1	2.0	2.0
Water (g)	41.8	48.0	48.0
Fat (g)	1.4	1.3	1.3
ITF (g)	0.0	8.0	8.0
Total weight per bap (g)	116.1	126.9	126.9
Energy per bap (kcal)	291	291	291

111

112 The energy was calculated assuming a contribution of 1.5 kcal/g from fructans^{34, 35}.

113 Thirteen apparently healthy adults (5 men and 8 women) who were non-smokers
114 were recruited by word of mouth to take part in this study. The study received ethical
115 approval from the faculty research ethics committee (approval number:
116 SBSREC1213/15) and all participants provided written informed consent. Exclusion
117 criteria included: pregnancy, current or history of gastrointestinal disorders, actively
118 trying to lose weight and not being over 18 years of age. Two participants withdrew
119 from the study, one because they were uncomfortable with the blood sampling (1
120 woman) and the other because they did not like the fixed lunch offered as part of the
121 study (1 woman). **Eleven participants were deemed sufficient to observe relevant**
122 **changes in our primary outcome (breath hydrogen) as identical ITF doses have been**
123 **reported to significantly increase breath hydrogen in a study with 10 participants³⁶.**
124 The characteristics of the 11 participants can be found in Table 3.

125

126 Table 3: participants' age, height and body weight.

Measurement	Mean	Range
Age (years)	30.3	20-58
Body weight (kg)	65.5	47.0-86.5
Height (m)	1.69	1.54-1.80
BMI (kg/m ²)	22.7	17.9-26.7

127

128 The design was a single-blind, cross-over study with a wash out period of a minimum
129 of 5 days. Participants attended the research facility on 3 test days during which they
130 consumed one of 3 breakfasts (control, FOS, inulin breads). The participants were
131 randomly allocated a sequential breakfast order based on a William's Latin square

132 design. The breakfasts consisted of a large glass of cold water, 30 g of jam and
133 either 2 control baps or 2 inulin or FOS enriched baps. A fixed lunch consisting of a
134 Baxter's vegetable soup and 2 small white bread rolls which participants were
135 instructed to finish was fed 3.5 hours after breakfast. After the last time point of the
136 day (450 minutes after breakfast), participants were free to eat and drink as they
137 wished but were required to record their food and drink intake in a food diary which
138 was used to estimate their energy intake using Netwisp 3.0 (Tinuviel software).

139

140 Breath hydrogen and methane excretion, self-reported satiety and finger prick blood
141 samples were taken at baseline (immediately before breakfast), 90 minutes, 210
142 minutes (immediately before lunch), 330 minutes and 450 minutes after breakfast.
143 Additionally, self-reported satiety was measured at 10 minutes (after breakfast) and
144 240 minutes (after lunch). These time intervals were selected to capture potential
145 changes in breath hydrogen and gut peptides over time throughout the
146 fasting/eating/digesting processes over the time period covering the first two meals
147 of the day. The time points 90 minutes after the meals were used because circulating
148 ghrelin reaches a nadir between 60 and 150 minutes post prandially with a median of
149 90 minutes³⁷.

150

151 Breath hydrogen and methane measurements were measured in duplicate using a
152 GastroCH₄eck Gastrolyzer (Bedfont Scientific Ltd., UK). To ensure that tidal breath
153 samples were analysed, participants were instructed to blow directly into the
154 mouthpiece connected to the instrument until the oxygen concentration reached 15
155 ppm at which point the hydrogen and methane concentrations were recorded.

156 Self-reported levels of hunger were captured using the SLIM category ratio scale³⁸
157 with the following anchors: greatest imaginable hunger, extremely hungry, very
158 hungry, moderately hungry, slightly hungry, neither hungry nor full, slightly full,
159 moderately full, very full, extremely full and greatest imaginable fullness.

160

161 Plasma active ghrelin and total PYY concentrations were determined in duplicate
162 using a Magpix analyser (Luminex corporation, Austin, USA) and a human metabolic
163 hormone magnetic bead panel (Milliplex Map Kit; HMHMAG-34K, Merck Millipore).
164 Finger prick blood samples were collected in potassium EDTA tubes (Microvette,
165 Sarstedt) and Pefabloc® SC (Sigma-Aldrich, Gillingham, U.K.) was added at a
166 concentration of 1 µg/µl of blood within 5 minutes of collection. Blood samples were
167 kept on ice and centrifuged for 10 min at 1000 g and 4°C, plasma was separated and
168 stored at -80°C until analysis.

169

170 The energy intake and area under the curves (breath hydrogen, PYY and ghrelin)
171 were analysed by repeated measures ANOVA. The satiety, PYY and ghrelin data
172 were analysed by factorial repeated measures ANOVA (factors: time and sample
173 type), where appropriate a Greenhouse-Geisser correction and a Bonferroni test
174 were applied. All statistical analysis were performed using SPSS v22 (IBM
175 Corporation, Armonk, NY).

176

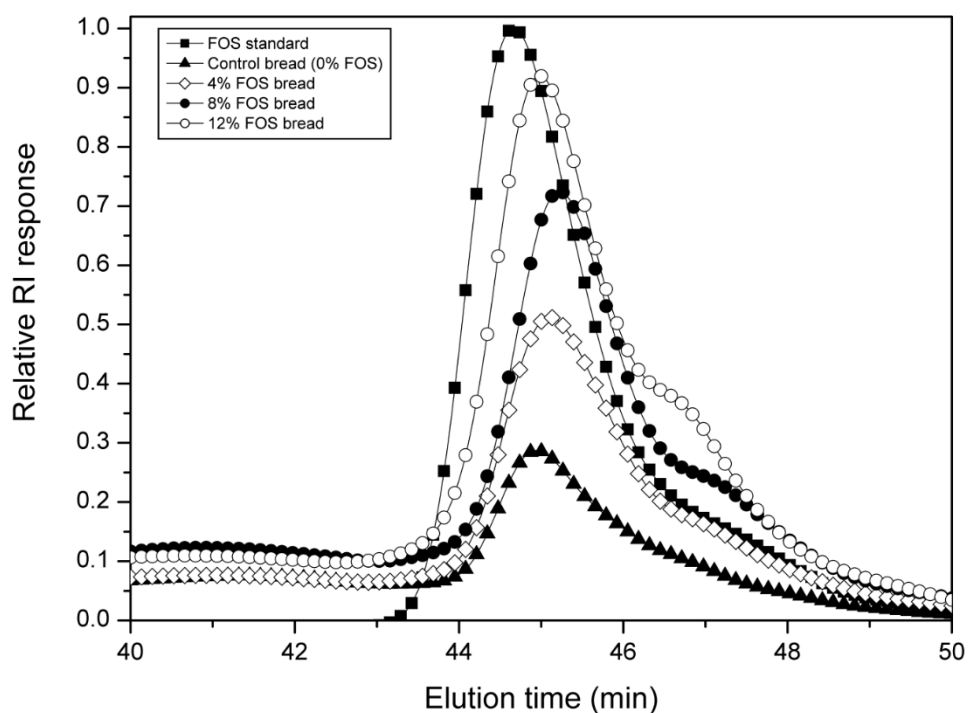
177

178

179 Results

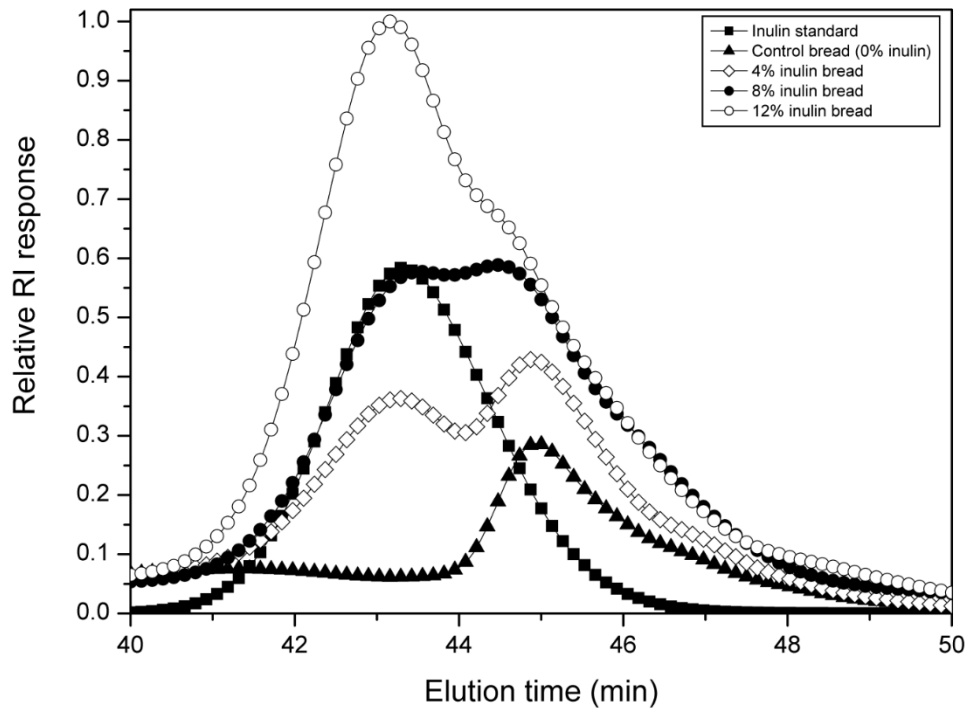
180 Degree of polymerisation:

181 The weight-average degree of polymerisation (DP) of FOS and inulin standards were
182 6 ± 2 and 19 ± 3 , respectively, which are in fair agreement with the manufacturer's
183 specifications. The results obtained from the crust and crumb of the breads were
184 identical and only the crust results are presented (Figure 1 for the FOS enriched
185 breads and Figure 2 for the inulin enriched breads).



186

187 Figure 1: Relative refractive index (RI) chromatograms of control bread, FOS
188 enriched breads (4%, 8% and 12%) and FOS standard. For clarity only 1 data point
189 in every 75 has been plotted.



190

191 Figure 2: Relative refractive index (RI) chromatograms of control bread, inulin
 192 enriched breads (4%, 8% and 12%) and inulin standard. For clarity only 1 data point
 193 in every 75 has been plotted.

194

195 From the chromatograms it is evident that some low molecular weight material was
 196 extracted from the control bread sample as indicated by the peak present in all
 197 breads between 44 and 48 minutes. In the bread samples, this peak merged with the
 198 FOS and inulin peaks observed at 44.7 minutes (FOS, Figure 1) and 43.3 minutes
 199 (inulin, Figure 2) and can be clearly seen as a shoulder in the inulin extracts. Data
 200 from GC-MS (not shown) after hydrolysis, reduction and acetylation indicated that
 201 this low molecular weight material extracted from all bread samples is rich in glucose
 202 and therefore most likely to be soluble starch.

203

204 The areas under the refractive index curves corresponding to the masses of FOS
205 and inulin extracted from the enriched breads peaks were consistent with the level of
206 ITF supplementation (Figures 1 and 2). The elution time of the FOS extracted from
207 the enriched breads (~ 44.7 minutes) was marginally greater than that of the FOS
208 standard solution at 45.1 minutes (Figure 1) indicating that a mild depolymerisation
209 had occurred during bread making. In contrast, there was no shift in elution time
210 observed for the inulin extracted from the inulin enriched breads when compared to
211 that of the inulin standard solution (Figure 2) indicating that under the same
212 processing conditions inulin chains did not undergo depolymerisation.

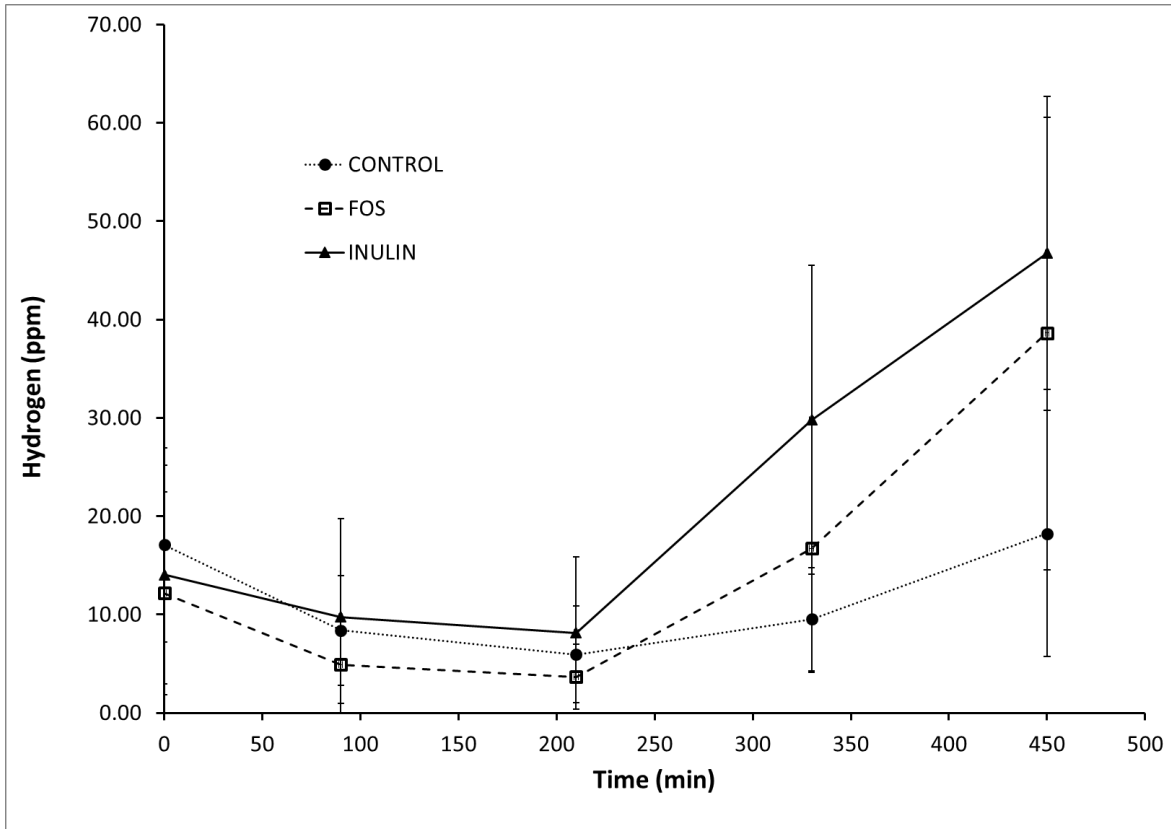
213

214 Feeding study:

215 Only one participant produced methane in greater quantities than hydrogen and in
216 excess of 20 ppm; therefore only the hydrogen results were analysed.

217 The differences in breath hydrogen excretion were significant for both factors: bread
218 type ($p=0.001$) and time ($p<0.001$), with the inulin bread resulting in a significantly
219 higher production of hydrogen than both the FOS and control breads (Figure 3). The
220 interaction bread type x time was also significant ($p=0.002$) as breath hydrogen
221 production increased for the inulin and FOS breads to a greater extent than that of
222 the control.

223



224

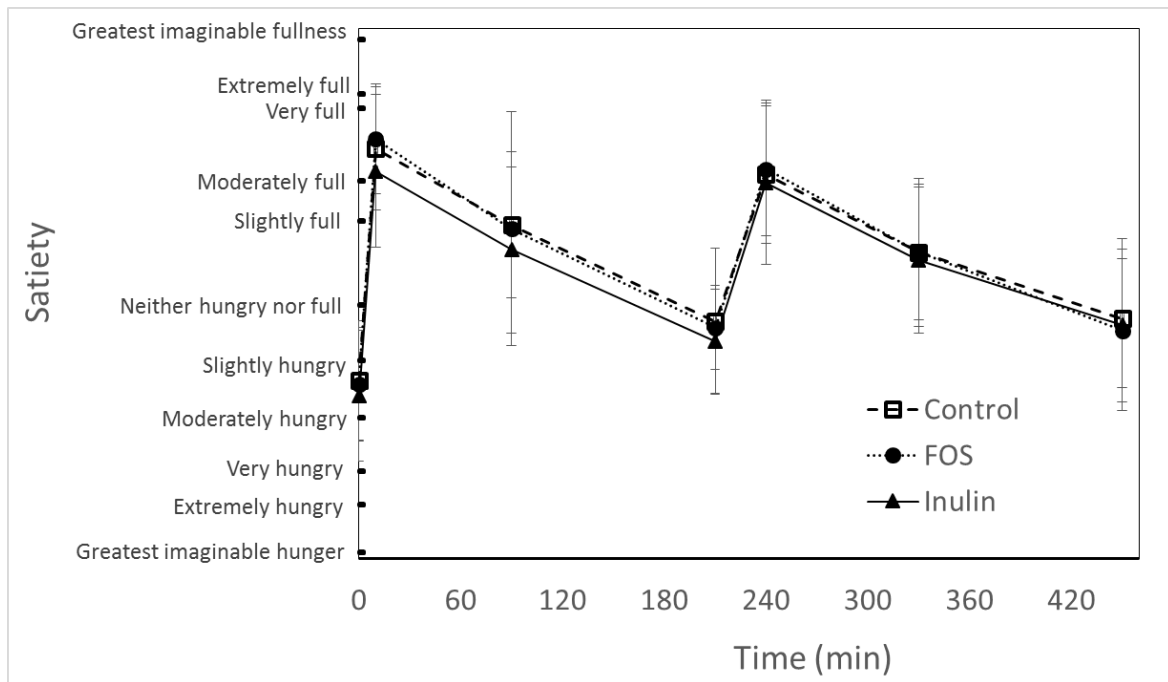
225 Figure 3: Breath hydrogen before and after breakfast (control, 12% FOS, 12% inulin
 226 breads) and fixed lunch. Data from 11 participants, error bars represent 1SD.

227

228 Differences in area under the curve were significant for bread type ($p=0.007$) with the
 229 inulin bread presenting a greater AUC (8404.5 ± 1152.9 ppm.min) than the control
 230 (4589.4 ± 648.5 ppm.min) or FOS (6082.7 ± 1042.4 ppm.min) breads.

231

232 There was no significant difference in satiety with respect to bread type ($p=0.129$)
 233 but there were significant differences observed with respect to time ($p<0.001$)
 234 reflecting the impact of meals (breakfast and fixed lunch) on hunger levels (Figure 4).
 235 The interaction bread type x time was not significant ($p=0.988$).

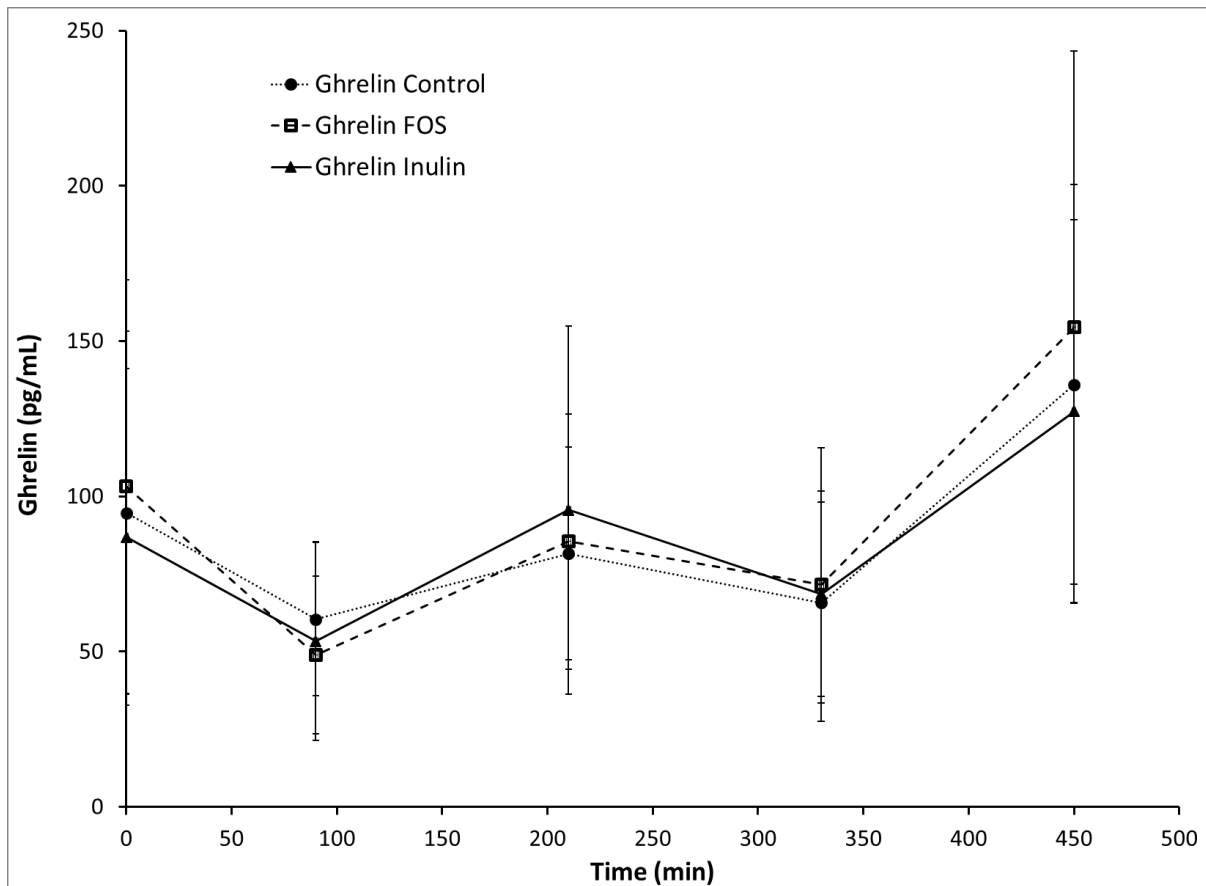


236

237 Figure 4: Self-reported satiety rating over time before and after breakfast (control,
 238 FOS or inulin breads) and lunch (fixed). Data from 11 participants, error bars
 239 represent 1SD.

240

241 The differences in ghrelin concentrations were significant for time ($p < 0.001$)
 242 reflecting the impact of the meals on ghrelin levels (Figure 5); however, there were
 243 no significant difference observed for bread type ($p = 0.684$). The interaction bread
 244 type x time was also not significant ($p = 0.592$). There were no significant difference in
 245 ghrelin AUC between bread types ($p = 0.829$).

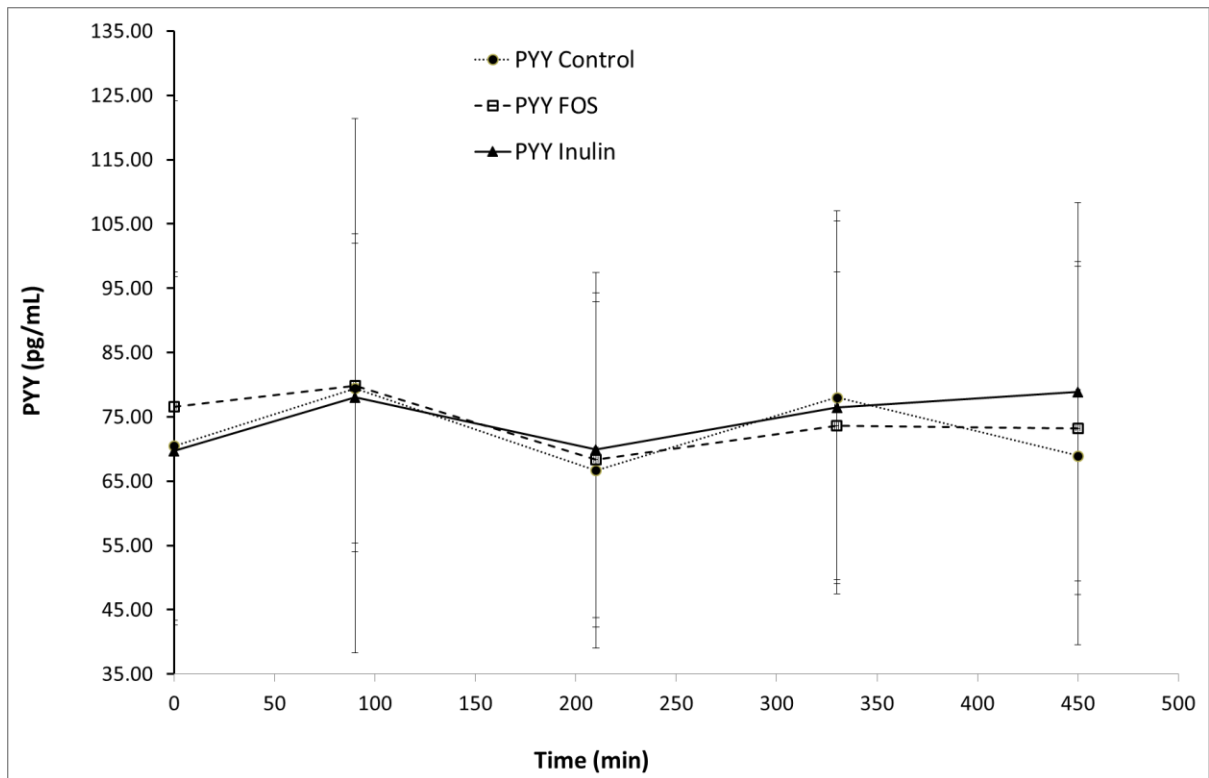


246

247 Figure 5: Active ghrelin concentration with time after breakfast (control, FOS and
 248 inulin breads) and fixed lunch. Data from 11 participants, error bars represent 1SD.

249

250 Samples from 2 participants contained concentrations of PYY below the detection
 251 limit of the assay so statistical analysis was restricted to 9 participants. Although the
 252 impact of meals can be observed (Figure 6), there were no significant differences in
 253 PYY levels for bread type ($p=0.793$) or time ($P=0.221$). There was no significant
 254 difference in PYY AUC for bread type ($p=0.811$).



255

256 Figure 6: PYY concentration before and after breakfast (control, FOS and inulin
 257 breads) and fixed lunch. Data from 9 participants, error bars represent 1SD.

258

259 There was no significant differences in reported energy intake for the rest of the test
 260 day ($p=0.944$), energy intake on the day after the test day ($p=0.240$) or overall
 261 energy intake ($p=0.544$) between the breads (Table 4).

262

263

264 Table 4: Average energy intake (standard deviation; n=11) for the remaining of the
265 test day, day following the test day and overall energy intake for the control, FOS
266 and inulin breads.

	Control	FOS	Inulin
Remaining of test day (kcal)	854.1 (330.0)	896.9 (310.1)	888.8 (421.6)
Day after test day (kcal)	1788.8 (357.7)	1458.4 (506.2)	1592.6 (350.4)
Overall (kcal)	2642.9 (487.7)	2355.3 (700.3)	2497.8 (645.5)

267

268

269 Discussion

270 Degree of polymerisation: the difficulty in estimating the DP of inulin due to the weak
271 light scattering signal³⁹ and the co-elution with soluble starch makes it impossible to
272 estimate the absolute degree of polymerisation for inulin extracted from bread⁴⁰. The
273 elution time can however be used as a qualitative indication of the degree of
274 polymerisation because in size exclusion chromatography molecules are separated
275 by their size (hydrodynamic volume). Larger molecules are excluded from the pores
276 in the column packing and therefore elute more quickly⁴¹. Making allowances for the
277 merging of the fructans and soluble starch peaks, it is apparent that inulin has not
278 been depolymerised during the bread making process, but FOS has undergone
279 some degradation. Previous work, albeit on dry inulin samples and not in bread,
280 suggested that high temperatures up to 195 °C would degrade inulin²⁷. In solutions,
281 the stabilities of both inulin and FOS have been shown to be influenced by
282 temperature, heating time and pH³⁰, however, heating time and temperature only
283 contributed to depolymerisation for pH ≤ 5³⁰. Typically, pH in white bread is

284 approximately 5 – 5.4⁴². Fructooligosaccharides of DP = 3 have been shown to be
285 more prone to degradation than those of DP = 5 in food matrices with low pH³¹;
286 moreover, FOS of low DP would appear to be more susceptible than inulin²⁸ and this
287 may explain why inulin and FOS behave differently through the bread making
288 process.

289

290 Feeding study: an increased concentration of hydrogen in the breath is commonly
291 used as an indirect marker of increased gut fermentation⁴³. A number of studies
292 have reported increased concentrations of exhaled hydrogen following ingestion of
293 FOS^{8, 10, 33, 36} with effects of similar order of magnitude as those reported here (15 to
294 30 ppm) for similar doses (10 g to 16 g). Interestingly, only 1 time point was recorded
295 in those studies at 240 min⁸ and 180 min¹⁰ after the test meals. In this study, there
296 was no evidence of increased gut fermentation 3 or 4 hours after the ingestion of ITF
297 enriched breads, this may be due to the different medium used to administer the ITF;
298 Hess et al⁸ used hot cocoa beverages and it could be hypothesized that the resulting
299 digestion process and food transit would be faster resulting in a more rapid increase
300 in breath hydrogen. Karalus et al¹⁰ used chocolate crisp bars, however, participants
301 were also given the same bars the night before the test breakfasts (used as the
302 baseline); the increase in breath hydrogen may have been partly due to the slow on-
303 going fermentation of the night bars rather than that of the breakfast bars. This would
304 be consistent with the present results which show that breath hydrogen was still
305 rising 450 minutes after ingestion of the ITF enriched breads. The fermentation of
306 ITF produces short chain fatty acids that may suppress appetite through binding to
307 the G protein coupled free fatty acid receptor (FFAR) 2 on colonic L cells and
308 stimulating the release of the anorexic gut peptides, PYY and GLP-1^{44, 45}. The ability

309 of a single dose of ITF to stimulate the release of PYY or GLP-1 probably depends
310 primarily on the magnitude of increase in luminal SCFA concentrations following
311 fermentation⁴⁵. Recently, it was reported that a 10 g dose of inulin failed to stimulate
312 the release of PYY whereas a 10 g dose of inulin-propionate ester that resulted in an
313 approximately 60% greater increase in the luminal concentration of propionate did⁴⁵.
314 In a dose escalation study, the consumption of 15 g/day of FOS failed to increase
315 postprandial secretion of PYY, whereas doses ≥ 35 g dose were effective³. In the
316 present study we found no change in circulating PYY after consumption of our test
317 breads enriched with 16 g of FOS or inulin. It is possible that the 16 g dose failed to
318 raise luminal SCFA concentrations sufficiently to stimulate the release of PYY. Also,
319 breath hydrogen seemed to be still rising at our final measurement point so our
320 measurements of PYY may not have coincided with the time of maximal
321 fermentation.

322

323 The ITF enriched breads failed to suppress the release of the orexigenic gut peptide,
324 ghrelin. In an acute cross-over study, a 24 g dose of inulin incorporated into a high
325 fructose corn syrup (HFCS) test drink suppressed plasma ghrelin in comparison to a
326 HFCS control drink⁴. The higher dose and different medium of delivery may explain
327 the contrast with our results. Energy intake and subjective ratings of appetite were
328 not significantly altered by consumption of the ITF enriched breads. This is
329 consistent with a number of other acute/short-term feeding studies that have
330 reported no effect of 10 or 16 g doses of ITF on short-term energy intake or ratings
331 of appetite^{8, 11}. In contrast to the lack of effect of acute/short-term supplementation
332 on energy intake and satiety, studies feeding ITF for ≥ 2 weeks provide some
333 evidence of an increase in satiety and a reduction in energy intake^{2, 6, 40}.

334

335

336 Conclusion

337 The current study provides evidence that bread may be a suitable vehicle to increase
338 inulin intake as inulin chains remain intact during bread making. Moreover significant
339 increases in breath hydrogen production were observed suggesting that the inulin
340 was fermented in the gut. Consumption of the FOS enriched bread also increased
341 breath hydrogen production compared to the control bread although, this did not
342 reach statistical significance. It is difficult to assess whether this is linked to the
343 modest depolymerisation of FOS that occurred during bread making. Despite some
344 evidence of fermentation, the inulin and FOS enriched breads failed to stimulate the
345 secretion of ghrelin and PYY, increase satiety or decrease energy intake. It is
346 possible that greater quantities of ITF enriched breads or longer periods of
347 consumption are needed to influence appetite and energy intake.

348

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352

353

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