# 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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# 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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Abstract:

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

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

## Introduction

There has recently been considerable interest in the potential satiety inducing properties of inulin type fructans (ITF) with a view to facilitate weight management[1]. Indeed, a number of studies have investigated the impact of ITF (fructooligosaccharides and inulin) on satiety regulating gut hormones[2-5], satiety[2, 3, 5-11], energy intake[2, 3, 5-8, 10, 11] and weight/BMI[9, 12] with mixed findings. The discrepancy between reported results may originate from different study designs and/ or the small number of participants. A recent systematic review of published trials concluded that there was limited data to suggest that long-term administration of ITF contributed to weight reduction[13]. Considering that many consumers have been shown to be receptive to nutrition and health claims associated with ITF enriched breads[14], it is not surprising that the incorporation of ITF into staple foods such as bread has been used as a tool to facilitate intake[15-24]. A review of the textural, rheological and sensory properties of ITF enriched bread concluded that low fortification levels should be feasible[25], however possible issues were identified around the integrity of ITF chains during bread making[26] as heat[27, 28] and yeast[29] have been shown to impact on the molecular integrity of ITF chains. In particular, high temperatures (195 °C) have been shown to alter the structure of dry inulin[27] whereas in solutions, the effect of temperature has been shown to be pH dependent[28, 30]. Similarly, the percentage of ITF retention has been shown to be both temperature and matrix dependant in a study investigating the kinetic rates of loss of ITF chain integrity at different temperatures in buffer, tomato juice or orange juice[31]. Despite these well documented effects of temperature and matrix, the effect of bread making remains unknown. The aim of this study was therefore to assess whether ITF chains and their properties are affected during the bread making process. Fructooligosaccharides

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

## Materials and Methods

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

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

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

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 |

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

eluted with distilled water at a flow rate of 0.7 mL/min. The eluent was detected on-line by a DAWN EOS light scattering detector (Wyatt Technology, Santa Barbara, U.S.A.) and a rEX differential refractometer (Wyatt Technology, Santa Barbara, U.S.A.). The refractive index increment, *dn/dc* was taken to be 0.131 mL/g[32].

Feeding study: the breakfast composition with nutrient content and associated energy for the test breakfasts are presented in Table 2. As several studies have reported that an ITF intake of 16 g significantly increased breath hydrogen[8, 33] or modulated the secretion of gut peptides[5], this amount was therefore chosen as an appropriate dose to be ingested as part of the enriched breakfast.

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 |

The energy was calculated assuming a contribution of 1.5 kcal/g from fructans[34, 35].

Thirteen apparently healthy adults (5 men and 8 women) who were non-smokers were recruited by word of mouth to take part in this study. The study received ethical approval from the faculty research ethics committee (approval number: SBSREC1213/15) and all participants provided written informed consent. Exclusion criteria included: pregnancy, current or history of gastrointestinal disorders, actively trying to lose weight and not being over 18 years of age. Two participants withdrew from the study, one because they were uncomfortable with the blood sampling (1 woman) and the other because they did not like the fixed lunch offered as part of the study (1 woman). Eleven participants were deemed sufficient to observe relevant changes in our primary outcome (breath hydrogen) as identical ITF doses have been reported to significantly increase breath hydrogen in a study with 10 participants[36]. The characteristics of the 11 participants can be found in Table 3.

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 |

The design was a single-blind, cross-over study with a wash out period of a minimum of 5 days. Participants attended the research facility on 3 test days during which they consumed one of 3 breakfasts (control, FOS, inulin breads). The participants were 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 minutes[37].

150

151 Breath hydrogen and methane measurements were measured in duplicate using a
152 Gastro$CH_4$eck 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.

Self-reported levels of hunger were captured using the SLIM category ratio scale[38] with the following anchors: greatest imaginable hunger, extremely hungry, very hungry, moderately hungry, slightly hungry, neither hungry nor full, slightly full, moderately full, very full, extremely full and greatest imaginable fullness.

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

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

## Results

### Degree of polymerisation:

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

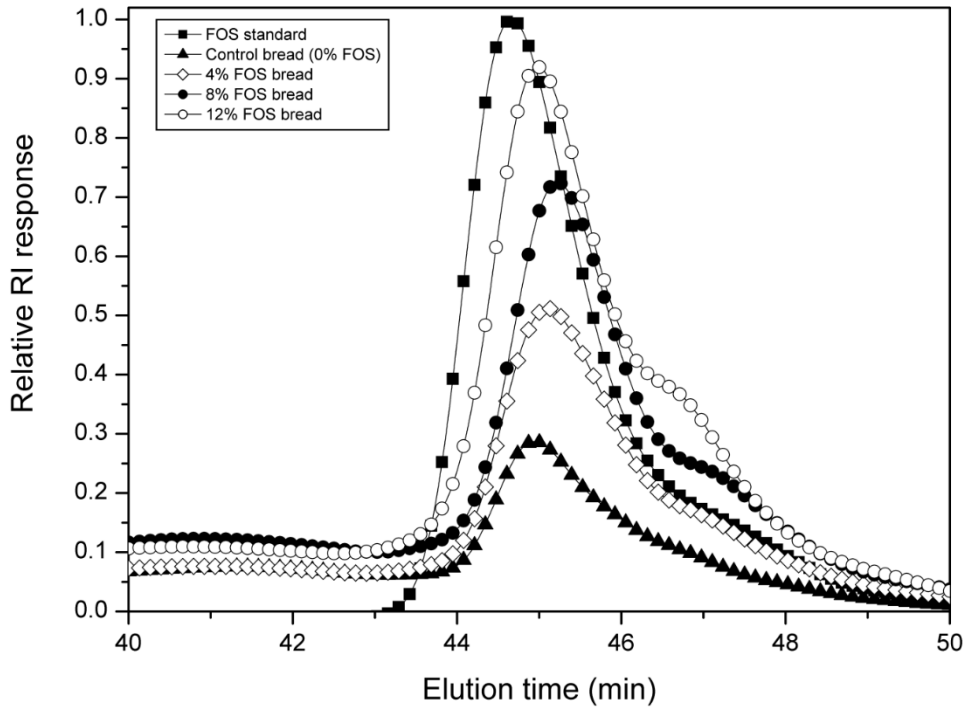
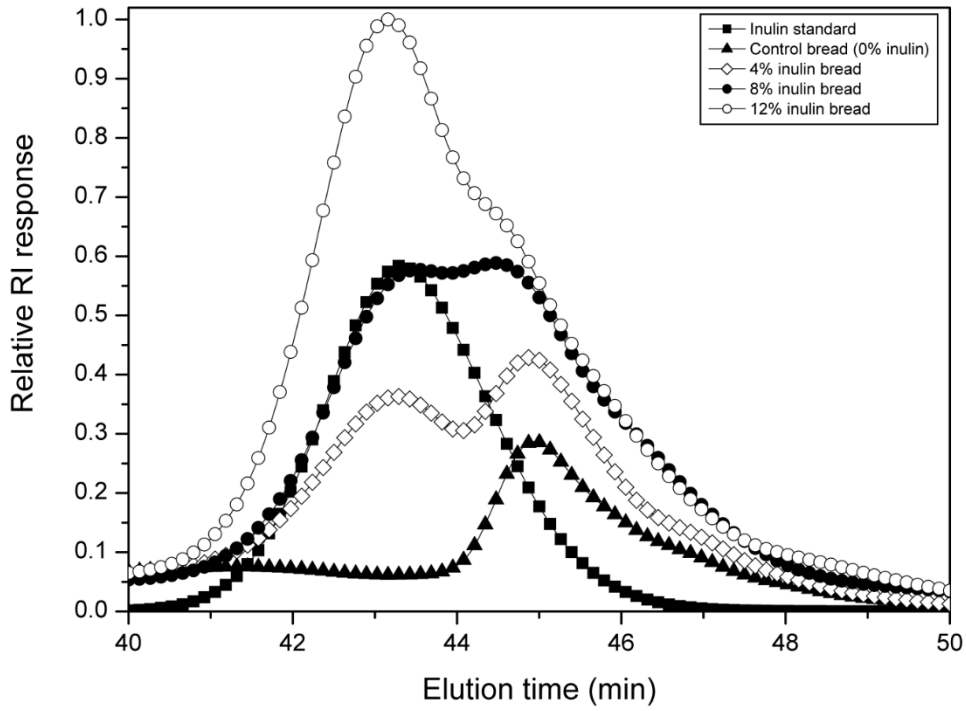



Figure 1: Relative refractive index (RI) chromatograms of control bread, FOS enriched breads (4%, 8% and 12%) and FOS standard. For clarity only 1 data point in every 75 has been plotted.




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.

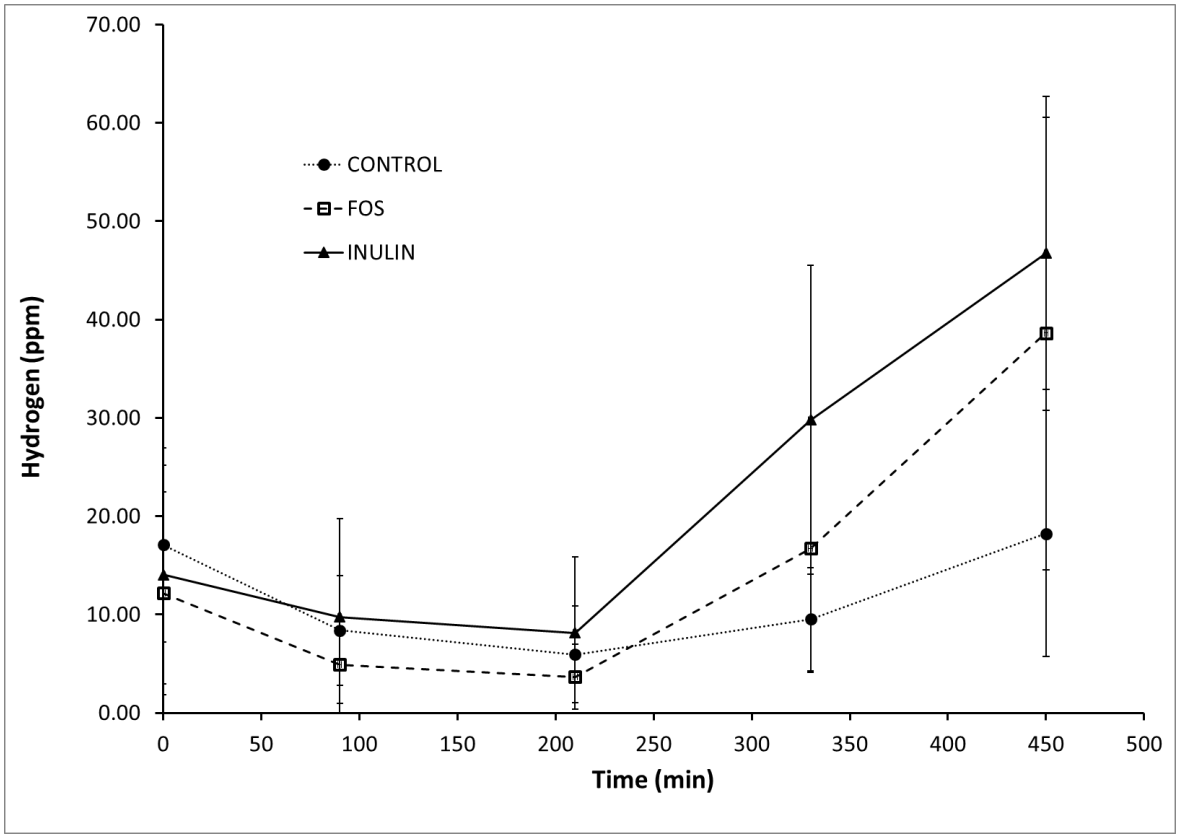
203

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

Feeding study:

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

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




224

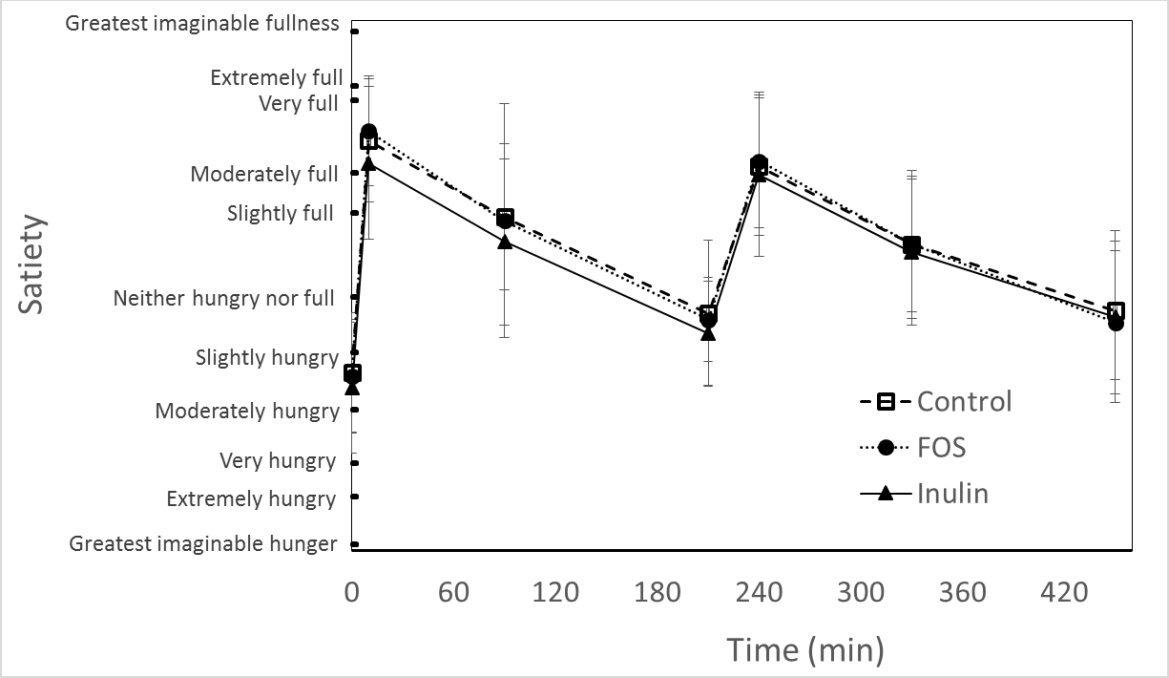
225 Figure 3: Breath hydrogen before and after breakfast (control, 12% FOS, 12% inulin
226 breads) and fixed lunch. Data from 11 participants, error bars represent 1SD.

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 ($p=0.829$).

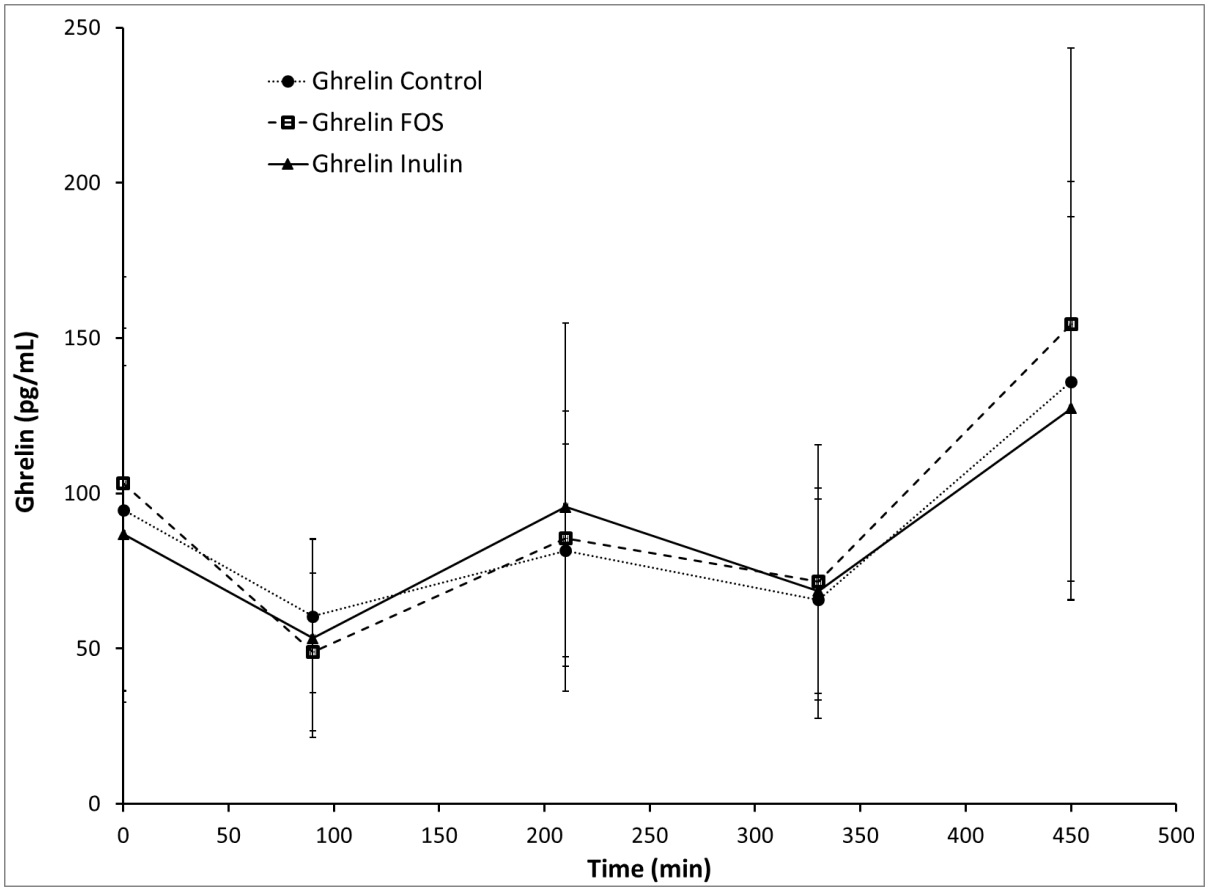



Figure 5: Active ghrelin concentration with time after breakfast (control, FOS and inulin breads) and fixed lunch. Data from 11 participants, error bars represent 1SD.

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

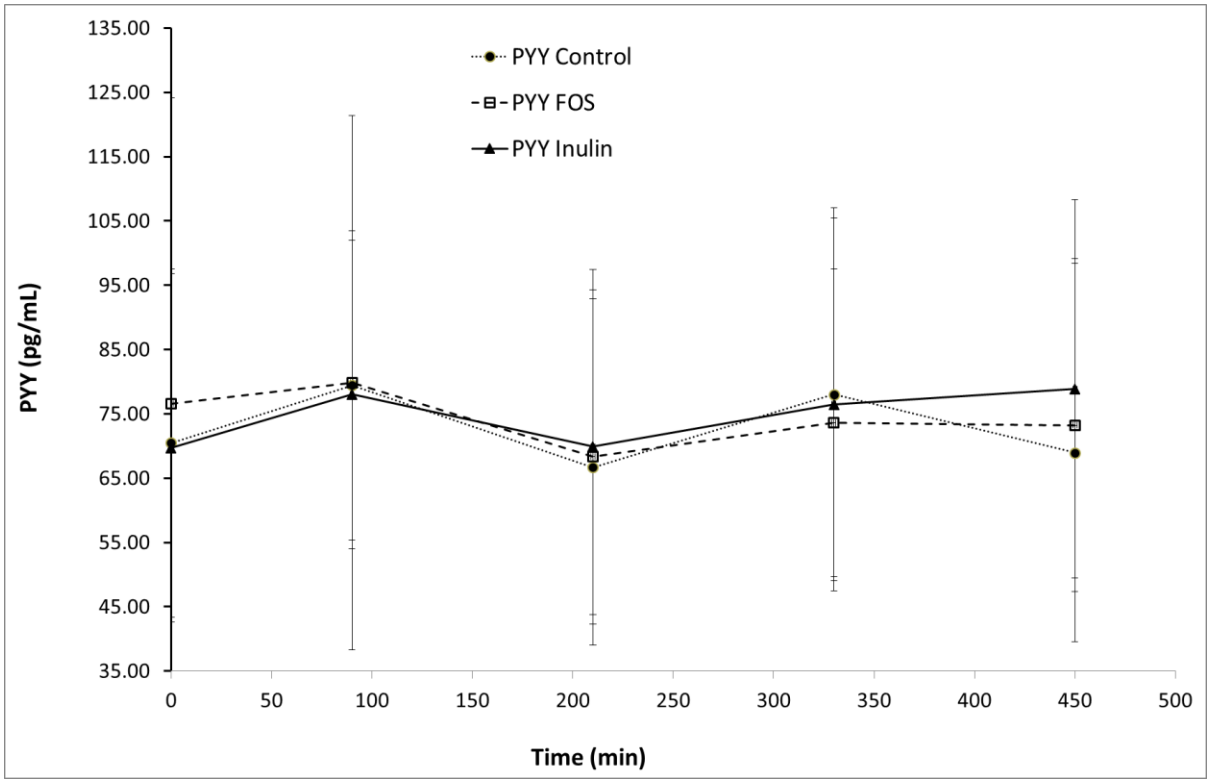



Figure 6: PYY concentration before and after breakfast (control, FOS and inulin breads) and fixed lunch. Data from 9 participants, error bars represent 1SD.

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

Table 4: Average energy intake (standard deviation; n=11) for the remaining of the test day, day following the test day and overall energy intake for the control, FOS 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) |

## Discussion

Degree of polymerisation: the difficulty in estimating the DP of inulin due to the weak light scattering signal [39] and the co-elution with soluble starch makes it impossible to estimate the absolute degree of polymerisation for inulin extracted from bread[40]. The elution time can however be used as a qualitative indication of the degree of polymerisation because in size exclusion chromatography molecules are separated by their size (hydrodynamic volume). Larger molecules are excluded from the pores in the column packing and therefore elute more quickly[41]. Making allowances for the merging of the fructans and soluble starch peaks, it is apparent that inulin has not been depolymerised during the bread making process, but FOS has undergone some degradation. Previous work, albeit on dry inulin samples and not in bread, suggested that high temperatures up to 195 °C would degrade inulin[27]. In solutions, the stabilities of both inulin and FOS have been shown to be influenced by temperature, heating time and pH[30], however, heating time and temperature only contributed to depolymerisation for pH ≤ 5 [30].Typically, pH in white bread is

284 approximately 5 – 5.4 [42]. Fructooligosaccharides of DP = 3 have been shown to be
285 more prone to degradation than those of DP = 5 in food matrices with low pH[31];
286 moreover, FOS of low DP would appear to be more susceptible than inulin[28] 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 fermentation[43]. 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 min[8] and 180 min[10] 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 al[8] 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 al[10] 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

of a single dose of ITF to stimulate the release of PYY or GLP-1 probably depends primarily on the magnitude of increase in luminal SCFA concentrations following fermentation[45]. Recently, it was reported that a 10 g dose of inulin failed to stimulate the release of PYY whereas a 10 g dose of inulin-propionate ester that resulted in an approximately 60% greater increase in the luminal concentration of propionate did[45]. In a dose escalation study, the consumption of 15 g/day of FOS failed to increase postprandial secretion of PYY, whereas doses ≥ 35 g dose were effective[3]. In the present study we found no change in circulating PYY after consumption of our test breads enriched with 16 g of FOS or inulin. It is possible that the 16 g dose failed to raise luminal SCFA concentrations sufficiently to stimulate the release of PYY. Also, breath hydrogen seemed to be still rising at our final measurement point so our measurements of PYY may not have coincided with the time of maximal fermentation.

The ITF enriched breads failed to suppress the release of the orexigenic gut peptide, ghrelin. In an acute cross-over study, a 24 g dose of inulin incorporated into a high fructose corn syrup (HFCS) test drink suppressed plasma ghrelin in comparison to a HFCS control drink[4]. The higher dose and different medium of delivery may explain the contrast with our results. Energy intake and subjective ratings of appetite were not significantly altered by consumption of the ITF enriched breads. This is consistent with a number of other acute/short-term feeding studies that have reported no effect of 10 or 16 g doses of ITF on short-term energy intake or ratings of appetite[8, 11]. In contrast to the lack of effect of acute/short-term supplementation on energy intake and satiety, studies feeding ITF for ≥2 weeks provide some evidence of an increase in satiety and a reduction in energy intake [2, 6, 40].

## Conclusion

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

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