Impact of the source of fermentable carbohydrate on SCFA production by human gut microbiota *in vitro* - a systematic scoping review and secondary analysis

Hannah C. Harris[1,2], Douglas J. Morrison[2,], Christine A. Edwards[1],

[1]Human Nutrition, School of Medicine, Dentistry and Nursing, University of Glasgow, Glasgow UK; [2]Scottish Universities Environmental Research Centre, University of Glasgow, East Kilbride UK.

Keywords:

Fermentation, dietary fibre, colonic bacteria, short chain fatty acids, systematic review

Short Title:

Systematic review carbohydrate source and SCFA in vitro

Corresponding Author:

Professor Christine Edwards

Human Nutrition

School of Medicine, Dentistry and Nursing

College of Medical, Veterinary and Life Sciences,

University of Glasgow, New Lister Building,

Alexandra Parade,

Glasgow G31 2ER

Email: Christine.edwards@glasgow.ac.uk

Footnote: HC Harris current position: Quadram Institute Biosciences, Norwich, UK

ABSTRACT

Short chain fatty acids (SCFA) are produced by bacterial fermentation of non-digestible carbohydrates (NDC) and have many potential tissue and SCFA specific actions, from providing fuel for colonic cells to appetite regulation. Many studies have described the fermentation of different carbohydrates, often using *in vitro* batch culture. As evidence-based critical evaluation of substrates selectively promoting production of individual SCFA is lacking, we performed a systematic scoping literature review. Databases were searched to identify relevant papers published between 1900 and 12/06/2016. Search terms included *In vitro batch fermentation* and *In vitro short chain fatty acid production.* Articles were considered for essential criteria allowing equivalent comparison of SCFA between NDC. Seventy seven articles were included in the final analysis examining 29 different carbohydrates. After 24-hour fermentation, galacto-oligosaccharide ranked highest for butyrate and total SCFA production and second for acetate production. Rhamnose ranked highest for propionate production. The lowest SCFA production was observed for kiwi fibre, polydextrose, and cellulose. This review demonstrates that choosing a substrate to selectively enhance a specific SCFA is difficult, and the molar proportion of each SCFA produced by individual substrates may be misleading. Instead the rate and ratio of SCFA production should be evaluated in parallel.

198 words

Key Words: Short chain fatty acids, NDC, batch fermentation, colon model

INTRODUCTION

Short chain fatty acids (SCFA), mainly acetate, propionate, and butyrate, are produced through bacterial fermentation of non-digestible carbohydrates (NDC), proteins, and other substrates that enter the colon. The composition of the diet, how it is prepared, and gastrointestinal transit time can all influence the type and quantity of fermentable substrate reaching the colon. Mucin and material sloughed off from the intestinal walls may also contribute to substrates for colonic fermentation (Cummings and Macfarlane, 1991). The physicochemical properties of each NDC, the colonic environment and host microbial activity together determine the rate and extent of NDC fermentation and SCFA production in the colon (Macfarlane and Macfarlane, 2003; Walker *et al.*, 2005; Edwards, 2017).

Observations of direct metabolic effects, in addition to receptor mediated effects on diverse cell-types and tissues, driven by SCFA have led to increased interest in recent years. SCFA are the natural ligands for free fatty acid receptors 2 and 3 (FFAR2 and 3), located on different cell types throughout the body, including colonic enteroendocrine L-cells (Le Poul *et al.*, 2003) and adipocytes (Brown *et al.*, 2003). Within the colon, SCFA activate an L-cell receptor mediated release of the anorexigenic gut hormones peptide YY (PYY) and glucagon like peptide 1 (GLP-1) (Lin *et al.*, 2012).

SCFA also act as metabolic substrates, however due to the inaccessibility of the colon, and rapid absorption of the SCFA, it is difficult to perform mechanistic human studies (McNeil, Cummings and James, 1978; Boets *et al.*, 2015). Acetate can be sequestrated into hepatic lipogenesis and cholesterol production (den Besten *et al.*, 2013), which may be inhibited by propionate (Wolever, Spadafora and Eshuis, 1991; Wolever *et al.*, 1995). Propionate potentially has roles in gluconeogenesis, although direct evidence in humans is largely lacking (De Vadder and Mithieux, 2014; Boets *et al.*, 2017). Propionate delivered directly to

the colon via an inulin propionate ester has been shown to supress appetite, prevent weight gain (Chambers *et al.*, 2015), and improve insulin sensitivity in overweight individuals (Chambers *et al.*, 2019), which is supported by improved β-cell function *in vitro* (Pingitore et al., 2016). Butyrate is largely utilised at the colonic epithelium as an energy source (Roediger, 1980). Butyrate has also been shown *in vitro* and in mice to have potentially beneficial roles in tumour development (Hinnebusch *et al.*, 2002; Bindels *et al.*, 2012). The mechanisms are likely multifaceted involving histone deacetylase inhibition (Chriett *et al.*, 2019), FFAR (Chang *et al.*, 2014), and inflammation through T-cell regulation (Furusawa *et al.*, 2013).

Assessing SCFA production in humans is problematical due to the inaccessibility of the proximal colon where the highest rates of fermentation occur. Faecal measurements of SCFA production have proven to be an unreliable proxy for production because 95% of SCFA are absorbed before excretion in the faeces (McNeil, Cummings and James, 1978). Therefore, *in vitro* batch or continuous culture fermentation models are often used to estimate SCFA production from NDC fermentation. The batch culture model is simple and low cost, with some limitations such as the build-up of metabolites. Continuous models do not have this limitation but are more challenging to establish, maintain, and are inherently low throughput. Due to their ease, cost effectiveness, and high throughput, batch culture fermentations are commonly used to screen NDC for their capacity to produce SCFA (Payne *et al.*, 2012; Williams *et al.*, 2014). Due to the lack of consensus within the research community for the batch fermentation system, differences in methodology makes comparing studies challenging. These differences include; inoculum size, carbohydrate dose, sampling times, and vessel size, which need to be ‘normalised’, making direct comparisons between studies difficult.

A clearer description of the evidence base from *in vitro* studies is required to allow selective manipulation of the production of individual SCFA in the colon, by choosing specific NDC. This led us to undertake a systematic scoping review of *in vitro* batch fermentations to determine whether the evidence supports selective SCFA production by fermentation of specific NDCs.

METHODS

Data collection

The search engines included PubMed, Web of Science, SCOPUS, and Medline Ovid. Searches were performed from the earliest possible year (1900, 1864, 1960 and 1947 respectively), to 12th June 2016. Search terms were discussed and agreed by the authors and included *in vitro colonic fermentation, in vitro batch fermentation, in vitro human fermentation, in vitro carbohydrate fermentation, human carbohydrate fermentation, faecal fibre fermentation, in vitro fibre fermentation, in vitro short chain fatty acid production, in vitro faecal fibre fermentation, short chain fatty acid fermentation* and*, in vitro volatile fatty acid production*, all variations of spelling and word truncations were incorporated into the search. Boolean operators were used where appropriate. Journal articles were limited to those using human adults and in the English language. References listed in articles were screened to identify additional papers. The quality of the studies was judged against pre-set inclusion and exclusion criteria (Table 1).

Criteria for analysis

Studies were excluded if methodological information regarding the substrate mass added to the fermentation vessel was absent, or if an experimental pre-digestion step was used without

declaring the remaining mass of substrate added for fermentation. Where the method was not clearly outlined (i.e. culture volumes and substrate quantities) the corresponding author was contacted. Lack of response led to the article being rejected from the analysis.

Data Analysis

Only studies with non-pooled faecal fermentations were included due to potential differences between pooled and non-pooled sample data (Aguirre, Ramiro-Garcia, Koenen, & Venema, 2014). Substrates were grouped based on substrate type. Many investigators presented their data using SCFA concentration in the culture supernatant, which is dependent on the mass of substrate and volume used, therefore data were harmonised for comparison. For this, SCFA data provided were converted into a rate of production term defined as mmoles SCFA produced per gram of substrate per day (mmol/g carbohydrate/day or mmol/g carbohydrate/hour) which accounted for mass of substrate added and fermenter volume. SCFA data were also assessed as a molar fraction of total SCFA production (%). Total SCFA production was calculated as the sum of acetate, propionate, and butyrate production. Once data were converted into a rate term, a Shapiro-Wilk test was conducted to assess data normality using IBM SPSS version 22. Due to non-normal distribution for some of the substrates at each time point, all data was treated as non-normally distributed. For each substrate the median and interquartile range (IQR) was calculated.

For a substrate to undergo statistical analysis three separate median values from a minimum of two individual studies were required. To account for the sigmoid pattern of SCFA production during fermentation, SCFA measurements at time points other than 24 hours were considered; 1-5 hours (early fermentation), 6-9 hours (mid fermentation) and, 10-23 hours (mid-late fermentation).

A selectivity index was devised to identify whether a substrate selectively led to the production of an individual SCFA. To calculate this index, substrates were ranked for the top and bottom five for both rate and ratio for each of the SCFA. Substrates in the top five for both rate and ratio of production were deemed highly selective. If a substrate ranked in the top five for acetate production, but did not rank in the top five for molar proportion it would not be considered highly selective. Substrates in the bottom five for both rate and ratio were deemed to have poor selectivity. Substrates ranking in the bottom five for production, but not for proportion would not be considered as having poor selectivity for a substrate. Only a small number of substrates with fermentation results at 0-5 hours were identified, therefore the selectivity index was not calculated.

RESULTS

Data Selection

Search engine and reference list screening generated 18,599 articles for further analysis. Six authors were contacted regarding seven articles. Of these, one author responded regarding two articles. In total, 135 articles fulfilled the inclusion criteria (Table 1), 77 of which did not pool the stool samples and were included for further analysis (Figure 1).

Rationale for combining substrate classes

Substrates were grouped based on source as well as similarities in chemical and physicochemical properties. For example, sugars and disaccharides were grouped by their common name, e.g. glucoses in one group, and lactoses in another. For more complex polysaccharides and fibres, the grouping increased with complexity, initially being broadly grouped e.g. starch, pectin, cellulose. When sufficient replicate data were available, groups were further categorised e.g. raw (no experimental pre-digestion step or purchased in the raw form) and for resistant starch (underwent an experimental pre-digestion step, or purchased as

resistant starch), fructooligosaccharide (FOS) and inulin which were assessed independently. If multiple fibres were assessed in the same fermentation bottle, these were grouped as a separate mixed fibre group. Substrates which did not fit into any existing group were formed into a separate group. These groups were then required to fulfil the criteria for analysis to be considered in the final data analysis.

SCFA produced from fermentation

1-5 hours (early fermentation)

There were sufficient data for six substrates fulfilling the inclusion criteria when SCFA were measured between 1 and 5 hours of fermentation. During this stage of fermentation, guar gum generated the highest rate and proportion of propionate and butyrate (see supplementary data).

SCFA production between 6 and 9 hours (mid fermentation)

There were sufficient data for 13 substrates where SCFA had been measured between 6 and 9 hours of fermentation (Tables 2 and 3).

Lactulose was highly selective for acetate resulting in the highest rate and proportion of acetate production (0.95 ± 0.90 mmol/g carbohydrate/hour, 81.97 ± 6.59%) (Table 2, and 3). Cellulose fermentation produced low concentrations of all SCFA tested ranking either 12^{th} or 13^{th} (of 13) for each SCFA and total SCFA. Of note however, cellulose fermentation resulted in highest proportion of propionate (Table 2, Table 3).

Proportionally, starch led to highest percentage of butyrate produced with raw starch (purchased as raw or not pre-digested) ranking highest (25.00 ± 15.3 %), and resistant starch (purchased as resistant or pre-digested) ranking second (19.18 ± 5.02 %), this was also associated with a high selectivity index for butyrate. Resistant starch however ranked the

lowest for acetate (56.38 ± 12.6 %) percentage and had a low selectivity index for acetate (Table 2,3).

SCFA production between 10 - 23 hours (mid - late fermentation)

Data were available for 15 substrates with SCFA measured between 10-23 hours of fermentation (Tables 4 and 5).

No substrates consistently resulted in the highest production of the SCFA. Guar gum ranked second for production for all the SCFA (Table 4). Maize and pea fibre consistently ranked the lowest for production of all SCFA with the rate of total SCFA production for maize fibre being 0.15 ± 0.03 mmol/g carbohydrate/hour, and 0.046 ± 0.16 mmol/g carbohydrate/hour for pea fibre (Table 4).

Pectic oligosaccharide led to the highest proportion of acetate (77.06 ± 4.54 %) and the lowest proportion of propionate (8.07 ± 6.08 %) (Table 4, Table 5). Seaweed derivatives ranked second for propionate proportion (33.33 ± 12.53 %), which was 48.07 % lower than rice bran which ranked the highest (81.4 ± 22.25 %). In contrast, rice bran ranked 15th (of 15) for acetate (14.65 ± 16.41 %) and butyrate (4.17 ± 18.98 %) proportion. Production and proportion however were linked for the propionate generated by guar gum. Guar gum ranked second for production (0.22 ± 0.02 mmol/g carbohydrate/hour) and third for proportion (31.42 ± 16.13 %) and was therefore considered a highly selective propiogenic substrate (Table 8).

24 hour SCFA production

A total of 29 substrates were evaluated for comparison after 24 hours of *in vitro* fermentation.

Galactooligosaccharide (GOS) ranked first for butyrate (2.36 ± 2.50 mmol/g carbohydrate/day) and total SCFA production (13.69 ± 16.18 mmol/g carbohydrate/day) (Table 6). Rhamnose was considered highly selective for propionate ranking the highest for production (4.75 ± 1.14 mmol/g carbohydrate /day) and second for percentage (34.28 ± 18.06 %). Galactose was had high selectivity for acetate ranking the highest for acetate production (11.35 ± 7.25 mmol/g carbohydrate/day) and percentage (90.55 ± 16.14 %)(Table 6, 7 and 8).

When comparing the rank order of rate and ratio of SCFA production at 24 hours, there was little similarity in the rank order of substrates (Table 6, 7, 8). This highlights the challenges in assessing SCFA production and/or yield from bacterial fermentation.

DISCUSSION

In this work we sought to gain insight into possible routes to selectively increase the production of individual SCFA in the colon through selection of particular NDC. Pulling together the literature on *in vitro* fermentation of NDCs highlighted the challenge of deciphering studies with differing methods and outcome units making it difficult to predict which NDCs promote individual SCFA. The frequent use of batch cultures to screen fibres for their fermentation characteristics highlights the need for standardisation of methods and or reporting of results to allow a valid overview of the evidence. In this study, standardisation of the published data across these disparate studies allowed us to compare directly the SCFA produced by the fermentation of a wide range of NDCs. The results suggest that readily fermentable NDC have rather similar SCFA production profiles, but the fermentability of NDCs varies widely which does affect the SCFA yield.

There have been previous attempts to standardise *in vitro* batch fermentation experiments (Barry and Hoebler, 1995; Edwards *et al.*, 1996; COST Action FA1005, 2015), yet large variations still exist between methodologies used by research teams. One of the main differences between studies is the decision to pool stool samples to provide a single inoculum or not.

A previous study compared the effects of pooling stool samples vs non-pooled samples using the continuous culture TIM-2 model system. Within this study no differences in SCFA production profiles between individual, or pooled slurries were observed. In contrast, differences in the bacterial profiles were found. It was found that the pooling the slurry led to the production of a dominant bacterial phenotype different to each of the individual samples, thus producing a bacterial profile not representative of any donor. This was demonstrated for *Roseburia* (a starch utiliser) when compared to the pooled homogenate, two individuals had a fold change of 63.5 and 18.9 in population (Aguirre *et al.*, 2014). In addition to altering the bacterial populations, pooling samples does not take into account the range of colonic bacterial composition and / or activity of the individual donors, as a result of external factors, and the impact on SCFA production (Qin *et al.*, 2010). Due to the bias of pooling the stool samples on the bacteria within the sample slurry, only non-pooled sample data were included for analysis within this article.

Methodological considerations

In this review, we examined if harmonising the published data creating a single comparable unit describing the rate of SCFA production (mmol/g carbohydrate/day or mmol/g carbohydrate/hour) enabled direct comparison of studies. Thirty-four studies lacked the basic information on the fermentation methodology for the determination of absolute SCFA production. SCFA concentration in the medium is “fermenter dependent”, and can vary based

on mass of substrate, and volume of medium. Thus, we call on the research community to publish a basic set of information for all NDC fermentation studies that would allow a rate term comparison to be conducted, and studies to be compared transparently. The basic set of information required for generating the rate term is as follows:

1. The mass of substrate added to the fermentation vessel
2. The final volume of the fermentation vessel
3. The amount of SCFA produced

Observations from scoping review

Some substrates were identified as being selective for the production of a specific SCFA, particularly where associations have been made in feeding trials. This was demonstrated by rhamnose which led to the production of the highest rate of propionate production and ranked second for proportion after 24 hours of fermentation, indicating that it is highly specific for propionate, although not exclusively propiogenic. Human feeding studies have shown that consumption of a 25 g dose of rhamnose per day for four weeks increased serum propionate concentration and was also associated with a decrease in serum triglycerides (Vogt *et al.*, 2004, 2006). In contrast, a more recent study assessing 25 g of rhamnose supplementation (after a six day run in period), did not lead to any observed changes in feelings of satiety, or serum concentrations of SCFA, but did identify a reduction in plasma insulin (Darzi *et al.*, 2016).

Substrates ranking highest for SCFA production – a measure of overall fermentability - and proportion of each SCFA produced were not always the same, thus caution must be used when interpreting SCFA data. This was demonstrated by GOS which was highly selective for

acetate, and yielded high butyrate and total SCFA production indicating that it is highly fermentable, however. GOS only ranked in the top 5 for acetate proportion. This suggests if only proportion is considered, the potential role of GOS in yielding high concentrations of butyrate would unlikely be acknowledged, as it did not rank within the top 5 for butyrate production(Table 6 -8).

Polydextrose and cellulose consistently generated low rates of SCFA production. This has been supported by previous human feeding trials. Consumption of 21 g/day of polydextrose by healthy adult men for 21 days led to a reduction in SCFA production versus no additional fibre control (Boler *et al.*, 2011). This differs from *in vitro* assessment using continuous culture techniques which have identified polydextrose as a fermentable substrate, which increases SCFA production (particularly acetate) and has prebiotic potential (Probert *et al.*, 2004). These confounding results may be due to differences in methodology with the continuous culture being performed for a longer duration, compared to the batch culture, allowing potentially for microbial adaptation to the substrate. Spiller et al., (1980), also identified that cellulose supplementation for three weeks (14 g/day) did not alter faecal SCFA concentrations (Spiller *et al.*, 1980).

There were a number of instances in which substrates produced low rates of SCFA production (Tables 2, 4, 6) but ranked high for SCFA proportion (Tables 3, 5, 7). Cellulose, ranked 12th (out of 13) for rate of propionate production between 6-9 hours, yet first for proportion of propionate. This highlights that both the absolute and relative production of SCFA are required to fully assess if a substrate is selective for an individual SCFA. This further demonstrates the need for authors to provide enough information in their published results to assess absolute production as well as molar SCFA proportions.

Ranking the rate of production showed that the top five producers, particularly for propionate and butyrate did not differ significantly, particularly at the 'mid' time points of the fermentation (6-9 hours). This indicates that total SCFA is more important than the molar proportion of SCFA produced in determining the final amount of an individual SCFA produced. However, as this review demonstrates, there is a relatively small effect of the NDC type on selective SCFA production. This may go some way to explain why many dietary intervention studies using different NDC to target specific SCFA production are inconclusive.

These results suggest that rather than using a specific carbohydrate source to increase SCFA production, it is beneficial to increase overall intake of fermentable carbohydrates. Animal studies, which often detect high levels of SCFA production have shown this where the test NDC comprises 5 – 20 % of the animal's diet, equating to doses of approximately 20.9 – 83.7 g/d for men and 16.3 – 65.3 g/d for women (Morrison and Preston, 2016). Considering current UK fibre (NSP) intake is approximately 13.8 g/d for individuals aged 19–64 substantial increases in fibre intake (closer to current recommendations of 30g/d) may be required for the consistent beneficial effects observed within animal trials (Drew et al, 2018).

In conclusion, fermentable substrates appear rather uniform in their capacity to change SCFA production, particularly during early fermentation. This means that dietary NDC sources are unlikely to be helpful in studies of receptor physiology *in vivo,* where selective targeting with individual SCFA is required to unravel the physiological significance of each SCFA in human studies. Increasing SCFA by using targeted synthetic delivery approaches or direct instillation may be more suitable to human mechanistic studies but this may restrict our knowledge of effects at physiological doses.

Acknowledgments: This research was funded by a BBSRC DRINC Doctoral Training Grant BB/H532091/1.

Author Contributions: H.C.H., C.A.E. and D.J.M. designed the review, and wrote the manuscript, H.C.H. performed the review and analysis. All authors contributed to writing the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References:

Adiotomre, J. *et al.* (1990) 'Dietary fiber: in vitro methods that anticipate nutrition and metabolic activity in humans.', *The American journal of clinical nutrition*, 52(1), pp. 128–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2163190.

Aguirre, M. *et al.* (2014) 'To pool or not to pool? Impact of the use of individual and pooled fecal samples for in vitro fermentation studies', *Journal of Microbiological Methods*. 2014/09/10. Top Institute of Food and Nutrition (TIFN), PO Box 557, AA Wageningen, Netherlands: Elsevier, 107, pp. 1–7. doi: 10.1016/j.mimet.2014.08.022.

Barry, J. and Hoebler, C. (1995) 'Estimation of the fermentability of dietary fibre in vitro: a European interlaboratory study', *British Journal of …*, pp. 303–322. Available at: http://journals.cambridge.org/production/action/cjoGetFulltext?fulltextid=875720 (Accessed: 18 October 2013).

den Besten, G. *et al.* (2013) 'Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids.', *American journal of physiology. Gastrointestinal and liver physiology*, 305(12), pp. G900-10. doi: 10.1152/ajpgi.00265.2013.

Bindels, L. B. *et al.* (2012) 'Gut microbiota-derived propionate reduces cancer cell proliferation in the liver.', *British journal of cancer*. Nature Publishing Group, 107(8), pp. 1337–44. doi: 10.1038/bjc.2012.409.

Boets, E. *et al.* (2015) 'Quantification of in vivo colonic short chain fatty acid production from inulin', *Nutrients*. Translational Research in Gastrointestinal Disorders, KU Leuven, Leuven, Belgium: MDPI AG, 7(11), pp. 8916–8929. doi: 10.3390/nu7115440.

Boets, E. *et al.* (2017) 'Systemic availability and metabolism of colonic-derived short-chain fatty acids in healthy subjects: a stable isotope study', *Journal of Physiology*. Blackwell Publishing Ltd, 595(2), pp. 541–555. doi: 10.1113/JP272613.

Boler, B. M. V. *et al.* (2011) 'Digestive physiological outcomes related to polydextrose and soluble maize fibre consumption by healthy adult men', *British Journal of Nutrition*, 106(May 2011), pp. 1864–1871. doi: 10.1017/S0007114511002388.

Bourquin, L. D. *et al.* (1992) 'Short-Chain Fatty Acid Production and Fiber Degradation by Human Colonie Bacteria : Effects of Substrate and Cell Wall Fractionation procedures', *nutrient metabolism*, (October 1991), pp. 1508–1520.

Bourquin, L. D., Titgemeyer, E. C. and Fahey, G. C. (1993) 'Vegetable Fiber Fermentation by Human Fecal Short-Chain Fatty Acid Production during In Vitro Bacteria: Cell Wall Polysaccharide Disappearance and Fermentation and Water-Holding Capacity of Clnfermented Residues1', *The Journal of nutrition*, 123, pp. 860–869.

Bourquin, L. D., Titgemeyer, E. C. and Fahey, G. C. (1996) 'FERMENTATION OF VARIOUS DIETARY FIBER SOURCES by human fecal bacteria', *Nutrition Research*, 16(7), pp. 1119–1131. doi: 10.1016/0271-5317(96)00116-9.

Brown, A. J. *et al.* (2003) 'The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids', *Journal of Biological Chemistry*, 278(13), pp. 11312–11319. doi: 10.1074/jbc.M211609200.

Cardelle-Cobas, A. *et al.* (2012) 'In vitro fermentation of lactulose-derived oligosaccharides by mixed fecal microbiota.', *Journal of agricultural and food chemistry*, 60(8), pp. 2024–32. doi: 10.1021/jf203622d.

Carlson, J. *et al.* (2016) 'In vitro analysis of partially hydrolyzed guar gum fermentation differences between six individuals', *Food & Function*. Department of Food Science and Nutrition, University of Minnesota-Twin Cities, 1334 Eckles Ave, St. Paul, MN, United States: Royal Society of Chemistry, 7(4), pp. 1833–1838. doi: 10.1039/c5fo01232e.

Chambers, E. S. *et al.* (2015) 'Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults', *Gut*. Nutrition and Dietetic Research Group, Section of Investigative Medicine, Imperial College London, Hammersmith Hospital, 6th Floor Commonwealth Building, London, United Kingdom: BMJ Publishing Group, 64(11), pp. 1744–1754. doi: 10.1136/gutjnl-2014-307913.

Chambers, E. S. *et al.* (2019) 'Dietary supplementation with inulin-propionate ester or inulin improves insulin sensitivity in adults with overweight and obesity with distinct effects on the gut microbiota, plasma metabolome and systemic inflammatory responses: a randomised cross-over trial', *Gut*. BMJ Publishing Group, 68(8), pp. 1430–1438. doi: 10.1136/gutjnl-2019-318424.

Chang, P. V *et al.* (2014) 'The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition.', *Proceedings of the National Academy of Sciences of the United States of America*, 111(6), pp. 2247–52. doi: 10.1073/pnas.1322269111.

Cherbut, C. *et al.* (1991) 'Dietary fibre effects on intestinal transit in man: involvement of their physicochemical and fermentative properties', *Food Hydrocolloids*. Elsevier Ltd., 5(1–2), pp. 15–22. doi: 10.1016/S0268-005X(09)80281-6.

Cherbut, C. *et al.* (1997) 'Digestive and metabolic effects of potato and maize fibres in human subjects.', *The British journal of nutrition*, 77(1), pp. 33–46. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9059228.

Chriett, S. *et al.* (2019) 'Prominent action of butyrate over β-hydroxybutyrate as histone deacetylase inhibitor, transcriptional modulator and anti-inflammatory molecule', *Scientific Reports*. Nature Publishing Group, 9(1). doi: 10.1038/s41598-018-36941-9.

Christian, M. T. *et al.* (2003) 'Starch fermentation by faecal bacteria of infants, toddlers and adults: importance for energy salvage.', *European journal of clinical nutrition*, 57(11), pp. 1486–91. doi: 10.1038/sj.ejcn.1601715.

COST Action FA1005 (2015) *The Impact of Food Bioactives on Gut Health:, The Impact of Food Bioactives on Health: In Vitro and Ex Vivo Models*. doi: 10.1007/978-3-319-16104-4.

Cummings, J. and Macfarlane, G. (1991) 'The control and consequences of bacterial fermenation in the human colon', *Journal of Applied …*, (Wiggins 1983), pp. 443–459. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2672.1991.tb02739.x/abstract.

Daou, C. *et al.* (2014) 'In-vitro fermentation by human fecal bacteria and bile salts binding capacity of physical modified defatted rice bran dietary fiber', *Food and Nutrition Sciences*, 5(12), pp. 1114–1120.

Darzi, J. *et al.* (2016) 'L-rhamnose as a source of colonic propionate inhibits insulin secretion but does not influence measures of appetite or food intake', *Appetite*. Diabetes and Nutritional Sciences Division, School of Medicine, King's College London, Franklin-Wilkins Building, London, United Kingdom: Elsevier Ltd, 98, pp. 142–149. doi: 10.1016/j.appet.2015.12.011.

Dongowski, G. and Lorenz, a (1998) 'Unsaturated oligogalacturonic acids are generated by in vitro treatment of pectin with human faecal flora.', *Carbohydrate research*, 314(3–4), pp. 237–44. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10335591.

Edwards, C. *et al.* (1996) 'In vitro method for quantification of the fermentation of starch by human

faecal bacteria', *Journal of the …*, (71), pp. 209–217. Available at: http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1097-0010(199606)71:2%3C209::AID-JSFA571%3E3.0.CO;2-4/abstract (Accessed: 18 October 2013).

Edwards, C. A. (2017) 'Determinants and Duration of Impact of Early Gut Bacterial Colonization.', *Annals of nutrition & metabolism*. Karger Publishers, 70(3), pp. 246–250. doi: 10.1159/000466711.

Fardet, A. *et al.* (1997) 'In Vitro Fermentation of Beet Fibre and Barle y Bran , of their Insoluble Residues after Digestion and of Ileal Effluents', 315.

Fernandes, J., Rao, A. V. and Wolever, T. M. S. (2000) 'Different Substrates and Methane Producing Status Affect Short-Chain Fatty Acid Profiles Produced by In Vitro Fermentation of Human Feces 1 , 2', *Human Nutrition and Metabolism*, (January), pp. 1932–1936.

Furusawa, Y. *et al.* (2013) 'Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells.', *Nature*. Nature Publishing Group, 504(7480), pp. 446–50. doi: 10.1038/nature12721.

Gannasin, S. P. *et al.* (2015) 'In vitro prebiotic activities of tamarillo (Solanum betaceum Cav.) hydrocolloids', *Journal of Functional Foods*. Faculty of Food Science and Technology, Universiti Putra Malaysia, UPM Serdang, Selangor Darul Ehsan, Malaysia: Elsevier Ltd, 19, pp. 10–19. doi: 10.1016/j.jff.2015.09.004.

Gelissen, I. C. and Eastwood, M. a (1995) 'Taurocholic acid adsorption during non-starch polysaccharide fermentation: an in vitro study.', *The British journal of nutrition*, 74(2), pp. 221–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7547839.

Gietl, E. *et al.* (2012) 'Factors involved in the in vitro fermentability of short carbohydrates in static faecal batch cultures', *International Journal of Carbohydrate Chemistry*, 2012, pp. 1–10. doi: 10.1155/2012/197809.

Gomez, B. *et al.* (2014) 'Purification, characterization, and prebiotic properties of pectic oligosaccharides from orange peel wastes', *J Agric Food Chem*. 2014/09/11. Department of Chemical Engineering, Faculty of Science, University of Vigo (Campus Ourense), As Lagoas, Ourense, Spain: American Chemical Society, 62(40), pp. 9769–9782. doi: 10.1021/jf503475b.

Gomez, B. *et al.* (2016) 'Prebiotic potential of pectins and pectic oligosaccharides derived from lemon peel wastes and sugar beet pulp: A comparative evaluation', *Journal of Functional Foods*. Department of Chemical Engineering, Faculty of Science, University of Vigo (Campus Ourense), As Lagoas, Ourense, Spain: Elsevier Ltd, 20, pp. 108–121. doi: 10.1016/j.jff.2015.10.029.

Guillon, F., Barry, J. and Thibault, J. (1992) 'Effect of autoclaving sugar-beet fibre on its physico-chemical properties and its in-vitro degradation by human faecal bacteria', *Journal of the Science of Food …*, (Cummings 1983), pp. 69–79. Available at: http://onlinelibrary.wiley.com/doi/10.1002/jsfa.2740600112/abstract (Accessed: 11 January 2014).

Gulfi, M., Arrigoni, E. and Amadò, R. (2007) 'In vitro fermentability of a pectin fraction rich in hairy regions', *Carbohydrate Polymers*, 67(3), pp. 410–416. doi: 10.1016/j.carbpol.2006.06.018.

Gullon, Beatriz *et al.* (2015) 'Assessment of prebiotic potential of Akpan-yoghurt-like product and effects on the human intestinal microbiota', *Journal of Functional Foods*. CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquiteto Lobão Vital, Apartado 2511, Porto, Portugal: Elsevier Ltd, 19, pp. 545–553. doi: 10.1016/j.jff.2015.09.026.

Gullon, B *et al.* (2015) 'In vitro assessment of the prebiotic potential of Aloe vera mucilage and its impact on the human microbiota', *Food Funct*. 2014/12/17. Centro de Biotecnologia e Química Fina-

Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Doutor António Bernardino de Almeida, Porto, Portugal: Royal Society of Chemistry, 6(2), pp. 525–531. doi: 10.1039/c4fo00857j.

Gullon, P. *et al.* (2015) 'In vitro fermentation of lupin seeds (Lupinus albus) and broad beans (Vicia faba): dynamic modulation of the intestinal microbiota and metabolomic output', *Food Funct*. CBQF-Centro de Biotecnologia e Química Fina-Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquiteto Lobão Vital Apartado 2511, Porto, Portugal: Royal Society of Chemistry, 6(10), pp. 3316–3322. doi: 10.1039/c5fo00675a.

Hinnebusch, B. F. *et al.* (2002) 'The effects of short-chain fatty acids on human colon cancer cell phenotype are associated with histone hyperacetylation.', *The Journal of nutrition*, 132(5), pp. 1012–1017.

Hughes, S. a *et al.* (2007) 'In vitro fermentation by human fecal microflora of wheat arabinoxylans.', *Journal of agricultural and food chemistry*, 55(11), pp. 4589–95. doi: 10.1021/jf070293g.

Kedia, G. *et al.* (2009) 'In vitro fermentation of oat bran obtained by debranning with a mixed culture of human fecal bacteria.', *Current microbiology*, 58(4), pp. 338–42. doi: 10.1007/s00284-008-9335-1.

Khalil, N. a *et al.* (2014) 'In vitro batch cultures of gut microbiota from healthy and ulcerative colitis (UC) subjects suggest that sulphate-reducing bacteria levels are raised in UC and by a protein-rich diet.', *International journal of food sciences and nutrition*. Faculty of Home Economics, Minufiya University, Egypt, 65(1), pp. 79–88. doi: 10.3109/09637486.2013.825700.

Khan, K. M. and Edwards, C. A. (2005) 'In vitro fermentation characteristics of a mixture of Raftilose and guar gum by human faecal bacteria', *European Journal of Nutrition*, 44(6), pp. 371–376. doi: 10.1007/s00394-004-0537-4.

Kim, H. J. and White, P. J. (2009) 'In vitro fermentation of oat flours from typical and high beta-glucan oat lines.', *Journal of agricultural and food chemistry*, 57(16), pp. 7529–36. doi: 10.1021/jf900788c.

Kuda, T. *et al.* (2005) 'Inhibitory effects of laminaran and low molecular alginate against the putrefactive compounds produced by intestinal microflora in vitro and in rats', *Food Chemistry*, 91(4), pp. 745–749. doi: 10.1016/j.foodchem.2004.06.047.

Lin, H. V *et al.* (2012) 'Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms.', *PloS one*, 7(4), p. e35240. doi: 10.1371/journal.pone.0035240.

Macfarlane, S. and Macfarlane, G. T. (2003) 'Regulation of short-chain fatty acid production.', *The Proceedings of the Nutrition Society*, 62(1), pp. 67–72. doi: 10.1079/PNS2002207.

McBurney, M. I. and Thompson, L. U. (1989) 'Effect of human faecal donor on in vitro fermentation variables.', *Scandinavian journal of gastroenterology*, 24(3), pp. 359–67. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2544024 (Accessed: 16 March 2017).

McBurney, M. I. and Thompson, L. U. (1990) 'Fermentative characteristics of cereal brans and vegetable fibers.', *Nutrition and cancer*, 13(4), pp. 271–80. doi: 10.1080/01635589009514069.

McBURNEY, M. I. and Thompson, L. U. (1989) 'In Vitro Fermentabilities of Purified Fiber Supplements', *Journal of Food Science*, 54(2), pp. 347–350. doi: 10.1111/j.1365-2621.1989.tb03077.x.

McNeil, N. I., Cummings, J. H. and James, W. P. (1978) 'Short chain fatty acid absorption by the human large intestine.', *Gut*, 19(9), pp. 819 LP – 822. doi: 10.1136/gut.19.9.819.

Min, B. J. *et al.* (2015) 'Fermentation patterns of various pectin sources by human fecal microbiota', *Food and Nutrition Sciences*, 6(12), pp. 1103–1114. doi: 10.4236/fns.2015.612115.

Morrison, D. J. and Preston, T. (2016) 'Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism', *Gut Microbes*. Taylor & Francis, 7(3), pp. 1–12. doi: 10.1080/19490976.2015.1134082.

Mortensen, P. B. *et al.* (1990) 'The degradation of amino acids, proteins, and blood to short-chain fatty acids in colon is prevented by lactulose.', *Gastroenterology*. United States, 98(15), pp. 353–360. doi: S001650859000049X [pii].

Mortensen, P. B. *et al.* (1991) 'Fermentation to short-chain fatty acids and lactate in human faecal batch cultures. Intra- and inter-individual variations versus variations caused by changes in fermented saccharides.', *Scandinavian Journal of Gastroenterology*, 26(12), pp. 1285–1294. doi: 10.3109/00365529108998626.

Mortensen, P. B., Holtug, K. and Rasmussen, H. S. (1988) 'Short-chain fatty acid production from mono- and disaccharides in a fecal incubation system: implications for colonic fermentation of dietary fiber in humans.', *The Journal of nutrition*, 118(April), pp. 321–325.

Mortensen, P. B. and Nordgaard-Andersen, I. (1993) 'The dependence of the in vitro fermentation of dietary fibre to short-chain fatty acids on the contents of soluble non-starch polysaccharides', *Scand J Gastroenterol*, 28(5), pp. 418–422. doi: 10.3109/00365529309098242.

Nakata, T. *et al.* (2016) 'Inhibitory effects of laminaran and alginate on production of putrefactive compounds from soy protein by intestinal microbiota in vitro and in rats', *Carbohydrate Polymers*. Department of Food Science and Technology, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-city, Tokyo, Japan: Elsevier Ltd, 143, pp. 61–69. doi: 10.1016/j.carbpol.2016.01.064.

Olano-martin, E. *et al.* (2000) 'In vitro fermentability of dextran , oligodextran and maltodextrin by human gut bacteria', 44, pp. 247–255.

Oufir, L. E. *et al.* (2000) 'Relationships between transit time in man and in vitro fermentation of dietary fiber by fecal bacteria.', *European journal of clinical nutrition*, 54(8), pp. 603–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10951507.

Parkar, S. G. *et al.* (2012) 'In vitro utilization of gold and green kiwifruit oligosaccharides by human gut microbial populations.', *Plant foods for human nutrition (Dordrecht, Netherlands)*, 67(3), pp. 200–7. doi: 10.1007/s11130-012-0293-1.

Parkar, S. G., Trower, T. M. and Stevenson, D. E. (2013) 'Fecal microbial metabolism of polyphenols and its effects on human gut microbiota', *Anaerobe*. Elsevier Ltd, 23, pp. 12–19. doi: 10.1016/j.anaerobe.2013.07.009.

Payne, A. N. *et al.* (2012) 'Advances and perspectives in in vitro human gut fermentation modeling.', *Trends in biotechnology*. Elsevier Ltd, 30(1), pp. 17–25. doi: 10.1016/j.tibtech.2011.06.011.

Le Poul, E. *et al.* (2003) 'Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation.', *The Journal of biological chemistry*, 278(28), pp. 25481–25489. doi: 10.1074/jbc.M301403200.

Probert, H. M. *et al.* (2004) 'Polydextrose , Lactitol , and Fructo-Oligosaccharide Fermentation by Colonic Bacteria in a Three-Stage Continuous Culture System', 70(8), pp. 4505–4511. doi: 10.1128/AEM.70.8.4505.

Qin, J. *et al.* (2010) 'A human gut microbial gene catalog established by metagenomic sequencing', *Nature*, 464(7285), pp. 59–65. doi: 10.1038/nature08821.A.

Roediger, W. E. (1980) ‘Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man’, *Gut*, 21, pp. 793–798.

Rosendale, D. I. *et al.* (2012) ‘Characterizing kiwifruit carbohydrate utilization in vitro and its consequences for human faecal microbiota.’, *Journal of proteome research*, 11(12), pp. 5863–75. doi: 10.1021/pr300646m.

Rycroft, C. E., Jones, M. R., Gibson, G. R. and Rastall, R. A. (2001) ‘A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides.’, *Journal of applied microbiology*, 91(3), pp. 878–87. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11722666.

Rycroft, C. E., Jones, M. R., Gibson, G. R. and Rastall, R. a (2001) ‘Fermentation properties of gentio-oligasaccharides’, *Letters in applied microbiology*, 32, pp. 878–87. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11264744.

Salazar, N. *et al.* (2008) ‘Exopolysaccharides produced by intestinal Bifidobacterium strains act as fermentable substrates for human intestinal bacteria.’, *Applied and environmental microbiology*, 74(15), pp. 4737–45. doi: 10.1128/AEM.00325-08.

Salvador, V. *et al.* (1993) ‘Sugar composition of dietary fibre and short-chain fatty acid production during in vitro fermentation by human bacteria.’, *The British journal of nutrition*, 70(1), pp. 189–97. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8399101.

Spiller, G. a *et al.* (1980) ‘Effect of purified cellulose, pectin, and a low-residue diet on fecal volatile fatty acids, transit time, and fecal weight in humans.’, *The American journal of clinical nutrition*, 33(4), pp. 754–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7361693.

Thompson, L. U. *et al.* (2011) ‘In Vitro Digestion of RS4-Type Resistant Wheat and Potato Starches, and Fermentation of Indigestible Fractions’, *Cereal Chemistry*, 88(1), pp. 72–79. doi: 10.1094/CCHEM-07-10-0098.

Titgemeyer, E. C. *et al.* (1991) ‘Fermentability of various fiber sources by human fecal bacteria in vitro.’, *The American journal of clinical nutrition*, 53(6), pp. 1418–24. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1852091.

De Vadder, F. and Mithieux, G. (2014) ‘Dietary fibers induce metabolic benefits via the activation of intestinal gluconeogenesis ’, *Obesite*. Institut de la Santé et de la Recherche Médicale, U855, Lyon, France: Springer-Verlag France, 9(4), pp. 280–285. doi: 10.1007/s11690-014-0451-8.

Vera-Pingitore, E. *et al.* (2016) ‘Screening and characterization of potential probiotic and starter bacteria for plant fermentations’, *LWT - Food Science and Technology*. Centro de Referencia para Lactobacilos (CERELA), CONICET, Chacabuco 145, Tucumán, Argentina: Academic Press, 71, pp. 288–294. doi: 10.1016/j.lwt.2016.03.046.

Vogt, J. a. *et al.* (2004) ‘L-Rhamnose increases serum propionate after long-term supplementation, but lactulose does not raise serum acetate’, *American Journal of Clinical Nutrition*, 80, pp. 1254–1261.

Vogt, J. a *et al.* (2006) ‘L-rhamnose and lactulose decrease serum triacylglycerols and their rates of synthesis, but do not affect serum cholesterol concentrations in men.’, *The Journal of nutrition*, 136(8), pp. 2160–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16857835.

Walker, A. W. *et al.* (2005) ‘pH and Peptide Supply Can Radically Alter Bacterial Populations and Short-Chain Fatty Acid Ratios within Microbial Communities from the Human Colon’, 71(7), pp. 3692–3700. doi: 10.1128/AEM.71.7.3692.

Wang, H., Shi, Y. and Le, G. (2014) ‘Rapid microwave-assisted synthesis of polydextrose and identification of structure and function’, *Carbohydrate Polymers*. Key Laboratory of Industrial

Biotechnology Ministry of Education, School of Food Science and Technology, Jiangnan University, Wuxi 214122, Jiangsu, China: Elsevier Ltd, 113, pp. 225–230. doi: 10.1016/j.carbpol.2014.07.012.

Wang, X. and Gibson, G. R. (1993) 'Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine.', *The Journal of applied bacteriology*, 75(4), pp. 373–80. doi: 10.1111/j.1365-2672.1993.tb02790.x.

Weaver, G. A. *et al.* (1989) 'Constancy of glucose and starch fermentations by two different human faecal microbial communities.', *Gut*. England, 30(1), pp. 19–25.

Weaver, G. a *et al.* (1992) 'Cornstarch fermentation by the colonic microbial community yields more butyrate than does cabbage fiber fermentation; cornstarch fermentation rates correlate negatively with methanogenesis.', *The American journal of clinical nutrition*, 55(1), pp. 70–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1309475.

Williams, C. F. *et al.* (2014) 'Comparative Analysis of Intestinal Tract Models', *Annual Review of Food Science and Technology*, 6(February), p. 150223151639004. doi: 10.1146/annurev-food-022814-015429.

Wolever, T. M. S. *et al.* (1995) 'Propionate inhibits incorporation into plasma lipids in humans13 of colonic and'.

Wolever, T. M., Spadafora, P. and Eshuis, H. (1991) 'Interaction between colonic acetate and propionate in humans.', *The American journal of clinical nutrition*. American Society for Nutrition, 53(3), pp. 681–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2000822 (Accessed: 26 July 2016).

Yu, Z.-T. *et al.* (2013) 'Trametes versicolor extract modifies human fecal microbiota composition in vitro.', *Plant foods for human nutrition (Dordrecht, Netherlands)*, 68(2), pp. 107–12. doi: 10.1007/s11130-013-0342-4.

Zhang, X. *et al.* (2013) 'Fermentation in vitro of EGCG, GCG and EGCG3"Me isolated from Oolong tea by human intestinal microbiota', *Food Research International*. Elsevier Ltd, 54(2), pp. 1589–1595. doi: 10.1016/j.foodres.2013.10.005.

Zhao, X.-H. and Lin, Y. (2009) 'Resistant starch prepared from high-amylose maize starch with citric acid hydrolysis and its simulated fermentation in vitro', *European Food Research and Technology*, 228(6), pp. 1015–1021. doi: 10.1007/s00217-009-1012-5.

Zhu, C. and Zhao, X. (2012) 'In vitro fermentation of a retrograded maize starch by healthy adult fecal extract and impacts of exogenous microorganisms on three acids production', *Starch-Stärke*, pp. 330–337. doi: 10.1002/star.201200100.

Zhu, X. *et al.* (2013) 'Puri fi cation and Fermentation in Vitro of Sesaminol Triglucoside from Sesame Cake by Human Intestinal Microbiota'.

Table 1: Inclusion and exclusion criteria for further analysis of articles identified based on abstracts

Inclusion Criteria	Exclusion criteria
Batch fermentation	Not a batch fermentation (i.e. continuous culture)
Initial pH 6 ≤ 8	pH stat controlled experiment[1]
24-hour time point	Initial pH 6 > 8
Use of a fresh faecal slurry	Additional bacteria added (i.e. use of bacterial pellet, probiotic)
Samples from healthy adults	Samples from children, infants, disease states or animals
Data on acetate, propionate and butyrate[4]	Gastrointestinal disorders
Volume of fermentation system given[4]	Use of antibiotics (within the study or participants use within the previous fortnight)
Mass of substrate fermented given[4]	Manipulation of the diet[3]

1. Where the fermentation system is conducted with the continuous use of an acid or a base to maintain a constant pH
2. If SCFA production was presented as a ratio only, total SCFA concentration must be provided
3. Includes; supplements provided to the donor, exclusion or inclusion of foods from the diet of the sample donor
4. To produce a rate term of SCFA production, the minimum information required was: mass of substrate fermented, final fermentation volume and amount of each SCFA produced

Table 2: The top and bottom five ranked producers of acetate, propionate, butyrate, and total production at 6-9 hours of fermentation (mmol/g carbohydrate/ hour)

Top 5	**Ranked on Acetate**		**Ranked on Propionate**		**Ranked on Butyrate**		**Ranked on Total**	
1	Lactulose (1-3)[1]	0.95 (0.90)	Sugarbeet fibre (12, 18 22 - 24)	0.17 (0.15)	Guar gum (9-11)	0.11 (0.07)	Lactulose (1-3)	1.09 (1.14)
2	Glucose (1,3-8)	0.73 (0.66)	Guar gum (9-11)	0.14 (0.16)	Raw starch (6,14)	0.08 (0.07)	Glucose (1,3-8)	0.94 (0.86)
3	Guar gum (9-11)	0.54 (0.28)	Glucose (1,3-8)	0.13 (0.15)	Pectin (7,8,10,12,13)	0.08 (0.09)	Guar gum (9-11)	0.81 (0.34)
4	Pectin (7,8,10,12,13)	0.51 (0.42)	Pectin (7,8,10,12,13)	0.11 (0.09)	Resistant starch (7, 19 - 21)	0.08 (0.05)	Pectin (7,8,10,12,13)	0.70 (0.56)
5	Ispaghula (7,8,10)	0.37 (0.32)	Ispaghula (7,8,10)	0.11 (0.08)	Glucose (1,3-8)	0.08 (0.18)	Sugarbeet fibre (12, 18 22 - 24)	0.64 (0.26)
Bottom 5								
9	Raw starch (6,14)	0.25 (0.15)	Lactulose (1-3)	0.08 (0.10)	Ispaghula (7,8,10)	0.05 (0.09)	FOS (4, 11, 13, 25 - 27)	0.44 (0.58)
10	Wheat bran (7,8, 15 - 17)	0.24 (0.37)	Heat-treated sugarbeet fibre (15, 18)	0.07 (0.05)	Wheat bran (7, 8, 15 - 17)	0.05 (0.09)	Wheat bran (7, 8, 15 - 17)	0.33 (0.52)
11	Heat-treated sugarbeet fibre (15, 18)	0.20 (0.18)	Wheat bran (7,8, 15 - 17)	0.07 (0.07)	Oat bran (12, 16, 17)	0.04 (0.18)	Heat-treated sugarbeet fibre (15, 18)	0.32 (0.26)
12	Resistant starch (7, 19 - 21)	0.16 (0.21)	Cellulose (2, 7, 8, 10)	0.04 (0.05)	Heat-treated sugarbeet fibre (15, 18)	0.03 (0.05)	Resistant starch (7, 19 -21)	0.31 (0.27)
13	Cellulose (2, 7, 8, 10)	0.12 (0.14)	FOS (4, 11, 13, 25 - 27)	0.04 (0.13)	Cellulose (2, 7, 8, 10)	0.02 (0.04)	Cellulose (2, 7, 8, 10)	0.19 (0.22)

[1] Number corresponds to reference, [2] Median (IQR). FOS: Fructooligosaccharide. Top 1-5 = high to low, Bottom 9-13 = low to high. References 1. (Mortensen, Holtug and Rasmussen, 1988), 2. (Mortensen *et al.*, 1990), 3. (Cardelle-Cobas *et al.*, 2012), 4. (Olano-martin *et al.*, 2000), 5. (Zhu *et al.*, 2013), 6. (Weaver *et al.*, 1989), 7. (Mortensen and Nordgaard-Andersen, 1993), 8. (Mortensen *et al.*, 1991), 9. (McBurney and Thompson, 1989), 10. (McBURNEY and Thompson, 1989), 11. (Khan and Edwards, 2005), 12. (Titgemeyer *et al.*, 1991), 13. (Min *et al.*, 2015), 14. (McBurney and Thompson, 1990), 15. (Cherbut *et al.*, 1991), 16. (McBurney and Thompson, 1990), 17. (Bourquin *et al.*, 1992), 18. (Guillon, Barry and Thibault, 1992), 19. (Zhu and Zhao, 2012), 20. (Zhao and Lin, 2009), 21. (Thompson *et al.*, 2011), 22. (Oufir *et al.*, 2000), 23. (Fardet *et al.*, 1997), 24. (Barry and Hoebler, 1995), 25. (Zhang *et al.*, 2013), 26. (P. Gullon *et al.*, 2015), 27. (Beatriz Gullon *et al.*, 2015).

Table 3: Top and bottom five ranked producers of acetate, propionate, and butyrate production at 6-9 hours of fermentation based on molar proportion (%)

Top 5	**Ranked on Acetate**		**Ranked on Propionate**		**Ranked on Butyrate**	
1	Lactulose (1-3)[1]	81.97 (6.59)[2]	Cellulose (2, 7, 8, 10)	26.85 (10.37)	Raw starch (6,14)	25.0 (15.3)
2	Pectin (7,8,10,12,13)	77.59 (11.64)	Resistant starch (7, 19 -21)	24.42 (9.65)	Resistant starch (7, 19 -21)	19.18 (5.02)
3	Glucose (1,3-8)	72.71 (16.3)	Heat-treated sugarbeet fibre (15, 18)	22.71 (4.20)	FOS (4, 11, 13, 25 - 27)	16.97 (9.00)
4	Oat bran (9, 18, 12)	72.73 (31.8)	Ispaghula (7,8,10)	21.86 (12.30)	Wheat bran (7,8, 15 - 17)	15.00 (10.90)
5	Sugarbeet fibre (12, 18 22 - 24)	69.39 (30.6)	Guar gum (9-11)	21.05 (13.35)	Sugarbeet fibre (12, 18 22 - 24)	12.24 (17.10)
Bottom 5						
9	Heat-treated sugarbeet fibre (15, 18)	66.33 (3.02)	Wheat bran (7,8, 15 - 17)	17.01 (4.15)	Ispaghula (7,8,10)	9.53 (6.60)
10	Ispaghula (7,8,10)	65.43 (11.35)	FOS (4, 11, 13, 25 - 27)	15.03 (11.55)	Heat-treated sugarbeet fibre (15, 18)	9.26 (5.80)
11	Cellulose (2, 7, 8, 10)	63.29 (8.28)	Glucose (9-11)	13.61 (7.77)	Oat bran (12, 16, 17)	9.09 (19.53)
12	Guar gum (9-11)	57.97 (20.4)	Pectin (7,8,10,12,13)	13.37 (5.25)	Glucose (9-11)	9.08 (11.03)
13	Resistant starch (7, 19 -21)	56.38 (12.6)	Lactulose (1-3)	9.85 (4.42)	Lactulose (1-3)	6.27 (4.08)

[1] Number corresponds to reference, [2] Median (IQR). FOS: Fructooligosaccharide. Top 1-5 = high to low, Bottom 9-13 = low to high. Reference numbers correspond to those in the legend of table 2.

Table 4: Top and bottom five ranked producers of acetate, propionate, butyrate, and total production at 10-23 hours of fermentation (mmol/g carbohydrate/ hour)

Top 5	**Ranked on Acetate**		**Ranked on Propionate**		**Ranked on Butyrate**		**Ranked on Total**	
1	Pectic oligosaccharide (28, 29)[1]	0.43 (0.22)[2]	Rice bran (16, 34)	0.88 (0.59)	FOS (13, 25, 28, 29, 32, 33)	0.13 (0.09)	Rice bran (16, 34)	1.08 (0.63)
2	Guar gum (9, 10, 30)	0.40 (0.28)	Guar gum (9, 10, 30)	0.22 (0.02)	Guar gum (9, 10, 30)	0.11 (0.03)	Guar gum (9, 10, 30)	0.71 (0.28)
3	Sugarbeet fibre (12, 18, 22 -24, 31)	0.32 (0.24)	Sugarbeet fibre (12, 18, 22 -24, 31)	0.10 (0.12)	Resistant starch (20, 21)	0.09 (0.04)	Pectic oligosaccharide (28, 29)	0.56 (0.23)
4	FOS (13, 25, 28, 29, 32, 33)	0.26 (0.21)	Oat bran (12, 16, 17)	0.09 (0.10)	Pectic oligosaccharide (28, 29)	0.08 (0.04)	Sugarbeet fibre (12, 18, 22 -24, 31)	0.46 (0.45)
5	Oat bran (12, 16, 17)	0.26 (0.27)	Resistant starch (20, 21)	0.08 (0.04)	Oat bran (12, 16, 17))	0.08 (0.10)	Oat bran (12, 16, 17)	0.45 (0.44)
Bottom 5								
11	Rice bran (16, 34)	0.16 (0.14)	Heat-treated sugarbeet fibre (15, 18)	0.05 (0.05)	Pectin (10, 12, 13, 28, 29)	0.04 (0.01)	Wheat bran (15 – 17, 31)	0.29 (0.14)
12	Heat-treated sugarbeet fibre (15, 18)	0.15 (0.17)	Seaweed derivatives (35, 36)	0.05 (0.14)	Heat-treated sugarbeet fibre (15, 18)	0.04 (0.03)	Heat-treated sugarbeet fibre (15, 18)	0.23 (0.23)
13	Seaweed derivatives (35, 36)	0.09 (0.15)	FOS (13, 25, 28, 29, 32, 33)	0.05 (0.03)	Maize fibre (15, 31, 37)	0.03 (0.00)	Maize fibre (15, 31, 37)	0.15 (0.03)
14	Maize fibre (15, 31, 37)	0.08 (0.02)	Maize fibre (15, 31, 37)	0.04 (0.01)	Pea fibre (12, 15, 31)	0.02 (0.03)	Pea fibre (12, 15, 31)	0.46 (0.16)
15	Pea fibre (12, 15, 31)	0.08 (0.09)	Pea fibre (12, 15, 31)	0.04 (0.04)	Seaweed derivatives (35, 36)	0.00 (0.01)	Seaweed derivatives (35, 36)	0.15 (0.38)

[1] Number corresponds to reference, [2] Median (IQR). FOS: Fructooligosaccharide. Seaweed derivatives: alginate, laminarin etc. Top 1-5 = high to low, Bottom 11-15 = low to high. References continued from Table 2 legend. 28. (Gomez *et al.*, 2014), 29. (Gomez *et al.*, 2016), 30. (Carlson *et al.*, 2016), 31. (Salvador *et al.*, 1993), 32. (Rycroft, Jones, Gibson and R. a Rastall, 2001), 33. (Gannasin *et al.*, 2015), 34. (Daou *et al.*, 2014), 35. (Kuda *et al.*, 2005), 36. (Nakata *et al.*, 2016), 37. (Cherbut *et al.*, 1997).

Table 5: Top and bottom five ranked producers of acetate, propionate, and butyrate production at 10-23 hours of fermentation based on molar proportion (%)

Top 5	Ranked on Acetate		Ranked on Propionate		Ranked on Butyrate	
1	Pectic oligosaccharide (28, 29)[1]	77.06 (4.54)[2]	Rice bran (16, 34)	81.4 (22.25)	Resistant starch (20, 21)	29.21 (15.8)
2	Pectin (10, 12, 13, 28, 29)	72.31 (7.13)	Seaweed derivatives (35, 36)	33.33 (12.53)	FOS (13, 25, 28, 29, 32, 33)	19.96 (18.98)
3	FOS (13, 25, 28, 29, 32, 33)	70.32 (14.5)	Guar gum (9, 10, 30)	31.42 (16.13)	Wheat bran (15 – 17, 31)	19.48 (5.42)
4	Sugarbeet fibre (12, 18, 22 -24, 31)	65.68 (22.15)	Maize fibre (15, 31, 37)	27.03 (1.61)	Raw starch (6, 14, 19, 38)	18.44 (12.5)
5	Heat-treated sugarbeet fibre (15, 18)	64.1 (3.53)	Resistant starch (20, 21)	23.27 (11.8)	Oat bran (12, 16, 17)	17.31 (16.78)
Bottom 5						
11	Guar gum (9, 10, 30)	56.49 (26.50)	Wheat bran (15 – 17, 31)	18.75 (8.45)	Pectic oligosaccharide (28, 29)	12.95 (5.06)
12	Maize fibre (15, 31, 37)	55.56 (5.53)	Raw starch (6, 14, 19, 38)	17.01 (12.05)	Pectin (10, 12, 13, 28, 29)	12.14 (6.35)
13	Seaweed derivatives (35, 36)	50 (16.73)	Pectin (10, 12, 13, 28, 29)	15.27 (4.2)	Sugarbeet fibre (12, 18, 22 - 24, 31)	11.79 (13.25)
14	Resistant starch (20, 21)	48.24 (27.2)	FOS (13, 25, 28, 29, 32, 33)	11.62 (10.45)	Seaweed derivatives (35, 36)	8.33 (20.83)
15	Rice bran (16, 34)	14.65 (16.41)	Pectic oligosaccharide (28, 29)	8.07 (6.08)	Rice bran (16, 34)	4.17 (18.98)

[1] Number corresponds to reference, [2] Median (IQR). Median (IQR). FOS: Fructooligosaccharide, Seaweed derivatives: alginate, laminarin. Top 1-5 = high to low, Bottom 11-15 = low to high. References continue from Table 4 legend. 38. (Khalil *et al.*, 2014)

Table 6: Top and bottom five ranked producers of acetate, propionate, butyrate, and total production at 24 hours of fermentation (mmol/g carbohydrate/ day)

Top 5	Ranked on Acetate		Ranked on Propionate		Ranked on Butyrate		Ranked on Total	
1	Galactose (1, 39, 40)[1]	11.35 (7.25)[2]	Rhamnose (1, 39, 43)	4.75 (1.14)	GOS (3, 32, 39, 41)	2.36 (2.50)	GOS (3, 32, 39, 41)	13.69 (16.18)
2	GOS (3, 32, 39, 41)	8.47 (12.62)	Arabinose (1, 39, 40)	3.18 (2.49)	Pectic oligosaccharide (28, 29)	1.55 (0.09)	Galactose (1, 39, 40)	12.32 (6.61)
3	Lactose (1, 3, 39, 40)	7.81 (8.50)	Guar gum (9 – 11, 30, 43, 50, 51)	2.90 (0.72)	Raw starch (6, 14, 19, 38, 40, 43, 54, 55)	1.28 (1.34)	Rhamnose 1, 39, 43)	11.14 (5.63)
4	Pectic oligosaccharide (28, 29)	7.48 (1.31)	Xylose (1, 39)	2.71 (2.81)	Lactose (1, 3, 39, 40)	1.18 (1.41)	Pectic oligosaccharide (28, 29)	9.87 (1.51)
5	Lactulose (2, 3, 39 – 43)	7.04 (16.91)	GOS (3, 32, 39, 41)	2.43 (1.76)	Guar gum (9 – 11, 30, 43, 50, 51)	1.00 (0.63)	Arabinose (1, 39, 40)	9.76 (7.25)
Bottom 5								
25	Pea fibre (12, 15, 31)	1.50 (2.04)	Modified pectin (52, 53)	0.42 (1.27)	Pea fibre (12, 15, 31)	0.25 (0.63)	Pea fibre (12, 15, 31)	2.25 (3.29)
26	Oat bran (12, 16, 17, 44, 45)	0.58 (3.71)	Cellulose (7, 8, 10, 33, 49)	0.35 (0.57)	Xylose (1, 39)	0.19 (0.87)	Oat bran (12, 16, 17, 44, 45)	0.99 (6.20)
27	Kiwi fibre (46, 47)	0.45 (0.22)	Oat bran (12, 16, 17, 44, 45)	0.25 (1.21)	Oat bran (12, 16, 17, 44, 45)	0.17 (1.25)	Cellulose (7, 8, 10, 33, 49)	0.93 (2.39)
28	Polydextrose (40, 48)	0.43 (3.96)	Kiwi fibre (46, 47)	0.12 (0.05)	Polydextrose (40, 48)	0.14 (0.86)	Kiwi fibre (46, 47)	0.68 (0.32)
29	Cellulose (7, 8, 10, 33, 49)	0.31 (1.42)	Polydextrose (40, 48)	0.05 (1.74)	Kiwi fibre (46, 47)	0.13 (0.06)	Polydextrose (40, 48)	0.62 (6.55)

[1] Number corresponds to reference, [2] Median (IQR).GOS: Galactooligosaccharide FOS: Fructooligosaccharide. Top 1-5 = high to low, Bottom 25-29 = low to high. References continue from legend of Table 5: 39. (Gietl *et al.*, 2012), 40. (Wang and Gibson, 1993), 41. (Rycroft, Jones, Gibson and R. A. Rastall, 2001), 42. (Kim and White, 2009), 43.(Fernandes, Rao and Wolever, 2000), 44. (Kedia *et al.*, 2009), 45. (Bourquin, Titgemeyer and Fahey, 1993), 46. (Rosendale *et al.*, 2012), 47. (Parkar *et al.*, 2012), 47. (Wang, Shi and Le, 2014), 49. (Yu *et al.*, 2013), 50. (Adiotomre *et al.*, 1990), 51. (Bourquin, Titgemeyer and Fahey, 1996), 52. (Dongowski and Lorenz, 1998), 53. (Gulfi, Arrigoni and Amadò, 2007), 54. (Weaver *et al.*, 1992), 55. (Christian *et al.*, 2003)

Table 7: The top and bottom five ranked producers of acetate, propionate, and butyrate, production at 24 hours of fermentation based on molar proportion (%)

Top 5	Ranked on Acetate		Ranked on Propionate		Ranked on Butyrate	
1	Galactose (1, 39, 40)[1]	90.55 (16.14)[2]	Modified pectin (52, 53)	37.39 (53.40)	Polydextrose (40, 48)	22.58 (9.73)
2	Xylose (1, 39)	83.41 (29.59)	Rhamnose (1, 39, 43)	34.28 (18.06)	Inulin (33, 40, 41, 47, 56 – 60)	22.02 (9.51)
3	Lactulose (2, 3, 39 – 43)	80.50 (16.48)	Guar gum (9 – 11, 30, 43, 50, 51)	34.28 (8.61)	Oat bran (12, 16, 17, 44, 45)	20.16 (9.73)
4	Lactose (1, 3, 39, 40)	80.0 (22.58)	Arabinose (1, 39, 40)	27.90 (1.13)	Raw starch (6, 14, 19, 38, 40, 43, 54, 55)	19.79 (8.35)
5	GOS (3, 32, 39, 41)	77.89 (22.02)	Cellulose (7, 8, 10, 33, 49)	25.00 (16.45)	Corn bran (12, 16, 17)	19.78 (15.69)
Bottom 5						
25	Oat bran (12, 16, 17, 44, 45)	59.30 (5.02)	Lactulose (2, 3, 39 – 43)	11.19 (7.98)	Gum arabic	8.46 (2.93)
26	Cellulose (7, 8, 10, 33, 49)	56.25 (24.24)	Lactose (1, 3, 39, 40)	11.09 (6.23)	Rhamnose (1, 39, 43)	5.41 (14.41)
27	Guar gum (9 – 11, 30, 43, 50, 51)	54.77 (4.70)	Pectic oligosaccharide (28, 29)	8.54 (0.21)	Xylose (1, 39)	3.40 (4.13)
28	Modified pectin (52, 53)	54.16 (47.40)	Polydextrose (40, 48)	8.48 (16.94)	Arabinose (1, 39, 40)	3.56 (8.60)
29	Rhamnose (1, 39, 43)	45.99 (18.15)	Galactose (1, 39, 40)	7.13 (8.37)	Galactose (1, 39, 40)	2.83 (8.27)

[1] Number corresponds to reference, [2] Median (IQR). GOS: Galactooligosaccharide Top 1-5 = high to low, Bottom 25-29 = low to high. References continued from Table 6: 56. (Parkar, Trower and Stevenson, 2013), 57. (Salazar *et al.*, 2008), 58. (Hughes *et al.*, 2007), 59. (B Gullon *et al.*, 2015), 60. (Chambers *et al.*, 2015), 61. (Gelissen and Eastwood, 1995)

Table 8: SCFA selectivity after 24 hours of fermentation.

High selectivity				
Acetate	Galactose	GOS	Lactose	Lactulose
Propionate	Rhamnose	Arabinose	Guar gum	
Butyrate	Raw Starch			
Low selectivity				
Acetate	Oat bran	Cellulose		
Propionate	Polydextrose			
Butyrate	Xylose			

Highly selective grouped as are top five for rate and ratio, low selectivity grouped as are bottom five for rate and ratio

Table 9: The top and bottom 5 ranking substrates for the propionate: acetate and butyrate: acetate ratio after 24 hours of fermentation.

Top 5	P:A		B:A	
1	Rhamnose	0.75 (0.65)	Inulin	0.36 (0.27)
2	Guar gum	0.64 (0.22)	Oat bran	0.34 (0.14)
3	Cellulose	0.44 (0.67)	Polydextrose	0.33 (0.12)
4	Arabinose	0.43 (0.47)	Corn bran	0.32 (0.26)
5	Sugarbeet fibre	0.40 (0.22)	Raw starch	0.30 (0.50)
Bottom 5				
25	Lactulose	0.14 (0.16)	Lactulose	0.11 (0.18)
26	Lactose	0.14 (0.11)	Lactose	0.11 (0.31)
27	Polydextrose	0.13 (0.29)	Arabinose	0.06 (0.14)
28	Pectic oligosaccharide	0.11 (0.01)	Xylose	0.05 (0.05)
29	Galactose	0.08 (0.12)	Galactose	0.03 (0.11)

Median (IQR). Ratio calculated by dividing the propionate or butyrate rate by the acetate rate.

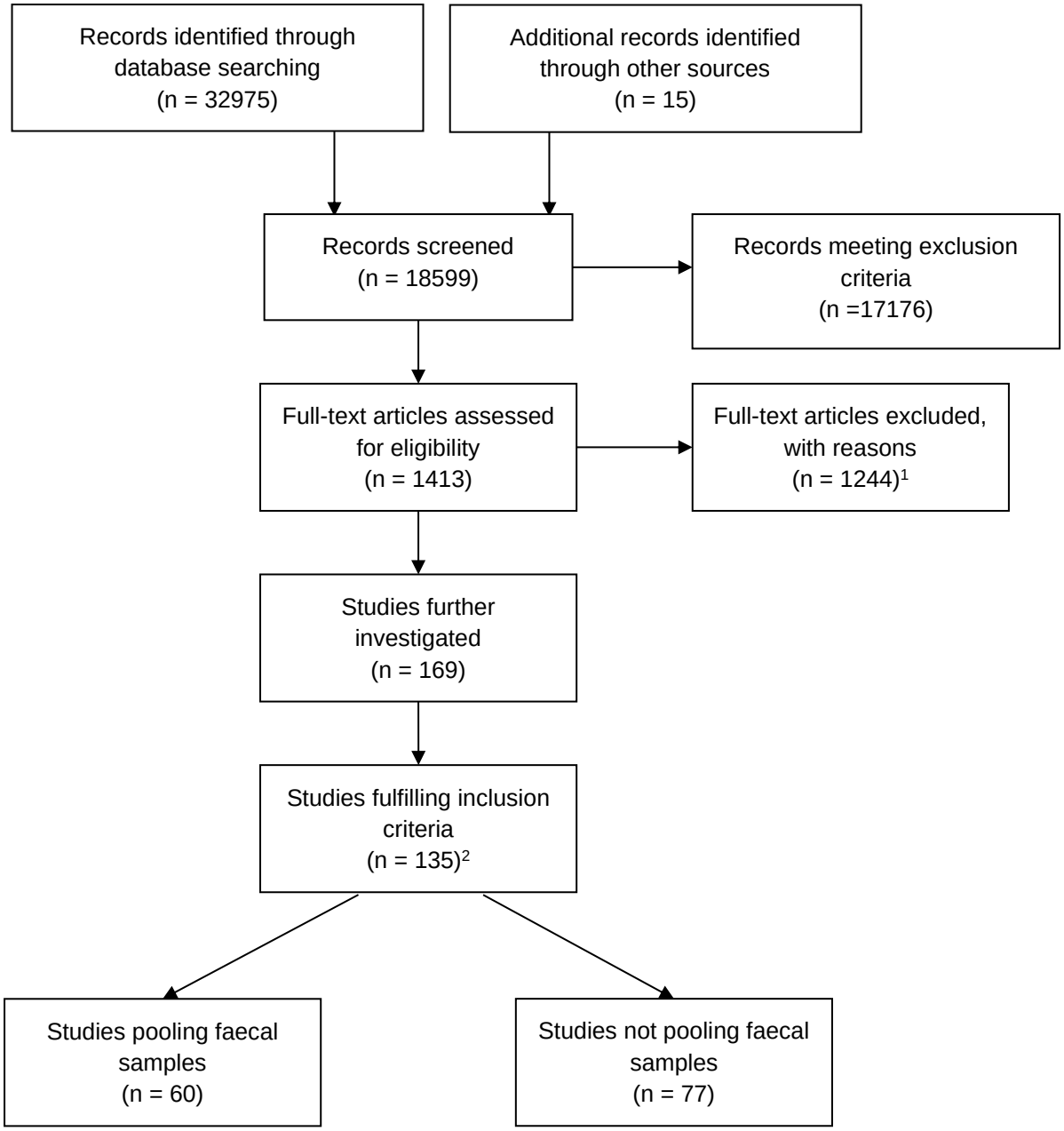

Figure 1: Flow diagram based on PRISMA Scoping review guidelines outlining the process of elimination of articles investigated

Two articles (Mortensen *et al.*, 1991; Barry and Hoebler, 1995) reported data using the pooled and non-pooled fermentation method.

1. Articles which fulfil the exclusion criteria outlined in Table 1.
2. Articles which fulfil the inclusion criteria and provide all the information required to form the rate unit