PREBIOTIC POTENTIAL OF A WIDE SELECTION OF TUBERS, GRAINS, AND PULSES IN COMPARISON TO FRUCTO-OLIGOSACCHARIDE

by

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LIST OF ABBREVIATIONS

bp	Base pairs
˚C	Degrees Celsius
DNA	Deoxyribonucleic acid
FOS	Fructooligosaccharide
g	Gram(s)
GC	Gas chromatography
GI	Gastrointestinal
h	hour
L	Liter(s)
µL	Microliter(s)
µmol	Micromole(s)
mg	Milligram
mL	Milliliter(s)
mM	Millimolar
min	Minute(s)
OTU	Operational Taxonomic Units
PCR	Polymerase chain reaction
s	Second
SCFA	Short Chain Fatty Acid

ABSTRACT

The most common food and supplement prebiotic fiber is inulin – most commonly extracted from chicory root. Fructo-oligosaccharide (FOS) is a smaller version of inulin, both containing mainly fructose units with β-1,2 linkages. FOS/inulin has been used, and studied, as a prebiotic for decades. The potential of alternative prebiotics intrinsic in whole foods, such as in tubers, grains, vegetables, and pulses – the world's most common staple crops – are not as commonly recognized as prebiotics, though have this potential if fermentable in the gut. If such alternative sources of prebiotic ingredients could be established it would allow for cheaper, possibly more effective, and more diverse food product development options beyond FOS/inulin.

This study demonstrates the potential of tubers, grains, and pulses as prebiotics in relation to their *in vitro* human fecal fermentation rate, short chain fatty acid (SCFA) production, and microbiota indicators of alpha diversity and impact on key bacterial genera. Fecal samples were obtained from three diverse healthy human donors and used as the initial bacterial inoculum to simulate conditions in the human gut (colon). Substrates (n=35), after undergoing an upper gastrointestinal tract simulated digestion, were fermented by each individual donors' inoculum separately, and measurements after 6, 12, and 24 h of fermentation were made on gas production, SCFA metabolite production, and microbiome composition.

The results of this study establish high fermentability and potential prebiotic effects of dietary fibers from tubers, grains, and pulses. Whole foods, ground and cooked the same way, produced dietary fibers that were largely insoluble, but surprisingly fermentable with high SCFA levels, mostly slow fermentation profiles indicating high tolerability, and mostly promoting diverse microbiota responses compared to FOS. Generally, whole food fibers had higher fermentability than similar isolated fibers. Overall, the processing steps, such as atmospheric or pressure cooking, tested in some pulses did not detract, or add to, the prebiotic abilities of the substrates. Each food fiber substrate had unique effects on the gut microbiota parameters tested. Gut microbiome compositional responses to the same substrate varied significantly among the three donors, but notably SCFA metabolite responses were similar among donors.

CHAPTER 1. LITERATURE REVIEW

1.1 Human gut microbiota

The human digestive tract contains trillions of bacteria, archaea, eukaryotes, and viruses; collectively known as the gut microbiota and the genetic material thereof, the microbiome. Bacteria, by count, constitute the vast majority of the microorganisms, approximately the same order of magnitude of bacteria as human cells; bacteria outnumber archaea and eukaryotes by 2 to 3 orders of magnitude (Sender et al., 2016). There are approximately 23,000 human genes, however the microorganisms within the human host have approximately 3 million genes (Zhao, 2013). The larger proportion of the genes in the gut microbiota are related to carbohydrate metabolism in comparison to the human genome, and therefore the gut microbiota possess a far more diverse metabolic capacity for carbohydrates (Gosalbes et al., 2011). Undigested carbohydrates by human enzymes in the digestive tract are available for the gut microbiota. Long-term and short-term dietary intake can influence the structure and activity of the gut microbiota (David et al., 2014).). This daily influence of diet on the microbiota provides an opportunity for accessing and improving food choices by consumers and production to address this growing area of research.

However, accessing positive impacts of dietary supplementation on the microbiome has been a challenge. Interindividual variation and variation over a lifetime from infancy to elder age presents a challenge in defining a 'healthy microbiome'. Additionally, data across several studies suggest enterotypes of the microbiome in an individual based on the predominant presence of either Bacteriodes, Prevotella, or Ruminococcus (Chen et al., 2017), which could explain how the same substrate leads to different microbiota community responses. Community composition, bacterial diversity, functional metagenomic, and transcriptomic changes in these bacteria, and also metabolic outputs – specifically short chain fatty acids (SCFA) - could all be useful approaches in defining changes and accessing impact. Ultimately, such changes in the gut microbiome would ideally be identified with associated changes in host physiology (C. A. Lozupone et al., 2012; Tremaroli & Bäckhed, 2012)

1.2 The human colonic environment, gut microbiota influence on the host

The microbes residing in the digestive tract have many mechanistically established impacts on the human host. A vital impact is on the immune system. The human gastrointestinal (GI) tract houses the largest part of the human immune system, which monitors the most widely exposed organ system to the environment with a surface of 200 m^2 (Lopetuso et al., 2013). One of the primary modes of action of the gut microbiota on the human host is the production of short chain fatty acid (SCFA) metabolites, and their triggering of key immunomodulatory, neuronal and hormonal, and metabolic functions. SCFAs are final energy state metabolites from anaerobic fermentation. The predominant SCFAs produced are acetate, propionate, and butyrate (X. Wu et al., 2018). Additional mechanisms of the gut bacteria on host health are related to diversity of the microbiota and specific microbes that have been correlated with a health state leading to a state of dysbiosis, an imbalance in microbial communities ((Kriss et al., 2018).

SCFAs directly and indirectly act via local and systemic effects on human physiology. Locally they provide the preferred energy source for human colonocytes, which enables optimum colonocyte function (Topping & Clifton, 2001). SCFAs also maintain and promote gut barrier function by up-regulating genes related to tight junction proteins and increasing mucus production in colonocytes (Stefka et al., 2014). SCFAs promote anti-inflammatory cytokine pathways that begin in the gut submucosa and have systemic effects. There are broad implications of reducing host inflammatory responses related to metabolism and other health and disease states. SCFAs modulating T cell expression has been shown to lower systematic inflammation, which subsequently improves glucose homeostasis and has moderating effects on type 2 diabetes and obesity. Gut microbiota SCFA production has been shown to improve glucose metabolism in the blood in rat model and human models (Pingitore et al., 2017). SCFAs have been shown to improve β cell function in the pancreas, which produces insulin, and thereby improve glucose homeostasis, and that dietary fibers that stimulate the gut microbiota to produce SCFAs could have implications for the control of type 2 diabetes, via mechanisms of decreasing blood glucose concentration, improving insulin sensitivity, and increasing glucagon-like peptide-1 (GLP-1) (Puddu et al., 2014).

Further on systemic effects, SCFAs have been shown to act as signal transduction molecules which activate G-protein coupled receptors in the brain, liver, and other organs (Kasubuchi et al., 2015). SCFAs have been shown to induce the epigenetic effect of regulating histone deacetylase (HDAC) inhibition, which was related to decreasing the incidence of colon

cancer (Sun et al., 2019). Also, SCFAs were recently shown to trigger host production of serotonin through an established cellular mechanism, and potentially being an important factor related to depression with over 90% of the serotonin in the human body produced in the enterochromaffin cells that are a part of the epithelial lining of the GI tract (Caspani et al., 2019; Yano et al., 2015).

The three main SCFAs (acetate, propionate, butyrate) each have been studied extensively regarding their individual functions related to human health. Acetate supplementation, in a mice model, was shown to reduce blood pressure and cardiac fibrosis, and down-regulated genes involved in heart and kidney health, and inflammation (Marques Francine Z. et al., 2017). Acetate and propionate were shown to stimulate GLP-1 and peptide YY (PYY) production in specialized gut enteroendocrine cells (L cells), which increased satiety (González Hernández et al., 2019). Butyrate is a widely studied SCFA metabolite with wide-reaching effects on the body including anti-inflammatory property, inhibiting activation of NF-kB, and anti-cancer effect from inducing apoptosis of tumor cells (Lopetuso et al., 2013). Butyrate via diet supplementation has been shown to improve insulin sensitivity and reduction of adiposity in a dietary-obese mice model (Gao et al., 2009). In a Caco2 cell model, butyrate was shown to improve intestinal barrier function (Cj et al., 2015). Mucosal-associated Clostridial bacteria, containing the major butyrate producers, are considered to have a direct and pronounced impact on the immune system due in part their location close to the epithelial layer (Lopetuso et al., 2013).

1.3 Gut microbiota and human health

One of the most critical and central interactions between the gut microbiota and human physiology is in regulating the human adaptive and innate immune systems. Pro- and anti-inflammatory cytokines are triggered by production of SCFA metabolites by the gut microbiota, thereby modulating inflammatory responses. (Blander et al., 2017)

Fermentation rate of a fiber determines where and how far distally along the GI tract SCFA production by the gut microbiota occurs, and relates to where SCFAs, with their rapid absorption by the epithelium are taken up in the colon (Cummings, 1981). GI diseases related to inflammation such as ulcerative colitis and colon cancer occur more in the distal colon, which could be due to an accumulation of toxic metabolites such ammonia (Macfarlane et al., 1992). Although, it is very difficult to measure SCFA and metabolic activity of gut bacteria along the human GI tract due to experimental limitations, a sustained fermentation rate for substrates would enable SCFA

production and uptake across the entirety of the lower GI tract, and could be beneficial to reducing incidence of colonic diseases (Rose et al., 2007).

Diversity is often used in comparing community ecology between and within the samples, and is related to a healthy microbiome. Alpha and beta diversity are the most common diversity measures between and within samples and treatments. Alpha diversity is a measurement of evenness and/or richness of the bacterial ecology within a sample. Richness is a measure of how many different microbes exist in a sample, and evenness refers to how equally abundant the microbes are in that sample. There are different calculation methods for alpha diversity of a sample, such as Shannon, Simpson, Chao1, and Faith Phylogenetic Distance indices. Shannon, which is shown below, is a commonly used alpha diversity measure as it considers evenness as well as the richness of a sample. On the other hand, beta diversity is the measure of the extent of change in the microbial community due to treatments, such as different dietary fibers.

There are widely accepted correlations of alpha diversity to health states, although there is an ongoing debate on its importance. High alpha diversity is thought be beneficial to the host for several reasons as more diversity enables more metabolic functional capacity of the microbiome, which is found in functional redundancy amongst different species that makes the colonic epithelium more resistant to opportunistic pathogens by being more diverse where less under-utilized resources would be available. Low gut microbial diversity has been linked to metabolic syndrome, type 2 diabetes, inflammatory bowel disease, and colorectal cancer (Martens, 2016), and importantly high fiber diets have been shown to improve alpha diversity in mice models (Sonnenburg et al., 2016). In humans, a study reported a correlation in 169 obese Danish individuals, compared to 123 non-obese, between low bacterial alpha diversity with increased adiposity, insulin resistance, more inflammation (Le Chatelier et al., 2013). Additionally, with regards to vascular health, increased gut microbiome alpha diversity lowered arterial stiffness (Menni et al., 2018).

1.4 Prebiotics and dietary fibers

The prebiotic effect is defined as “the selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host” (M. Roberfroid et al., 2010). Dietary fiber is defined by the American Association of Cereal Chemists as “the edible part of plants and analogous carbohydrates that are

resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the human large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, blood cholesterol attenuation, and/or blood glucose attenuation." (DeVries, 2003). Dietary fibers are unable to be digested by human enzymes in the small intestine, and therefore pass through the digestive tract and become available to the microbiota in the distal small intestine and large intestine for metabolism via fermentation (Sims & Monro, 2013). Fermentable dietary fibers that provide metabolic substrate available to the gut microbiota have been shown to improve microbiome diversity (Sonnenburg et al., 2016).

1.4.1 Inulin

Inulin is a longer form of fructo-oligosaccharides (FOS) and FOS/inulin has been widely studied and used as a prebiotic for decades (Welters et al., 2002). Inulin is a storage polysaccharide common in many plants, predominantly in roots, and especially in chicory, garlic, and leek. Inulin is extracted from roots using a hot water diffusion process, then purified and dried; the final powder usually contains 10% free sugars. (Niness, 1999)

The term inulin refers to all β (2→1) fructans of varying lengths. Chicory inulin has a degree of polymerization (DP) of 2 to 60 with an average DP of 12, and when it undergoes enzymatic hydrolysis FOS has a DP of 2 to 8, with an average DP of 4 (M. B. Roberfroid, 2007). A glucose molecule often is linked at the terminal end of each fructan by an α (1→2) bond. Human digestive enzymes are unable to break down β bonds and therefore FOS/inulin is available to the gut microbiota exclusively for utilization as a carbon source (Niness, 1999).

Inulin has been used as a prebiotic and supplement in the food industry for decades. The main reasons for the wide industry acceptance of inulin are its ease of use in processed foods due to its high solubility and small structure, as well as its neutral and acceptable flavor and mouthfeel. FOS/inulin is the most common added fiber to processed foods, mainly used to increase fiber amount of products but also improve gut health. Beneficial food physical properties are also associated with FOS/inulin. It has been used to increase viscosity in dairy products like yogurt, biscuits, and spreads. (Kolida et al., 2002; M. B. Roberfroid, 2007). Inulin commercially is also used as a fat replacer due to a mouthfeel effect and as a sugar replacer (Shoaib et al., 2016).

Bifidobacteria, an early established probiotic, has been shown to be stimulated by inulin and FOS (Kolida et al., 2002). Selected inulin studies with benefits to health are shown in Table 1.

FOS/inulin is rapidly digested by the gut bacteria and fast gas production occurs during fermentation, which can be intolerable to some individuals (Cummings & Macfarlane, 1991). Additionally, its rapid fermentation rate suggest microbial metabolic by-products would not effectively reach the distal colon. FOS/inulin has been shown to have prebiotic effects, and subsequent wide health impacts, both in in vivo and *in vitro* experimental models, and is the earliest and most consistent commonly studied substrate for its prebiotic effect. The widely accepted probiotics, Lactobacillus and Bifidobacteria, have been shown to be stimulated and increased by inulin supplementation. Also, inulin has been shown to improve stool bulking, frequency, and consistency (Shoaib et al., 2016).

Animal studies have shown consistent effects of inulin supplementation; however, human studies have had mixed results (Vandeputte et al., 2017). Inulin has been shown to decrease gut mucosal lesions and mucosal inflammation in animal models, due to SCFA stimulation (Guarner, 2005). FOS/inulin has had mixed results on biomarkers related to colon cancer (CRC); animal models have shown protective effects on CRC biomarkers, however humans studies have not yielded positive results (M. J. Clark et al., 2012) (Boutron-Ruault et al., 2005). Several studies have investigated FOS/inulin potential in improving diabetic conditions in animal models (Q. Zhang et al., 2018). Inulin, and other oligosaccharides, have also been shown to enhance absorption of calcium, magnesium, and iron in relation to bone health (Shoaib et al., 2016). Selected studies of inulin on human health are shown in Table 1; chosen based on recency and health impact with a preference on *in vivo* human studies.

Table 1. Selected inulin studies related to health benefits.

Substrate Specifications	Experimental Design	Results of Supplementation
Inulin fed at 5% wt/wt of the diet compared to saline in the control group (K. Li et al., 2019)	Leptin resistant db/db mice, Type 2 Diabetes model	Lowering of; pro-inflammatory cytokines (TNF-α), plasma LPS, IL-6), body weight Modulation of the gut microbiota
Inulin-type fructans (Catry et al., 2018)	Apolipoprotein E knockout mice, measuring artery nitric oxide	Reversal of endothelial dysfunction Modulation of gut microbiota
Inulin supplementation 10 g/day, 16 days (Ramirez-Farias et al., 2008)	12 Human volunteers, inulin supplementation 10 g/d for a 16-d period vs. control	Increase in *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*
Inulin vs. wheat dextrin vs. guar gum (Noack et al., 2013)	*In vitro* batch fecal fermentation pooled from 3 healthy human donors	Inulin resulted in higher butyrate production

1.4.2 Tubers and roots

A tuber is a non-grain structure in some plant species that is used for nutrient storage. Tubers have two broad types, stem and root. Stem tubers are usually for seasonal energy storage and possible reproduction; examples include potato and yam. Root tubers are for longer term storage of nutrients from one year to the next; examples include sweet potato and cassava. Root crops are grown all around the world and are staple foods in many regions, and are second only to cereal grains as a global carbohydrate source (Hoover, 2001) (FAO, 2020). Tubers have not received much attention as a potential prebiotic food source category, however, they have relatively high total dietary fiber with potential prebiotic properties.

Tubers and roots on average are 16-24% starch on a wet weight basis with low quantities of proteins and lipids (Hoover, 2001). Total dietary fiber contents are in the range of 2-4% on an "as eaten" basis (B. W. Li et al., 2002). Types of fibers in tubers and roots are principally pectins, xyloglucans (part of hemicelluloses), and cellulose (Salvador et al., 2000). Tubers protein content is 1-2% on a dry weight basis. The starch granules of tubers are mostly simple, with cassava and large taro root having a mixture of simple and compound starch granules (Hoover, 2001). Ferulic acid, a phenolic compound, in tubers and roots is involved in cross-linking between polysaccharides within the cell wall and between cells. The presence of phenolics in tubers have been studied along with beneficial health effects, particularly around; anti-inflammatory and anti-

tumor effects (Chandrasekara & Josheph Kumar, 2016). Selected studies on tubers, the gut microbiome, and health benefits are found in Table 2, chosen based on recency and health impact with a preference on *in vivo* human studies.

Table 2. Selected studies on tubers and the gut microbiome related to health.

Substrate	Study Design	Results
Color-fleshed, anthyocyanin-containing, potatoes anti-colitic effect (Reddivari et al., 2020)	Ulcerative colitis mice model (DSS); supplementation with either purple or red potato	Purple fleshed potato supplementation improved over red potato ZO-1 gene expression – improving barrier function
Malanga tubers vs. Potato (B. L. Graf et al., 2018)	Mice fed a high fat diet with either 20% Malanga or 20% Potato	Malanga consumption increased alpha diversity in mice compared to potato
Potato powder (Y. Wu et al., 2019)	Healthy Rats fed potato powder for 7 weeks	increased SCFAs, increased abundance of *Bifidobacterium, Ruminococcus_1*
Potato fiber (Panasevich et al., 2015)	Dietary intervention in dogs	SCFA increased in feces, increase of *Faecalibacterium*

1.4.3 Cereal grains

Grains refer to the dry seeds of plants and two broad categories of grains are cereals and legumes or pulses, however the term ‘grains’ more commonly refers to cereal grains. Cereal grains are from the *Poaceae* or *Gramineae* family, or grass family such as rice, wheat, and corn. Cereal grains are composed of three layers; bran, endosperm, and germ. The bran layer contains, mostly insoluble fiber consisting of matrix bound complex hemicellulose cross-linked with phenolics (Beloshapka et al., 2016). The endosperm is the largest part of the grain and it is mostly comprised of starch, a polysaccharide composed of glucose units linked together with α-1,4 and α-1,6 glycosidic linkages. A portion of starch, resistant starch (RS), is resistant to digestion in the small intestine by human starch digestive enzymes, thereby allowing for its fermentation by the gut microbiota. The germ layer contains the developing embryo of the plant and has a higher protein and fat content.

Bran insoluble fibers are mainly composed by arabinoxylans, cellulose, lignin, structural proteins, and esterified phenolics (Selvendran, 1984). Arabinoxylan is the chemical make-up or

backbone of most of the fiber from cereals such as wheat, maize, rice, sorghum, and pearl millet (Collins et al., 2010; Crittenden et al., 2002). Arabinoxylan is a polysaccharide made up of a β-(1-4)-linked D-xylose backbone that has glycosidic bonds at O-2 and/or O-3 by side chains composed of single arabinose units or multiple units of arabinose, xylose and/or galactose (Izydorczyk & Biliaderis, 1995).

Many epidemiological studies have shown consistently that increased whole grain consumption correlates with decreased negative health conditions, such as cardiovascular disease, type 2 diabetes, colorectal cancer, and obesity (Kyrø et al., 2013). However, the mechanism of the whole grain response is not fully understood (Eriksen et al., 2020); polyphenols within the grain have established anti-oxidant capabilities, and fiber content benefits have been focused on hinderance of cholesterol re-uptake in the colon for improved cardiovascular health. Selected studies on cereal grains, the gut microbiome, and health are found in Table 3, chosen based on recency and health impact with a preference on *in vivo* human studies.

Table 3. Selected studies on cereal grains and the gut microbiome related to health.

Substrate	Design	Results
Whole grain Consumption vs. refined (Roager et al., 2019)	Human subjects (n=50); dietary supplementation for 8 weeks of 179 g/d of whole grains treatment	Whole grain reduced; IL-6, C-reactive protein. Whole grain increased *Faecalibacterium.*
Whole grain vs. fruits and vegetables vs. refined grains (Kopf et al., 2018)	Human subjects (n=49), randomized parallel, 6 week dietary supplementation	Whole grain consumption reduced; pro-inflammatory TNF-α, LPS Binding Protein. No changes in OTUs observed or SCFA between treatments.
Whole grain wheat, whole grain rye, compared to refined wheat (Vuholm et al., 2017)	Dietary supplementation in obese humans (n-71), randomized parallel trial for 6 weeks; 145 g/d whole grain wheat, 124 g/d whole grain rye groups vs. refined control	Fecal butyrate increased in whole grain wheat. No overall notable changes in gut microbiota composition.
Whole grain vs. refined (Vetrani et al., 2016)	Humans with metabolic syndrome (n=54) 40 g/d dietary fiber treatment group	Treatment reduced; TNF-α, high sensitivity C-reactive protein (hs-CRP). Treatment increased; plasma propionate and butyrate levels.
Whole grain barely, whole grain brown rice, and whole grain blend (Martínez et al., 2013)	4 week cross over human trial (n=28)	All Treatments increased microbiota diversity. Fiber blend lowered IL-6. No changes in fecal SCFA.

1.4.4 Pulse grains

Legumes are plants in the family Fabaceae, also called Leguminosae, and the dried seed of a legume plant is called a pulse (Rochfort & Panozzo, 2007). Most legumes have symbiotic nitrogen-fixing bacteria in root nodules and the ability to fix atmospheric nitrogen; thereby reducing the need for fertilizer (FAO, 2016). Increased pulse consumption is advocated as a part of the Mediterranean diet as compared to the standard American diet. Other epidemiological observations and intervention studies have shown positive health effects of legumes. Legumes have been a staple human food for millennia, especially as an essential source of protein. Some common examples include lentils, soybeans, beans, peas, chickpeas, and peanuts.

Monocotyledons, or monocots, and dicotyledons, or dicots, make up two classifications of flowering plants. Cereals are monocots and pulses are dicots. Pulses consist of three parts;

endosperm or cotyledon, the embryo or germ, testa or the seed coat. Whole or slit lentils/pulses have the seed coat layer, while peeled or skinned seeds do not have the outer layer. The seed coat is composed mainly of fiber and phenolics as well as minerals, and its function is for protection of the seed. The cotyledon stores nutrients for the seed, such as protein, lipids, and carbohydrates. Flavonoids are mostly present in the seed coat and non-flavonoid phenolics are in the cotyledon. Microstructures of pulses and cereals are integral to not only the physical, sensory, and rheological behaviors (Tiwari & Singh, 2012), but also digestibility – which could have implications for the gut microbiota in terms of resistant starch content.

As dicots, the dietary fiber composition of pulses is similar to tubers and roots, with main fiber polysaccharides comprised of pectins, xyloglucans, and cellulose (Salvador et al., 2000). Pulses also have oligosaccharides in their structure, specifically α-linked galactooligosaccharides (GOS), which are not digested by human digestive enzymes. Chickpea seeds, in 19 cultivars, were shown to have α-GOS content varying from 6.4 to 8.7% (Xiaoli et al., 2008). Pulses, in general, have a relatively high protein content varying between 18-32% on a dry weight basis (Boye et al., 2010), and pulse protein content is almost twice that of cereal grains (Rochfort & Panozzo, 2007). Pulses have bioactive compounds, such as polyphenols, carotenoids, and tocopherols, and the majority of such compounds and polyphenols are in the seed coat. Therefore, pulses with intact seed coats would provide greater health benefits than if removed (Padhi & Ramdath, 2017).

The overall beneficial health effects of pulses have centered around polyphenols and resistant starch (Rochfort & Panozzo, 2007), and relatively not as much focus has been placed on the possible fiber prebiotic effects of pulses. In addition to the dietary fibers of pulses, their prebiotic effect could also be due to polyphenols within the pulses structure, and even their relatively high protein content, or a combination of all of the elements in pulses. Pulses have several structural features that make them ideal candidates be explored for health benefits such as low-caloric density and high nutrient-density (S. Clark & Duncan, 2017). Pulses have broad and well-established beneficial health effects in human consumption, ranging from satiety, low glycemic index, cardiovascular disease (i.e., lowering blood pressure and cholesterol, inflammation), and effectiveness in reducing body weight (Padhi & Ramdath, 2017).

Overall studies investigating the impact of pulses on the gut microbiota and associated health implications are limited. Lentils have gained interest for prebiotic properties in recent rat studies highlighting effects of reducing colitis and obesity markers (Johnson et al., 2015; Siva et

al., 2018). Selected studies on pulses and the gut microbiome related to health are shown in Table 4, chosen based on recency and health impact with a preference on *in vivo* human studies.

Table 4. Selected studies on pulses and the gut microbiome related to health.

Substrate	Study Design	Results
Red lentils, dose dependent 5, 10 or 20% cooked (D. Graf et al., 2019)	Healthy mice	20% lentil group had the greatest impact on increased; α diversity, fecal SCFA, tight junction proteins (ZO-1, Claudin, E-Cadherin)
Rice bran 30 g/d compared to Navy bean 35 g/d (Sheflin et al., 2017)	Overweight/obese humans with a prior history of colorectal cancer (n=29)	Only rice bran led to increased SCFA in the stool.

1.5 Processing effects on dietary fiber and prebiotic properties

Food processing effects of tubers/roots, cereal grains, and pulse grains have often been connected to increases in postprandial glycemic response, and generally associated with decreased health value of the subsequent food products. However, limited studies have investigated post-production food products on the gut microbiota and compared these effects to the pre-production food. This study element was incorporated into this thesis research, and could have important implications for the food industry as product innovation pipelines and processing methods could possibly be designed for improved gut microbiome health effects. While one would not want to decrease fiber value to the gut microbiome by processing, certain processing techniques (e.g., extrusion) could also be used to open up fiber structures for making substrates more fermentable and increasing value.

Processing effects on cereals primarily include various heat treatment/cooking methods and physical modifications by introduction of high shear and/or pressure. Additionally, grains may be pre-processed, such as removing the bran layer and germ of whole grains to make refined grain products. Similarly, in legumes, dehulling or peeling removes the outer seed coat, analogous to the bran layer. Cooking changes food matrices and three-dimensional structures, as well as modifies food components and compounds through chemical reactions. Gelatinization of starch through cooking processes increases its digestibility in the upper GI, and greatly reduces resistant starch available to the gut microbiota (Carmody et al., 2019). Similarly, with regards to human digestive

enzymes, cooking processes can change and reveal previous indigestible carbon sources for the microbiota via opening up structures and reducing chemical hinderances. It is largely unknown how these cooking and processing methods change the metabolic ability of the diverse gut microbiota. Additionally, the implications of resistant starch and its positive health associations in humans are generally known, but not to how different processes retain or diminish resistant starch content in regards to changes in gut microbial fermentation and subsequent SCFA metabolites on human health.

Johnson et al. (2015) compared the presence of prebiotic compounds, raffinose family oligosaccharides and other OSs and resistant starch (RS), in lentils pre- and post-cooking. Resistant starch was shown to increase after cooking and cooling, including after additional reheating. The cooking and cooling process fostered a breakdown and then re-alignment of amylose chain interactions into more compact and less digestible three-dimensional structures, making them indigestible and substrate for the gut microbiota. (Johnson et al., 2015)

Cooking of meat has been hypothesized to generate carcinogens such as heterocyclic amines and polycyclic aromatic hydrocarbons. The gut microbiota has the ability to convert such pre-carcinogens into carcinogens. Cooking effects have been shown to generate compounds like xenobiotics which could negatively impact the gut microbiota. (Nogacka et al., 2019) Notably, individual differences have been shown in gut microbiota to metabolize carcinogenic compounds due to heat processing, and in a portion of people could modulate the exposure to carcinogenic risk from the same compounds produced in cooked meat products (Vanhaecke et al., 2008).

Other processing effects have been studied with regards to their potential effect on the gut microbiota. Perez-Burillo et. al. (2018) compared common thermal treatments, including frying, boiling, grilling, roasting, and toasting to 5 different common food items; banana, bread, chicken, chickpea, and pepper. Furosine, 5-(hydroxymethyl)furfural (HMF), and furfural were used as indicators of heat treatment development since they are intermediaries of Mailliard browning. The presence of furfural breakdown products was shown to be increased in more intense heat treatment like grilling compared to baking, however this depended on the type of food processed. Overall the cooking processes impacted the gut microbiota composition differently relative to their raw counterparts with regards to specific operational taxonomic units (OTUs). At the phyla level and taxa level, bacteria were significantly correlated to the production of furosine, HMF, and furfural compounds. (Pérez-Burillo et al., 2018)

High-temperature, high-shear extrusion processing has been studied related to its potential to promote prebiotic properties of grains, and overall has shown positive changes in gut microbiota metabolites and community composition. Extrusion exposes flour components to high pressure and shear, and subsequent changes to physical and chemical structures could make the cell wall matrix fibers more available to the gut microbiota. Extrusion was shown in sorghum flour to improve microbiota structure and function in an obese rat model. Rats showed reduced inflammatory biomarkers and an increase in Bacteriodetes (phylum level) when fed extruded sorghum in comparison to a high fat diet. However, no comparison was made between non-extruded sorghum flour to isolate the effects of processing. (Brahma et al., 2017; de Sousa et al., 2019). Whole grain oats of varying moisture contents, when extruded, had improved microbiota effects, in an *in vitro* model, in terms of gut bacteria amounts and SCFA production (Brahma et al., 2017). Extruded flaxseed was not shown to have significant effect on swine microbiota, however improved omega-3 fatty acid content of pork meat (Holman et al., 2014).

1.6 Overview

In summary, food components have varying effects on the structure and function of the human gut microbiota. Various food sources, linkages and processing steps all have established effects on the gut microbiota, often along with host physiological health impact. Previous studies establish a groundwork for further investigation on tubers, grains, and pulses in comparison to FOS. Whole food fiber sources would be better, or at least comparable, prebiotic sources in comparison to FOS.

1.7 Study objectives

1. Explore prebiotic efficacy/potential of a wide selection of tubers/roots, pulses, and cereals in comparison to industry leading prebiotic fiber fructo-oligosaccharide (FOS) that would be;
    a. More tolerable than FOS (a lower gas production)
    b. Increase SCFA production by the microbiota

c. Stimulate growth and increase of bacterial groups of interest that have been associated with positive health

d. Increase alpha diversity, comparable to FOS

2. Processing effects on select pulses in comparison to non, or alternatively, processed pulses on gut microbiota composition and gas and SCFA production.

1.8 Rationale of study

- Establishing additional whole food-based prebiotic ingredients would allow more diverse product development choices for improved taste, texture, and processing.
- Establishing additional whole food-based prebiotic ingredients that would be a more cost-effective alternative compared to added, and more expensive, prebiotics like FOS/inulin.

CHAPTER 2. PREBIOTIC POTENTIAL OF A WIDE SELECTION OF TUBERS, GRAINS, AND PULSES RELATIVE TO FRUCTO-OLIGOSACCHARIDE

2.1 Introduction

Dietary fibers are classified based on water solubility into either soluble or insoluble fibers. Examples of soluble fiber include pectins, beta-glucans, xyloglucans, a variety of hydrocolloid polysaccharides, inulin, and some fabricated fiber components (e.g., resistant maltodextrins). Examples of insoluble fiber include cellulose and hemicelluloses. Matrix bound fibers, like in plant cell wall structures that exist in whole foods, are also insoluble as they are physically retained in the cell wall. Soluble fibers are nearly all rapidly and more easily fermentable by the gut microbiota (Jonathon et al., 2012). Whereas, insoluble fibers have been considered as barely fermentable; and therefore health benefits to the human host has been limited to effects on stool formation and laxation (Gemen de Vries & Slavin, 2011). Due this early understanding in the field of dietary fiber and microbiome studies, an emphasis was placed on soluble fiber structures and isolating soluble fiber components out of otherwise insoluble matrices to increase their value and make them potential prebiotic fibers. The term "prebiotic" recently was defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" by a sub-committee of the International Scientific Association of Probiotics and Prebiotics (ISAPP) (Hutkins et al., 2016). Industry leading prebiotics in the functional food area are oligosaccharides such as lactulose, FOS, galactooligosaccharides (GOS), isomaltooligosaccharides (IMO), xylooligosaccharises (XOS), and arabinoxylanooligosaccharides (AXOS) (Sanchez et al., 2009).

Soluble fibers, such as inulin and FOS, are rapidly fermented in the cecum and proximal colon, therefore less fiber substrate reaches the distal colon (Cummings & Macfarlane, 1991). This leads to bloating and makes such ingredients potential intolerable if eaten in large amounts over a short amount of time. Insoluble fibers, although often only partial fermentable by the gut microbiota, are slower fermenting and provide a more gradual and sustainable delivery of fiber substrate across the entire length of the GI tract, and would therefore have improved prebiotic function distally in the large intestine.

The use of unprocessed, unrefined, whole foods in this thesis study were evaluated in comparison with a industry leading prebiotic, FOS. Whole food dietary fibers are generally

considered to be bulking fibers to improve laxation, and not good sources of fermentable fibers. The potential of their being highly fermentable suggests a new term "whole food prebiotics" that could be used to differentiate intrinsic prebiotics opposed to purified or isolated prebiotics that are currently available to consumers. The whole food prebiotic approach presented a unique study design challenge compared to isolated structures, because of the presence of many different whole food macronutrient contents and complex linkages of polysaccharides in food matrices. This presented a difficulty in identifying structure-function relationships between carbohydrate linkage types and gut microbiota responses. However, when the food matrix is broken down to isolate or purify the components, then the potential impact of the food matrix as a whole can no longer be studied. In an overall effort in the investigation of dietary fiber and gut microbiota interactions to define specific causal relationships between specific food structures and targeted bacteria, an overlooked feature has been that of whole food prebiotics. Whole food prebiotics could be a challenge to define in the regulatory definition of prebiotics for the food industry, however, studies such as presented here may support a broader understanding of prebiotics.

Tubers, grains, and pulses were studied compared to FOS. Overall, previous studies support the fermentability of each of these food groups, however, none have compared fermentation properties and microbiota responses across these broad categories in the same experimental design, and compared to a known prebiotic (in this case, FOS, which is the base unit of inulin). This approach is aligned with determining the prebiotic nature of various food source ingredients with application to the food industry. Results from this study, will better inform food industry product development pipelines that seek to increase gut fermentation effectiveness in humans and help collate broader comparisons between substrates relative to the most studied prebiotic supplements.

Most of the dietary fibers tested in this thesis research were processed in the whole food form, and not as extracted, isolated fiber components. Thus, fibers were maintained in a form resembling an actual human eating experience as whole foods were prepared and digested in a similar basic way as occurs in practice. Raw materials were cooked and treated to an *in vitro* simulated upper GI tract digestion procedure, and then dialyzed, freeze-dried, and applied in equal amount to a human fecal *in vitro* fermentation assay. Three donors with different gut microbiota communities and fiber responses were chosen and tested separately.

2.2 Materials and methods

2.2.1 Dietary fiber preparation

Tubers/roots were shipped by PepsiCo and arrived as raw unprocessed whole foods. They were cut, dried, and then milled using an Eberbach Mill (model E3703, Belleville, MI) to coarse flours. Prior to upper GI simulated digestion, all food samples were milled to a fine flour using a cyclone mill (UDY Corporation, Fort Collins, CO) with a 0.4 mm brass screen. Potato Starch (Penford Food Ingredients, Centennial, CO) and FOS from chicory root (F8052, Sigma-Aldrich, St. Louis, MO, USA) were not milled or subjected to upper GI simulated digestion.

2.2.2 Full list of substrates

Positive and negative controls:

1. Blank
2. FOS – no upper GI digestion

Tubers/roots series:

3. Cassava
4. Large Taro Root
5. Carrots
6. Red Beets
7. Red Radish
8. Sweet Potato
9. Yams
10. Hiccamah
11. Plantain, green
12. Plantain, ripe
13. Potato starch : control – no upper GI digestion

Grains series:

14. White Whole Grain Sorghum Flour (representing non-decorticated comparison for Pearl Millet)
15. White Coarse Sorghum Meal (representing de-corticated comparison for Pearl Millet)
16. Pearl Millet Whole (non-decorticated)
17. Pearl Millet (de-corticated)
18. Amaranth
19. Wheat bran
20. Rice bran
21. Corn bran

Pulses:

22. Whole Pea flour
23. Pea fiber
24. Whole Chickpea flour
25. Atmospheric Cooking Chickpea Flour – SODA
26. Pressure Cooking Chickpea Flour – SODA; Garbano Bic Sodio
27. Pressure Cooking Chickpea Flour – CA
28. Chickpea Flour Lime 0.3%
29. Whole Faba Bean
30. Whole lentil flour
31. Lentil Fiber
32. Lentil Flour Lentil Lime 0.3%
33. Atmospheric Cooking Lentil Flour – SODA
34. Atmospheric Cooking Lentil Flour - CA
35. Atmospheric Cooking Lentil Flour - Lime
36. Pressure Cooking Lentil Flour - CA
37. Pressure Cooking Lentil Flour - SODA

Substrate Notes:

Samples such as the tuber series (except Potato Starch) and grain series were milled through a brass 0.4 mm sized filter screen to obtain a uniform particle size for the upper GI digestion simulation.

2.2.3 Upper GI digestion simulation

Milled samples were then subjected to an upper GI simulated digestion, with extended enzymatic treatments to remove more starch from samples, as described by Tuncil et al. (2018). The digestion simulates the pH, enzymes, and duration of the stages in the upper human digestive tract. After an initial boiling step to cook the starch, stages in the digestion included pepsin (≥250 units/mg, P7000, Sigma-Aldrich, St. Louis, MO, U.S.A.) treatment at pH 2.5 for 30 min, followed by pancreatin (P-7545, Sigma-Aldrich) and amyloglucosidase (3260 units/mL, E-AMGDF, Megazyme International, Wicklow, Ireland) treatment at pH 6.9 for 6 hr. Samples were then dialyzed (MWCO: 6-8kDa, Fisher #08670F) and freeze dried, and ground, resulting in a powder of the undigested and mostly fiber food material from each sample.

2.2.4 Total dietary fiber (TDF) assay

The TDF assay was conducted on the substrates using the Megazyme © Total Dietary Fiber Assay K-TDFR-100A, based on AOAC Method 991.43 and AACC Method 32-07.01.

2.2.5 *In vitro* fecal fermentation

Fecal samples were collected from three healthy individuals who had not taken antibiotics at least 6 months prior; Donors X, Y and Z. Donors had fecal collections gathered on-site at Purdue University, and within 3 hours the fecal material inocula were added in the ex vivo fecal fermentation reaction tubes and sealed. Fecal donors were asked to eat their typical diets leading up to the collection. Donor X was a 29-year-old male born and raised in the USA, and ate a mostly plant-based diet. Donor Y was a 28-year-old female born and raised in the USA, and ate a typical American diet. Donor Z was a 31year-old male born and raised in Africa and ate a mostly traditional Kenyan inspired high plant-based diet. The protocol was approved by the Purdue IRB.

The *in vitro* fecal fermentation assay was done according to Lebet et al. (1998) in an anaerobic chamber (BACTRONEX Anaerobic Chamber; Shel Lab, Cornelius, OR) with 85% N_2, 5% CO_2, and 10% H_2 atmosphere, in triplicate. Each of the substrates (50 mg) post upper GI digestion were then weighed into test tubes for fecal fermentation and transferred into the anaerobic chamber, and 4 mL of buffer was added to each of the tubes, and 1 mL of the fecal inoculum was added. Tubes with no substrate were negative controls. Tubes were closed with rubber stoppers and sealed, and incubated at 37^{o}C in a shaking water bath. Then, each set of tubes at appropriate time points, 6, 12, or 24 h, were removed from the water bath and total gas production was measured with a graduated glass syringe through the rubber stopper (Lebet et al., 1998).

The buffer for the assay was a carbonate-phosphate buffer sterilized by autoclaving at 121^{o}C for 20 min and reduced by adding cysteine hydrochloride. The fecal inoculum was prepared by homogenizing the fecal samples with a sterilized carbonate-phosphate buffer [feces:buffer 1:3 (w/v)]. The buffer contained a trace mineral mixture to meet growth needs of various bacteria, including; $FeSO_4$, $MnSO_4$, $ZnSO_4$, $CoCl_2$, $NiCl_2$, $CuSO_4$.

2.2.6 Short chain fatty acid (SCFA) analysis

The tubes were opened and aliquots were taken from the fermentation tube for SCFA analysis and DNA isolation. SCFA samples were combined with an internal standard of 4-methylvaleric acid, centrifuged at 13,000 rpm for 10 min and the supernatant were injected into a gas chromatograph equipped with a fused silica capillary column (NukonTM, Supelco No: 40369-03A, Bellefonte, PA) and a flame ionization detector (GC-FID 7890A, Agilent Technologies, Inc., Santa Clara, CA). Also, there was an external standard for each SCFA and branched SCFAs (Supelco, Bellefonte, PA). Branched short chain fatty acids were also measured, but were insignificant and not included in the results.

2.2.7 DNA analysis and Bioinformatics

DNA extractions were done using FastDNA SPIN kit for feces (PC: 116570200) (MP Biomedical, Santa Ana, USA) according to the manufacturer's instructions with slight modifications. Samples after aliquoted were stored at -80°C until DNA extractions were done. DNA extraction was done on the blanks at time 0 h for baseline microbiota composition, and 24-h time point for each of the substrates in triplicate. PCR of the V4 region of the 16S rRNA gene, 515F806R with a 2x153 read length, and sequencing was performed on the Illumina MiSeq platform (Illumina, Inc. San Diego, CA). PCR amplification and sequencing were performed at University Illinois Chicago Sequencing Core.

Illumina-generated sequencing data was analyzed using QIIME version 2 (Bolyen et al., 2019). Raw sequences for each sample consist of 2 readings, forward and reverse, – 153 base pairs in each direction - of the targeted V4 region of the 16sRNA gene. The paired reads were joined, and quality filtering of the sequencing data was done at a cut-off of a Phred Quality score of 20. Data denoising was done using Deblur within Qiime. Taxonomic classifier used was Greengenes 13_8 (Bokulich et al., 2018). α-Diversity of the samples is presented by Shannon's index, along with Chao1 in the Appendix section. Beta diversity ordination plots are shown (Figures 15, 16 and 17) according to Weighted Unifrac Emperor ordination plots, measuring the difference in phylogenic relationship and their relative abundance; which highlights the extent of the impact of the treatment on community composition from on sample relative to another (C. Lozupone & Knight, 2005).

Taxonomic analysis at the genus level for each donor was used to create bar plots with relative abundances for genera present at above 1%; were made in RStudio using the qiime2R library. A heat map was constructed (Figure 14) by selecting the top 5 most increased genera, in relative abundance increase from the initial, listed in Appendix C Table A1. The percentage increase in relative abundance for each bacteria genus was plotted for each treatment for each donor to highlight distinct donor microbiome responses to the same treatment.

2.3 Results

Substrates were grouped for easy comparison, statistics and visualization in the broadest categories of tubers, grains, and pulses where appropriate. Other groupings of substrates to consider include processing conditions used, whole food flours compared to isolated food components (e.g., brans), and all whole food sources compared to FOS.

The three fecal donors; Donor X, Donor Y and Donor Z, have distinct responses to the same treatment in the microbiome analysis with regards to diversity and genera changes; highlighting donor specific effects. However, the three donors have similar responses to the same treatment with regards to SCFA and gas production and so average responses across all donors could be compared; highlighting treatment effect.

In order to account for non-fiber amount in each sample that would remain after upper GI digestion, inverse TDF proportions were multiplied by gas and SCFA values for a fiber normalized data set (shown in the Appendix A along with statistical differences in Appendix D). However, fiber normalized values seem to exaggerate differences in some substrates when graphed and not shown in Figures 1, 2, and 3.

2.3.1 Gas and SCFA production observations

Somewhat surprisingly, both gas and SCFA values across the three distinct donor's microbiotas were quite similar, as seen in the small standard error bars on the graphs (bars are averages of the three donors). This suggests that despite microbiota community structure differences for the three donors (as seen below), they responded functionally in terms of SCFA outputs similarly. This is a good sign for a commercial product, where say high fermentability of whole food fibers would result in similar responses among consumers. Since gas and SCFA are

both biproducts of metabolism by the microbiota, they would be expected be similar for each substrate, which is the case in these results. Total SCFA, acetate, propionate, and butyrate aligned for each substrate, implying that the substrates stimulated a proportional amount of each of the SCFAs. The fermentation gas and SCFA results are displayed in categories to be easier visualized, and positive and negative controls of FOS and blank were also graphed for comparison.

2.3.2 Gas production/fermentation rate

Gas measurements taken over time are an indication of fermentation rate, as well as extent of fermentation. FOS is the positive control, as it is a fast fermenting fiber. Inulin, which is somewhat larger than FOS but of the same composition, is similarly fast fermenting. A fast fermentation rate, such as FOS could indicate a potentially intolerable substrate, due to bloating caused by rapid gas production from bacterial fermentation. *In vitro* gas production rate gives inferences about a fiber's tolerability; for example, if a fiber is as rapidly fermented as FOS then it would be expected to cause bloating similar to FOS. A sustained or delayed fermentation rate could indicate a sustained bacterial fermentation distally along the colon. Measurements for each treatment, for each donor, was done in triplicate by a glass syringe puncturing each anaerobically sealed fermentation tube.

FOS was the fastest metabolized substrate with little gas production increase from 12 h to 24 h. Substrates that showed delayed fermentability would be expected to be metabolized slowly and more distally by the microbiome, thereby impacting structural and functional benefits of the fibers distally in the GI tract. Substrates that started at a lower gas production at 6 h, but then steadily increased at 12 and 24 h would be expected to be tolerable, as it is rapid gas production that causes discomfort. The 24 h gas production value and total SCFAs are good indicators of the extent of fermentability of the substrates to the microbiota.

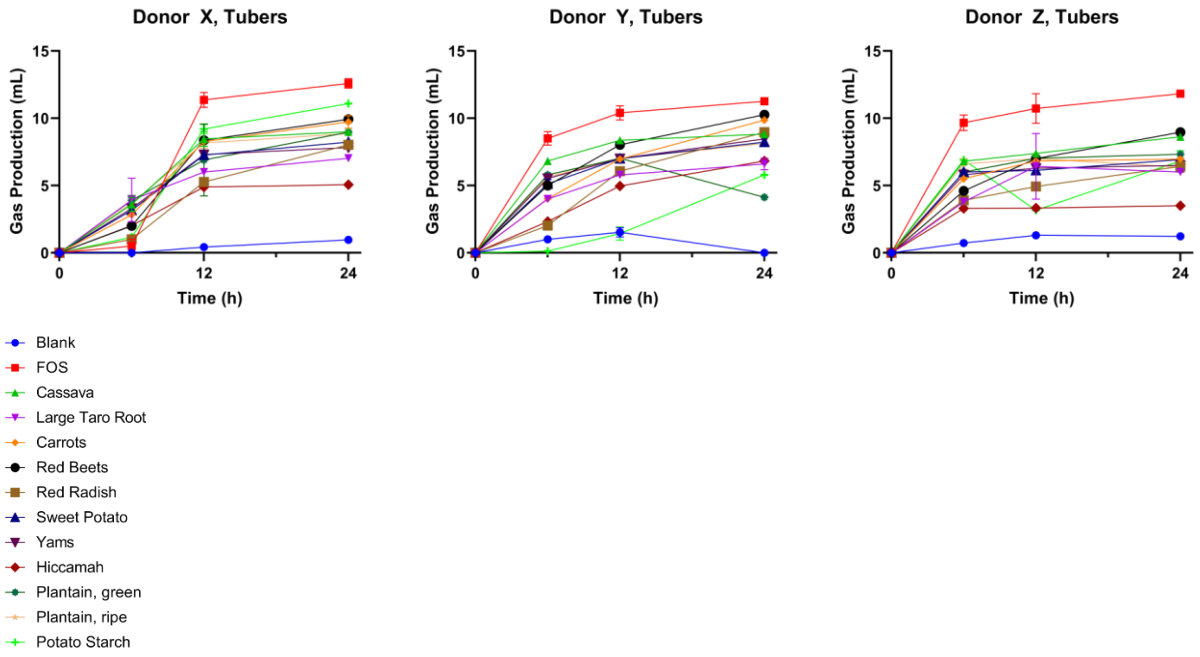

Figure 1. Gas production across 6, 12, 24 h timepoints for each donor separately, for the root/tuber series.

In Figure 1, the fermentation of roots/tubers over time show similar effects as a group across all donors. Cassava, Red Beets, Carrots were consistently relatively more rapidly fermented and accessible. Hiccamah was relatively less accessible by the microbiota than then other tubers, which otherwise mostly grouped together. Possibly the unique characteristics of Hiccamah specifically makes it less accessible to the microbiota relative to other tubers.

Tubers were shown in all donors to yield a responsive fermentation profile, especially relative to the blank, which would establish these substrates' prebiotic potential. Hiccamah had a relatively lower gas production in all of the donors, compared to the other tested substrates. Each of the substrates in the tubers category had a similar fermentation profile pattern overall, except for Potato Starch – which was an additional control. Yams, carrors, and cassava had consistently high, relative to other tubers, fermentation. Large Taro Root is consistently fermented near the middle of the category.

Potato Starch was an additional control in the roots/tuber category, and was not subjected to the upper GI digestion assay prior to fermentation. Since the starch lacks extensive fiber linkages in other substrates it would be expected to be fermented rapidly similar to FOS. Potato Starch response in Donor Y was delayed until 24 hours of fermentation, lower than expected gas

production response at hour 6. In Donor Z, Potato Starch had a lower 12 h gas production compared to the 6-hour time point. This could be due to the Potato Starch not being subjected to the upper GI digestion, which included a boiling step, and so the starch was left raw and mostly ungelatinized. However, in Donor X, the fermentation profile of Potato Starch was normative relative to other substrates, and has a different profile compared to Donor Y and Z. The microbiota community composition or function of Donor X was better able to metabolize potato starch at the 6 and 12 h time points.

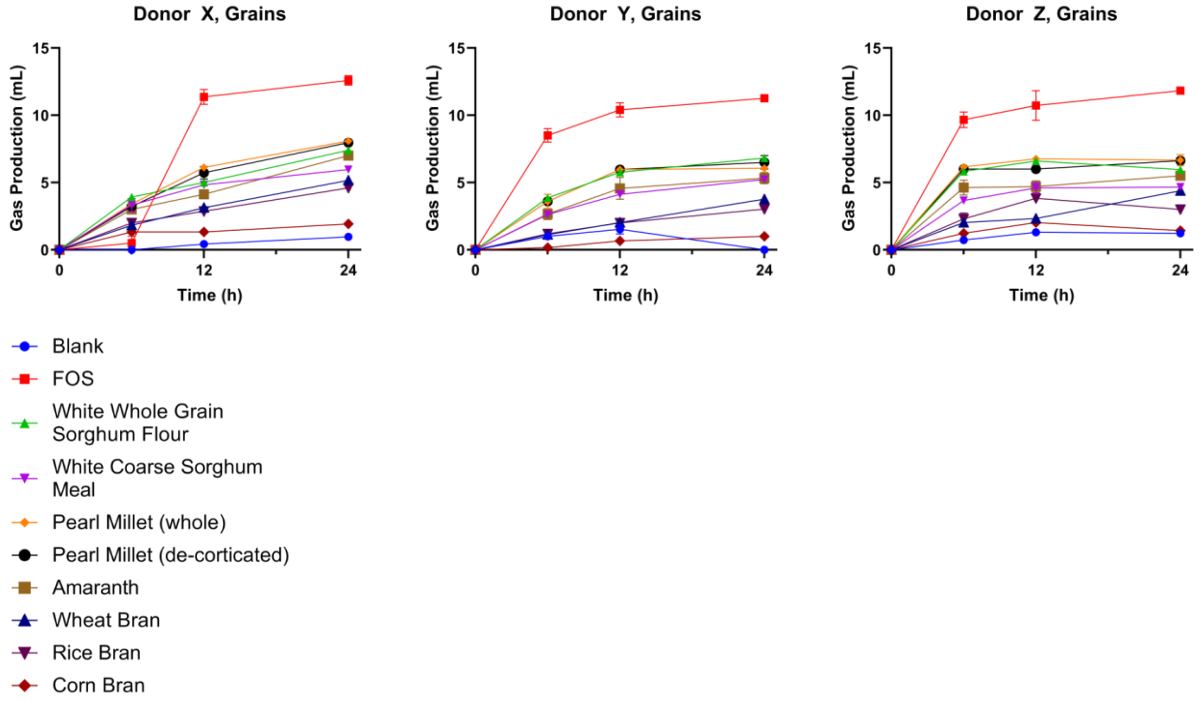

Figure 2. Gas production across 6, 12, 24 h timepoints for each donor separately for the grain series.

In Figure 2, within the grains category, the brans were less accessible to the microbiota compared to the other treatments, and specifically Corn Bran was the least fermentable in all donors. Pearl Millet – Whole and White Whole Grain Sorghum Flour were more readily fermentable, compared to other grains, in all donors. Pearl Millet – de-corticated was also fermented well in Donor Y and Z, but not as well in Donor X; possibly due to microbiota structural differences.

As expected, brans; Corn Bran, Wheat Bran, Rice Bran – had low fermentation results compared to other grains, this would be due to the extensive carbohydrate cross linking in the bran portion of the grain, specifically. Corn Bran was the lowest fermented substrate, and Rice Bran and Wheat Bran were both more easily fermented compared to corn bran. White Whole Grain Sorghum Flour, Peart Millet Whole and Pearl Millet De-corticated were the most easily fermented grains, followed by; Amaranth, and White Coarse Sorghum Meal.

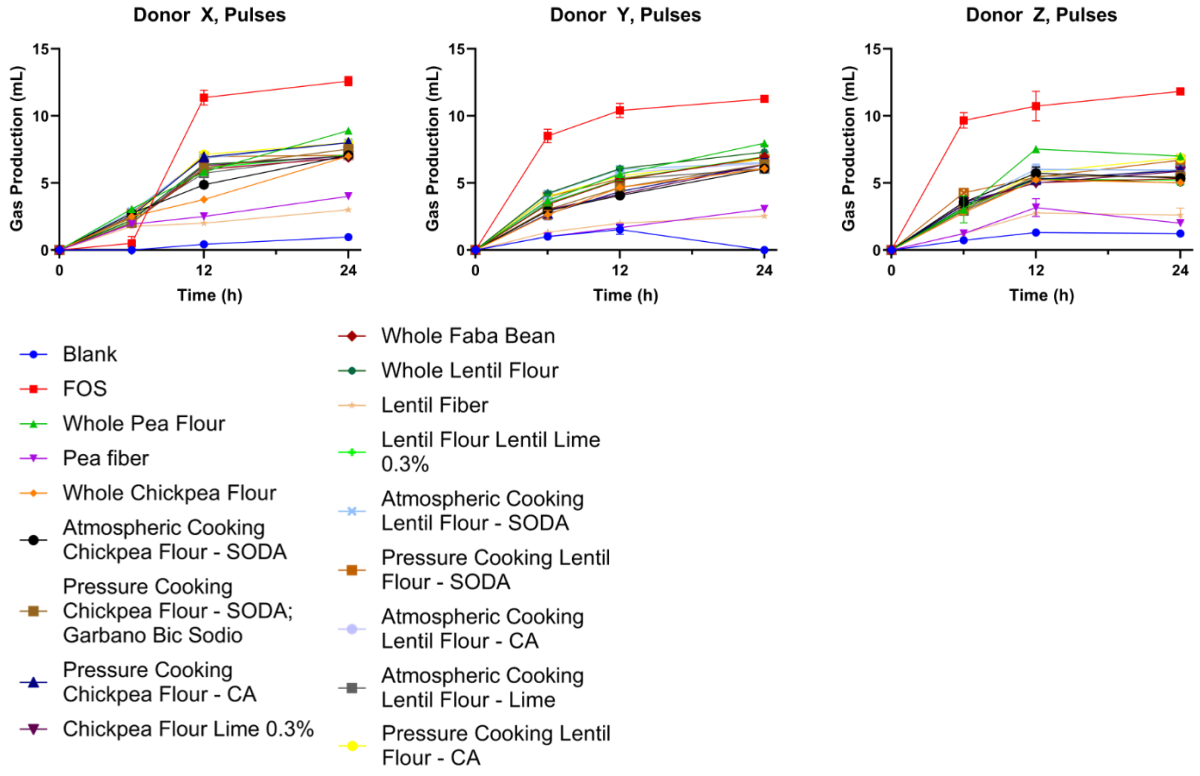

Figure 3. Gas production across 6, 12, 24 h timepoints for each donor separately for the pulse series.

In the pulse category shown in Figure 3, Pea Fiber and Lentil Fiber were both poorly fermented; this could be due to more concentrated and complex isolated fiber structures compared to whole flour forms of pulses. Whole Pea Flour was consistently the most fermentable pulse across all donors, however, all other pulses besides Pea and Lentil Fiber, grouped together in their fermentation profiles.

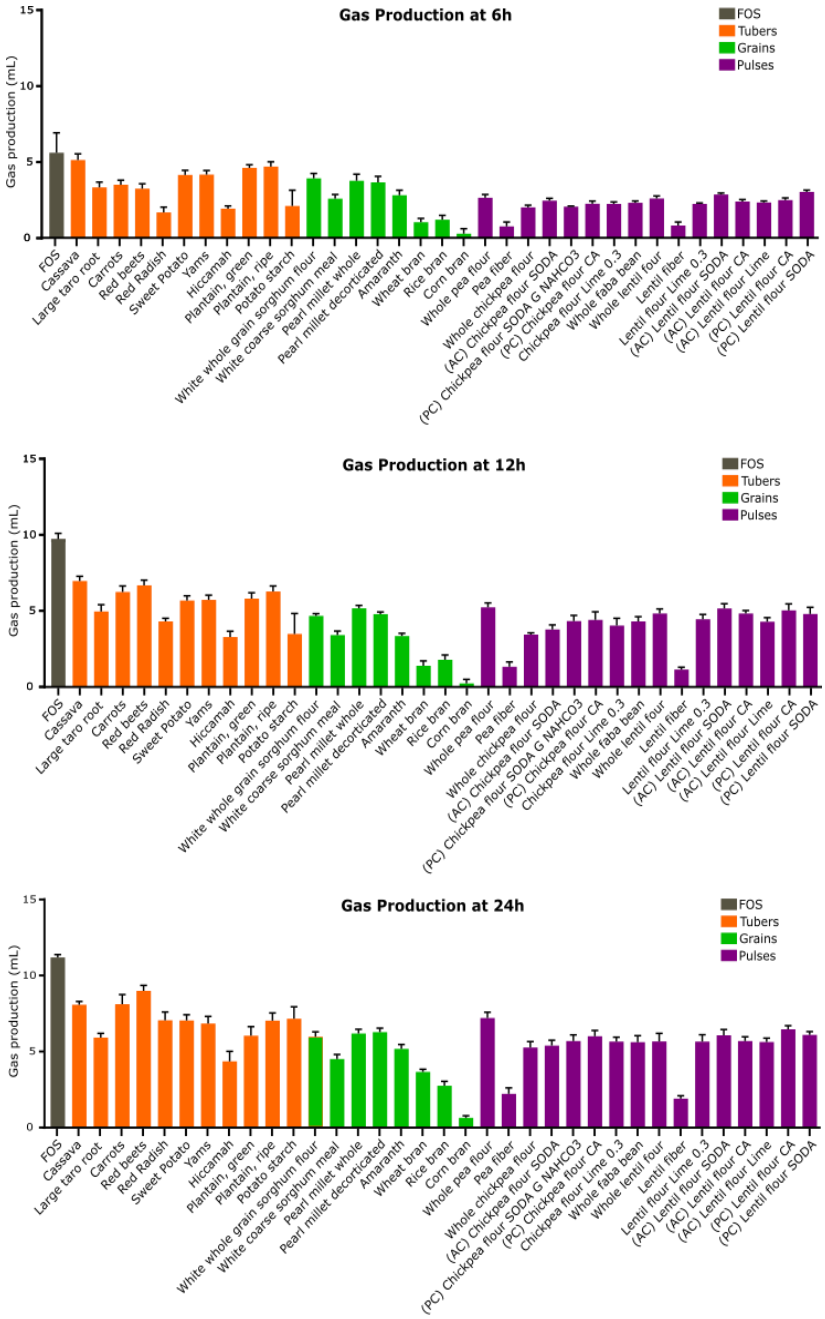

Figure 4. Gas production at 6, 12, and 24 h of fecal fermentation averaged across the 3 donors.

In Figure 4, averages the Gas Production measurements across all 3 donors in order to compare treatment effects rather than individual donor responses, standard Error of the Mean was used to generate the error bars and show similar variance across all donors for the same treatment. Since the blank, serving as the negative control with no substrate added to the fermentation tube,

values were different for each donor, perhaps due to residual fiber amount in the feces; the average of the blank were subtracted from each treatment to normalize the gas production amount by each donor in order to compare values at the same initial gas production relative to the other donor.

Figure 4 indicates that in the Gas Production data treatment effects, rather than donor specific effects, primarily cause the shared responses in all donors due to the small error bars. Additionally, whole food fibers from tubers/roots, cereals, and pulses fermented quite well overall relative to FOS.

2.3.3 SCFA production

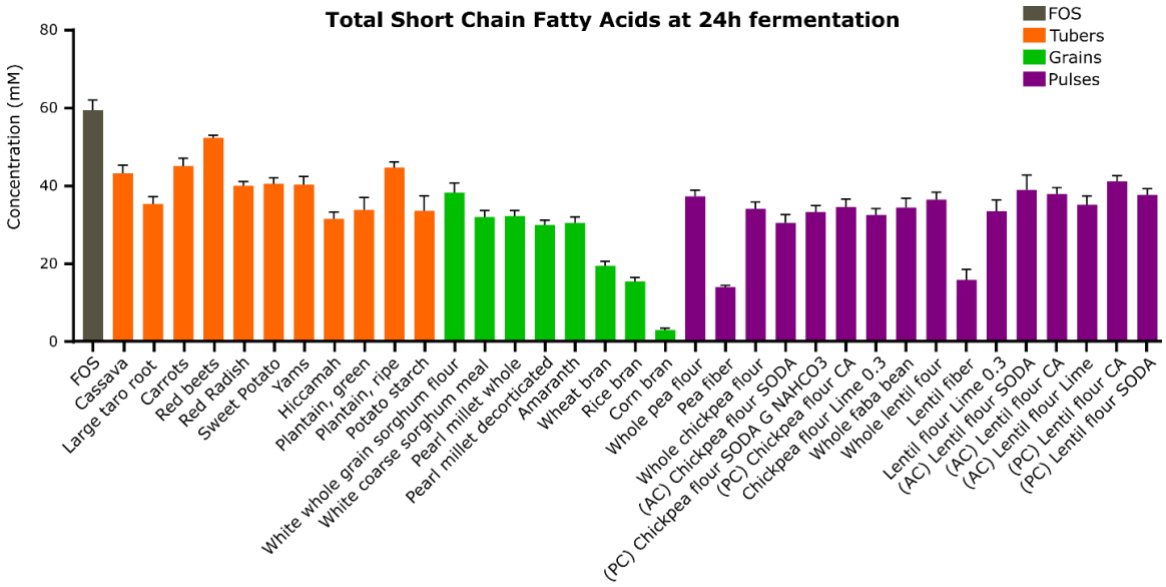

Figure 5. Total SCFA production at 24 h of fermentation averaged across 3 donors.

In Figure 5, averages the total SCFA measurements across all 3 donors in order to compare treatment effects rather than individual donor responses, standard Error of the Mean were used to generate the error bars and show similar variance across all donors for the same treatment. Since the blank, serving as the negative control with no substrate added to the fermentation tube, values were different for each donor, perhaps due to residual fiber amount in the feces; the average of the

blank were subtracted from each treatment to normalize the total SCFA production amount by each donor in order to compare values at the same initial SCFA production relative to the other donor.

Figure 5 indicates, similar to Figure 4, that in the SCFA Production data treatment effects, rather than donor specific effects, primarily cause the shared responses in all donors due to the small error bars. Additionally, Figure 5 shows that whole food fibers from tubers/roots, cereals, and pulses fermented quite well overall relative to FOS. In general, Gas Production and SCFA results mirror each other since they are both measuring metabolic biproducts of fermentation by the collective fecal microbiota. 24 h time points showing total SCFA output caused by the treatment provides an approximation of total accessibility, whereas the gas production fecal fermentation profile provides a rate of fermentation approximation across the GI tract, and also an indicator of tolerability *in vivo*.

Overall, tubers were somewhat more fermentable by the gut microbiota and yielded more SCFA production, followed by pulses, and then grains. Whole foods or flours also yielded more SCFA production by the microbiota and the fibers were likely more accessible to the microbiota than isolated fibers; for example, Carrot and Whole Grain Sorghum were more accessible compared to densely packed complex fibers in Corn Bran.

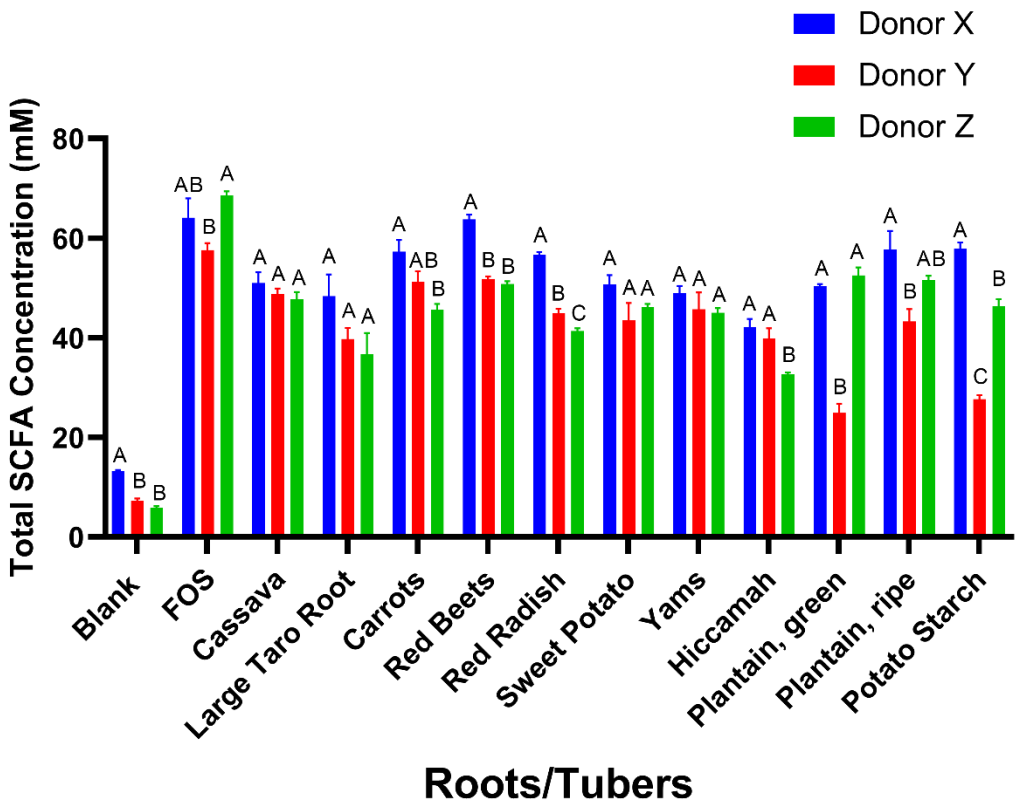

Figure 6. Total SCFA production at 24 h of fermentation for each donor separately for the root/tuber series.

In Figure 6, the mean and deviation for each donor is compared relative to the other donors' only within the same treatment, showing donor specific responses. Donor response that does not share a letter with the response of another donor in the same treatment are significantly different at a α=0.05 significance level.

Donor Y's microbiota often fermented less roots/tubers, and drastically less Potato Starch and Plantain, green relative to Donor X and Donor Z. Perhaps specific bacterial community composition or enzymatic preferences by the bacteria due to conditioning by diet or other lifestyle factors contributed to this unique response by Donor Y.

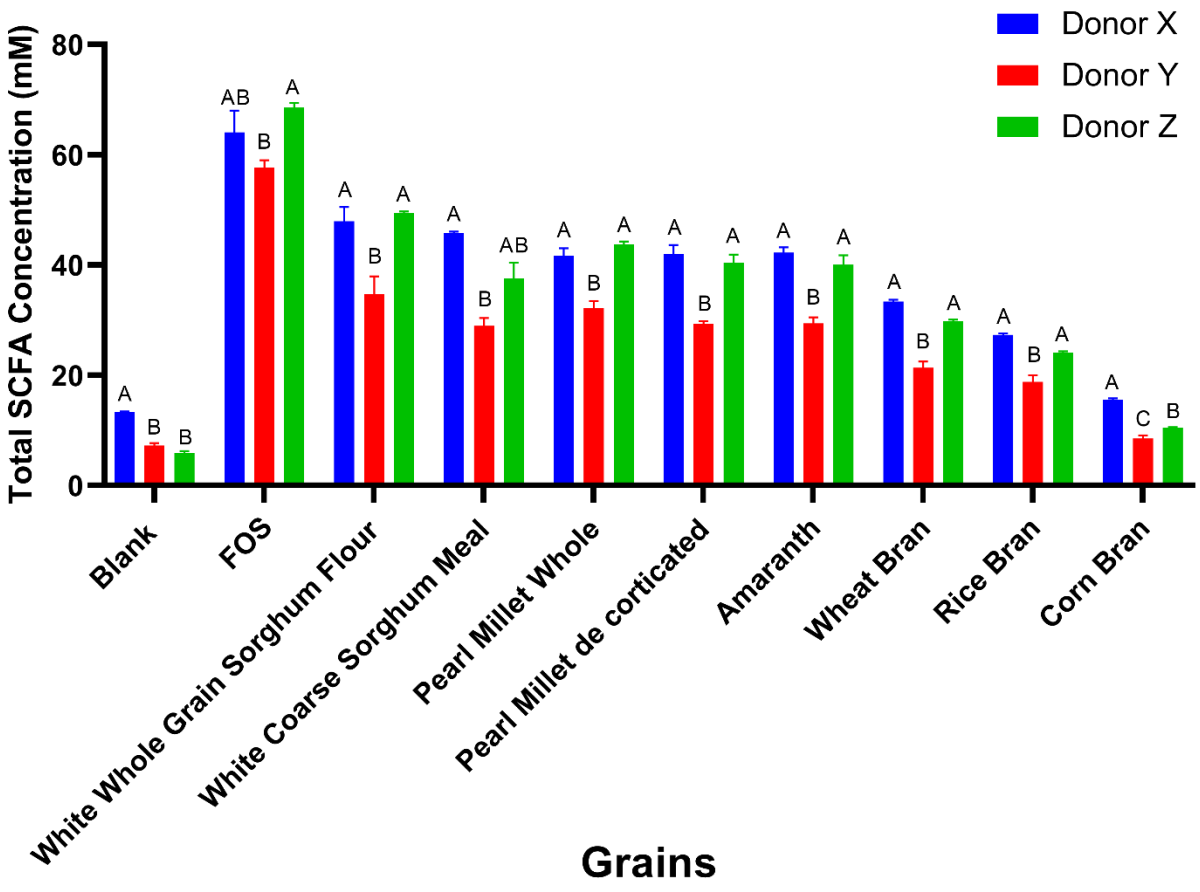

Figure 7. Total SCFA production at 24 h of fermentation for each donor separately for the grain series.

In Figure 7, the mean and deviation for each donor is compared relative to the other donors' only within the same treatment from the grains category, showing donor specific responses. Donor response that does not share a letter with the response of another donor in the same treatment are significantly different at a α=0.05 significance level. Donor Y microbiota also produced less total SCFA production compared to Donor X and Donor Z in the grains category consistently in all the treatments.

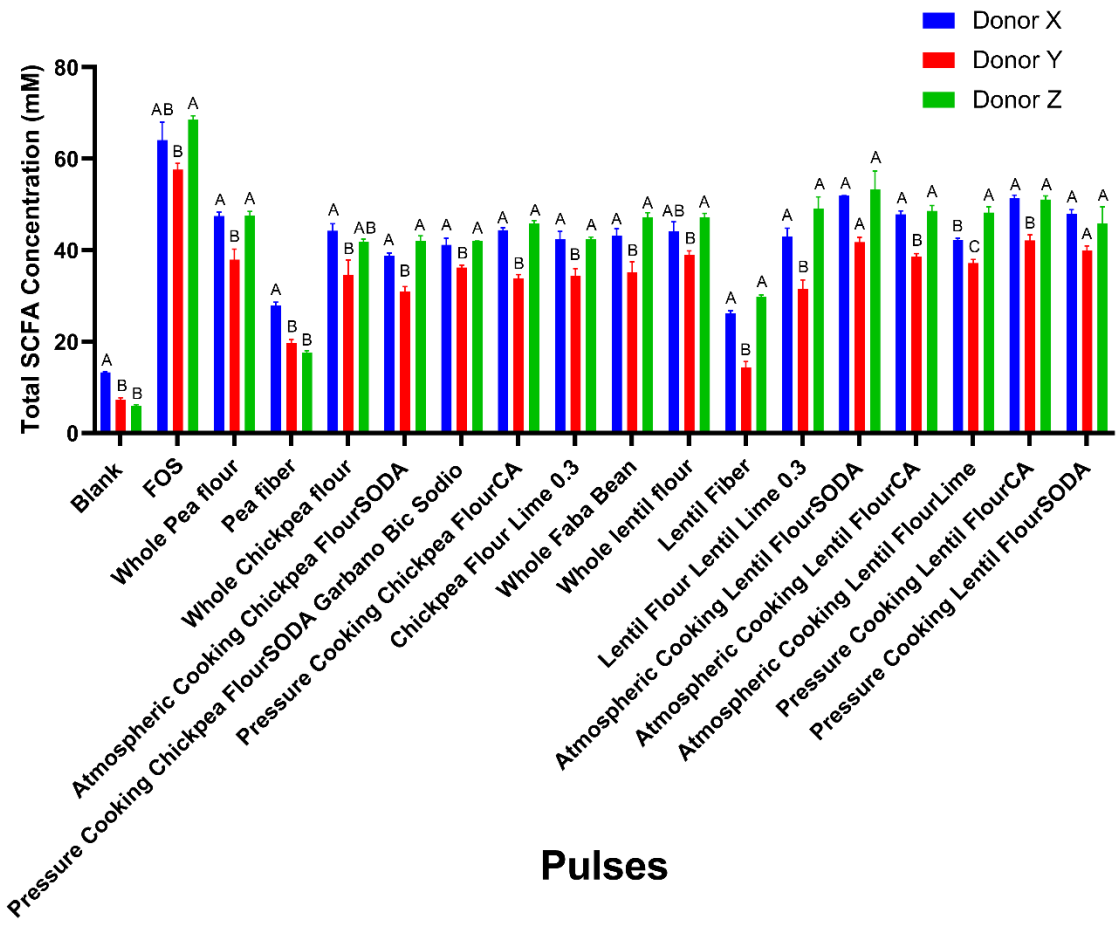

Figure 8. Total SCFA production at 24 h of fermentation for each donor separately for the pulse series.

In Figure 8, the mean and deviation for each donor is compared relative to the other donors' only within the same treatment for pulses, showing donor specific responses. Donor response that does not share a letter with the response of another donor in the same treatment are significantly different at a α=0.05 significance level. Donor Y microbiota produced less total SCFA than Donor X and Z in all treatments in the pulse category, except for Pea Fiber. It is possible that in the case of Donor Y, Pea Fiber would be a more efficacious prebiotic supplement compared to other pulses due to the relative ability to specifically metabolize Pea Fiber for Donor Y's microbiota. Overall, Donor Y consistently has a less metabolically active microbiota compared to Donor X and Donor Z.

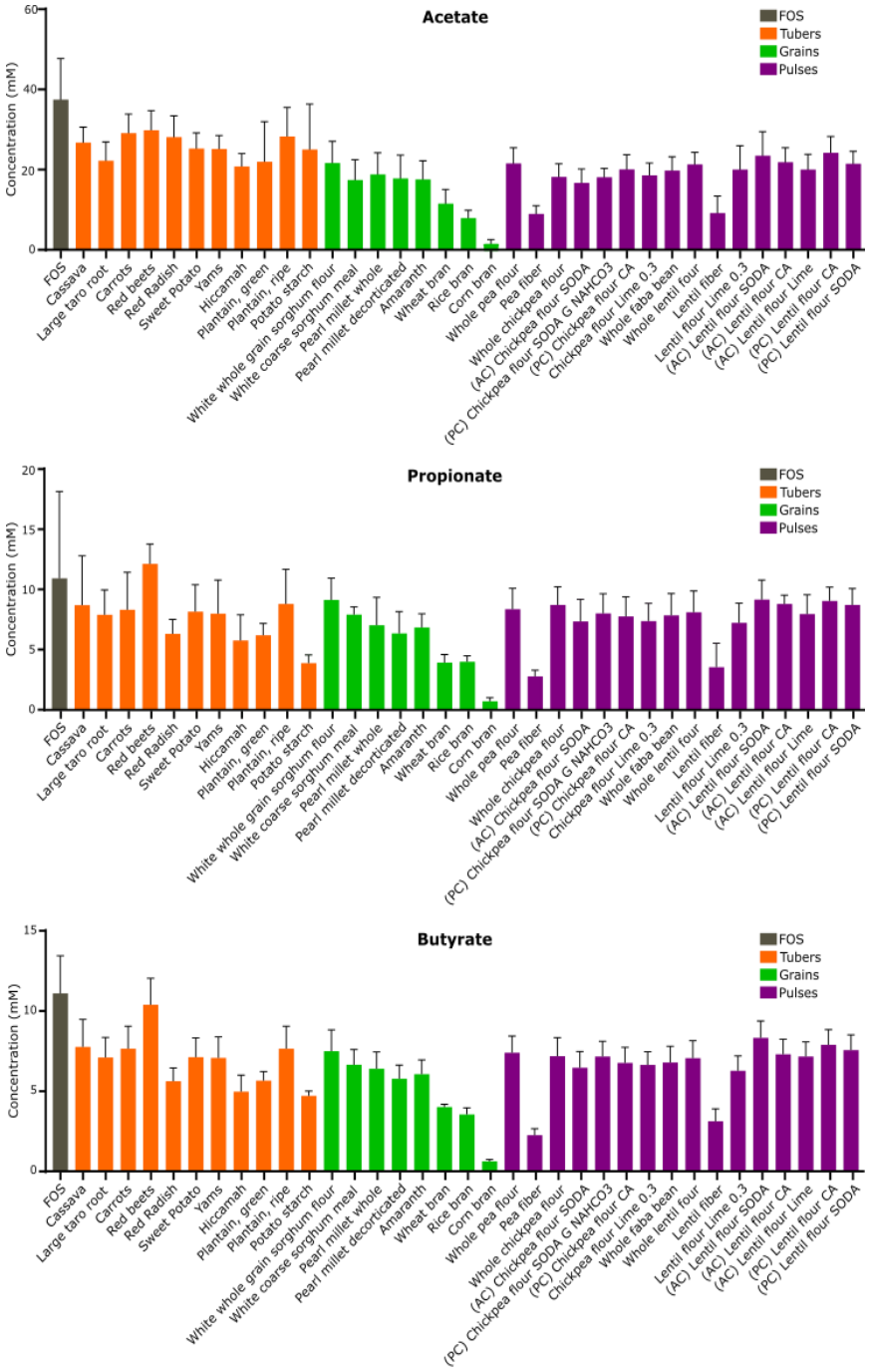

Figure 9. Acetate, propionate, and butyrate at 24 h fermentation averaged across 3 donors.

Figure 9 shows the concentration of Acetate, Propionate, and Butyrate as an average across 3 donors with error bars calculated by Standard Error of the Mean. As expected, acetate is the predominant SCFA produced by the microbiota, followed by propionate, and then by butyrate. Treatments that yield relatively high or low metabolic output by the microbiota for one SCFA also

consistently yield a similar result of the other SCFAs. Since, each SCFA has health benefits, often overlapping, total SCFA will be used to primarily compare treatment prebiotic potential efficacy.

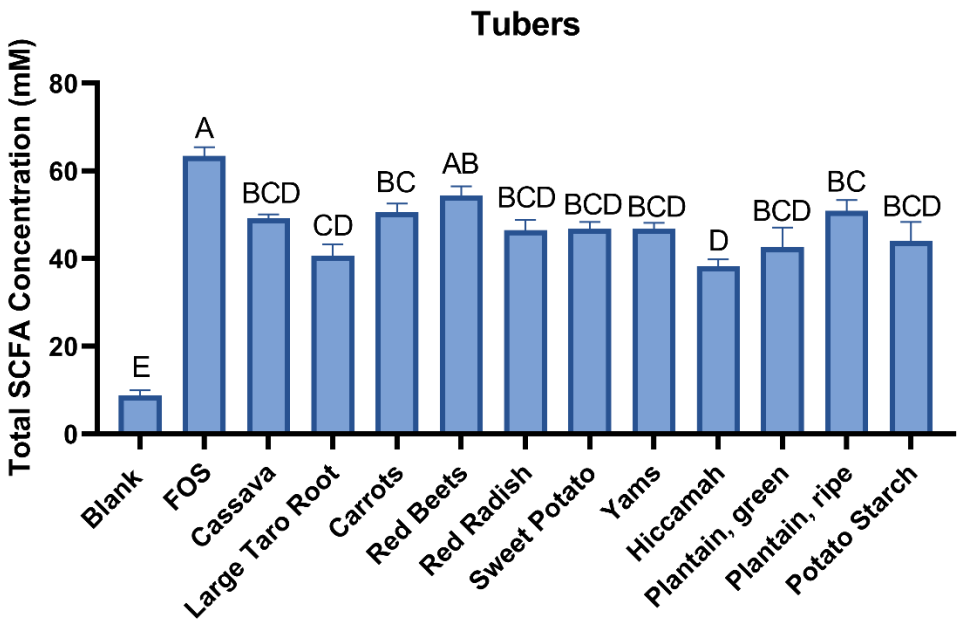

Figure 10. Total SCFA production at 24 h of fermentation averaged across all donors for the roots/tuber series.

In Figure 10, the mean and deviation for all donors within the same treatment are averaged and compared relative other treatment averages in total SCFA production for Roots/Tubers, showing treatment specific responses. Treatment responses that does not share a letter with the response of another treatment are significantly different at a α=0.05 significance level. As expected, FOS produced the most total SCFA since it is the most accessible substrate, however unexpected was how readily fermentable Red Beets as a treatment is relative to FOS with statistically insignificant difference in their total SCFA production. All tubers yielded more total SCFA than the Blank.

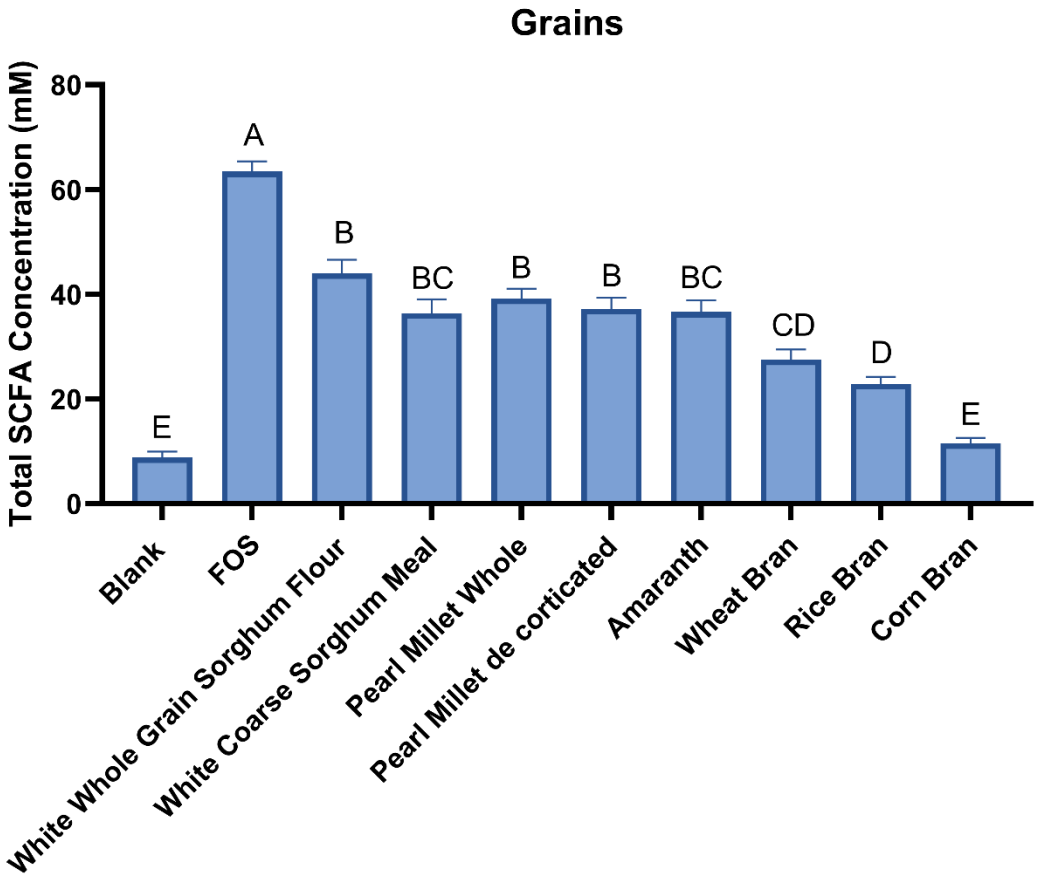

Figure 11. Total SCFA production at 24 h of fermentation for each donor separately for the grains series.

In Figure 11, the mean and deviation for all donors within the same treatment are averaged and compared relative other treatment averages in the pulse category, showing treatment specific responses. Treatment responses that does not share a letter with the response of another treatment are significantly different at a α=0.05 significance level. Corn Bran difference in total SCFA to the Blank was statistically insignificant, showing the extent towhich it is inaccessible by the microbiota.

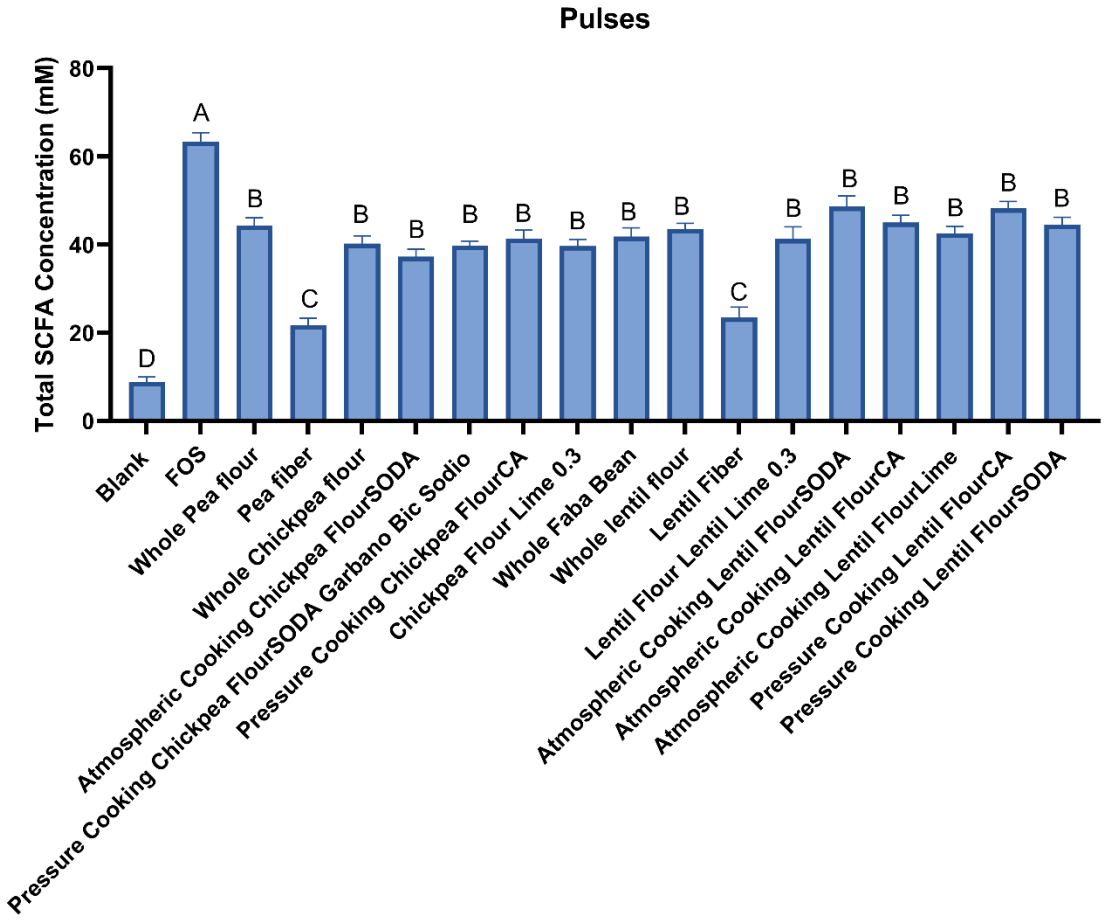

Figure 12. Total SCFA production at 24 h of fermentation for each donor separately for the pulse series.

In Figure 12, the mean and deviation for all donors within the same treatment are averaged and compared relative other treatment averages in the pulse category, showing treatment specific responses. Treatment responses that does not share a letter with the response of another treatment are significantly different at a α=0.05 significance level. All pulses tested, except for Pea Fiber and Lentil Fiber, had statistically insignificant differences in total SCFA production. The pulse category had various processing steps performed on treatments by our industry partner, PepsiCo. These processing effects had statistically insignificant impact on total SCFA. This finding could be useful to food companies as they access various processing steps on fermentation properties of food ingredients/products; and that the processing steps tested here were not negatively impactful to the total SCFA produced by the gut microbiota.

2.3.4 Gut microbiome analysis

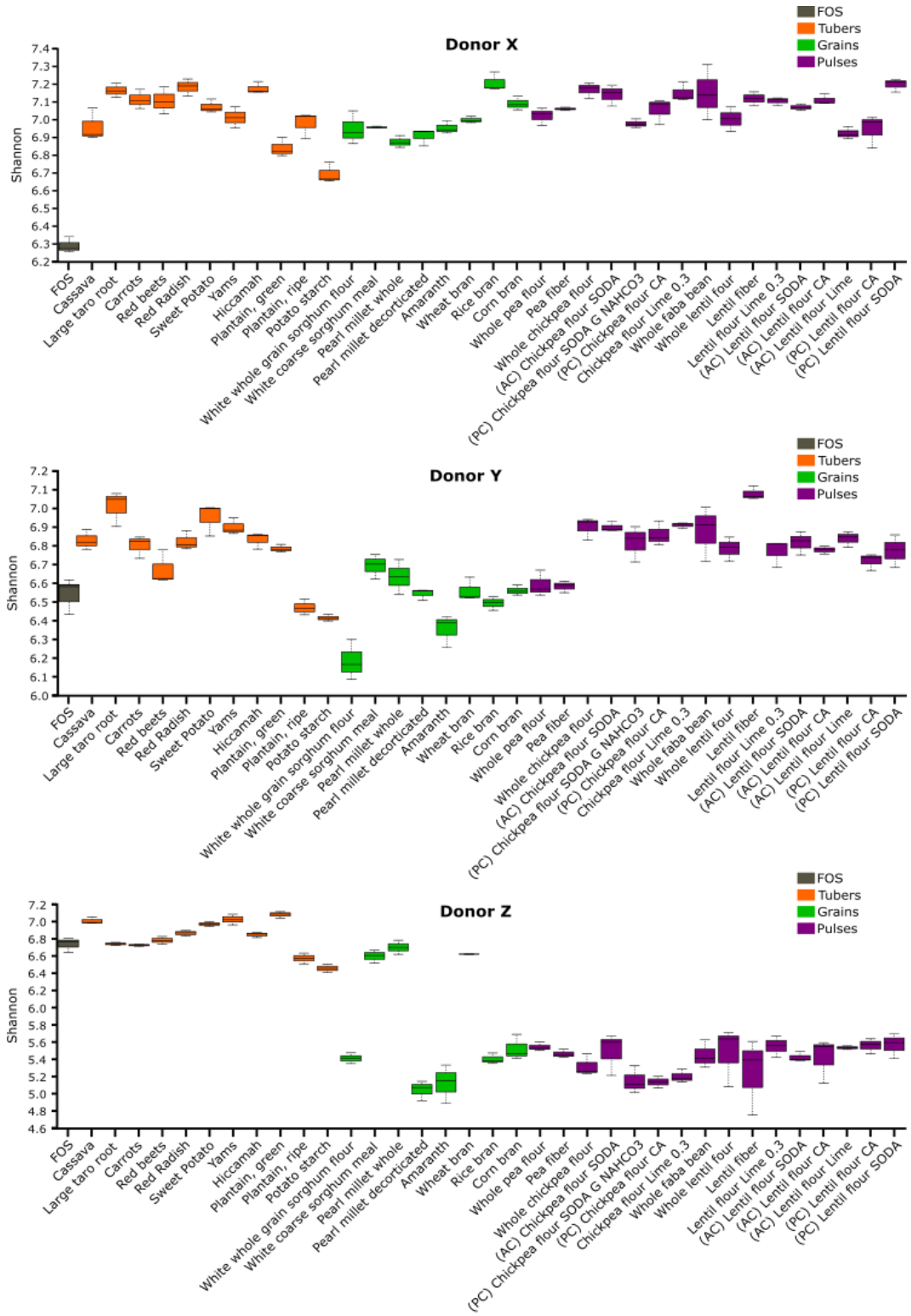

Figure 13. Shannon alpha diversity scores for Donor X, Y, and Z at 24 h of fermentation.

In Figure 13, alpha diversity scores and bacteria genera increases after 24 h of fermentation varied considerably among the 3 donors for the same substrates. Individual fecal microbiota from each donor, when provided the same fiber substrate, resulted in different bacterial group relative abundance increases. These inter-individual differences highlight the specific microbiota community structural dynamics when aiming to strategically manipulate the human gut microbiome using diet in order to increase a specific bacterial group of interest. Shannon Index was used as the principal metric for accessing alpha diversity since it considers richness and evenness, and also because other widely used alpha diversity metrics like Chao1, Faith, and Pielou Evenness overlap and mostly align with the Shannon Index. The Alpha Diversity

We hypothesized that alpha diversity would increase for the tested fibers compared to FOS, because they contain different fiber structures for support of different bacteria. Donor X alpha diversity increased in all substrate treatments relative to FOS (Figure 13). This could be due to the regular dietary pattern and community composition in Donor X specifically. Other Donors did not have as uniform of an increase of alpha diversity relative to FOS. Donor Y showed many substrates exceeding or close to the alpha diversity increase by FOS, with White Whole Grain Sorghum relatively lower alpha diversity. Donor Z has FOS increasing alpha diversity and all tubers and two grains also showing similar increase. Other grains and all the pulses had decreased alpha diversity relative to FOS. Overall, there was an increase in alpha diversity scores close to, or exceeding FOS in Donors X and Y with most treatments, and in Donor Z with the tubers/roots. Donor Z has a reduction in alpha diversity for the samples that cause a sharp relative rise in Shigella in Figure 15; as one particular group of bacteria decreased the evenness of the sample thereby lowering the alpha diversity.

Substrates that were low in accessibility by the microbiota overall, measured by gas and SCFA production, impacted the gut microbiome alpha diversity of the overall community. For example, corn bran, wheat bran, rice bran, and lentil fiber were not very well fermented, however increased alpha diversity in two of the three donors. This could be due to the complex fiber structures requiring more diverse bacteria to degrade and metabolize the substrates. Additionally, substrates low in the overall accessibility by the community could selectively stimulate specific groups of bacteria that would thereby increase the overall alpha diversity in the sample; these effects can be seen in the heat map as substrates stimulate the growth of specific bacteria even when the overall accessibility was low. These effects are donor specific due to the community

differences in each fecal inoculum as to which specific bacterial group gets stimulated in that community dynamic when presented the same substrate.

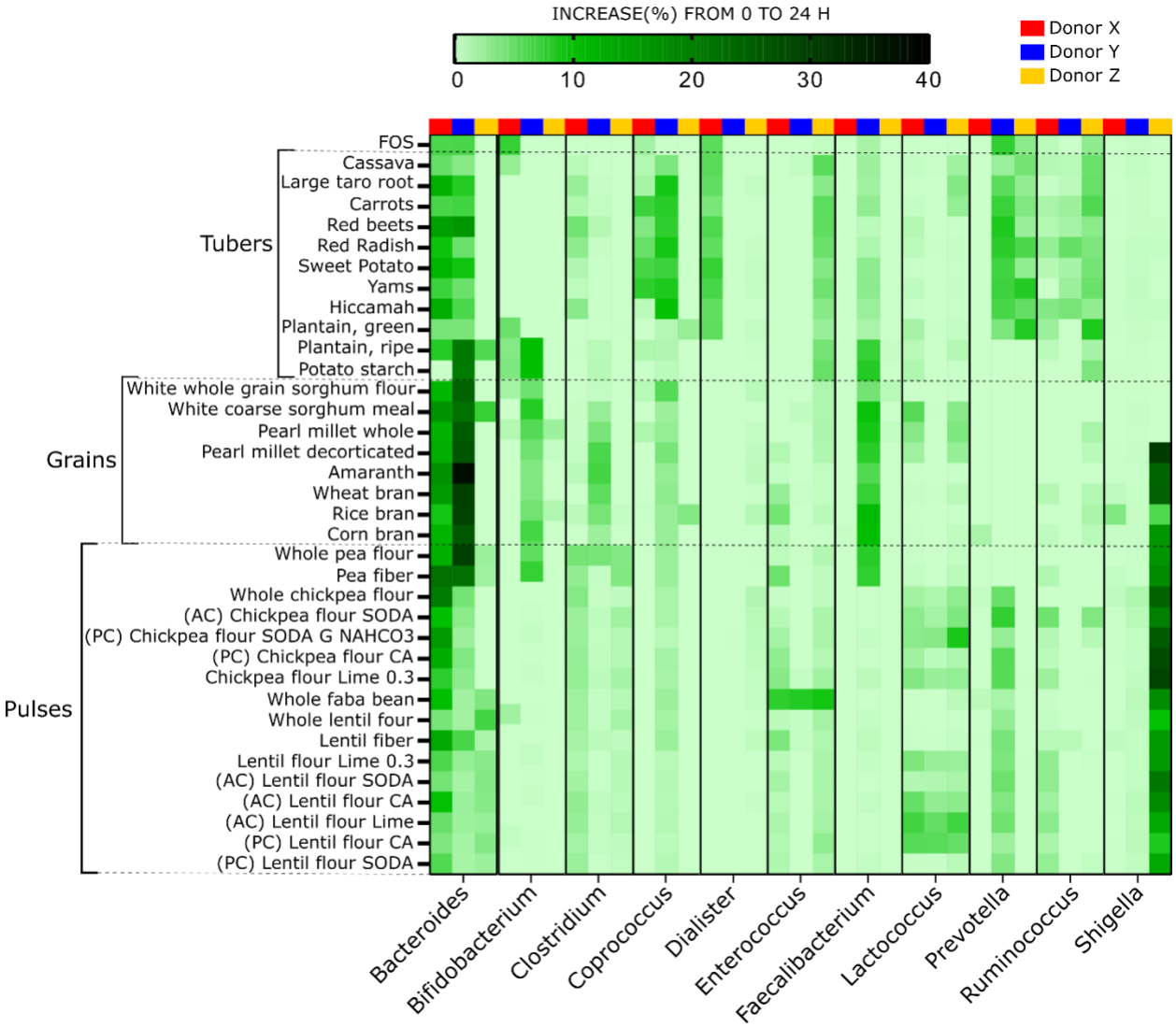

Figure 14. Heatmap of the top relative abundance bacterial genera increase from the initial to the 24 h time point in each donor.

The most increased bacterial groups at the genus level were arranged on a heat map in Figure 14 indicating the relative abundance increase from the initial inoculum for each donor (Table A1) that were observed in all donors. This highlights donor specific responses to the same treatment, noting how the similarity in the Donor SCFA and gas responses did not cause the same bacterial groups, alpha or beta diversity to shift in the same manner. This could imply a collective

enzymatic functional redundancy in the microbiota's ability to metabolize the substrate in the sample. Since the bacteria are present in each donor; the same substrate did not necessarily increase the same bacteria in all donors to the same degree; implying that ecological or competitive features in each microbiome sample determines which bacterial group increases relatively in the sample or not.

In Figure 14, Donor Z unique to the other donors had a sharp increase in the genus *Shigella* in relative abundance for most of the pulse samples and Pearl Millet de-corticated, Corn bran, Wheat Bran, and Amaranth – but none of the other grains. *Bacteroides*, in Donor Y, uniquely to the other donors, had a sharp increase as well in the grains series and Whole Pea Flour and Pea Fiber – but none of the other pulses, and ripe plantain, but not green plantain - but none of the other roots/tubers. These donor differences in how the same substrate affected the relative abundance in the samples would suggest community dynamics in each sample dictate the changes in bacteria in the sample over the individual treatment effect. Additionally, category effect was inconsistently observed; suggesting that similar food substrates with similar profiles do not yield the same bacterial changes. These observations would require additional reductionist testing in each group and donor to determine why particular bacterial ecological samples change in one donor versus another donor by the same substrate treatment. Also, drastic bacterial changes in one donor did not alter in one substrate vs. another drastic changes in the SCFA or gas production measurements, implying that the bacterial communities have functional redundant metabolic outputs and SCFA and gas measurements are more consistent measurements of microbiota activity regardless of the genus level changes in the sample.

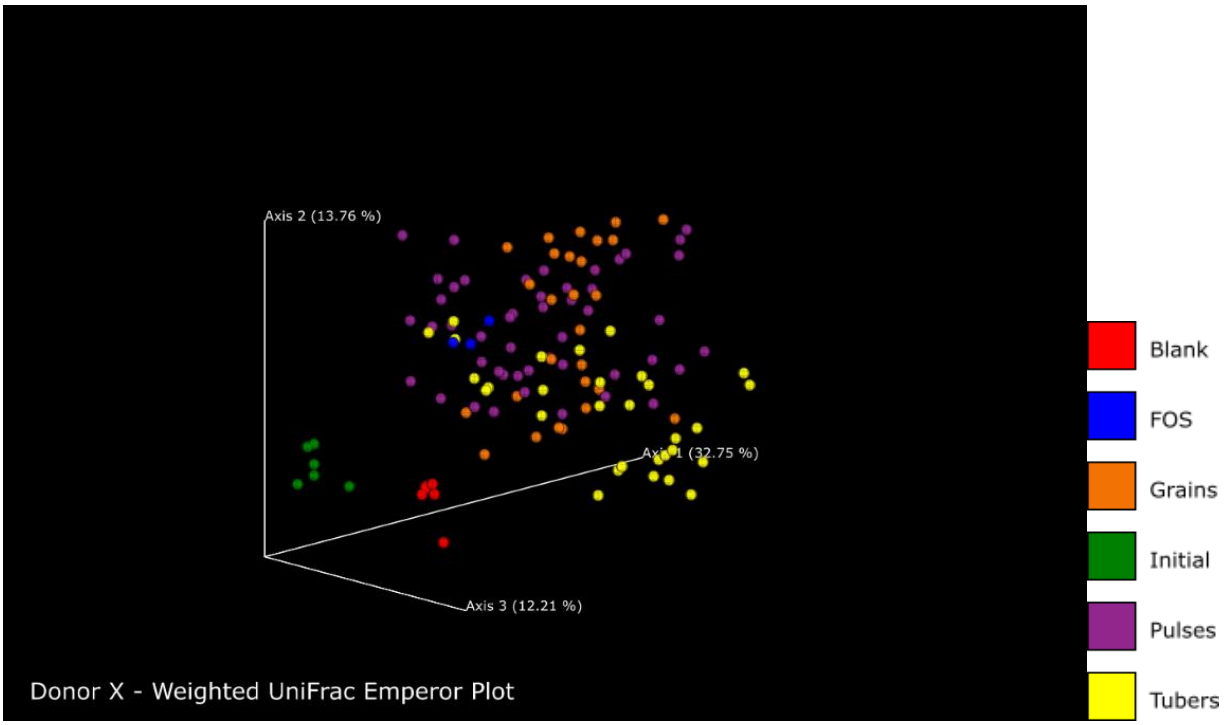

Figure 15. β- Diversity Donor X – Weighted UniFrac Emperor Plot by Category

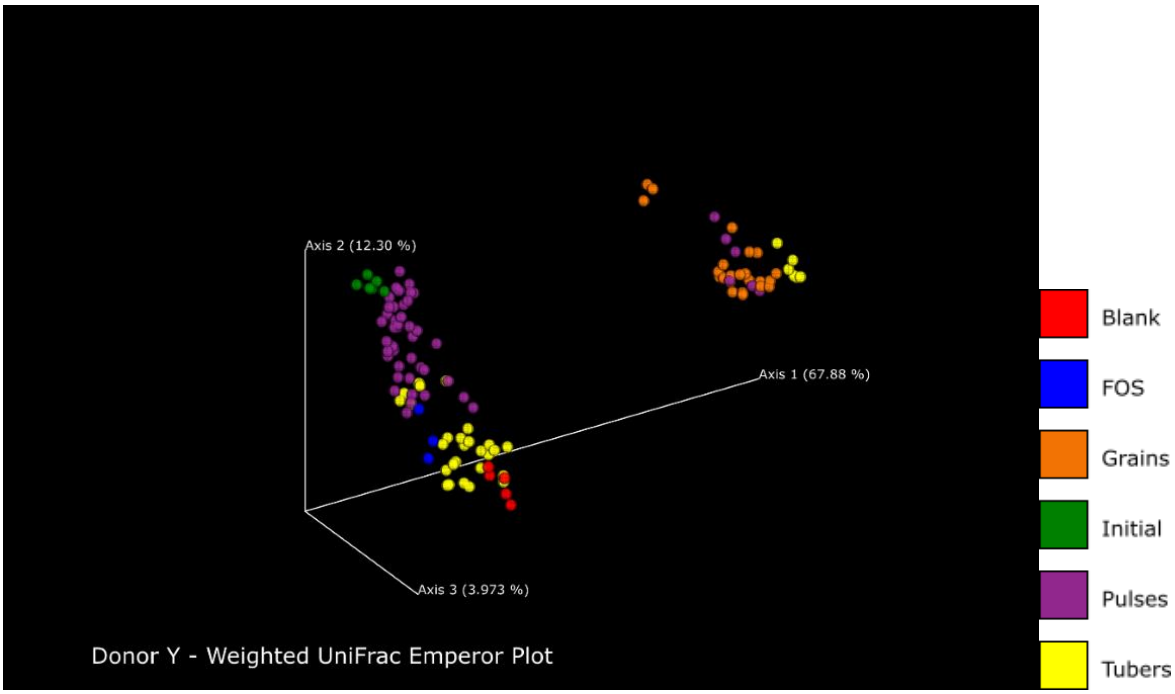

Figure 16. β- Diversity Donor Y – Weighted UniFrac Emperor Plot by Category

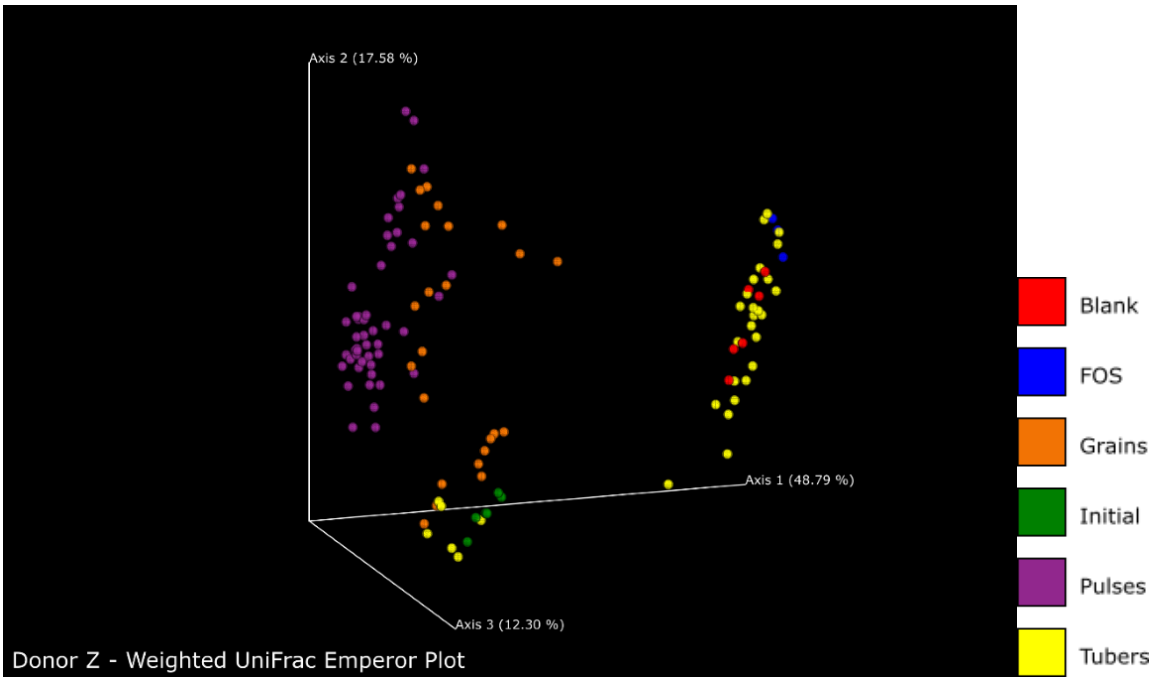

Figure 17. β- Diversity Donor Z – Weighted UniFrac Emperor Plot by category.

In Figure 17, beta diversity plots show an impact of the treatments – grouped in their respective categories - on the initial group microbiome community compared to the blank - which had no substrate, and FOS. The Weighted Beta Diversity Emperor plots considers, not just the bacterial species that are different, but also weights the magnitude of relative abundance change of a species compared to the other test group. Each donor's microbiome beta diversity reacted differently to the categories. In Figure 15, for Donor X, all test groups moved away from the initial and blank, without major grouping based on categories or against FOS. In Figure 16, for Donor Y, treatments in the pulse category impacted the microbiome in similar ways compared to the other categories; similarly, the grain category treatment also created a similar shift in the microbiome. In Figure 17, for Donor Z, pulse samples seemed to cause overall similar effect on the microbiome – distinct from other categories; also, tubers also had a strong shared effect on the samples. Overall, across the three donors, the initial and the blank appeared to be grouped close together in all the donor beta diversity plots, which was expected, while treatments categories in Donor Y and Z had similar microbiome shifts due to the category of the treatments – particularly for pulses and grains.

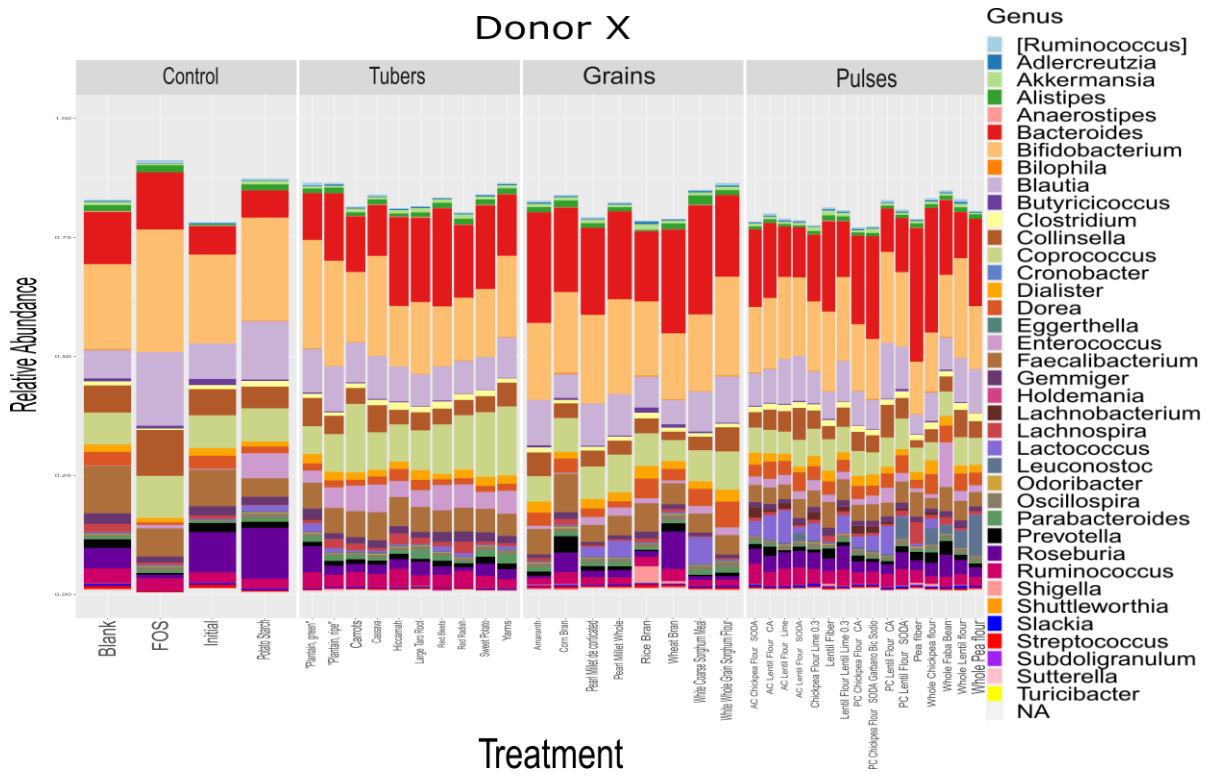

Figure 18. Bacterial relative abundance plots for Donor X at 24 h of fermentation

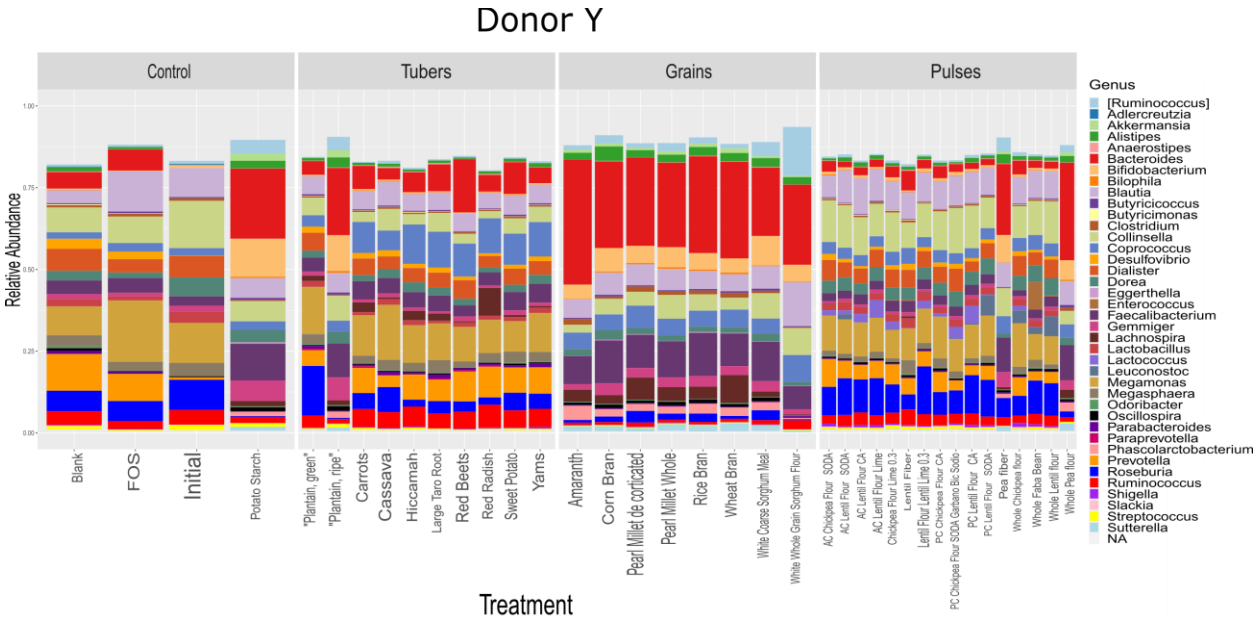

Figure 19. Bacterial relative abundance plots for Donor Y at 24 h of fermentation.

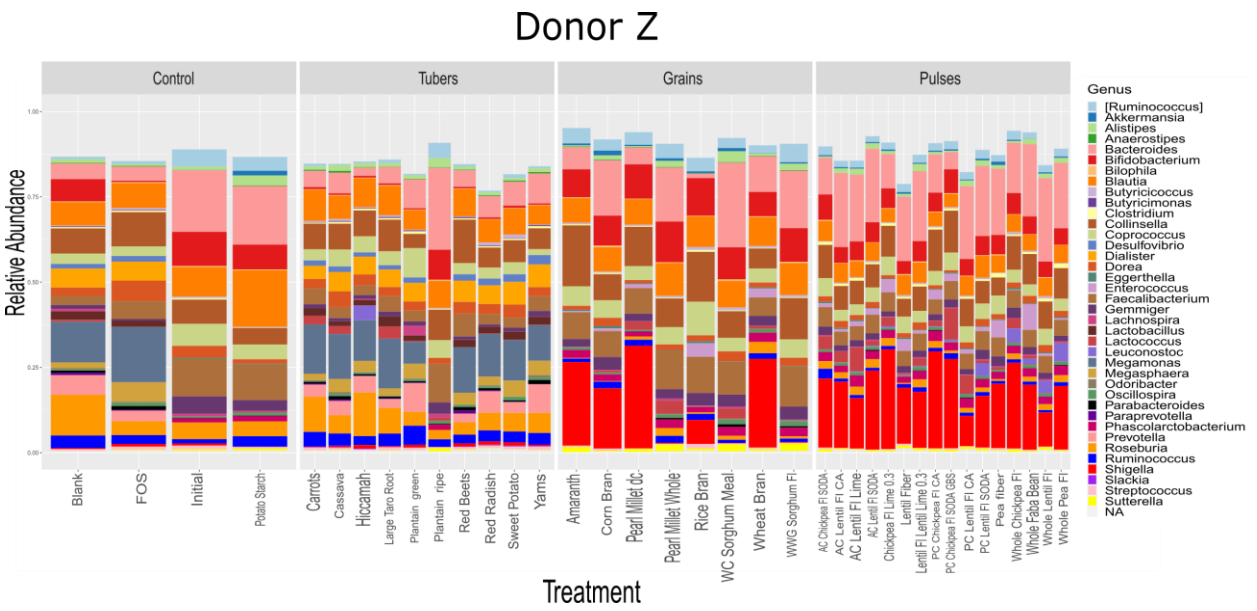

Figure 20. Bacterial relative abundance plots for Donor Z at 24 h of fermentation.

Figures 18, 19, and 20 show clear differences in the composition of the initial inoculum of each individual donor and also the distinct ways in which each treatment, and category, affected the composition differently. The relative abundance plot omits the genera with less than 1% relative abundance and also omits un-identified bacteria at the genus level.

Donor X has relatively stable or comparable genus level proportional responses to various substrates, whereas Donor Y and Donor Z have overwhelming shifts in relative abundance due to treatments in a particular category (Figure 18). Donor X has a few unique increases such as for Rice Bran increasing *Sutterella* distinctly compared to other treatments.

Donor Y microbiome response highlights *Prevotella* increasing in many tubers and pulses, but not in any of the grain treatments (Figure 19). Also, *Prevotella*, increases in all the tubers but not in the Plantain, ripe, and not in the Potato Starch treatment; this could be due to starch stimulating other bacterial genera that would outcompete *Prevotella*. In Donor Y, *Parabacteroides* and *Paraprevotella* are both, perhaps synergistically, increased in the grains collectively, Pea Fiber, Whole Pea Flour, and Plantain - ripe, and Potato Starch. *Bacteroides* also increases in the grains and Pea Fiber and Whole Pea Flour (not in the other pulses) and Potato Starch and Plantain, ripe. Interestingly, in Donor Y, Potato Starch, Plantain – ripe, the grains

collectively, Pea Fiber and Whole Pea Flour all stimulated similar proportions of bacterial genera compared to other treatments.

Donor Z microbiome shifted due to pulses collectively to drastically increase *Shigella*, also observed with Wheat Bran, Pearl Millet de-corticated (but not for Pearl Millet Whole), Corn Bran and Amaranth. Roots/Tubers, except for Plantain – ripe, increased *Prevotella* clearly, not not in the other treatment groups (Figure 20). *Bacteroides* increased in many pulses and many grains as well in Donor Z.

2.4 Discussion

The prebiotic potential of the whole foods tested when compared to FOS is a unique comparison that expands the impact of whole food and intact fibers beyond widely held views that health implications centered around laxation benefits only due to low fermentability. Microbiota accessibility of these mostly insoluble substrates was demonstrated by gas and SCFA production, and compositional changes were observed by increasing alpha diversity for most substrates and shifting relative abundancies of specific genera and impacting β diversity. The increase in SCFA production, relative to the blank - except for Corn Bran, of the whole foods tested in the study by itself suggests direct human host *in vivo* beneficial effect; as SCFAs have a consistently shown mechanistic-supported health impact on the human host (Topping & Clifton, 2001). SCFAs health impact is established; aside from ongoing debate, and conflicting correlation studies, on the benefit on human health of specific bacteria or bacterial groups or alpha diversity metrics. The fiber sources tested in this study all fermented at a slower rate that FOS, suggesting them to be more tolerable to consumers, and more conducive to gut health in the distal colon.

In order to identify whether the responses by the microbiota were a result of particular components, or the collective interlinking of them in the whole food form requires further study to test various isolated dietary fibers and perhaps other components in the food matrix compared to whole food controls. Since there are various macromolecular and micronutrient differences in each of the whole foods, without further comparisons it is difficult to deduce whether the fiber component alone, or the broader food matrix, is the reason for the high fermentability and mostly high alpha diversity response. Notably, the whole food fibers tested were much more fermentable by gut microbiota than isolated bran-based cereal and pulse fibers.

Whole food sources of fibers have been tested as prebiotics in various experimental designs; with substrates in a whole food matrix form or isolated fiber components or isolated polyphenols. However, one of the main benefits of this study is the comparison between broad categories of treatment sources and relative to the industry leading prebiotic supplement FOS. Broccolli, tested in its whole food form, has been shown to influence mice microbiota and reduce inflammation (Hubbard et al., 2017). Polyphenols impact on the gut microbiota is a growing area of study (Calderón-Pérez et al., 2020). Phenolic compounds often crosslink fibers in a plant cell matrix, for example, in brans, ferulic acid crosslink arabinoxylans (Kale et al., 2013). When the complex fiber matrix is disrupted to isolate a fiber or phenolic to study specific effects of a particular structure then the impact of the whole complexed structure is lost. Ferulic linkages between fiber structures in a matrix where shown to be the cause to increase commensal *Clostridium*, which have the enzymatic ability to catalyze such linkages (Zhang et al., 2019). Sorghum bran with more phenolics, and therefore more linkages, were shown to suppress colitis in a rat DSS model greater than isolated cellulose (Ritchie et al., 2017). Three-dimensional matrix models, or 'whole food prebiotics', may confer greater health benefits relative to isolated fiber components *in vivo* with greater investigation, which would eventually lead to subsequent shifts in the food and supplement industry.

The *in vitro* fermentation results demonstrate clear donor specific differences in bacterial growth response to the same substrate even though SCFA metabolic outputs and gas production were relatively comparable among the three donors. This suggests a functional redundancy in the different microbiota communities that results in similar overall abilities to ferment the complex structures of whole food fibers. Reproducibility in gut microbiome related studies has been an ongoing challenge in human gut microbiome studies, however this study shows good consistency in the metabolic production of SCFA and metabolism – measured by gas production. *In vivo* supplementation studies examining the gut microbiota and diet have a limitation in SCFA measurements produced by the gut microbiota due to by re-uptake by the epithelium; and possible more consistent correlations between fiber consumption and SCFA exist in *in vitro* fermentation studies than with in vivo.

Long-term dietary trends, whether in high or low habitual dietary fiber consumers, have been shown in a human intervention study to impact which bacterial groups increase when the same fiber supplement is consumed (Healey et al., 2018). Fiber amount and type differences in the

diets of the individuals could explain the unique donor responses in Donor X, Y, and Z. Additional considerations include gender, as Donor Y was the only female donor; gender differences have been shown in the microbiota of mice, however there is mixed results in human populations (Org et al., 2016). The results of this study suggest *in vitro* fermentation studies, which have controlled microbiota experimental conditions, also need to take into consideration long-term dietary patterns of the fecal donors (Kolodziejczyk et al., 2019), and suggests future work to cater specific prebiotic supplementation tailored to an individual's baseline microbiota composition in order to address a particular dysbiotic state or address an overlying pathology (Zmora et al., 2016).

The influence of the gut microbiota on human health is a growing area of research; and diet has been identified as a key modulator of the gut microbiota structure and function (David et al., 2014). The leading prebiotic ingredient in the food and supplement industry is inulin – which is composed of fructo-oligosaccharides. FOS/inulin has been used, and studied, as a prebiotic for decades, however, no studies have investigated in a systematic way the potential of alternative – non-isolated - prebiotics such as whole food roots, tubers, grains, and pulses – the world's most common staple crops - as prebiotics in comparison to FOS. Fiber structures complexed in a food matrix in tubers, grains, and pulses, have been historically wrongly considered to be mostly inaccessible by the gut microbiota due to being insoluble, with limited to prebiotic effectiveness *in vivo* to aid in laxation and bulking (Ayua et al., 2020).

This study shows the efficacy of tubers, grains, and pulses as potential prebiotics in relation to FOS measured by fermentation rate, short chain fatty acid production, and effect on human fecal microbiota composition measured by alpha diversity and other specific bacterial changes via an *in vitro* fecal fermentation assay using feces from three healthy human donors. Implications from this study could be used by the food industry to expand the prebiotic ingredients in food product development. Also, compared were processing variations such as atmospheric or pressure cooking, showing no overall major impacts on fermentation outputs. This experimental approach could be used to further test final food products or ingredients to better inform dietary choices or for product development pipelines around prebiotic ingredients.

2.5 Conclusion and implications

This study establishes clear health promoting potential, via the gut microbiota, of globally cheap and accessible food crops relative to the industry leading prebiotic ingredient supplement.

The prebiotics market, globally, was $3.2 billion in 2016, and is projected to reach $6.0 billion in 2022 (Gaurav, 2017). The selected food crops of tubers/roots, grains, and pulses were chosen for their interest to the PepsiCo team and range from widely available to novel food ingredients. The study establishes their utility as potential prebiotic fiber-contributing ingredients due to their high and comparable fermentability to FOS, a known prebiotic. As whole foods, they provide clear benefits as they are regular food ingredients with intrinsic fibers that are highly fermentable, probably tolerable, and beneficial to gut health.

With regards to processing effects in increasing the fermentative abilities of the substrates, there was not a considerable difference between substrates that were cooked, atmospheric or pressure, or otherwise processed with unique processing steps in the samples by PEPSICO relative to non-processed similar substrates, for Lentil Flours and Chickpea Flours. The different processing conditions applied to the chickpea and lentil fibers (processed by our industry partner PepsiCo at Frito-Lay, Plano, TX) did not have any significant effect on SCFA production (Figure 12). While increases in accessibility were expected but not observed, it is helpful for companies to note that the processing in this study did not impede fermentation.

The study shows a variety of tuber/root, grain, and pulse ingredients for a product development pipeline around gut health and tolerability. Benefits at least lay the framework for further exploration of 1) increased consumption tolerance in comparison to inulin, 2) sustained and more distal delivery of microbial metabolites – like SCFA, in comparison to inulin, and 3) increased diversity of the microbiome. In future work, one or more human pilot supplementation studies would determine the impact of food materials and processing on amount of fiber needed to achieve measurable outcomes in a human system relative to inulin, including health biomarkers like serum inflammatory cytokines (e.g. IL-6), lipopolysaccharide binding protein, and C-reactive protein. Additional experimentation is needed to determine which food matrix structures, components, or linkages cause specific changes in each donor; which would potential highlight enzymatic advantages by a particular group of bacteria or the ecological community structure and function overall that determines donor specific differential responses.

APPENDIX A. TOTAL DIETARY FIBER

Analysis for the Total Dietary Fiber Assay was performed according to the Megazyme kit and the manufacture instructions, based on AOAC Method 991.43 “Total, Soluble, and Insoluble Dietary Fiber in Foods” and AACC Method 32-07-01 “Determination of Soluble, Insoluble, and Total Dietary Fiber in Foods and Food Products”; and simplifies the AACC total dietary fiber (TDF) method, 32-05.01, and the AACC soluble/insoluble dietary fiber method, 32-21.01.

Blank	1	0	0	
FOS	2	54.13905055	98.91559982	1.011
Cassava	3	5.419036778	70.372	1.421
Large Taro Root	4	29.34063408	73.51720538	1.36
Carrots	5	22.25207931	84.15832627	1.188
Red Beets	6	17.06715143	55.82364892	1.791
Red Radish	7	39.85386613	87.34230179	1.145
Sweet Potato	8	16.60749179	68.50791738	1.46
Yams	9	9.585504085	49.46114285	2.022
Hiccamah	10	22.55999427	88.625	1.128
Plaintain, green	11	28.63847454	91.80733706	1.089
Plaintain, ripe	12	14.00808641	82.816875	1.207
Potato Starch	13	2.90271838	100	1
White Whole Grain Sorghum Flour	14	6.586170532	28.5543814	3.502
White Coarse Sorghum Meal	15	10.71292932	48.83000041	2.048
Pearl Millet Whole (non-decorticated)	16	7.486421925	31.04241249	3.221
Pearl Millet (de-corticated)	17	10.00834405	47.77018913	2.093
Amaranth	18	7.551222367	44.43705837	2.25
Wheat bran	19	48.52997836	78.72990394	1.27
Rice bran	20	34.73851624	72.56701538	1.378
Corn bran	21	79.40588811	89.58125294	1.116
Whole Pea flour	22	9.268548346	52.94902286	1.889
Pea fiber	23	65.48457826	82.32382772	1.215
Whole Chickpea flour	24	12.51142015	50.2385695	1.991
Atmospheric Cooking Chickpea Flour – SODA	25	18.63862557	62.56344904	1.598

Pressure Cooking Chickpea Flour – SODA; Garbano Bic Sodio	26	18.67008311	57.15201892	1.75
Pressure Cooking Chickpea Flour – CA	27	16.63467723	67.68106239	1.478
Chickpea Flour Lime 0.3%	28	20.50679569	61.38866615	1.629
Whole Faba Bean	29	9.719427219	35.95467013	2.781
Whole lentil flour	30	9.689151079	53.92046537	1.855
Lentil Fiber	31	33.836601	65.06018374	1.537
Lentil Flour Lentil Lime 0.3%	32	15.62682497	57.06816853	1.752
Atmospheric Cooking Lentil Flour – SODA	33	20.10918707	64.12289735	1.56
Atmospheric Cooking Lentil Flour - CA	34	14.960567	48.97286571	2.042
Atmospheric Cooking Lentil Flour - Lime	35	20.60250983	59.38004176	1.684
Pressure Cooking Lentil Flour - CA	36	12.12012764	41.19891726	2.427
Pressure Cooking Lentil Flour - SODA	37	13.81267638	45.54136957	2.196

Error in the Nitrogen Analyzer step forced to use approximations based on similar substrates for protein content for the following samples; 3, 10, 21, 30, and 36.

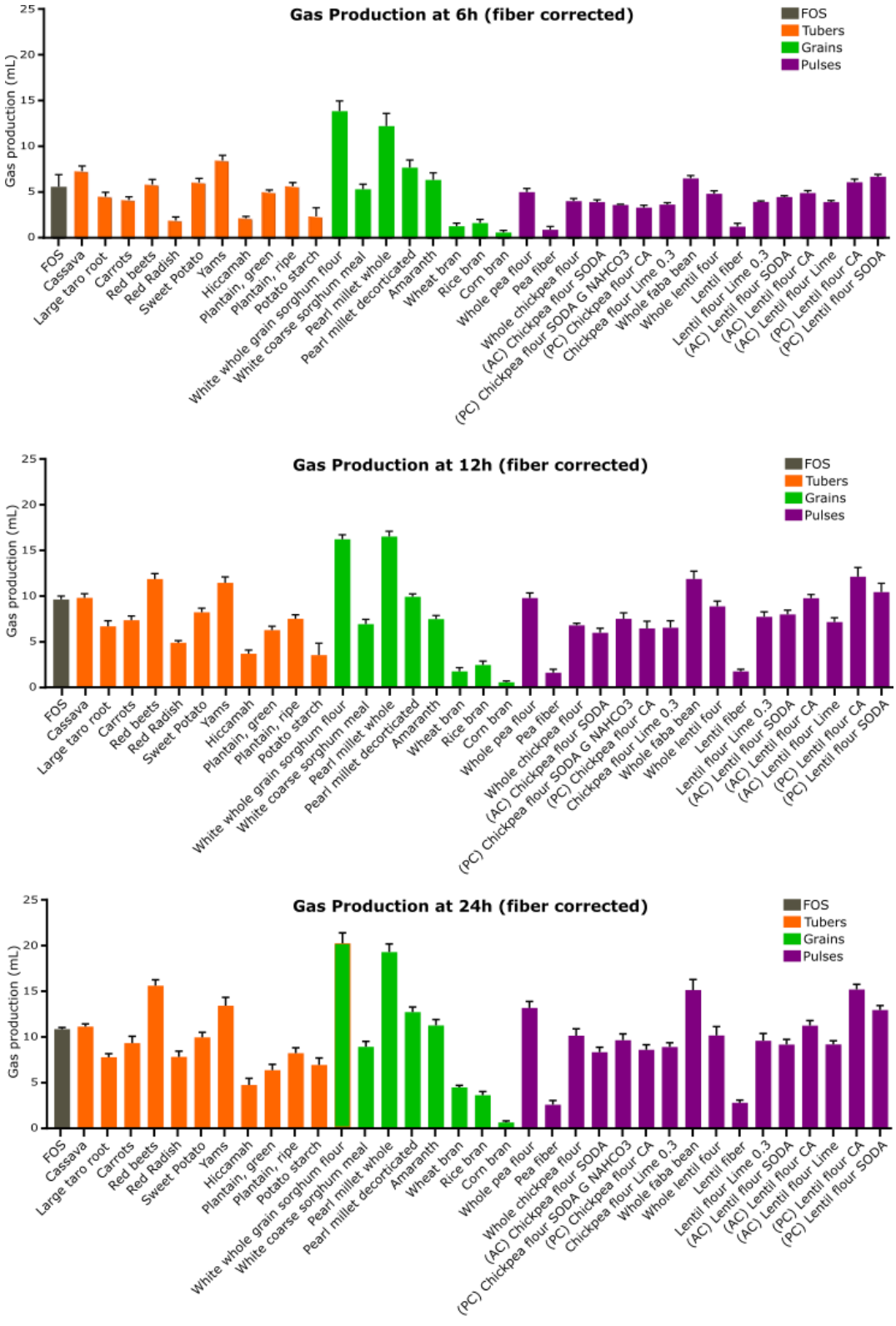

Figure A1. Gas production at 24 h of fermentation average across 3 donors normalized for total dietary fiber in each sample.

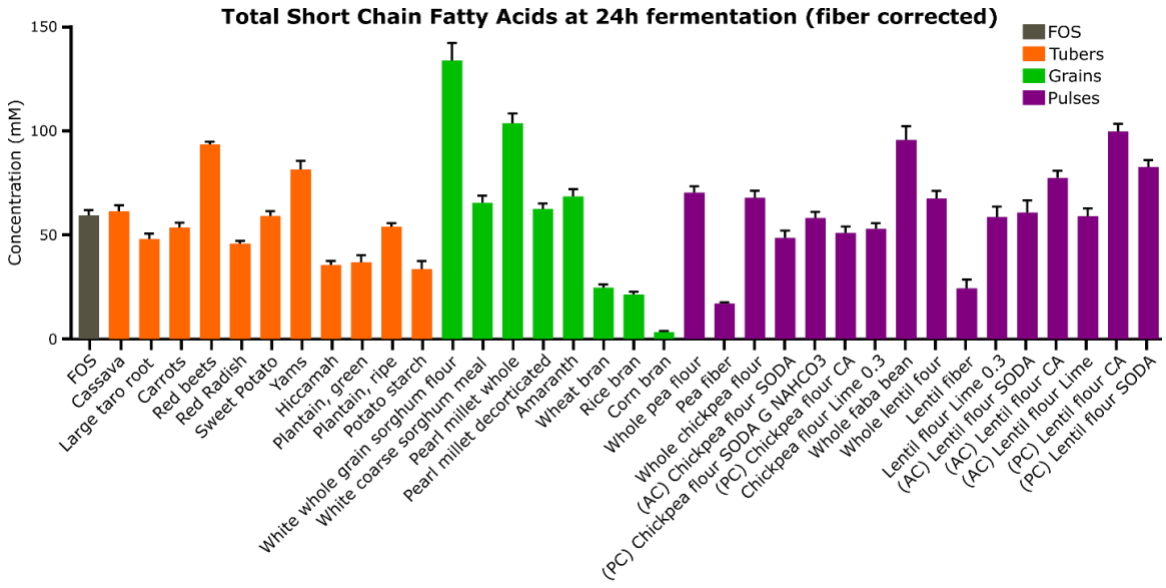

Figure A2. Total SCFA production at 24 h of fermentation average across 3 donors normalized for total dietary fiber in each sample.

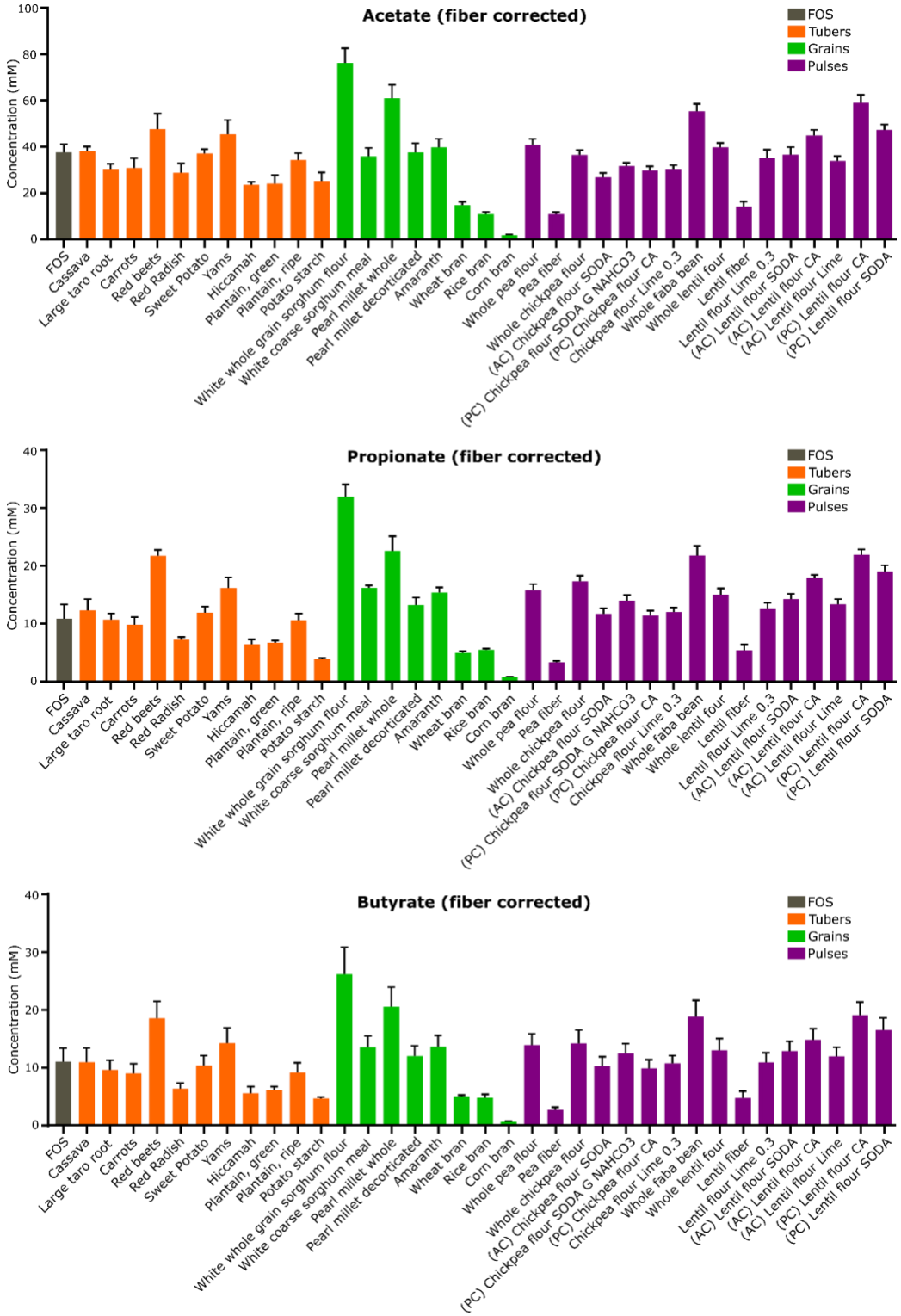

Figure A3. Acetate, propionate, and butyrate at 24 h fermentation averaged across 3 donors normalized for total dietary fiber in each sample.

APPENDIX B. CHAO1 ALPHA DIVERSITY SCORES

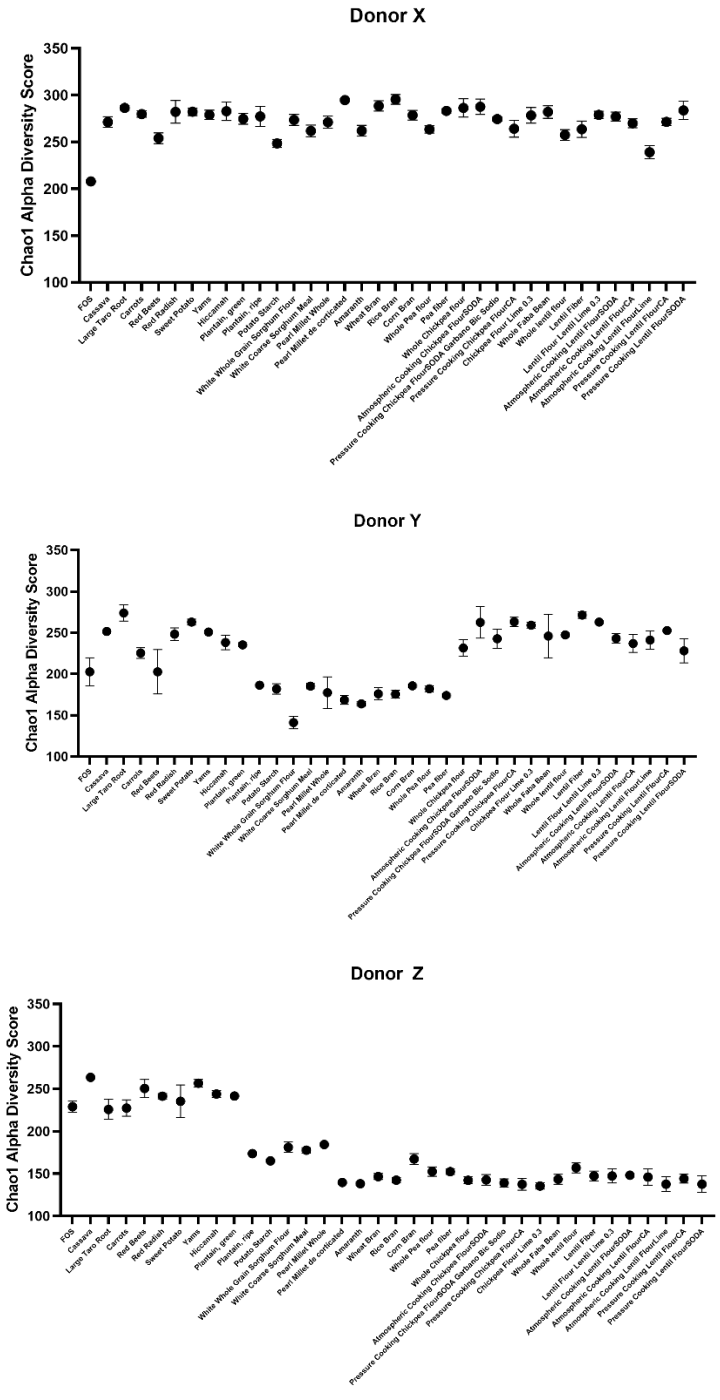

Figure A4. Chao1 Alpha Diversity Scores for each donor

APPENDIX C. BACTERIAL RELATIVE ABUNDANCES MOST INCREASED FOR EACH DONOR

Table A1. Bacterial Genera Changes in Each Donor:

The top 5 bacterial genera that were increased the most overall due to fecal fermentation was chosen for each donor.

Donor X

k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae;g__Enterococcus
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus

Donor Y

k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Faecalibacterium
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Coprococcus
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium

Donor Z

k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Shigella
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Megamonas
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Dialister

The Megamonas genera for Donor Z was removed since it was only substantially present in that donor, and not present in the other donors.

APPENDIX D. ANOVA STATISTICAL OUTCOMES FOR FIGURES LISTED

Table A2. Full ANOVA Statistical Outcomes

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Pairwise comparisons for One-Way ANOVA

SHORT CHAIN FATTY ACIDS-1-ACETATE

2-PROPIONATE

3-BUTYRATE

4-TOTAL SCFA

SHANNON INDEX 5-SHAHNON-DONOR X

6-SHAHNON-DONOR Y

7-SHAHNON-DONOR Z

FIBER CORRECTED DATA 8- ACETATE

9- PROPIONATE

10-BUTYRATE

11-TOTAL SCFA

12-GAS 6h

13-GAS 12h

14-GAS 24h

1-ACETATE

Treatment	N	Mean	Grouping									
FOS	9	37.50	A									
Red Beets	8	29.83	A	B								
Carrots	8	29.13	A	B	C							
Plantain, ripe	9	28.30	A	B	C							
Red Radish	8	28.13	A	B	C	D						
Cassava	9	26.78		B	C	D	E					
Sweet Potato	9	25.28		B	C	D	E	F				
Yams	8	25.18		B	C	D	E	F				
Potato Starch	9	25.03		B	C	D	E	F				
Pressure Cooking Lentil FlourCA	9	24.23		B	C	D	E	F				
Atmospheric Cooking Lentil FlourSODA	9	23.45		B	C	D	E	F				
Large Taro Root	8	22.21		B	C	D	E	F				
Plantain, green	9	22.00		B	C	D	E	F				
Atmospheric Cooking Lentil FlourCA	9	21.87		B	C	D	E	F				
White Whole Grain Sorghum Flour	9	21.67		B	C	D	E	F				
Whole Pea flour	9	21.56		B	C	D	E	F				
Pressure Cooking Lentil FlourSODA	9	21.46		B	C	D	E	F				
Whole lentil flour	9	21.337		B	C	D	E	F				
Hiccamah	9	20.83		B	C	D	E	F	G			
Pressure Cooking Chickpea FlourCA	9	20.06			C	D	E	F	G			
Atmospheric Cooking Lentil FlourLime	9	20.03			C	D	E	F	G			
Lentil Flour Lentil Lime 0.3	9	20.02			C	D	E	F	G			
Whole Faba Bean	9	19.80			C	D	E	F	G			
Pearl Millet Whole	9	18.82				D	E	F	G			
Chickpea Flour Lime 0.3	9	18.56					E	F	G			
Whole Chickpea flour	9	18.24					E	F	G	H		
Pressure Cooking Chickpea FlourSODA Garbano Bic Sodio	9	18.114					E	F	G	H	I	
Pearl Millet de corticated	9	17.85					E	F	G	H	I	
Amaranth	8	17.58					E	F	G	H	I	J
White Coarse Sorghum Meal	8	17.42					E	F	G	H	I	J
Atmospheric Cooking Chickpea FlourSODA	9	16.71						F	G	H	I	J
Wheat Bran	8	11.54							G	H	I	J
Lentil Fiber	9	9.19								H	I	J	K
Pea fiber	9	8.993									I	J	K
Rice Bran	8	7.936										J	K
Corn Bran	9	1.647											K

2-PROPIONATE

Treatment	N	Mean	Grouping							
Red Beets	8	12.130	A							
FOS	9	10.92	A	B						
Atmospheric Cooking Lentil FlourSODA	8	9.166	A	B	C					
White Whole Grain Sorghum Flour	9	9.121	A	B	C					
Pressure Cooking Lentil FlourCA	9	9.036	A	B	C					
Atmospheric Cooking Lentil FlourCA	9	8.793	A	B	C					
Plantain, ripe	9	8.783	A	B	C					
Pressure Cooking Lentil FlourSODA	9	8.708	A	B	C					
Whole Chickpea flour	9	8.704	A	B	C					
Cassava	9	8.70	A	B	C					
Whole Pea flour	9	8.363	A	B	C					
Carrots	8	8.31	A	B	C					
Sweet Potato	9	8.161	A	B	C					
Whole lentil flour	9	8.102	A	B	C					
Pressure Cooking Chickpea FlourSODA Garbano Bic Sodio	9	8.003		B	C	D				
Yams	9	7.999		B	C	D				
Atmospheric Cooking Lentil FlourLime	9	7.959		B	C	D	E			
White Coarse Sorghum Meal	8	7.900		B	C	D	E	F		
Large Taro Root	7	7.884	A	B	C	D	E	F		
Whole Faba Bean	9	7.842		B	C	D	E			
Pressure Cooking Chickpea FlourCA	9	7.759		B	C	D	E	F		
Chickpea Flour Lime 0.3	9	7.369		B	C	D	E	F	G	
Atmospheric Cooking Chickpea FlourSODA	9	7.340		B	C	D	E	F	G	
Lentil Flour Lentil Lime 0.3	9	7.227		B	C	D	E	F	G	
Pearl Millet Whole	9	7.021		B	C	D	E	F	G	
Amaranth	8	6.834			C	D	E	F	G	
Pearl Millet de corticated	9	6.341			C	D	E	F	G	H
Red Radish	8	6.311			C	D	E	F	G	H
Plantain, green	9	6.184			C	D	E	F	G	H
Hiccamah	9	5.758			C	D	E	F	G	H
Rice Bran	8	3.994				D	E	F	G	H	I
Wheat Bran	8	3.929					E	F	G	H	I
Potato Starch	9	3.869						F	G	H	I
Lentil Fiber	9	3.541							G	H	I
Pea fiber	9	2.779								H	I
Corn Bran	9	0.7156									I

Means that do not share a letter are significantly different.

3-BUTYRATE

Treatment	N	Mean	Grouping				
FOS	9	11.10	A				
Red Beets	8	10.40	A	B			
Atmospheric Cooking Lentil FlourSODA	8	8.33	A	B	C		
Pressure Cooking Lentil FlourCA	9	7.892	A	B	C	D	
Cassava	9	7.77	A	B	C	D	
Plantain, ripe	9	7.66	A	B	C	D	
Carrots	8	7.65	A	B	C	D	
Pressure Cooking Lentil FlourSODA	9	7.571	A	B	C	D	
White Whole Grain Sorghum Flour	9	7.50	A	B	C	D	
Whole Pea flour	9	7.40	A	B	C	D	
Atmospheric Cooking Lentil FlourCA	9	7.304	A	B	C	D	
Whole Chickpea flour	9	7.19	A	B	C	D	
Pressure Cooking Chickpea FlourSODA Garbano Bic Sodio	9	7.169	A	B	C	D	
Atmospheric Cooking Lentil FlourLime	9	7.163	A	B	C	D	
Sweet Potato	9	7.14	A	B	C	D	
Large Taro Root	7	7.10	A	B	C	D	
Yams	9	7.08	A	B	C	D	
Whole lentil flour	9	7.07	A	B	C	D	
Whole Faba Bean	9	6.80	A	B	C	D	
Pressure Cooking Chickpea FlourCA	9	6.769	A	B	C	D	
White Coarse Sorghum Meal	8	6.659	A	B	C	D	
Chickpea Flour Lime 0.3	9	6.644	A	B	C	D	
Atmospheric Cooking Chickpea FlourSODA	9	6.46	A	B	C	D	
Pearl Millet Whole	9	6.40	A	B	C	D	E
Lentil Flour Lentil Lime 0.3	9	6.284	A	B	C	D	E
Amaranth	8	6.073	A	B	C	D	E
Pearl Millet de corticated	9	5.781	A	B	C	D	E
Plantain, green	9	5.667	A	B	C	D	E
Red Radish	8	5.633	A	B	C	D	E
Hiccamah	9	4.98		B	C	D	E
Potato Starch	9	4.722		B	C	D	E
Wheat Bran	8	4.020			C	D	E
Rice Bran	8	3.553			C	D	E
Lentil Fiber	9	3.137			C	D	E
Pea fiber	9	2.269				D	E
Corn Bran	9	0.630					E

Means that do not share a letter are significantly different.

4-TOTAL SCFA

Treatment	N	Mean	Grouping							
FOS	9	54.66	A							
Red Beets	8	46.245	A	B						
Carrots	8	42.47		B	C					
Plantain, ripe	9	42.15		B	C					
Atmospheric Cooking Lentil FlourSODA	8	40.44		B	C	D	E			
Cassava	9	40.43		B	C	D				
Pressure Cooking Lentil FlourCA	9	39.44		B	C	D	E			
Red Radish	8	38.36		B	C	D	E	F		
Sweet Potato	9	38.03		B	C	D	E	F		
Yams	8	37.66		B	C	D	E	F		
Atmospheric Cooking Lentil FlourCA	9	36.26		B	C	D	E	F		
Pressure Cooking Lentil FlourSODA	9	35.84		B	C	D	E	F		
Whole Pea flour	9	35.56		B	C	D	E	F		
White Whole Grain Sorghum Flour	9	35.26			C	D	E	F		
Potato Starch	9	35.20			C	D	E	F		
Whole lentil flour	9	34.73			C	D	E	F		
Plantain, green	9	33.86			C	D	E	F		
Atmospheric Cooking Lentil FlourLime	9	33.78			C	D	E	F		
Whole Faba Bean	9	33.08			C	D	E	F		
Pressure Cooking Chickpea FlourCA	9	32.61			C	D	E	F		
Large Taro Root	8	32.56			C	D	E	F		
Lentil Flour Lentil Lime 0.3	9	32.49			C	D	E	F		
Whole Chickpea flour	9	31.47				D	E	F		
Pressure Cooking Chickpea FlourSODA Garbano Bic Sodio	9	31.05				D	E	F		
Chickpea Flour Lime 0.3	9	30.93				D	E	F		
Pearl Millet Whole	9	30.44				D	E	F		
Hiccamah	9	29.48					E	F	G	
Atmospheric Cooking Chickpea FlourSODA	9	28.53						F	G	
Pearl Millet de corticated	9	28.49						F	G	
Amaranth	8	28.43						F	G	
White Coarse Sorghum Meal	8	28.21						F	G	
Wheat Bran	8	19.36							G	H
Rice Bran	8	14.72								H
Lentil Fiber	9	14.71								H
Pea fiber	9	12.974								H
Corn Bran	9	2.748								I

Means that do not share a letter are significantly different.

5-SHANNON-DONOR X

Treatment	N	Mean	Grouping										
Rice Bran	3	7.2069	A										
PC Lentil Flour SODA	3	7.1965	A	B									
Red Radish	3	7.1832	A	B	C								
Hiccamah	3	7.1752	A	B	C								
Whole Chickpea flour	3	7.1680	A	B	C								
Large Taro Root	3	7.1632	A	B	C	D							
Chickpea Flour Lime 0.3	3	7.1489	A	B	C	D							
Whole Faba Bean	3	7.1483	A	B	C	D							
AC Chickpea Flour SODA	3	7.1403	A	B	C	D	E						
Lentil Fiber	3	7.1176	A	B	C	D	E	F					
Carrots	3	7.1126	A	B	C	D	E	F					
AC Lentil Flour CA	3	7.1109	A	B	C	D	E	F					
Red Beets	3	7.1054	A	B	C	D	E	F	G				
Lentil Flour Lentil Lime 0.3	3	7.1036	A	B	C	D	E	F	G				
Corn Bran	3	7.0895	A	B	C	D	E	F	G	H			
Sweet Potato	3	7.0719	A	B	C	D	E	F	G	H			
AC Lentil Flour SODA	3	7.06919	A	B	C	D	E	F	G	H			
Pea fiber	3	7.05981	A	B	C	D	E	F	G	H	I		
PC Chickpea Flour CA	3	7.0548	A	B	C	D	E	F	G	H	I		
Whole Pea flour	3	7.0207	A	B	C	D	E	F	G	H	I	J	
Yams	3	7.0118		B	C	D	E	F	G	H	I	J	
Whole Lentil flour	3	7.0024			C	D	E	F	G	H	I	J	
Wheat Bran	3	6.9976			C	D	E	F	G	H	I	J	
Plantain, ripe	3	6.9774				D	E	F	G	H	I	J	
PC Chickpea Flour SODA Garbano Bic Sodio	3	6.9764				D	E	F	G	H	I	J	
Cassava	3	6.9600					E	F	G	H	I	J	
White Coarse Sorghum Meal	3	6.95663					E	F	G	H	I	J	
Amaranth	3	6.9531					E	F	G	H	I	J	
White Whole Grain Sorghum Flour	3	6.9464						F	G	H	I	J	
PC Lentil Flour CA	3	6.9451						F	G	H	I	J	
AC Lentil Flour Lime	3	6.9223							G	H	I	J	
Pearl Millet de corticated	3	6.9065								H	I	J	
Pearl Millet Whole	3	6.8737									I	J	K
Plantain, green	3	6.8389										J	K
Potato Starch	3	6.6927											K
FOS	3	6.2891											L

Means that do not share a letter are significantly different.

6-SHANNON-DONOR Y

Treatment	N	Mean	Grouping											
Lentil Fiber	3	7.0779	A											
Large Taro Root	3	7.0093	A	B										
Sweet Potato	3	6.9505	A	B	C									
Chickpea Flour Lime 0.3	3	6.90835	A	B	C	D								
Whole Chickpea flour	3	6.8987	A	B	C	D								
Yams	3	6.8987	A	B	C	D								
AC Chickpea Flour SODA	3	6.8986	A	B	C	D								
Whole Faba Bean	3	6.8774	A	B	C	D	E							
PC Chickpea Flour CA	3	6.8596	A	B	C	D	E							
AC Lentil Flour CA	3	6.8370		B	C	D	E							
Hiccamah	3	6.8314		B	C	D	E							
Cassava	3	6.8280		B	C	D	E							
Red Radish	3	6.8219		B	C	D	E							
PC Chickpea Flour SODA Garbano Bic Sodio	3	6.8176		B	C	D	E							
AC Lentil Flour Lime	3	6.8159		B	C	D	E							
Carrots	3	6.7999		B	C	D	E	F						
Whole Lentil flour	3	6.7847			C	D	E	F						
Plantain, green	3	6.7840			C	D	E	F						
AC Lentil Flour SODA	3	6.7770			C	D	E	F	G					
PC Lentil Flour SODA	3	6.7723			C	D	E	F	G					
Lentil Flour Lentil Lime 0.3	3	6.7675			C	D	E	F	G					
PC Lentil Flour CA	3	6.7183				D	E	F	G	H				
White Coarse Sorghum Meal	3	6.6918				D	E	F	G	H	I			
Red Beets	3	6.6730					E	F	G	H	I	J		
Pearl Millet Whole	2	6.6331					E	F	G	H	I	J	K	
Whole Pea flour	3	6.5903						F	G	H	I	J	K	
Pea fiber	3	6.5817						F	G	H	I	J	K	
Corn Bran	3	6.5597							G	H	I	J	K	L
Wheat Bran	3	6.5591							G	H	I	J	K	L
FOS	3	6.5464								H	I	J	K	L
Pearl Millet de corticated	3	6.5421								H	I	J	K	L
Rice Bran	3	6.4922									I	J	K	L
Plantain, ripe	3	6.4688										J	K	L
Potato Starch	3	6.4132											K	L
Amaranth	3	6.3541												L	M
White Whole Grain Sorghum Flour	3	6.1823													M

Means that do not share a letter are significantly different.

7-SHANNON-DONOR Z

Treatment	N	Mean	Grouping				
Plantain, green	3	7.0786	A				
Yams	3	7.0219	A	B			
Cassava	3	7.0056	A	B			
Sweet Potato	2	6.9683	A	B	C		
Red Radish	3	6.8622	A	B	C		
Hiccamah	3	6.8439	A	B	C		
Red Beets	3	6.7786	A	B	C		
Large Taro Root	2	6.7369	A	B	C		
FOS	3	6.7348	A	B	C		
Carrots	3	6.72279	A	B	C		
Pearl Millet Whole	3	6.6983	A	B	C		
White Coarse Sorghum Meal	3	6.61870	A	B	C		
White Whole Grain Sorghum Flour	3	6.5952		B	C		
Plantain, ripe	3	6.5698		B	C		
Potato Starch	3	6.4529			C		
PC Lentil Flour SODA	3	5.5661				D	
PC Lentil Flour CA	3	5.5559				D	
Lentil Flour Lentil Lime 0.3	3	5.5499				D	
Whole Pea flour	3	5.5408				D	
AC Lentil Flour Lime	3	5.5311				D	
Corn Bran	3	5.5194				D	
AC Chickpea Flour SODA	3	5.489				D	E
Whole Lentil flour	3	5.472				D	E
Pea fiber	3	5.4613				D	E
Whole Faba Bean	3	5.4446				D	E
AC Lentil Flour SODA	3	5.4208				D	E
AC Lentil Flour CA	3	5.417				D	E
Wheat Bran	3	5.4077				D	E
Rice Bran	3	5.3999				D	E
Whole Chickpea flour	3	5.3161				D	E
Lentil Fiber	3	5.247				D	E
Chickpea Flour Lime 0.3	3	5.1965				D	E
PC Chickpea Flour SODA Garbano Bic Sodio	3	5.1463				D	E
PC Chickpea Flour CA	3	5.1323				D	E
Amaranth	3	5.121				D	E
Pearl Millet de corticated	3	5.0419					E

Means that do not share a letter are significantly different.

8-FIBER CORRECTED DATA-ACETATE

Treatment	N	Mean	Grouping									
White Whole Grain Sorghum Flour	9	75.88	A									
Pearl Millet Whole	9	60.64	A	B								
Pressure Cooking Lentil FlourCA	9	58.82	A	B								
Whole Faba Bean	9	55.06		B	C							
Red Beets	9	47.50		B	C	D						
Pressure Cooking Lentil FlourSODA	9	47.12		B	C	D	E					
Yams	9	45.26		B	C	D	E	F				
Atmospheric Cooking Lentil FlourCA	9	44.66		B	C	D	E	F				
Whole Pea flour	9	40.72			C	D	E	F	G			
Amaranth	8	39.57			C	D	E	F	G			
Whole lentil flour	9	39.57			C	D	E	F	G			
Cassava	9	38.06			C	D	E	F	G			
FOS	9	37.50			C	D	E	F	G			
Pearl Millet de corticated	9	37.35				D	E	F	G			
Sweet Potato	9	36.90				D	E	F	G			
Atmospheric Cooking Lentil FlourSODA	9	36.57				D	E	F	G			
Whole Chickpea flour	9	36.30				D	E	F	G			
White Coarse Sorghum Meal	8	35.68				D	E	F	G			
Lentil Flour Lentil Lime 0.3	9	35.08				D	E	F	G			
Plantain, ripe	9	34.16				D	E	F	G			
Atmospheric Cooking Lentil FlourLime	9	33.72				D	E	F	G			
Pressure Cooking Chickpea FlourSODA Garbano Bic Sodio	9	31.69				D	E	F	G	H		
Carrots	9	30.77				D	E	F	G	H		
Chickpea Flour Lime 0.3	9	30.23				D	E	F	G	H		
Large Taro Root	8	30.22				D	E	F	G	H		
Pressure Cooking Chickpea FlourCA	9	29.64					E	F	G	H		
Red Radish	9	28.63						F	G	H	I	
Atmospheric Cooking Chickpea FlourSODA	9	26.71							G	H	I	J
Potato Starch	9	25.03							G	H	I	J
Plantain, green	9	23.95							G	H	I	J
Hiccamah	9	23.51							G	H	I	J
Wheat Bran	8	14.66								H	I	J
Lentil Fiber	9	14.12								H	I	J
Rice Bran	8	10.936									I	J
Pea fiber	9	10.924										J
Corn Bran	9	1.839										

K
K
K
K
K

Means that do not share a letter are significantly different.

9- FIBER CORRECTED DATA -PROPIONATE

Treatment	N	Mean	Grouping
White Whole Grain Sorghum Flour	9	31.94	A
Pearl Millet Whole	9	22.62	B
Pressure Cooking Lentil FlourCA	9	21.931	B C
Whole Faba Bean	9	21.81	B C
Red Beets	8	21.73	B C
Pressure Cooking Lentil FlourSODA	9	19.12	B C D
Atmospheric Cooking Lentil FlourCA	9	17.956	B C D E
Whole Chickpea flour	9	17.33	B C D E F
White Coarse Sorghum Meal	8	16.178	B C D E F G
Yams	9	16.17	C D E F G
Whole Pea flour	9	15.79	C D E F G
Amaranth	8	15.378	C D E F G
Whole lentil flour	9	15.03	D E F G
Atmospheric Cooking Lentil FlourSODA	8	14.295	D E F G
Pressure Cooking Chickpea FlourSODA Garbano Bic Sodio	9	14.003	D E F G
Atmospheric Cooking Lentil FlourLime	9	13.403	D E F G H
Pearl Millet de corticated	9	13.27	D E F G H
Lentil Flour Lentil Lime 0.3	9	12.663	E F G H I
Cassava	9	12.36	E F G H I
Chickpea Flour Lime 0.3	9	12.004	E F G H I J
Sweet Potato	9	11.91	E F G H I J
Atmospheric Cooking Chickpea FlourSODA	9	11.732	E F G H I J K
Pressure Cooking Chickpea FlourCA	9	11.464	F G H I J K L
FOS	9	10.92	G H I J K L
Large Taro Root	7	10.72	F G H I J K L
Plantain, ripe	9	10.60	G H I J K L
Carrots	8	9.87	G H I J K L M
Red Radish	8	7.226	H I J K L M N
Plantain, green	9	6.735	I J K L M N
Hiccamah	9	6.497	I J K L M N
Rice Bran	8	5.503	J K L M N
Lentil Fiber	9	5.44	K L M N
Wheat Bran	8	4.990	L M N
Potato Starch	9	3.869	M N
Pea fiber	9	3.376	M N
Corn Bran	9	0.799	N

Means that do not share a letter are significantly different.

10- FIBER CORRECTED DATA -BUTYRATE

Treatment	N	Mean	Grouping					
White Whole Grain Sorghum Flour	9	26.27	A					
Pearl Millet Whole	9	20.62	A	B				
Pressure Cooking Lentil FlourCA	9	19.16	A	B	C			
Whole Faba Bean	9	18.91	A	B	C			
Red Beets	8	18.62	A	B	C			
Pressure Cooking Lentil FlourSODA	9	16.62	A	B	C	D		
Atmospheric Cooking Lentil FlourCA	9	14.92		B	C	D	E	
Yams	9	14.32		B	C	D	E	
Whole Chickpea flour	9	14.31		B	C	D	E	
Whole Pea flour	9	13.98		B	C	D	E	
Amaranth	8	13.67		B	C	D	E	F
White Coarse Sorghum Meal	8	13.64		B	C	D	E	F
Whole lentil flour	9	13.12		B	C	D	E	F
Atmospheric Cooking Lentil FlourSODA	8	12.99		B	C	D	E	F
Pressure Cooking Chickpea FlourSODA Garbano Bic Sodio	9	12.54		B	C	D	E	F
Pearl Millet de corticated	9	12.10		B	C	D	E	F
Atmospheric Cooking Lentil FlourLime	9	12.06		B	C	D	E	F
FOS	9	11.10		B	C	D	E	F	G
Cassava	9	11.04		B	C	D	E	F	G
Lentil Flour Lentil Lime 0.3	9	11.01		B	C	D	E	F	G
Chickpea Flour Lime 0.3	9	10.82		B	C	D	E	F	G
Sweet Potato	9	10.42		B	C	D	E	F	G
Atmospheric Cooking Chickpea FlourSODA	9	10.33		B	C	D	E	F	G
Pressure Cooking Chickpea FlourCA	9	10.00			C	D	E	F	G
Large Taro Root	7	9.66		B	C	D	E	F	G
Plantain, ripe	9	9.25			C	D	E	F	G
Carrots	8	9.09			C	D	E	F	G
Red Radish	8	6.449				D	E	F	G
Plantain, green	9	6.171				D	E	F	G
Hiccamah	9	5.62					E	F	G
Wheat Bran	8	5.107					E	F	G
Rice Bran	8	4.896					E	F	G
Lentil Fiber	9	4.82					E	F	G
Potato Starch	9	4.722					E	F	G
Pea fiber	9	2.756						F	G
Corn Bran	9	0.703							G

Means that do not share a letter are significantly different.

11- FIBER CORRECTED DATA -TOTAL SCFA

Treatment	N	Mean	Grouping													
White Whole Grain Sorghum Flour	9	134.10	A													
Pearl Millet Whole	9	103.88		B												
Pressure Cooking Lentil FlourCA	9	99.91		B	C											
Whole Faba Bean	9	95.78		B	C	D										
Red Beets	8	93.79		B	C	D										
Pressure Cooking Lentil FlourSODA	9	82.86			C	D	E									
Yams	8	81.60			C	D	E	F								
Atmospheric Cooking Lentil FlourCA	9	77.53				D	E	F	G							
Whole Pea flour	9	70.50					E	F	G	H						
Amaranth	8	68.62					E	F	G	H	I					
Whole Chickpea flour	9	67.94					E	F	G	H	I					
Whole lentil flour	9	67.71					E	F	G	H	I	J				
White Coarse Sorghum Meal	8	65.49					E	F	G	H	I	J	K			
Pearl Millet de corticated	9	62.73						F	G	H	I	J	K			
Cassava	9	61.46							G	H	I	J	K			
Atmospheric Cooking Lentil FlourSODA	9	60.82							G	H	I	J	K			
FOS	9	59.52							G	H	I	J	K			
Sweet Potato	9	59.23							G	H	I	J	K			
Atmospheric Cooking Lentil FlourLime	9	59.19							G	H	I	J	K			
Lentil Flour Lentil Lime 0.3	9	58.76							G	H	I	J	K			
Pressure Cooking Chickpea FlourSODA Garbano Bic Sodio	9	58.24								H	I	J	K			
Plantain, ripe	9	54.00								H	I	J	K	L		
Carrots	8	53.57								H	I	J	K	L		
Chickpea Flour Lime 0.3	9	53.06								H	I	J	K	L		
Pressure Cooking Chickpea FlourCA	9	51.10									I	J	K	L	M	
Atmospheric Cooking Chickpea FlourSODA	9	48.77									I	J	K	L	M	
Large Taro Root	8	48.05										J	K	L	M	
Red Radish	8	45.89											K	L	M	
Plantain, green	9	36.86												L	M	N
Hiccamah	9	35.62												L	M	N
Potato Starch	9	33.62													M	N
Wheat Bran	8	24.75														N
Lentil Fiber	9	24.38														N
Rice Bran	8	21.34														N
Pea fiber	9	17.055														
Corn Bran	9	3.341														

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Means that do not share a letter are significantly different.

12- FIBER CORRECTED DATA -GAS 6h

Treatment	N	Mean	Grouping												
White Whole Grain Sorghum Flour	9	13.81	A												
Pearl Millet Whole	9	12.21	A												
Yams	8	8.466		B											
Pearl Millet de corticated	9	7.699		B	C										
Cassava	9	7.310		B	C	D									
Pressure Cooking Lentil FlourSODA	9	6.709		B	C	D	E								
Whole Faba Bean	9	6.520		B	C	D	E	F							
Amaranth	9	6.376		B	C	D	E	F							
Pressure Cooking Lentil FlourCA	9	6.122		B	C	D	E	F	G						
Sweet Potato	9	6.082		B	C	D	E	F	G						
Red Beets	9	5.852		B	C	D	E	F	G						
Plantain, ripe	9	5.686		B	C	D	E	F	G						
FOS	9	5.62		B	C	D	E	F	G						
White Coarse Sorghum Meal	9	5.347			C	D	E	F	G	H					
Plantain, green	9	5.046			C	D	E	F	G	H	I				
Whole Pea flour	9	5.036			C	D	E	F	G	H	I				
Atmospheric Cooking Lentil FlourCA	9	4.946			C	D	E	F	G	H	I				
Whole lentil flour	9	4.863			C	D	E	F	G	H	I	J			
Large Taro Root	8	4.557				D	E	F	G	H	I	J	K		
Atmospheric Cooking Lentil FlourSODA	9	4.505				D	E	F	G	H	I	J	K		
Carrots	9	4.185					E	F	G	H	I	J	K	L	
Whole Chickpea flour	9	4.069					E	F	G	H	I	J	K	L	
Lentil Flour Lentil Lime 0.3	9	3.972					E	F	G	H	I	J	K	L	
Atmospheric Cooking Lentil FlourLime	9	3.967					E	F	G	H	I	J	K	L	
Atmospheric Cooking Chickpea FlourSODA	9	3.960					E	F	G	H	I	J	K	L	
Chickpea Flour Lime 0.3	9	3.692						F	G	H	I	J	K	L	M
Pressure Cooking Chickpea FlourSODA Garbano Bic Sodio	9	3.6549						F	G	H	I	J	K	L	M
Pressure Cooking Chickpea FlourCA	9	3.365							G	H	I	J	K	L	M
Potato Starch	9	2.422								H	I	J	K	L	M
Hiccamah	9	2.207									I	J	K	L	M
Red Radish	9	1.959										J	K	L	M
Rice Bran	9	1.700											K	L	M
Wheat Bran	9	1.355												L	M
Lentil Fiber	9	1.298												L	M
Pea fiber	9	0.958													M
Corn Bran	9	0.657													

Means that do not share a letter are significantly different.

13- FIBER CORRECTED DATA -GAS 12h

Treatment	N	Mean	Grouping												
Pearl Millet Whole	9	16.669	A												
White Whole Grain Sorghum Flour	9	16.370	A												
Pressure Cooking Lentil FlourCA	9	12.24		B											
Whole Faba Bean	9	11.981		B	C										
Red Beets	9	11.976		B	C										
Yams	7	11.567		B	C	D									
Pressure Cooking Lentil FlourSODA	9	10.533		B	C	D	E								
Pearl Millet de corticated	9	10.018		B	C	D	E	F							
Cassava	9	9.895		B	C	D	E	F	G						
Whole Pea flour	9	9.877		B	C	D	E	F	G						
Atmospheric Cooking Lentil FlourCA	9	9.863		B	C	D	E	F	G						
FOS	9	9.719		B	C	D	E	F	G	H					
Whole lentil flour	9	8.957			C	D	E	F	G	H	I				
Sweet Potato	9	8.299				D	E	F	G	H	I				
Atmospheric Cooking Lentil FlourSODA	9	8.052					E	F	G	H	I	J			
Lentil Flour Lentil Lime 0.3	9	7.802					E	F	G	H	I	J			
Pressure Cooking Chickpea FlourSODA Garbano Bic Sodio	9	7.576					E	F	G	H	I	J			
Plantain, ripe	9	7.573					E	F	G	H	I	J			
Amaranth	9	7.544					E	F	G	H	I	J			
Carrots	9	7.416					E	F	G	H	I	J			
Atmospheric Cooking Lentil FlourLime	9	7.217						F	G	H	I	J			
White Coarse Sorghum Meal	9	6.979						F	G	H	I	J			
Whole Chickpea flour	9	6.850							G	H	I	J	K		
Large Taro Root	9	6.751							G	H	I	J	K		
Chickpea Flour Lime 0.3	9	6.583								H	I	J	K	L	
Pressure Cooking Chickpea FlourCA	9	6.512									I	J	K	L	
Plantain, green	8	6.320									I	J	K	L	
Atmospheric Cooking Chickpea FlourSODA	9	6.033									I	J	K	L	
Red Radish	9	4.932										J	K	L	M
Hiccamah	9	3.707											K	L	M
Potato Starch	9	3.56												L	M
Rice Bran	9	2.460													M
Wheat Bran	9	1.760													
Lentil Fiber	9	1.754													
Pea fiber	9	1.616													
Corn Bran	9	0.554													

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Means that do not share a letter are significantly different.

14- FIBER CORRECTED DATA -GAS 24h

Treatment	N	Mean	Grouping												
White Whole Grain Sorghum Flour	9	20.58	A												
Pearl Millet Whole	9	19.615	A												
Red Beets	9	15.883		B											
Pressure Cooking Lentil FlourCA	9	15.453		B											
Whole Faba Bean	9	15.39		B											
Yams	9	13.636		B	C										
Whole Pea flour	9	13.409		B	C										
Pressure Cooking Lentil FlourSODA	9	13.175		B	C	D									
Pearl Millet de corticated	9	12.932		B	C	D	E								
Amaranth	9	11.452			C	D	E	F							
Atmospheric Cooking Lentil FlourCA	9	11.435			C	D	E	F							
Cassava	9	11.321			C	D	E	F							
FOS	9	11.044			C	D	E	F							
Whole lentil flour	9	10.344			C	D	E	F	G						
Whole Chickpea flour	9	10.306			C	D	E	F	G						
Sweet Potato	9	10.137			C	D	E	F	G						
Pressure Cooking Chickpea FlourSODA Garbano Bic Sodio	9	9.818				D	E	F	G	H					
Lentil Flour Lentil Lime 0.3	9	9.754				D	E	F	G	H					
Carrots	9	9.506					E	F	G	H					
Atmospheric Cooking Lentil FlourLime	9	9.337						F	G	H					
Atmospheric Cooking Lentil FlourSODA	9	9.322						F	G	H					
White Coarse Sorghum Meal	9	9.079						F	G	H					
Chickpea Flour Lime 0.3	9	9.068						F	G	H					
Pressure Cooking Chickpea FlourCA	9	8.750						F	G	H					
Atmospheric Cooking Chickpea FlourSODA	9	8.489						F	G	H					
Plantain, ripe	9	8.369						F	G	H	I				
Red Radish	9	7.964						F	G	H	I	J			
Large Taro Root	8	7.923						F	G	H	I	J			
Potato Starch	9	7.056							G	H	I	J	K		
Plantain, green	9	6.486								H	I	J	K		
Hiccamah	9	4.827									I	J	K	L	
Wheat Bran	9	4.558										J	K	L	
Rice Bran	9	3.705											K	L	M
Lentil Fiber	9	2.852												L	M
Pea fiber	9	2.632												L	M
Corn Bran	9	0.682													M

Means that do not share a letter are significantly different.

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