View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by DigitalCommons@University of Nebraska

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Dissertations, Theses, & Student Research in Food Science and Technology

Food Science and Technology Department

11-2019

Effect of Processing on Microbiota Accessible Carbohydrates in Whole Grains

Caroline Smith
University of Nebraska - Lincoln, csmith159@huskers.unl.edu

Follow this and additional works at: https://digitalcommons.unl.edu/foodscidiss

Smith, Caroline, "Effect of Processing on Microbiota Accessible Carbohydrates in Whole Grains" (2019). *Dissertations, Theses, & Student Research in Food Science and Technology*. 102.
https://digitalcommons.unl.edu/foodscidiss/102

This Article is brought to you for free and open access by the Food Science and Technology Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Dissertations, Theses, & Student Research in Food Science and Technology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

EFFECT OF PROCESSING ON MICROBIOTA ACCESSIBLE CARBOHYDRATES IN WHOLE GRAINS

by

Caroline Smith

A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Food Science and Technology

Under the Supervision of Professor Devin J. Rose

Lincoln, Nebraska

November, 2019

EFFECT OF PROCESSING ON MICROBIOTA ACESSIBLE CARBOHYDRATES IN WHOLE GRAINS

Caroline Smith, M.S.

University of Nebraska, 2019

Advisor: Devin J. Rose

There is potential to increase microbiota accessible carbohydrates (MAC) in whole grains through food processing. Therefore, different processing conditions for boiling and extrusion of whole wheat flour were studied for their effect on MAC. Processing conditions had a minimal effect on fermentation response using 2 fecal samples of disparate composition. However, the processing method had an impact on the fermentation of non-digestible carbohydrates and subsequent short chain fatty acids production, but only for a microbiome with high diversity and a predominance of microbes associated with dietary fiber intake. Subsequently, five processing methods: boiling, extrusion, unleavened bread, yeast bread, and sourdough bread, were studied for their effects on *in vitro* fermentation of whole wheat flour using the fecal microbiota from 10 subjects. Different grain processing operations caused varying shifts in microbiota composition and activity. Notably, sourdough bread supported a diverse microbial community that produced more butyrate and propionate. Conversely, extrusion led to decreases in diversity and butyrate and propionate production, but enabled increase carbohydrate fermentation. These differences were attributed to changes in the microstructure of the grains. In order to better understand the effects of grain processing on host health, two microbiomes that ranged in diversity and were from subjects who habitually consumed high and low levels of dietary fiber and whole grains, were inoculated into germ free mice. These mice were then fed isocaloric western diets supplemented with cellulose (control), boiled whole wheat, extruded whole wheat, or extruded

brown rice. The mice harboring the microbiome from the low dietary fiber consumer had increases in diversity due to whole grains and the whole grain source and processing had differential effects. The mice harboring the microbiome from the high dietary fiber consumer was more diverse and was minimally affected by treatment. These mice had reduced body fat and body weight gain with boiled whole wheat compared to extruded whole wheat while there was no difference between brown rice and whole wheat. Both microbiomes had reduced glucose tolerance with extruded grains. Thus, both the digestion and fermentation are important factors of the effect of whole grains and food processing on host health.

ACKNOWLEDGMENTS

I would like to express my immense gratitude to Dr. Rose for providing an opportunity to work and study in his lab. His guidance, support, and leadership motivated me to explore cereal science in more depth and allowed me to develop into a better scientist. I am so grateful to have had him as my advisor and mentor.

I would like to thank the members of my committee Dr. Amanda Ramer-Tait, Dr. Randy Wehling, and Dr. Jeyam Subbiah for their helpful suggestions and guidance that allowed me to develop professionally. I would like to thank Tushar Verma for his help with extrusion. I am thankful for the assistance of Dr. Yibo Xian, Rafael Segura Munoz, and Alex Rancine for their help and guidance in my *in-vivo* experiments. I would like to thank Robert Schmaltz; Tammy May; Stacy Wiles; and the Ramer-Tait lab; Ashley, Paul, Maria, Mika, Morgan, and Anthony; and the genomic mouse facility staff for their guidance and assistance with the mouse experiment. I would also like to thank Mallory Van Haute for her help with 16S sequencing. I would like to thank the pilot plant staff; Steve Weier, Russell Parde, Thomas Dobesh, and Sarah Herzinger; for their help with freeze drying and extrusion and Marc Walter for helping me in wheat quality and in the milling lab. I am thankful to my lab mates past and present; Paridhi, Sandrayee, Rachana Poudel, Sviatoslav, Kristina, Trang, Shu en , Hollman, Lei, and Sujun (Willow). Your help in the lab, support through my graduate degree, and friendship that I hope will last a lifetime have made my time in Nebraska wonderful.

I would like to thank my parents. Mom and Dad, thank you for your endless love, continuous support, and guidance. I am eternally grateful for everything you have done to get me to where I am today. Finally, thank you to all of my friends and family for your love and support throughout graduate school and my life.

LIST OF TABLES vii

LIST OF FIGURES viii

CHAPTER 1 REVIEW OF EFFECT OF PROCESSING ON MICROBIOTA ACCESSIBLE CARBOHYDRATES IN WHOLE GRAINS 1

1.1. ABSTRACT 1

1.2. INTRODUCTION 2

1.3. WHOLE GRAINS IN THE DIET 4

1.4. PROCESSING TO MODIFY CARBOHYDRATE FERMENTABILITY 8

1.5. REFRENCES 16

CHAPTER 2 : DETERMINING THE EFFECT OF PROCESSING METHOD AND CONDITIONS ON MICROBIOTA ACCESSIBLE CARBOHYDRATES IN WHOLE GRAINS 32

2.1. ABSTRACT 32

2.2. INTRODUCTION 33

2.3. MATERIALS AND METHODS 35

2.3.1. *Whole wheat processing* 35

2.3.2. *in vitro digestion* 36

2.3.3. *Fecal samples for in vitro fermentation* 37

2.3.4. *Dietary patterns* 38

2.3.5. *In vitro fermentation* 38

2.3.6. *Microbiota accessible carbohydrates (% of non-digestible carbohydrate) analysis* 39

2.3.7. *Short chain fatty acid analysis* 40

2.3.8. *Statistical analysis* 41

2.4. RESULTS 41

2.4.1. *Microbiota composition of fecal samples and dietary patterns* 41

2.4.2. *Whole wheat flour composition* 41

2.4.3. *Boiling* 42

2.4.4. *Extrusion* 42

2.4.5. *Processing techniques* 42

2.5. DISCUSSION 43

2.6. CONCLUSION 46

CHAPTER 3 PROCESSING HAS DIFFERENTIAL EFFECTS ON MICROBIOTA ACCESSIBLE CARBOHYDRATES IN WHOLE GRAINS 63

3.1. ABSTRACT 63
3.2. INTRODUCTION 64
3.3. MATERIALS AND METHODS 67
3.3.1. *Whole wheat processing* 68
3.3.2. *In vitro digestion* 70
3.3.3. In vitro fermentation 70
3.3.4. Microbiota composition 72
3.3.5. Carbohydrate analysis 72
3.3.6. Short chain fatty acids 73
3.3.7. Statistical analysis 74
3.4. RESULTS 74
3.5. DISCUSSION 77
3.6. CONCLUSION 81
3.7. REFERENCES 83

CHAPTER 4 EFFECT OF WHOLE GRAIN TYPE AND PROCESSING METHOD ON HOST HEALTH IN CONTRASTING MICROBIOMES 99
4.1. ABSTRACT 99
4.2. INTRODUCTION 100
4.3. MATERIALS AND METHODS 103
4.3.1. Whole grain processing 103
4.3.2. Experimental diets 104
4.3.3. Fecal samples 104
4.3.4.Dietary patterns of fecal donors 105
4.3.5.Mouse Experiment 105
4.3.6.Intraperitoneal glucose tolerance test 106
4.3.7.Cecal short chain fatty acids 106
4.3.8.Characterization of the fecal microbiota composition 107
4.3.9.In vitro digestibility of diets 108
4.3.10.Statistical analysis 109
4.4. RESULTS AND DISCUSSION 109
4.4.1.Fecal microbiome characteristics and dietary patterns 109
4.4.2.Changes in the microbiome 110
4.4.3.Diffrences in short chain fatty acids 114

4.4.4.Changes in physiology 114

4.4.5.Diffrences in diet in vitro digestibility 118

4.5. CONCLUSIONS..................... 119

4.6. REFERENCES 121

LIST OF TABLES

Table 2.1. Non-digestible carbohydrates (NDC) and residual starch content in processed and digested whole wheat products….…………………………………………………………….57

Table 2.2. ANOVA results (mean squares) for boiled wheat……..……………………………58

Table 2.3. ANOVA results (mean squares) for extruded wheat.………………………………..59

Table 4.1. Formulation and composition of experimental diets (g).……………………….……..131

LIST OF FIGURES

Figure 2.1. α-Diversity of subject fecal samples used for *in vitro* fecal fermentation by (A) observed operational taxonomical units (OTU), (B) Faith's Phylogenic Diversity, and (C) Pielou Evenness……………..………………………………………………………………….54

Figure 2.2. Heat Tree depicting taxonomic significant differences between the two microbial communities; green is more abundant in subject 1 while gold is more abundant in subject 2 (Wilcoxon rank sum test, p< 0.05)…………………………..……………………………….55

Figure 2.3. Average grams of dietary fiber (A), serving of whole grains, and servings of refined grains (B) expressed per 1000 kcal. …………………………………………………………..56

Figure 2.4. Effect of boiling conditions on change in fermentable carbohydrates due to moisture content and subject after 12 hours of *in vitro* fecal fermentation. Letters denote Tukey honest significant difference at p <0.05results (mean squares) for boiled wheat. ……………………...60

Figure 2.5. Effect of extrusion screw speed revolutions per minute (rpm) on butyrate production 2 hours of *in vitro* fecal fermentation. Letters significant difference at p <0.05………………..61

Figure 2.6. Average Effect of processing technique and subject on (A) fermentable carbohydrates (% of NDC fermented) and (B) short chain fatty acids (SCFA) after 12 hours of *in vitro* fecal fermentation. Letters denote Tukey honest significant difference at p <0.05……….62

Figure 3.1. Carbohydrates in dialysis retentate of processed grain; error bars show standard error; different letters denote a significant difference (p<0.05). ……..…………………………...90

Figure 3.2. Characteristics of subject fecal samples. Genera relative abundance in fecal samples (A) and differences in α- diversity with Faith Phylogenic Diversity (B)..……………..…………...91

Figure 3.3. Change in microbiota α-diversity during *in vitro* fermentation (12 h - 0 h) of processed wheat samples by subject. Error bars show standard error across all 10 subjects; different letters denote a significant difference (p<0.05)…………….……………………….....92

Figure 3.4. Bray Curtis distance from fecal sample after 12 hours of *in vitro* fecal fermentation by subject and process; error bars show standard error; different letters denote a significance difference (p<0.05)………………………………………..………………….……………93

Figure 3.5. Log fold change of relative abundance of genera that significantly varied by processing method after 12 hours of *in vitro* fermentation by processing method; different letters denote a significance difference (p<0.05)…………………………...……………………......94

Figure 3.6. Percentage of microbiota accessible carbohydrates (MAC) after 12 hours of *in vitro* human fecal fermentation of whole wheat flour by subject and by processing method compared to after 0h of fermentation; error bars show standard error; different letters denote a significance difference (p<0.05)………………………………………...…………….……..……..95

Figure 3.7. Short chain fatty acid (SCFA) production after 12 h of *in vitro* human fecal fermentation of whole wheat flour by subject and process for (A) acetate (B) propionate (C) butyrate and (D) total SCFA; error bars show standard error; different letters denote a significance difference (p<0.05)…………….……………………………..…………………….....96

Figure 3.8. (A) significant partial correlations with subject and processing method as partial variables between Butyrate and % carbohydrates fermented (p 0.0174) (B) correlation between Faith’s Phylogenetic Diversity and % carbohydrates fermented (p 0.0109). ……...………......97

Figure 3.9. Illustration of hypothesis for transformation of carbohydrates though processing, digestion and fermentation…………………………………………………...…….………....98

Figure 4.1. Heat Tree depicting taxonomic significant differences between the two microbial communities; green is more abundant in microbiome A while gold is more abundant in microbiome B (Wilcoxon rank sum test, p< 0.05……………………..………..……………...132

Figure 4.2. α-Diversity of subject fecal samples by (A) observed operational taxonomical units (OTU), (B) Faith's Phylogenic Diversity, and (C) Pielou Evenness.…………….…..…………...133

Figure 4.3. Dietary patterns of subjects adjusted for total caloric intake for (A) grams of dietary fiber per 1000 kCal, and (B) servings of whole grains and refined grains per 1000 kCal. of variance (mean squares) of measured variables on whole wheat flour…..……..……………...134

Figure 4.4. α-Diversity of humanized mice by (A) observed operational taxonomical units (OTU), (B) Faith's Phylogenic Diversity, and (C) Pielou Evenness.……………….…..………...135

Figure 4.5. β-Diversity of human fecal samples and humanized mice over the course of the study (w0: before dietary intervention, w1: 1 week after dietary intervention, and w11: 11 weeks after dietary intervention) using Bray Curtis diversity metric.……………………..……..………...136

Figure 4.6. β-Diversity of human fecal samples and humanized mice over the course of the study. Red denotes microbiome A and blue denotes microbiome B. Ring denotes fecal microbiome, sphere denotes humanized mice microbiome before dietary intervention, diamond denotes microbiome after 1 week of dietary intervention, and square denotes microbiome after 11 week of dietary intervention using Bray Curtis diversity metric………...………………….137

Figure 4.7. Change in α-diversity after 11 weeks of dietary intervention for microbiome A and B using (A) Faith's Phylogenic Diversity (B) observed operational taxonomical units (OTU), and (C) Pielou Evenness……………………………………………………...……..…..………...138

Figure 4.8. Linear Discriminant Analysis Effective Size (LEfSe) for the differentially abundant genera, using Kruskal-Wallis (KW) sum-rank test with an FDR adjusted p value of 0.05 for both

microbiome mice based on dietary treatment C (control), WD (western diet), WD + BWW (western diet + boiled whole wheat), WD + EWW (western diet + extruded whole wheat), WD + EBR (western diet + extruded brown rice)……….……………………………..…………...139

Figure 4.9. Cecal short chain fatty acids (SCFA) based on mouse microbiome and dietary treatment C (control), WD (western diet), WD + BWW (western diet + boiled whole wheat), WD + EWW (western diet + extruded whole wheat), WD + EBR (western diet + extruded brown rice). Error bars show standard error…….………………………………….…..………...140

Figure 4.10. Change in body composition based on percent body fat due to change in percent body fat for microbiome A (A) and microbiome B (B) due to dietary treatment C (control), WD (western diet), WD + BWW (western diet + boiled whole wheat), WD + EWW (western diet + extruded whole wheat), WD + EBR (western diet + extruded brown rice). Error bars express standard error..…………………………………………………………...……..………...141

Figure 4.11. Change in body weight for microbiome A (A) and microbiome B (B) due to dietary treatment C (control), WD (western diet), WD + BWW (western diet + boiled whole wheat), WD + EWW (western diet + extruded whole wheat), WD + EBR (western diet + extruded brown rice). Error bars express standard error…………….……………………...……………...142

Figure 4.12. Glucose intolerance for microbiome A (A) and microbiome B (B) due to dietary treatment C (control), WD (western diet), WD + BWW (western diet + boiled whole wheat), WD + EWW (western diet + extruded whole wheat), WD + EBR (western diet + extruded brown rice). Area under the curve of glucose intolerance response for microbiome A (C) and microbiome B (D). Error bars express standard error…………………………………...…...143

Figure 4.13. *In vitro* starch digestibility profile of experiment diets C (control), WD (western diet), WD + BWW (western diet + boiled whole wheat), WD + EWW (western diet + extruded

whole wheat), WD + EBR (western diet + extruded brown rice). Rapidly available glucose (RAG), the glucose available after 20 minutes, slowly available glucose (SAG), the glucose available after 120 minutes, and resistant starch (RS), the starch not digested within 120 minutes……………………………………………………………………………….…………..144

CHAPTER 1 REVIEW OF EFFECT OF PROCESSING ON MICROBIOTA ACCESSIBLE CARBOHYDRATES IN WHOLE GRAINS

1.1. ABSTRACT

The gut microbiome is a complex part of the human body. A lack of diversity and imbalances in the gut microbiome have been shown to be associated with disease. Non-digestible carbohydrates, which are found in foods such as whole grains, are the main energy source for the microbiome. Many of the health benefits of fiber are attributed to their fermentation by the microbiota and the metabolites produced, particularly short chain fatty acids (SCFA). However, not all non-digestible carbohydrates are fermentable, or are microbiota accessible carbohydrates (MAC), especially in whole grains. Moreover, most people do not eat enough fiber or whole grains, suggesting that there is not enough MAC in the typical western diet. Still, whole grains and dietary fiber have been shown to influence microbiota composition and reduce the risk of disease. Food processing is a possible approach to increase MAC in grains. Grains are typically processed before consumption by techniques including but not limited to boiling, baking, extrusion, flaking, and puffing. Processing is widely known to change the structure of food. This review will examine previous studies that looked at whole grains and the effect of processing on whole grain structure, the microbiome, its metabolites, and/or physiological health markers. Overall, it is clear that processing leads to complex changes in structure and thus the fermentability of carbohydrates.

1.2. INTRODUCTION

The gut microbiome is a complex part of the human body. There are as many microorganisms in the intestinal tract as there are human cells in the body (Sender, Fuchs, & Milo, 2016). The adult microbiome is primarily comprised of the phyla Bacteroidetes and Firmicutes (Backhed, Ley, Sonnenburg, Peterson, & Gordon, 2005). The species population and level of diversity is highly variable between people due a myriad of factors including host genetics, diet, environment, and life events (i.e. antibiotic use) (Nicholson, et al., 2012). While the ideal microbiota composition is not yet determined, many studies have characterized positive elements such as diversity. Imbalances in gut microbial populations have been shown to be associated with disease including inflammatory bowel disease (IBD), osteoarthritis, obesity, and type II diabetes (Manichanh, et al., 2006; Frank, et al., 2007; Gore, et al., 2008). Additionally, maintaining a diverse microbiota in the gut helps prevent harmful bacteria such as pathogens from colonizing (Colon & Bird, 2015).

Around the world, about 1 in 5 deaths are attributed to poor dietary habits (Wang, et al., 2014). In the US, one in ten adults are diabetic, and over one in three adults are obese (Hales, Fryar, Carroll, Freedman, & Ogden, 2018). Cardiovascular disease is the leading cause of death worldwide and claims the lives of one in three Americans. These diseases are a drain on the economy and cost the US economy more than $600 billion dollars each year (Benjamin, Muntner, & Bittencourt, 2019).

Non-digestible carbohydrates, commonly from dietary fiber sources such as whole grains, are the main potential energy source for the microbiome. Unlike human somatic cells, the microbes in the gut are capable of hydrolyzing a wide variety of glycosidic linkages, allowing these carbohydrates to act as food for the microbiome (Gill, et al., 2006). It is believed that many

of the health benefits of fiber are a result of their fermentation by the microbiota and the metabolites produced, particularly short chain fatty acids (SCFA). SCFA provide energy to other bacteria, provide energy to epithelial tissues, inhibit the growth of pathogenic bacteria, and maintain tissue integrity by preventing the fermentation of proteins into toxins such as ammonia, phenols and hydrogen sulfide (Cummings & Macfarlane, 1991; Topping & Clifton, 2001; Donohoe, et al., 2011). Moreover, SCFA are absorbed by the host and have pleiotropic local effects on intestinal function as well as systemic roles in insulin secretion, lipid metabolism, and inflammation (Kasubuchi, Hasegawa, Hiramatsu, Ichimura, & Kimura, 2015; Den Besten, et al., 2013; Puertollano, Kolida, & Yaqoob, 2014). Furthermore, SCFA have been found to be associated with reduced risk of many western diseases including osteoarthritis, obesity, type II diabetes and cardiovascular disease (Fung, Cosgrove, Lockett, Head, & Topping, 2012; Topping & Clifton, 2001).

However, not all carbohydrates that reach the colon are fermentable. While over 75% of the dietary fibers in fruits and vegetables are generally available for fermentation by the microbiota, only about one third of the dietary fibers in grains are fermentable. Sonnenburg and Sonnenburg (2014) proposed the term microbiota accessible carbohydrates (MAC), which is defined as “carbohydrates that are metabolically available to gut microbes.” Since these carbohydrates must be metabolized by the microbiota, the quantity of MAC in any given food is dependent upon the interaction between the microbiota in question and the dietary fibers in the food. Thus, while whole grains are an excellent source of dietary fiber, they may not be a good source of MAC.

While on the rise, the levels of whole grain consumption, dietary fiber consumption, and healthy eating are much lower than desired. Most Americans only eat approximately 1 serving of

whole grains and 15 grams of dietary fiber per day (Albertson, Reicks, Joshi, & Gugger, 2015). This is only a fraction of the recommended 3 servings of whole grains and 28 grams of dietary fiber per day (US Department of Health and Human Services, 2017). Moreover, only 1.5% of American adults achieve an ideal healthy diet score and 41% of Americans have a poor diet score from the American Heart Association. These diet scores are based on the American Heart Association's 2020 Strategic Impact Goals for diet where elements are scored with 50 points possible. Survey responders' diets are classified as poor (<20/50), intermediate (20-39.9/50) or ideal (>40/50) (Rehm, Peñalvo, Afshin, & Mozaffarian, 2016). However, there have been some improvements in the last decade where poor diet scores decreased by 18%, and the average number of whole grains consumed increased by 35% in adults (Albertson, Reicks, Joshi, & Gugger, 2015).

While an improvement, these metrics lag behind the ideal amount even though whole grain consumption has been recommended in the dietary guidelines since the guidelines began in 1980 and have been believed to be an important part of the diet since the 1960s (Cho, Qi, Fahey, & Klurfeld, 2013).

1.3. WHOLE GRAINS IN THE DIET

Despite potentially low availability of fermentable carbohydrates, whole grains are an important source of dietary fiber in the diet. The US Food and Drug Administration (FDA) defines whole grains as "cereal grains that consists of intact, ground, cracked, milled, or flaked fruit of the grains whose principal components—the starchy endosperm, germ, and bran—are present in the same relative proportions as they exist in the intact grain." The bran and germ, which comprise 14-16% and 2-4% of the wheat kernel, respectively, contain the majority of biologically active compounds found in grains. This includes high concentrations of B vitamins

(thiamin, niacin, riboflavin, and pantothenic acid), minerals (Ca, Mg, K, P, Na, and Fe), phytochemicals (phytates and antioxidants) lipids, and dietary fiber (Miller, Prakash, & Decker, 2002). The fiber in bran is primarily made up of cell wall polysaccharides, which consist of arabinoxylans (64%), cellulose (29%), and mixed-linkage β-glucan (6%) in wheat bran. The endosperm, which makes up 81-84% of the wheat kernel, contains starch (50-75%), storage proteins (8-18%), and cell wall polysaccharides (Slavin, 2004; Posner, 2000). For people of all ages, the most common whole grain sources are ready to eat cereals, yeast breads/rolls, oatmeal, and popcorn (Reicks, Jonnalagadda, Albertson, & Joshi, 2014). In western countries such as the US, cereal foods supply 50% of the dietary fiber to the diet. The remaining half consists mostly of fruits and vegetables and other minor sources (Rodriquez, Jimenez, Fernandez-Bolanos, Guillen, & A, 2006). Moreover, whole grain intake and total dietary fiber intake have been shown to be correlated (Reicks, Jonnalagadda, Albertson, & Joshi, 2014).

The health benefits of whole grains are thought to be due to many potential factors. Whole grains are an excellent source of vitamins, minerals, carotenoids, lignans, phytosterols, and polyphenols, which protect against disease and act as antioxidants (Adlercreutz & Mazur, 1997; Yankah & Jones, 2001; Soto-Vaca, Gutierrez, Losso, Xu, & Finley, 2012). Whole grains also increase fecal bulk due to water holding capacity of the dietary fiber fractions. This property protects against diseases such as colon cancer by potential mechanisms including the dilution and elimination of toxins, decreased transit time, and the reduction of intracolonic pressure (Bingham, et al., 2003; Stephen & Cummings, 1980).

Whole grains and dietary fiber have been shown to influence microbiota composition. De Filippo et. al. (2010) saw that children in rural Africa and South America who consumed a more traditional plant-based diet had higher microbiota diversity and increased abundances of

Xylanibacter and *Prevotella* compared with European children with low fiber Western diets (De Filippo, et al., 2010). In multiple human intervention studies, whole grains increase the abundance of *Bifidobacterium* (Carvalho-Wells, 2010; Christensen, Licht, Kristensen, & Baul, 2013). In addition to *Bifidobacterium*, in a 3-week double-blind, randomized, placebo-controlled crossover study; whole wheat breakfast cereal resulted in increased *Lactobacillus/ Enterococci* and *Bacteroides* compared to the pre-intervention baseline (Costabile, 2008). Mixed whole grains also increased the abundance of *Enterococcus* spp. and *Clostridium leptum* and increased stool frequency compared to refined grains in a 2-week randomized crossover study (Adams & Engstrom, 2000; Ross, et al., 2011). In a human intervention study, responders, or people that had reduced incremental blood glucose area, blood glucose area, and decreased insulin after dietary intervention, had a higher *Prevotella/Bacteroides* ratio compared to non-responders, or people who had the least improvements in blood glucose and insulin after consuming barley kernel bread (Kovatcheva-Datchary, et al., 2015). Lappi, et al. (2013) fed whole grain rye breads and refined wheat breads to subjects in a 12-week intervention study and saw increases in the abundance of *Bacteroides* and *Prevotella* on the whole grain rye breads (Lappi, et al., 2013). In a 4-week randomized crossover study, whole grain barley and brown rice led to increased diversity and increased Firmicutes and reduced Bacteroidetes compared with baseline. These changes in barley were mainly due to a higher abundance of *Blautia* and *Roseburia* and a lower abundance of *Bacteroides* (Martínez, et al., 2013).

In animal studies, the effects of whole grains on the microbiome, its metabolites, and physiological health markers have also been repeatedly studied. Whole barley increased diversity along with the abundance of *Akkermansia, Ruminococcus, Blautia,* and *Bilophila* in rats (Zhong, Nyman, & Fåk, 2015). Whole wheat increased *Lactobacillus* in obese mice (Garciamazcorro,

Ivanov, Mills, & Noratto, 2016). Whole rye increased diversity and *Neisseriaceae* and decreased *Clostridium* and the Firmicutes/ Bacteroidetes ratio (Ounnas, et al., 2016). Whole grains have been shown to increase cecal and fecal SCFA levels, especially butyrate and propionate (Garciamazcorro, Ivanov, Mills, & Noratto, 2016; Ounnas, et al., 2016; Zhong, Marungruang, & Nyman, 2015; Zhong, Nyman, & Fåk, 2015). Whole grains have been shown to also reduce weight gain and increased insulin sensitivity, cholesterol levels, and glucose clearance (Garciamazcorro, Ivanov, Mills, & Noratto, 2016; Ounnas, et al., 2016; Murtaza, et al., 2014).

Many studies have shown the benefits of eating whole grains and their impact on disease. Whole grain consumption is associated with reduced risk of cancer (Jacobs, Marquart, Slavin, & Kushi, 1998), cardiovascular disease (Mellen, Walsh, & Herrington, 2008; Mente, De Koning, Shannon, & Anand, 2009), type II diabetes mellitus (De Munter, Hu, Spiegelman, Franz, & Van Dam, 2007), and metabolic syndrome (Lutsey, et al., 2007), and all-cause mortality (Aune, et al., 2016). Whole grain consumption has been shown to be associated with many physiological makers for good health including improvements in blood pressure (Tighe, et al., 2010), cholesterol levels (Giacco, et al., 2010; Jensen, et al., 2006), glycemic control (Pereira, et al., 2002), inflammation (Motonen, et al., 2013; Vanegas, et al., 2017; Vitaglione, et al., 2014), and liver functions (Motonen, et al., 2013).

There are numerous reasons why people do not consume whole grains. The largest barrier to whole grain consumption is the negative perception of the sensory properties of whole grains (Arvola, 2007; Burgess-Champoux T, 2006; Dean, Raats, & Shepherd, 2007; Zhang, Malik, & Pan, 2010). A focus group study conducted in the UK found that in addition to sensory properties of whole grains, a lack of knowledge about of what they are, where to find them, how to use them, and their health benefits were barriers to increased consumption (McMackin, Dean,

Woodside, & McKinley, 2012). People that do eat whole grains tend to be older, higher income, higher educated, non-smokers that exercise (Adams & Engstrom, 2000; Cleveland, Moshfegh, Albertson, & Oldman, 2000; Johansson, Thelle, Slovoll, Bjoerneboe, & Drevon, 1999). Men also consume more whole grains than women, but this is due to increased food consumption (Lang & Jebb, 2003).

The lack of a healthy diet indicates that the level of MAC in the diet is much lower than necessary in order to maintain a thriving microbiome. When not fed MAC, gut microbes have been shown to die off at alarming rates in humanized mice. In this study, mice were fed a high MAC diet for 4 weeks and then were fed a low MAC diet for 7 weeks, during which they gave birth, before returning to a high MAC diet. Switching to a low MAC diet resulted in 60% of the taxa decreased at least two-fold in abundance. This was further pronounced in generations of poor diets where after four generations, 72% of the taxa were lost. However, it was observed that by switching back to a diet high in fermentable carbohydrates, it was possible to regain a portion of the lost taxa, suggesting that incorporating more MAC into the diet has great potential to improve the microbiome (Sonnenburg, et al., 2016).

1.4. PROCESSING TO MODIFY CARBOHYDRATE FERMENTABILITY

Cereal processing has been suggested as a potential strategy to increase the availability of carbohydrates in foods for fermentation (Poutanen, Sozer, & Della Valle, 2014; Slavin, Jacobs, & Marquart, 2000). Most grains are subject to some type of heat processing such as boiling, baking, extrusion, flaking, puffing, and more (Slavin, Jacobs, & Marquart, 2000). Processing technologies are widely accepted to change the structure and biological effects of bioactive components in food (Poutanen, Sozer, & Della Valle, 2014).

Many of the techniques that can be applied to grain can influence dietary fiber structure and thus potentially modify MAC. Particle size has been shown to be a factor for gut health. Coarse wheat bran increased the fecal bulking, delayed gastric emptying, and accelerated small-bowel transit time compared to finely ground wheat bran (McIntyre, Vincent, Perkins, & Spiller, 1997; Wrick, et al., 1983). Connolly, Lovegrove, and Tuohy (2010) compared two particle sizes of whole oat flakes using human fecal fermentation *in vitro*. The results showed that the larger particle size resulted in significant increases in *Bifidobacterium* together with increases in propionate and butyrate concentrations. Meanwhile, the smaller particle size significantly increased the level of members of the *Bacteroides-Prevotella* group and decreased *Bifidobacterium* (Connolly, Lovegrove, & Tuohy, 2010). Germination, or sprouting, has been shown to increase the activity of hydrolytic enzymes. During prolonged germination, dietary fiber significantly increased and soluble dietary fiber increased 3-fold (Koehler, Hartmann, Wieser, & Rychlik, 2007).

Whole grains are commonly boiled into foods such as porridge. Boiling is a minimal form of grain processing that involves lower temperatures (100 °C), atmospheric pressure, minimal shear, and an abundance of water. During boiling, water swells the starch granules resulting in the leaching of amylose, starch gelatinization, and an amorphous structure. Boiling also results in tissue softening in dietary fibers (Slavin, Jacobs, & Marquart, 2000). Arcila and Rose (2015) repeatedly boiled and froze whole wheat flour for up to 7 cycles. With each cycle, there was an increase in resistant starch that was countered by a decrease in slowly digestible starch. Moreover, each cycle increased individual and total SCFA after *in vitro* fermentation for 24 hours. This processing also increased the concentration of *Bifidobacterium* (Arcila & Rose, 2015). Boiling of whole grain rye flour (64 g), salt (0.58 g) with water (200 g) resulted in no

measured changes in β-glucan and arabinoxylan, but did result in swollen, intact cell walls (Johansson, Vázquez Gutiérrez, Landberg, Alminger, & Langton, 2018). Boiling of whole wheat flour or wheat bran has commonly been used as a control process for whole grain experiments (Arcila, Weier, & Rose, 2015; Bjorck, Asp, & Lundquist, 1984; Bjorck, et al., 1984). Some grains such as rice can be parboiled, or partially boiled and dried. Bird (2000) studied the difference in parboiled brown rice and boiled white rice substituted with heat-stabilized rice bran to equate the amount of fiber in pig diets. The pigs fed parboiled brown rice had significantly more fecal output in the first week, greater digesta mass, and higher total and individual SCFA in the feces and the colon (Bird, et al., 2000).

Extrusion, a form of processing that uses pressure, mechanical shear and higher temperatures (>120 °C) to process grains into foods such as breakfast cereals and crispy snacks. This process results in the disruption of cell wall structures (dietary fibers), through increased porosity and fragmentation, and gelatinization and breakdown of starch (Robin, Dubois, Pineau, Schuchmann, & Palzer, 2011). In extruded rye, β-glucan and arabinoxylan separated and distributed thought a starch matrix as small fragments. The starch granules were also completely disrupted, resulting in a homogeneous starch phase, which encapsulated small fragments of cell walls and aleurone layers and a thinner lamella (Johansson, Vázquez Gutiérrez, Landberg, Alminger, & Langton, 2018). Moreover, extrusion has been shown to convert insoluble fiber into soluble fiber in different cereals (Kahlon, Berrios, Smith, & Pan, 2006; Ralet, Thibault, & Della Valle, 1990; Robin, Dubois, Pineau, Schuchmann, & Palzer, 2011; Wang, et al., 2014).

The effect of extrusion on digestibility has also been reported. Sun compared raw and extruded barley, pea, and potato starch wheat bran *in vitro* and in pigs. Extrusion increased the proportion of rapidly digestible starch, and reduced the proportion of slowly digestible starch and

resistant starch *in vitro*. In the raw material, barley was more rapidly digested, but there were no significant differences between the extruded cereals. When fed to pigs, extrusion increased the digestibility of starch, but this was dependent upon cereal source as barley was not significantly affected by extrusion. There was a difference in the amount of substrate fermented in the large bowl based on carbohydrate source and processing. Barley was not affected by extrusion but fermentation was reduced in peas and potato starch (Sun, Lærke, Jørgensen, & Knudsen, 2006). Fadel (1988) also saw that extrusion increased starch digestibility in the ilium but saw no difference in the fecal digestibility (Fadel, Newman, Newman, & Graham, 1988). Sandberg (1986) studied extrusion cooking on a high fiber wheat and rice mixture was compared to a boiled product by ileostomy in 7 humans. There was no significant difference in fiber, starch, fat, water or ash content in the ileostomy contents (Sandberg, Andersson, Kivistö, & Sandström, 1986).

The effect of extrusion on the fermentability of carbohydrates by the gut microbiome has also been studied. Arcila, Weier, and Rose (2015) found that extrusion resulted in up to a 55% increase in soluble fiber, an 114% increase in total NSP consumption, and a significant increase in SCFA production. Aoe (1990) found that extruded wheat bran was more available to the gut microbes in rats than native bran based on the neutral detergent fiber. Furthermore, the cell walls of the extruded bran were more disrupted after passage (Aoe, Nakaoka, Ama, Ohta, & Ayan, 1990). Kraler (2015) studied native, fermented and extruded wheat bran in piglets. There were no significant differences in performance parameters such as body weight gain and feed intake, but the amount of goblet cells in the ileum was higher when pigs were on the native and extruded wheat bran, compared to fermented bran. Moreover, the processed wheat bran had less SCFA than the native bran (Kraler, et al., 2015).

Bread making is the most common processing technique for whole wheat worldwide and how most people consume whole grains (Reicks, Jonnalagadda, Albertson, & Joshi, 2014). During the bread making process, a dough is formed through the addition of water and mechanical agitation (kneading), which creates a continuous matrix of protein (gluten) with starch granules and cell wall particles (containing dietary fiber) embedded within. Following dough development, bread making commonly involves fermentation of the dough by either *Saccharomyces cerevisiae* for yeast leavened bread or a use of co-cultures of lactic acid bacteria and yeasts that develop from the flour and environment (Minervini, De Angelis, Di Cagno, & Gobbetti, 2014). During fermentation, yeast metabolizes mono and disaccharides (glucose, galactose, sucrose and when needed maltose) into ethanol and carbon dioxide (among other minor, albeit important, flavor compounds) to leaven the dough. The bacteria in a sourdough culture, which are commonly lactic acid bacteria (LAB), have a symbiotic relationship with the yeast. The bacteria break down more complex carbohydrates (larger sugars and for some species, starch) to simpler sugars for the yeast to ferment. LAB additionally produce lactic and acetic acid that lower the pH of the co culture (Corsetti, et al., 1998). During baking, volatilization of carbon dioxide and steam further leaven the bread until the protein structure is set and starch is gelatinized.

The breadmaking process has been shown to increase non-digestible carbohydrate extractability (Cleemput, Booij, Hessing, Gruppen, & Dekcour, 1997). Bread making techniques were compared in whole grain rye bread in yeast-fermented crispbread, unfermented crispbread, and sourdough bread. In all breads, β-glucan and arabinoxylan were distributed as small fragments throughout the starch matrix, which suggest that fiber was degraded. The yeast-fermented crispbread and unfermented crispbread had highly swollen starch granules where the

unfermented crispbread appeared to have a higher degree of starch gelatinization and a thinner lamella. Additionally, the unfermented crispbread had larger pieces of bran and intact cell structures compared with the breads. For sourdough, starch granules were less swollen and surrounded by more leaked amylose, which was proposed to act as a protective layer during digestion (Johansson, Vázquez Gutiérrez, Landberg, Alminger, & Langton, 2018). Pre-fermentation of wheat bran also resulted in changes in microstructure. Salmenkallio-Marttila (2001) studied spontaneous fermentation, yeast fermentation, and sourdough fermentation (addition of yeast and lactic acid bacteria) on the characteristics of bread including microstructure. While the different bran fermentations did not noticeably affect the microstructure of the bran particles or cell wall fragments, there were differences in the starch microstructure. Longer fermentation times (16 h) resulted in increased swelling of the starch granules together with increased leaching of amylose from the starch granules. Yeast fermentation did not change the degree of swelling, but did result in the protein matrix being separated from the large starch granules, which was thought to be due to the reduction of the adhesion between gluten and starch. Sourdough fermentation resulted in the highest degree of starch granule swelling and leached amylose (Salmenkallio-Marttila, 2001). A sourdough style fermentation of bran reduced the average degree of arabinose substitution, which indicated that the water extractable arabinoxylans in the fermented bran were less substituted with arabinose (Manini, et al., 2014). A lower degree of arabinose substitution has been previously shown to be more degradable by the microbiota (Damen, et al., 2011; Karppinen, Kiiliäinen, Liukkonen, Forssell, & Poutanen, 2001).

In addition to changes in structure, bread making techniques have been shown to influence the health benefits of whole grains. Sourdough fermentation has been shown to

increase the concentration of folates, the concentration of free phenolic acids, the concentration of soluble arabinoxylans, and the release bioactive peptides (Coda, Di Cagno, Gobbetti, & Rizzello, 2014; Katina, et al., 2012; Manini, et al., 2014). Sourdough fermentation has been shown to improve the glycemic index of barley and wheat bread (De Angelis, et al., 2007; Liljeberg & Björck, 1996; Maioli, et al., 2008). This is thought to be due to the formation of organic acids, such as lactic acid, or due to the degree of starch gelatinization (Poutanen, Flander, & Katina, 2009). Sourdough breads have been shown to have higher levels of resistant starch compared with breads baked with commercial yeast (Scazzina, 2009). The increase in resistant starch is also thought to be due to the presence of lactic acid. Liljeberg, Åkerberg, & Björck (1996) observed an increase in resistant starch when lactic acid concentrations increased.

Fermentation has been shown to influence the microbiome and carbohydrate fermentation. Mateo-Anson (2011) studied SCFA production *in vitro* on whole meal wheat bran with bran that was native, fermented with baker's yeast, or fermented with baker's yeast and enzyme treated. Fermentation of wheat (with or without enzyme addition) induced a higher production of butyrate and less production of propionate compared to that with native bran. The native bran and fermented and enzyme treated bran were fed to 8 men and their blood plasma was collected. There were no differences in SCFA in plasma, likely due to its utilization in the colon (Mateo-Anson, et al., 2011). Nordlund (2013) used bioprocessing, a combination of hydrolytic enzymes and yeast, on rye bran to include in bran enriched breads. The result of the study showed bioprocessing had more soluble dietary fiber, soluble arabinoxylans, less furctans, less β-glucan than the non-bioprocessed bran bread. There were also changes in the physical structure of the bran. Bioprocessing appeared to degrade the aleurone cell walls and release starch granules compared with the intact aleurone cells, subaleurone and starchy endosperm of

the non-bioprocessed bran bread. When the breads were digested and fermented *in vitro*, bioprocessed bran was more fermentable due to a higher initial rate of SCFA formation and the increased degradation observed by microscopy (Nordlund, Katina, Aura, & Poutanen, 2013). Zhong (2015) studied the effect of malting barley in high fat diets of rats and found that rats consuming malted barley (and the cellulose control) had higher microbiota α-diversity than the unmalted barley. Moreover, there was a clear separation in the microbiota communities based on diet where unclassified *Clostridaceae, Rikenellaceae, RF32,* and *Clostridiales* were abundant in the malt group compared with the barley group and while the unmalted group was abundant in *Akkermansia, Bacteroides*, and unclassified *S24-7*. Compared to the control, the barley group had higher abundance of *Roseburia*. Furthermore, malting also increased butyric acid while acetic and propionic acid were high in the un-malted barley (Zhong, 2015).

Processing clearly changes the structure of food and has an effect on the microbiome. The effects of processing tend to improve the various metrics measured or not affect them significantly. However, comparing these processes across studies is difficult due to the various models used, whole grain sources, and conditions of processing. Moreover, most studies compare processing to its native state, a form in which whole grains are rarely consumed. Furthermore, most studies have focused on the change in digestibility rather than the change in fermentability. In conclusion, processing may be a promising strategy to increase MAC in foods for a healthier population.

1.5. REFRENCES

Adams, J. F., & Engstrom, A. (2000). Dietary intake of whole grains vs. recommendations. *Cereal Foods Worldwide, 45*, 75-79.

Adlercreutz, H., & Mazur, W. (1997). Phyto-oestrogens and western diseases. *Annals of Medicine 29*, 95-120.

Albertson, A. M., Reicks, M., Joshi, N., & Gugger, C. K. (2015). Whole grain consumption trends and associations with body weight measures in the United States: results from the cross sectional National Health and Nutrition Examination Survey 2001–2012. *Nutrition Journal, 15(1)*, 8.

Aoe, S., Nakaoka, T., Ama, M. Y., Ohta, F., & Ayan, Y. (1990). Availability of dietary fiber in extruded wheat bran and apparent digestibility in rats of coexisting nutrients. *Cereal Chemistry*, 145-148.

Arcila, J. A., & Rose, D. J. (2015). Repeated cooking and freezing of whole wheat flour increases resistant starch with beneficial impacts on in vitro fecal fermentation properties. *Journal of Functional Foods, 12*, 230-236.

Arcila, J. A., Weier, S. A., & Rose, D. J. (2015). Changes in dietary fiber fractions and gut microbial fermentation properties of wheat bran after extrusion and bread making. *Food Research International, 74*, 217-223.

Arvola A, L. (2007). Consumers' beliefs about whole and refined grain products in the UK, Italy and Finland. *Journal of Cereal Science, 46*, 197-202.

Aune, D., Keum, N., Giovannucci, E., Fadnes, L. T., Boffetta, P., Greenwood, D. C., & Norat, T. (2016). Whole grain consumption and risk of cardiovascular disease, cancer, and all cause and cause specific mortality: systematic review and dose-response meta-analysis of prospective studies. *BMJ, 353,* i2716.

Backhed, F., Ley, R., Sonnenburg, J., Peterson, D., & Gordon, J. (2005). Host-bacterial mutualism in the human intestine. *Science, 307*, 1915–1920.

Benjamin, E. J., Muntner, P., & Bittencourt, M. S. (2019). Heart Disease and Stroke Statistics—2019 Update: A Report From the American Heart Association. *Circulation, 139(10)*, e56-e528.

Bingham, S., Day, N., Luben, R., Ferrari, P., Slimani, N., Norat, T., . . . Boeing, H. (2003). Dietary fibre in food and protection against colorectal cancer. *The European Prospective Investigation into Cancer and Nutrition*, 1496–1501.

Bird, A. R., Hayakawa, T., Marsono, Y., Gooden, J. M., Record, I. R., Correll, R. L., & Topping, D. L. (2000). Coarse Brown Rice Increases Fecal and Large Bowel Short-Chain Fatty Acids and Starch but Lowers Calcium in the Large Bowel of Pigs. *The Journal of Nutrition, 130(7)*, 1780-1787.

Bjorck, I., Asp, N. G., & Lundquist, I. (1984). Digestion in vitro and In vivo; I extrusion cooking of wheat flours and starch. *Journal of Cereal Science, (2)*, 91-103.

Bjorck, I., Asp, N. G., Birked, A. C., Eliasson, L. B., Shjoberg, L. B., & Lundquist, I. (1984). Effects of prcessing on starch avalibility in vitro and in vivo; II drum drying of wheat flour. *Journal of Ceareal Science, (2)*, 165-178.

Burgess-Champoux T, M. L. (2006). Perceptions of children, parents and teachers regarding whole grain foods and implications for a school based intervention. *Journal of Nutrition Education, 38*, 230-237.

Carvalho-Wells, A. L., Helmolz, K., Nodet, C., Molzer, C., Leonard, C., McKevith, B., . . . Tuohy, K. M. (2010). Determination of the in vivo prebiotic potential of a maize-based whole grain breakfast cereal: a human feeding study. *British Journal of Nutrition, 104(9),* 1353-1356.

Cho, S. S., Qi, L., Fahey, G. C., & Klurfeld, D. M. (2013). Consumption of Cereal Fiber, Mixtures of Whole Grains and Bran, and Whole Grains and Risk Reduction in Type 2 Diabetes, Obesity, and Cardiovascular Disease. *American Journal of Clinical Nutrition, 98*, 594–619.

Christensen, E. G., Licht, T. R., Kristensen, M., & Baul, M. I. (2013). Bifidogenic effect of whole-grain wheat during a 12-week enrgy-restricted dietary intervention in post-menopausal women. *European Jornal of Clinical Nutrition, 67*(12), 1316-1321.

Cleemput, G., Booij, C., Hessing, M., Gruppen, H., & Dekcour, J. A. (1997). Solubilisation and changes in molecular weight distribution of arabinoxylans and protein in wheat flours during bread-making, and the effects of endogenous arabinoxylan hydrolysing enzymes. *Journal of Cereal Science, 26(1),* 55-66.

Cleveland, L. E., Moshfegh, A. J., Albertson, A. M., & Oldman, J. D. (2000). Dietary Intake of whole grains. *Journal of the American College of Nutrition, 19*, 331S-338S.

Coda, R., Di Cagno, R., Gobbetti, M., & Rizzello, C. G. (2014). Sourdough lactic acid bacteria: exploration of non-wheat cereal-based fermentation. *Food Microbiology, 37*, 51-58.

Colon, M., & Bird, A. (2015). The impact of diet and lifestyle on gut microbiota and human health. *Nutrients, 7(1)*, 17-44.

Connolly, M. L., Lovegrove, J. A., & Tuohy, K. M. (2010). In vitro evaluation of the microbiota modulation abilities of different sized whole oat grain flakes. *Anaerobe, 16(5)*, 483-488.

Corsetti, A., Gobbetti, M., Balestrieri, F., Paoletti, F., Russi, L., & Rossi, J. (1998). Sourdough lactic acid bacteria effects on bread firmness and stalin. *Journal of Food Science, 63(2)*, 347-351.

Costabile, A. K. (2008). Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, crossover study. *British Journal of Nutrition, 99(1)*, 110-120.

Cummings, J., & Macfarlane, G. (1991). The control and consequences of bacterial fermentation in the human colon. *Journal of Applied Bacteriology, 70*, 443-459.

Damen, B., Verspreet, J., Pollet, A., Broekaert, W. F., Delcour, J. A., & Courtin, C. M. (2011). Prebiotic effects and intestinal fermentation of cereal arabinoxylans and arabinoxylan oligosaccharides in rats depend strongly on their structural properties and joint presence. *Molecular Nutrition & Food Research, 55(12)*, 1862-1874.

De Angelis, M., Rizzello , C. G., Alfonsi, G., Arnault, P., Cappelle, S., Di Cagno, R., & Gobbetti, M. (2007). Use of sourdough lactobacilli and oat fibre to decrease the glycaemic index of white wheat bread. *British Journal of Nutrition, 98(6)*, 1196-1205.

De Filippo, C., Cavalieri, D., Di Paola, M., Poullet, J. B., Massart, S., Collini, S., . . . Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in

children from Europe and Rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*, 14691–14696.

De Munter, J. S., Hu, F. B., Spiegelman, D., Franz, M., & Van Dam, R. M. (2007). Whole grain, bran, and germ intake and risk of type 2 diabetes: a prospective cohort study and systematic review. *PLoS medicine, 4(8),* e261.

Dean, M., Raats, M. M., & Shepherd, R. (2007). Consumers and functional cereal products. In B. M. Hamaker (Ed.), T*echnology of functional cereal products* (pp. 3-22). Cambridge: Woodhead Publishing.

Den Besten, G., Van Eunen, K., Groen, A. K., Venem, A. K., Venema, K., Reijngoud, D. J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research, 54(9)*, 2325-2340.

Donohoe, D., Garge, N., Zhang, X., Sun, W., O'Connell, T., Bunger, M., & Bultman, S. (2011). The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metabolism, 13*, 517-526.

Fadel, J. G., Newman, C. W., Newman , R. K., & Graham, H. (1988). Effects of extrusion cooking of barley on ileal and fecal digestibilities of dietary components in pigs. *Canadian Journal of Animal Science, 68(3)*, 891-897.

Frank, D., Amand, A., Feldman, R., Boedeker, E., Harpaz, N., & Pace, N. f. (2007). Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proceedings of the National Academy of Sciences of the United States of America*, 13780-13785.

Fung, K., Cosgrove, L., Lockett, T., Head, R., & Topping, D. (2012). A review of the potential mechanisms for the lowering of colorectal oncogenesis by butyrate. *British Journal of Nutrition, 108*, 820-831.

Garciamazcorro, J. F., Ivanov, I., Mills, D. A., & Noratto, G. (2016). Influence of wholewheat consumption on fecal microbial community structure of obese diabetic mice. *PeerJ, 4(3).*

Giacco, R. C., Giacco, R., Clemente, G., Cipriano, D., Luongo, D., Viscovo, D., . . . Ciati, R. (2010). Effects of the regular consumption of wholemeal wheat foods on cardiovascular risk factors in healthy people. *Nutrition, Metabolism and Cardiovascular Diseases, 20(3)*, 186-194.

Gill, S., Pop, M., DeBoy, R., Eckburg, P., Turnbaugh, P., Samuel, B. (2006). Metagenomic analysis of the human distal gut microbiome. *Science, 312*, 1355-1359.

Gore, C., Munro, K., Lay, C., Bibiloni, R., Morris, J., Woodcock, A., . . . Tannock, G. (2008). Bifidobacterium pseudocatenulatum is associated with atopic eczema: A nested case-control study investigating the fecal microbiota of infants. *Journal of. Allergy and Clinical Immunology*, 135-140.

Hales, C. M., Fryar, C. D., Carroll, M. D., Freedman, D. S., & Ogden, C. L. (2018). Trends in obesity and severe obesity prevalence in US youth and adults by sex and age, 2007–2008 to 2015–2016. *JAMA 319*, 1723-1725.

Jacobs, D. R., Marquart, L., Slavin, J., & Kushi, L. H. (1998). Whole-grain intake and cancer: An expanded review and meta-analysis. *Nutrition and Cancer, 30(2)*, 85-96.

Jensen, M. K., Koh-Banerjee, P., Franz, M., Sampson, L., Grønbæk, M., & Rimm, E. B. (2006). Whole grains, bran, and germ in relation to homocysteine and markers of glycemic control, lipids, and inflammation. *The American Journal of Clinical Nutrition, 83(2)*, 275-283.

Johansson, D. P., Vázquez Gutiérrez, J. L., Landberg, R., Alminger, M., & Langton, M. (2018). Impact of food processing on rye product properties and their in vitro digestion. *European Journal of Nitrition, 57*, 1651–1666.

Johansson, L., Thelle, D., Slovoll, K., Bjoerneboe, G. E., & Drevon, C. H. (1999). Healthy dietary habits in relation to social determinants and lifestyle factors. *British Journal of Nutrition, 81*, 211-220.

Kahlon, T. S., Berrios, D. J., Smith, G. E., & Pan, J. L. (2006). Extrusion conditions modify hypocholesterolemic properties of wheat bran fed to hamsters. *Cereal Chemistry*, 152-156.

Karppinen, S., Kiiliäinen, K., Liukkonen, K., Forssell, P., & Pouranen, K. (2001). Extraction and in vitro fermentation of rye bran fractions. *Journal of Cereal Science, 34(3)*, 269-278.

Kasubuchi, M., Hasegawa, S., Hiramatsu, T., Ichimura, A., & Kimura, I. (2015). Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. *Nutrients, 7(4)*, 2839-2849.

Katina, K., Juvonen, R., Laitila, A., Flander, L., Nordlund, E., Kariluoto, S., . . . Poutanen, K. (2012). Fermented Wheat Bran as a Functional Ingredient in Baking. *Cereal Chemistry, 89*, 126-134.

Koehler, P., Hartmann, G., Wieser, H., & Rychlik, M. (2007). Changes of folates, dietary fiber, and proteins in wheat as affected by germination. *Journal of Agricultural and Food Chemistry, 55(12)*, 4678-4683.

Kovatcheva-Datchary, P., Nilsson, A., Akrami, R., Lee, Y. S., De Vadder, F., Arora, T., . . . Bäckhed, F. (2015).Dietary fiber-induced improvement in glucose metabolism is associated with increased abundance of Prevotella. *Cell Metabolism, 22(6)*, 971-982.

Kraler, M., Schedle, K., Schwarz, C., Domig, K. J., Pichler, M., Oppender, A., . . . Somoza, A. (2015). Fermented and extruded wheat bran in piglet diets: impact on performance, intestinal morphology, microbial metabolites in chyme and blood lipid radicals *Animal Feed Science and Technology,* 197, 121-129.

Lang, R., & Jebb, S. A. (2003). Who consumes whole grains and how much? *Proceedings of the Nutritional Society, 60*, 218A.

Lappi, J., Salojärvi, J., Kolehmainen, M., Mykkänen, H., Poutanen, K., De Vos, W. M., & Salonen, A. (2013). Intake of whole-grain and fiber-rich rye bread versus refined wheat bread does not differentiate intestinal microbiota composition in Finnish adults with metabolic syndrome. *The Journal of Nutrition, 143(5)*, 648-655.

Liljeberg, H. G., & Björck, I. M. (1996). Delayed gastric emptying rate as a potential mechanism for lowered glycemia after eating sourdough bread: studies in humans and rats using test products with added organic acids or an organic salt. *The American Journal of Clinical Nutrition, 64(6)*, 886-893.

Lutsey, P. L., Jacobs, D. R., Kori, S., Mayer-Davis, E., Shea, S., Steffen, L. M., . . . Tracy, R. (2007). Whole grain intake and its cross-sectional association with obesity, insulin

resistance, inflammation, diabetes and subclinical CVD: The MESA Study. *British Journal of Nutrition, 98(2)*, 397-405.

Maioli, M., Pes, G. M., Sanna, M., Cherchi, S., Dettori, M., Manca, E., & Farris, G. A. (2008). Sourdough-leavened bread improves postprandial glucose and insulin plasma levels in subjects with impaired glucose tolerance. *Acta Diabetologica, 45(2)*, 91-96.

Manichanh, C., Rigottier-Gois, L., Bonnaud, E., Gloux, K., Pelletier, E., Frangeul, L. (2006). Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut, 55*, 205-211.

Manini, F., Brasca, M., Plumed-Ferrer, C., Morandi, S., Erba, D., & Casiraghi, M. C. (2014). Study of the chemical changes and evolution of microbiota during sourdoughlike fermentation of wheat bran. *Cereal Chemistry, 91(4)*, 342-349.

Martínez, I., Lattimer, J. M., Hubach, K. L., Case, J. A., Yang, J., Weber, C. G., . . . Haub, M. D. (2013). Gut microbiome composition is linked to whole grain-induced immunological improvements. *The ISME journal, 7(2).*

Mateo-Anson, N., Havenaar, R., Vaes, W., Coulier, L., Venema, K., Selinheimo, E., . . . Haenen, G. R. (2011). Effect of bioprocessing of wheat bran in wholemeal wheat breads on the colonic SCFA production in vitro and postprandial plasma concentrations in men. *Food Chemistry, 128*, 404-409.

McIntyre, A., Vincent, R. M., Perkins, A. C., & Spiller, R. C. (1997). Effect of bran, ispaghula, and inert plastic particles on gastric emptying and small bowel transit in humans: the role of physical factors. *Gut, 40(2),* 223-227.

McMackin, E., Dean, M., Woodside, J. W., & McKinley, M. C. (2012). Whole grains and health: attitudes to whole grains against a prevailing background of increased marketing and promotion. *Public Health Nutrition: 16(4)*, 743-751.

Mellen, P. B., Walsh, T. F., & Herrington, D. M. (2008). Whole grain intake and cardiovascular disease: a meta-analysis. *Nutrition, Metabolism and Cardiovascular Diseases, 18(4)*, 283-290.

Mente, A., De Koning, L., Shannon, H. S., & Anand, S. S. (2009). A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. *Archives of Internal Medicine, 169(7)*, 659-669.

Miller, G., Prakash , A., & Decker, E. (2002). Whole-grain micronutrients. In L. Marquart, J. L. Slavin, & R. G. Fulcher (Eds.), *In Whole-grain foods in health and disease* (pp. 243-258). St Paul, MN: Eagan Press.

Minervini, F., De Angelis, M., Di Cagno, R., & Gobbetti, M. (2014). Ecological parameters influencing microbial diversity and stability of traditional sourdough. *International Journal of Food Microbiology, 171*, 136-146.

Motonen, J., Boeing, H., Fritsche, A., Schleicher, E., Joost, H. G., Schulze, M. B., . . . Pischon, T. (2013). Consumption of red meat and whole-grain bread in relation to biomarkers of obesity, inflammation, glucose metabolism and oxidative stress. *European Journal of Nutrition, 52(1)*, 337-345.

Murtaza, N., Baboota, R. K., Jagtap, S., Singh, D. P., Bhutani, S. M., Khare, P., . . . Bhutani, K. K. (2014). Finger millet bran supplementation alleviates obesity-induced oxidative stress,

inflammation and gut microbial derangements in high-fat diet-fed mice. *Journal of Nutrition, 112(9)*, 1447-1458.

Nicholson, J., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., & Pettersson, S. (2012). Host-gut microbiota metabolic interactions. *Science, 336*, 1262–1267.

Nordlund, E., Katina, K., Aura, A. M., & Poutanen, K. (2013). Changes in bran structure by bioprocessing with enzymes and yeast modifies the in vitro digestibility and fermentability of bran protein and dietary fibre complex. *Journal of Cereal Science, 58(1)*, 200-208.

Ounnas, F., Privé, F., Salen, P., Gaci, N., Tottey, W., & Calani, L. (2016). Whole rye consumption improves blood and liver n-3 fatty acid profile and gut microbiota composition in rats. *PLoS One, 11(2).*

Pereira, M. A., Jacobs, D. R., Pins, J. J., Raatz, S. K., Gross, M. D., Slavin, J. L., & Seaquist, E. R. (2002). Effect of whole grains on insulin sensitivity in overweight hyperinsulinemic adults. *The American Journal of Clinical Nutrition, 75(5)*, 848-855.

Posner, E. S. (2000). Wheat. In K. Kulp, & J. G. Ponte (Eds.), *Handbook of cereal science and technology* (2 ed., pp. 1-32). New York: Marcel Dekker.

Poutanen, K., Flander, L., & Katina, K. (2009). Sourdough and cereal fermentation in a nutritional perspective. *Food Microbiology, 26(7),* 693-699.

Poutanen, K., Sozer, N., & Della Valle, G. (2014). How can technology help to deliver more of grain in cereal foods for a healthy diet? *Journal of Cereal Science, 59(3)*, 327-336.

Puertollano, E., Kolida, S., & Yaqoob, P. (2014). Biological significance of short-chain fatty acid metabolism by the intestinal microbiome. *Current Opinion in Clinical Nutrition & Metabolic Care, 17(2)*, 139-144.

Ralet, M., Thibault, J., & Della Valle, G. (1990). Influence of extrusion-cooking on the physico-chemical properties of wheat bran. *Journal of Cereal Science*, 249-259.

Rehm , C. D., Peñalvo , J. L., Afshin , A., & Mozaffarian, D. (2016). Dietary intake among US adults, 1999-2012. *JAMA, 315*, 2542-2553.

Reicks, M., Jonnalagadda, S., Albertson, A. M., & Joshi, N. (2014). Total dietary fiber intakes in the US population are related to whole grain consumption: results from the National Health and Nutrition Examination Survey 2009 to 2010. *Nutrition Research, 34*, 226-234.

Robin, F., Dubois, C., Pineau, N., Schuchmann, H., & Palzer, S. (2011). Expansion mechanism of extruded foams supplemented with wheat bran. *Journal of Food Engineering, 107*, 80-89.

Rodriquez, R., Jimenez, A., Fernandez-Bolanos, J., Guillen, R., & A, H. (2006). Dietary fiber from vegtable products as source of functional ingredients. *Trends in Food Science and Technology, 17*, 3-15.

Ross, A. B., Bruce, S. J., Blondel-Lubrano, A., Oguey-Araymon, S., Beaumont, M., Bourgeois, A., & Kochhar, S. (2011). A whole-grain cereal-rich diet increases plasma betaine, and tends to decrease total and LDl-chloestrol compared with refined grain diet in healthy subjects. *British Journal of Nutrition, 105*(10), 1492-1502.

Salmenkallio-Marttila, M., Katina, K., & Autio, K. (2001). Effects of bran fermentation on quality and microstructure of high-fiber wheat bread. *Cereal Chemistry*, 429-435.

Sandberg, A. S., Andersson, H., Kivistö, B., & Sandström, B. (1986). Extrusion cooking of a high-fibre cereal product: Effects on digestibility and absorption of protein, fat, starch, dietary fibre and phytate in the small intestine. *British Journal of Nutrition, 55(2)*, 245-254.

Scazzina, F. D. (2009). Sourdough bread: Starch digestibility and postprandial glycemic response. *Journal of Cereal Science 49(3)*, 419-421.

Sender, R., Fuchs, S., & Milo, R. (2016). Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLOS Biology*.

Slavin, J. (2004). Whole grains and human health. *Nutrition Research Reviews, 17(1)*, 99-110.

Slavin, J. L., Jacobs, D., & Marquart, L. (2000). Grain Processing and Nutrition. *Critical Reviews in Food Science and Nutrition*, 309-326.

Sonnenburg, E. D., & Sonnenburg, J. L. (2014). Starving our microbial self: The deleterious consequences of a diet deficient in microbiota- accessible carbohydrates. *Cell Metabolism, 20(5)*, 779-786.

Sonnenburg, E. D., Smits, S. A., Tikhonov, M., Higginbottom, S. K., Wingreen, N. S., & Sonnenburg, J. L. (2016). Diet-Induced Extinctions in the Gut Microbiota Compound Over Generations. *Nature, 529(7585)*.

Soto-Vaca, A., Gutierrez, A., Losso, J., Xu, Z., & Finley, J. (2012). Evolution of phenolic compounds from color and flavor problems to health benefits. *Journal of Agriculture and Food Chemistry, 60*, 6658-6677.

Stephen, A., & Cummings, J. (1980). Mechanism of action of dietary fiber in the human colon. *Nature 284*, 283-284.

Sun , T., Lærke , H. N., Jørgensen, H., & Knudsen, K. E. (2006). The Effect of Extrusion Cooking of Different Starch Sources on the In Vitro and In Vivo Digestibility in Growing Pigs. *Animal Feed Science and Technology, 131(1-2)*, 67-86.

Tighe, P., Duthie, G., Vaughan, N., Brittenden, J., Simpson, W. G., Duthie, S., . . . Thies, F. (2010). Effect of increased consumption of whole-grain foods on blood pressure and other cardiovascular risk markers in healthy middle-aged persons: randomized controlled trial. *The American Journal of Clinical Nutrition, 92(4)*, 733-740.

Topping, D., & Clifton, P. (2001). Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews, 81*, 1031-1064.

US Department of Health and Human Services. (2017). Dietary Guidelines for Americans 2015-2020. *Skyhorse Publishing Inc.*

Vanegas, S. M., Meydani, M., Barnett, J. B., Goldin, B., Kane, A., Rasmussen, H., & Brown, C. (2017). Substituting whole grains for refined grains in a 6-wk randomized trial has a modest effect on gut microbiota and immune and inflammatory markers of healthy adult. *American Journal of Clinical Nutrition, 105*, 635-650.

Vitaglione, P., Mennella, I., Ferracane, R., Rivellese, A. A., Giacco, R., Ercolini, D., . . . Thielecke, F. (2014). Whole-grain wheat consumption reduces inflammation in a randomized controlled trial on overweight and obese subjects with unhealthy dietary and lifestyle behaviors: role of polyphenols bound to cereal dietary fiber. *The American Journal of Clinical Nutrition, 101(2),* 251-261.

Wang, D. D., Leung, C. W., Li, Y., Ding, E. L., Chiuve, S. E., Hu, F. B., & Hu, F. B. (2014). Trends in dietary quality among adults in the United States, 1999 through 2010. *JAMA Internal Medicine, 174*, 1587–1595.

Wang, W. M., Klopfenstein, C. F., & Ponte, J. (1993). Effects of twin-screw extrusion on the physical properties of dietary fiber and other components of whole wheat and wheat bran and on the baking quality of the wheat bran. *Cereal Chemistry*, 707-711.

Wrick, K. L., Robertson, J. B., Van Soest, P. J., Lewis, B. A., Rivers, J. M., Roe, D. A., & Hackler, L. R. (1983). The influence of dietary fiber source on human intestinal transit and stool output. *The Journal of Nutrition, 113(8)*, 1464-1479.

Yankah, V. V., & Jones, P. (2001). Phytosterols and health implications – efficacy and nutritional aspects. *Inform, 12*, 899-903.

Zhang, G., Malik, V. S., & Pan, A. (2010). Substituting brown rice for white rice to lower diabetes risk: a focus group study in Chinese adults. *Journal of American Dietetic Association, 110,* 1216-1221.

Zhong, Y., Marungruang, N., & Nyman, F. (2015). Effects of two whole-grain barley varieties on caecal SCFA, gut microbiota and plasma inflammatory markers in rats consuming low- and high-fat diets. *British Journal of Nutrition, 113(10)*, 1558-1570.

Zhong, Y., Nyman, M., & Fåk, F. (2015). Modulation of gut microbiota in rats fed high-fat diets by processing whole-grain barley to barley malt. *Molecular Nutrition & Food Research, 59(10)*, 2066-2076.

CHAPTER 2 : DETERMINING THE EFFECT OF PROCESSING METHOD AND CONDITIONS ON MICROBIOTA ACCESSIBLE CARBOHYDRATES IN WHOLE GRAINS

2.1. ABSTRACT

Fermentation of non-digestible carbohydrates (NDC) by the gut microbiota is important for human health. Whole grains are low in fermentable NDC, but there is potential to increase fermentable NDC through processing. Whole grain wheat flour was processed by boiling or extrusion under different processing conditions. Processed whole wheat products were then subjected to *in vitro* digestion followed by fermentation using the fecal microbiome from two subjects, where the microbiome from subject 1 was comparatively more diverse and characterized by microbes associated with diets rich in dietary fiber. The microbiome from subject 1 produced less acetate and more propionate and butyrate during fermentation of boiled wheat compared with extruded wheat. The microbiome from subject 2 did not show differences in short chain fatty acids (SCFA) production between processing methods. Higher screw speed during extrusion resulted in enhanced butyrate production during fermentation in both subjects. High moisture content during boiling of wheat resulted in more fermentable NDC during fermentation, but only for the microbiome from subject 1. Overall, processing method had an impact on the fermentation of NDC and subsequent SCFA production, but only for a microbiome with high diversity and predominance of microbes associated with dietary fiber intake. This information can be used to better understand how to use food processing for personalized nutrition and the promotion of a healthy microbiome.

2.2. INTRODUCTION

Whole grain intake has been shown to be associated with reduced risk of type 2 diabetes, cardiovascular disease, cancer, and obesity (Cho, Qi, Fahey, & Klurfeld, 2013). Whole grains are a major source of dietary fiber and non-digestible carbohydrates (NDC) in the diet (US Department of Health and Human Services, 2017). Fermentation of NDC by the microbiota results in several microbial metabolites, including short chain fatty acids (SCFA), that are absorbed by the host and have numerous local effects on intestinal function as well as systemic roles in insulin secretion, lipid metabolism, and inflammation, among others (Den Besten, et al., 2013; Kasubuchi, Hasegawa, Hiramatsu, Ichimura, & Kimura, 2015; Puertollano, Kolida, & Yaqoob, 2014). However, only a fraction of the NDC that reach the colon are available for fermentation by the microbiota (Daniel, Wisker, Rave, & Feldheim, 1997; Nyman, Asp, Cummings, J., & Wiggins, H., & Wiggins, 1986; Wisker, Knudsen, Daniel, Eggum, & Feldheim, 1997; Wisker, Daniel, Rave, & Feldheim, 1998). The fraction of NDC that are available for fermentation have been termed microbiota accessible carbohydrates (MAC) (Sonnenburg & Sonnenburg, 2014). Human feeding trials have shown that roughly one third of the NDC in grains are metabolically available for fermentation while fruits and vegetable have over 75% that are fermentable. Thus, the majority of NDC in grains are not fermented into metabolites including SCFA and the numerous health benefits of these products are not obtained.

There is a potential to increase the amount of fermentable NDC in whole grains through food processing. For instance, extrusion is a form of processing that uses pressure, mechanical shear and higher temperatures (>120 °C) to process grains into foods such as breakfast cereals and crispy snacks. The extrusion process results in gelatinization and partial depolymerization of starch together with fragmentation of cell wall structures (Robin, Dubois, Pineau, Schuchmann,

& Palzer, 2011). These cell wall structures contain most of the NDC. In extruded whole grain rye, for instance, small, fragmented cell walls and aleurone layers containing β-glucan and arabinoxylan were visualized embedded within a continuous, amorphous starch phase (Johansson, Vázquez Gutiérrez, Landberg, Alminger, & Langton, 2018). Extrusion has been shown to convert insoluble fiber to soluble fiber, which is traditionally thought to be more fermentable, in different cereals (Kahlon, Berrios, Smith, & Pan, 2006; Ralet, Thibault, & Della Valle, 1990; Robin, Dubois, Pineau, Schuchmann, & Palzer, 2011; Wang, et al., 2014).

In contrast, boiling is a minimal form of grain processing that involves lower temperatures (100 °C), atmospheric pressure, minimal shear, and an abundance of water. During boiling, water swells the starch granules resulting in the leaching of amylose, starch gelatinization, and an amorphous structure. In whole grain rye flour, boiling has been shown to have minimal influences on the NDC, β-glucan and arabinoxylan. Boiling did result in swollen cell walls, but the cell walls appeared to remain intact (Johansson, Vázquez Gutiérrez, Landberg, Alminger, & Langton, 2018). Boiling of whole wheat flour or wheat bran has commonly been used as a control process for whole grain experiments (Arcila, Weier, & Rose, 2015; Bjorck, Asp, & Lundquist, 1984; Bjorck, et al., 1984).

Changes in NDC structure that happen as a result of processing have been shown to change the fermentability of carbohydrates by the microbiota. Arcila (2015) studied the *in vitro* fermentability of extruded and boiled wheat bran. Extrusion resulted in a 114% increase in fermentable NDC and a significant increase in SCFA production compared to boiled wheat bran (Arcila, Weier, & Rose, 2015). Aoe (1990) found that extruded wheat bran was more available to the gut microbes in rats than native bran based on the neutral detergent fiber. Furthermore, the

cell walls of the extruded bran were more disrupted after passage (Aoe, Nakaoka, Ama, Ohta, & Ayan, 1990).

Conditions during grain processing, such as moisture content, and cooking time and temperature, may be varied depending on the finished product desired. For instance, cereal flours can be boiled under different moisture conditions to make thick [e.g., polenta or uglai (an African porridge)] or thin (e.g., grits or oji) porridges. Different processing conditions can also be used to change the sensory properties of extruded products such as crispy snacks, breakfast cereals, and pasta. Changing these processing conditions may affect the fermentation of NDC. Thus, the purpose of this study was to determine how different food processing methods, conditions during processing, and microbiome communities interact to affect the fermentation of NDC.

2.3. MATERIALS AND METHODS

2.3.1. *Whole wheat processing*

Milling. Hard red wheat was obtained from Ardent Mills (Denver, CO). Wheat kernels were dried at 40°C for 16 h before milling according to Doblado-Maldonado, Flores, & Rose (2013). All milled fractions obtained from the mill were mixed together to obtain whole wheat flour.

Boiling. Boiling was performed in a factorial design with two moisture contents and cooking times in triplicate in a randomized order. Water (530 g for high moisture content or 270 g for low moisture content) was brought to a boil on a gas range. Once boiling, the heat was reduced to a simmer and 1 g of salt and 100 g whole wheat flour were added under rapid manual stirring (2.5 or 5 min). The boiled wheat porridge was then cooled to cool to room temperature before being subject to *in vitro* digestion within 2 h.

Extrusion. Extrusion was performed in a factorial design with two moisture contents and two screw speeds in triplicate in a randomized order. Extrusion feed mixtures were prepared 24 h in advance. Whole wheat flour (1 kg) was mixed for 10 min with1% salt (w/w) and water to adjust to the correct moisture content [20% for low moisture content and 30% for high moisture content (dry weight basis)] in a stand mixer (c-100, Hobart). The mixtures equilibrated in closed containers at 4°C overnight. The moisture-adjusted whole wheat flours were the extruded using two screw speeds (250 and 125 rpm) on a lab-scale twin screw extruder with a single stage mixing zone, 3:1 compression ratio, 3 mm die diameter, and a 20:1 L/D ratio (CW Brabender Instruments, NJ, USA). The extruder was operated by a direct current drive unit with a 7.5 kW motor (Intelli-Torque, Pastic Corder Lab-station, C.W. Brabender). The flour was fed into the extruder using a volumetric feeder (FW 40 Plus, C. W. Brabender) set at a constant flow rate of ~50 g/min. Barrel temperatures were set at 60 °C (zone 1; inlet), 70 °C (zone 2), 120 °C (zone 3) , and 120 °C (zone 4; die assembly). The four processing condition combinations were run in random order in triplicate. The product was collected from the extruder die after stabilization and dried in a convection oven overnight at 70°C before being subjected to *in vitro* digestion within 4 d.

2.3.2. *In vitro digestion*

Samples were digested following the procedure of Yang, Keshavarzian, and Rose (2013) with minor modifications. Briefly, boiled and extruded products were stomached with an adjusted amount of water for 1 min based on the moisture content of the product (extrudate: 25 g coarsely ground extrudate and 300g of water, low moisture boiled wheat: 125 g of porridge and 200g of water, high moisture boiled wheat: 225 g porridge boiled wheat and 100g water). After stomaching, the pH was adjusted to 2.5 with 1 M HCl followed by the addition of 10 mL of 10%

(w/v) pepsin (P7000, Sigma, St Louis, MO) in 50 mM HCl. The slurry was then incubated for 30 min at 37 °C with orbital shaking at 150 rpm. Following pepsinolysis, 50 mL of 0.1 M sodium maleate buffer (pH 6, containing 1 mM $CaCl_2$) was added and the pH was adjusted to 6.9 with 1 M $NaHCO_3$. Next, 50 mL of 12.5% (w/v) pancreatin (P7545, Sigma) in sodium maleate buffer was added followed by 2 mL of amyloglucosidase (3260 U/mL, Megazyme, Bray, Ireland). The slurry was then incubated for 6 h at 37 °C with orbital shaking at 150 rpm. Following digestion, the material was transferred to dialysis tubing (molecular weight cutoff, 12,000-14,000) and dialyzed against distilled water for 4 d with a water change at least every 12 h (Yang, Keshavarzian, & Rose, 2013). The retentate from dialysis was collected and freeze dried.

2.3.3. *Fecal samples for in vitro fermentation*

Fresh fecal samples from 2 healthy adults with no history of gastrointestinal abnormalities, no prebiotic or probiotic consumption, and no antibiotic consumption within the past 6 months were collected. A fecal slurry was prepared under anaerobic conditions within 2 h of defecation by blending (Sunbeam 2774, Boca Raton, Florida, USA) each fresh fecal sample separately with sterile phosphate buffered saline pH 7.0 (1:9 w/v) containing 10% glycerol as a cryoprotectant for 1 min. The slurry was then filtered through 4 layers of sterile cheesecloth and then frozen at -80° until fermentation was performed. Two fecal slurries from fecal samples collected on different days were prepared from each subject.

The fecal samples from both subjects were analyzed for differences in the microbiome using 16 sRNA sequencing. A kit from Biovet (BioSprint 96 One-For-All Vet Kit, Quebec Canada) was used. Microbiome characterization was performed by amplicon sequencing of the V4 region of the 16S rRNA gene on the Illumina MiSeq platform using the MiSeq Reagent kit v2 (2 X 250 bp) following the protocol of Kozich et al. (2013). Sequences were demultiplexed

and barcodes were removed prior to sequence analysis with the QIIME 2 platform (Boylen et al., 2019). Sequence quality control, trimming and denoising was performed with DADA2 (Callahan et al., 2016). Forward and reverse reads were trimmed to maintain sequence qualities above a phred score of 30. Taxonomy was assigned using the Greengenes database (DeSantis et al., 2006). Reads were rarefied to a sampling depth of 9000 prior to analysis. Diversity of each fermented and fecal sample was determined using Qiime 2. Subject's fecal microbiome characteristics were determined using Microbiome analyst platform (https://www.microbiomeanalyst.ca). Through this platform, taxa with <2 reads, <10% in prevalence of sequencing read counts, and <10% variance based on the inner quartile range were removed leaving 98 taxa. The data was rarified to the minimum library size and scaled using total sum squaring. Heat tree analysis was conducted to depict taxonomic differences between microbial communities to show which taxa were more abundant for each microbiota using median abundance and non-parametric Wilcoxon Rank Sum test (FDR adjusted p cutoff of 0.05) (Foster, Sharpton, & Grünwald, 2017).

2.3.4. *Dietary patterns*

After collecting fecal samples, the two subjects were asked to complete a 24-hour dietary recall survey on three separate days within 2 weeks of each other. Dietary intake data were collected and analyzed using the Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool (National Cancer Institute, 2018). The average grams of dietary fiber, serving of whole grains, and servings of refined grains were expressed per 1000 kcal consumed.

2.3.5. *In vitro fermentation*

In vitro batch fecal fermentation was performed as described (Yang, Keshavarzian, and Rose 2013). Briefly, inside of an anaerobic hood (Bactron X, Sheldon manufacturing, Cornelius,

OR USA, containing 5% H2, 5% CO2, and 90% N2), 15 mg of the freeze dried material obtained after *in vitro* digestion and dialysis of the processed whole wheat flour was suspended in 1 mL of sterile fermentation medium containing (per L): peptone (2 g, Fisher Scientific, Waltham, MA), yeast extract (2 g, Fisher Scientific, Waltham, MA), bile salts (0.5 g, Oxoid, Cheshire, England), $NaHCO_3$ (2 g), NaCl (0.1 g), K_2HPO_4 (0.08 g), $MgSO_4 \cdot 7H_2O$ (0.01 g), $CaCl_2 \cdot 6H2O$ (0.01 g), L-cysteine hydrochloride (0.5 g, Fisher Scientific, Waltham, MA), hemin (50 mg dissolved in DMSO), Tween 80 (2 mL, Fisher Scientific, Waltham, MA), vitamin K (10 µL dissolved in ethanol, Alfa Aesar, Haverhill, MA), and 0.025% (w/v) resazurin solution (4 mL dissolved in water, Alfa Aesar, Haverhill, MA), capped, and hydrated for 1 h. Tubes were then inoculated with 0.1 mL of fecal slurry, capped, and incubated at 37 °C with orbital shaking (140 rpm) for 12 h before being flash frozen in liquid nitrogen. Samples that were immediately flash frozen in liquid nitrogen following inoculation were used as controls (0 h of fermentation). After freezing in liquid nitrogen, tubes were stored at -80 °C until analysis. Because most of the dietary fibers in whole grains are not soluble, separate fermentation tubes were prepared for analysis of fermentable NDC versus SCFA.

2.3.6. *Microbiota accessible carbohydrates (% of non-digestible carbohydrate) analysis*

To measure MAC (or the % of NDC fermented), a modified version of the procedure used by Basumallick and Rohrer (2015) was followed. Fermented samples were thawed and centrifuged. The supernatant was removed and stored on ice. The pellet was treated with 0.18 mL 12 M sulfuric acid for 1 h at 30° C with periodic vortex mixing followed by the addition of 1 mL of internal standard (3 mg/mL fucose in water). Then, the supernatant (previously stored on ice) was quantitatively transferred to the tube containing the acid-treated pellet using additional water (3.4 mL) to bring the final sulfuric acid concentration to 0.4 M. The samples were then

autoclaved at 121 °C for 1 h and then filtered with a 0.45 μm filter before injection. Sugars were separated on a high performance anion exchange chromatograph (Dionex CarboPac) equipped with a CarboPac PA1 column at 30 °C and 1 mL/min flow rate. The mobile phase was 200 mM NaOH for column cleaning followed by 10 min of equilibration of 3 mM NaOH before injection of 10 μL of sample. The sugars were detected over 22 min with pulsed amperometric detection (Dionex ICS-5000+ SP). Chromatographs were analyzed for the peak areas of the sugars with Chromeleon 7.2 software. Sugar residues were quantified by calculating response factors for each sugar relative to fucose using injections of pure standards. The concentration of each sugar residue was corrected for its weight as it occurs in a polysaccharide (0.88*weight for pentose sugars and 0.9*weight for hexose sugars). Fermentable NCD were calculated by dividing the difference between the initial carbohydrates at time 0 h and the final carbohydrates at time 12 h by the initial carbohydrates at time 0 h and expressing the results as a percentage (Basumallick & Rohrer, 2015).

2.3.7. *Short chain fatty acid analysis*

SCFA were extracted and measured by gas chromatography as described (Hartzell, Maldonado-Gómez, Hutkins, & Rose, 2013). In brief, 0.4 mL of fermented sample, 0.1 mL of 7 mM 2-ethylbutyric acid in 2 M potassium hydroxide, 0.2 mL of 9 M sulfuric acid, and ~0.1 g of sodium chloride was mixed together. Then, 0.5 mL of diethyl ether was added and mixed. The top layer was injected into a gas chromatograph (Clarus 580, PerkinElmer, MA USA) equipped with a capillary column (Nukol, 30 m × 0.25 mm inner diameter × 0.25 μm film thickness, Supelco, Bellefonte, PA) and flame ionization detector. SCFA were quantified by calculating response factors for each SCFA relative to 2-ethylbutyric acid as an internal standard.

2.3.8. ***Statistical analysis***

Processing methods were each analyzed separately for changes due to conditions using analysis of variance (ANOVA) using factors of subject, moisture content, and screw speed/cook time together with their interactions (SAS version 9.4). After analysis of the processing conditions, the replicates of each condition were averaged and the two processes were compared using ANOVA with factors of subject and processing method. For factors that were significant in the ANOVA, Tukey's honest significant difference was used to determine significant differences among least square means.

2.4. RESULTS

2.4.1. ***Microbiota composition of fecal samples and dietary patterns***

α- Diversity varied between the two subjects based on the metric used (Figure 2.1). The microbiota from subject 1 had higher phylogenetic diversity and more observed operational taxonomical units than the microbiome from subject 2. The microbiota from subject 2 was more even than the microbiota from subject 1. At the genus level, the microbiota from subject 1 was enriched in of *Provetella*, *Ruminococcus,* (Ruminococcaceae), *Oscillospira*, and *Akkermansia*. The microbiome from subject 2 was more abundant in *Veillonella*, *Streptococcus*, *Escherichia*, and *Bifidobacterium* (Figure 2.2). Subject 1 had a diet high in dietary fiber and whole grains while subject 2 had a diet deficient in dietary fiber and whole grains (Figure 2.3).

2.4.2. ***Whole wheat flour composition***

Whole wheat flour contained 76.5% (±1.5) carbohydrates, consisting of 62.6% (± 0.8) starch and 13.9% (±1.5) non-starch polysaccharides. Processing method changed the NDC and residual starch in the digested material (Tables 2.1). However, the conditions of processing within a given method did not have a significant effect on the NDC or residual starch.

2.4.3. *Boiling*

For boiled wheat, fermentable NDC varied depending on moisture content and subject (Figure 2.4). The microbiome from subject 1 was able to ferment 12% more NDC when the wheat was boiled at the lower moisture compared with the higher moisture. However, the microbiome from subject 2 did not show any differences in fermentation of NDC among processing moisture contents. There were no significant effects of cooking time on fermentable NDC for either microbiome. SCFA production was not significant affected by boiling conditions for either microbiome (Table 2.2).

2.4.4. *Extrusion*

In contrast to boiling, extrusion conditions affected SCFA production and not fermentable NDC during fermentation (Table 2.3). A higher screw speed was associated with an increase in butyrate production for both microbiomes (Figure 2.5). Other SCFA were not affected by extrusion conditions.

2.4.5. *Processing techniques*

Food processing technique had significant on fermentable NDC when using the microbiome from subject 1 but not subject 2 (Fig 2.6). The microbiome from subject 1 was able to ferment 31% more NDC from boiled wheat compared with extruded wheat. While fermentable NDC did not differ between microbiomes for extruded wheat, the microbiome from subject 1 was able to ferment significantly more NDC from boiled wheat than the microbiome from subjects 2.

Food processing technique and the microbiome affects SCFA production after 12 h *of in vitro* fecal fermentation (Figure 2.6). The microbiome from subject 1 produced more butyrate while the microbiome of subject 2 produced more acetate. Processing technique affected butyrate and propionate production depending on the microbiome. The microbiota from subject 1

produced more butyrate on boiled wheat, but the microbiota from subject 2 did not differ in butyrate between the techniques. Additionally, the microbiota of subject 1 produced more propionate with extruded wheat while the microbiota of subject 2 showed no difference between boiling and extrusion. The microbiota of subject 2 produced more acetate with boiled wheat, while the microbiota of subject 1 produced more acetate with extruded wheat. However, the change in acetate levels due to processing technique was more pronounced for the microbiome of subject 1.

2.5. DISCUSSION

MAC was dependent upon food processing technique and the microbiome. The microbiome from subject 1 showed differences in fermentable NDC and SCFA production depending on processing technique, while the microbiome from subject 2 was unable to distinguish between techniques. These differences in response may be attributed to differences in the microbiome. The microbiome of subject 1 was richer and more phylogenetically diverse while the microbiome of subject 2 had a more even distribution of the taxa present. Additionally, the microbiome of subject 1 was characterized by microbes associated with a diet rich in dietary fiber and complex carbohydrate fermentation including *Prevotella* (De Filippo, et al., 2010; Wu, et al., 2011), and *Ruminococcus (*Ruminococcaceae) (Biddle, Stewart, Blanchard, & Leschine, 2013; Chassard, Delmas, Robert, Lawson, & Bernalier-Donadille, 2012). The microbiome from subject 1 was also characterized by other beneficial microbes associated with leanness such as *Oscillospira* (Konikoff & Gophna, 2016) and *Akkermansia* (Everard, et al., 2013). Moreover, subject 1 had a diet high in dietary fiber and whole grains which is likely responsible for these characteristics. Meanwhile, subject 2 had a diet deficient in dietary fiber and whole grains. Moreover, the microbiome of subject 2 was more abundant in less beneficial microbes that range

in potential host effects, such as *Streptococcus* and *Escherichia* (De Vos, et al., 2009). The microbiome of subject 2 did have more *Bifidobacterium,* a bacterial genus shown to increase in abundance with dietary fiber intervention in humans (Kund & Bach, 2015), but this is also one of the most abundant bacteria in human stool (Backhed, Ley, Sonnenburg, Peterson, & Gordon, 2005). Therefore, the microbiome from subject 1 likely saw difference due to processing method because the microbiome was richer and phylogenetically diverse, which would result in a more varied and effective community of carbohydrate fermenters.

Grain processing technique showed different effects depending on the microbiome. In subject 1, boiling resulted in increased fermentable NDC and butyrate production, while extrusion increased the propionate and acetate production. These differences could be due to the change in structure that occurs as a result of food processing. During boiling, many changes occur to the starch in whole wheat flour including the swelling of starch granules, leaching of amylose, starch gelatinization, and an amorphous structure. However, the NDC in wheat remains largely unchanged during boiling, even after 30 min (Anderson & Clydesdale, 1980). Additionally, boiled wheat resulted in more resistant starch than extruded wheat. Johansson, Vázquez-Gutiérrez, Landberg, Alminger, and Langton (2018) studied the microstructure of boiled and extruded whole grain rye. In this study, the boiled product had more swollen cell walls that remained largely intact and enclosed starch granules. These cells were separated by the continuous phase of loose starch granules and leached starch (of which amylose would be the primary component). On the other hand, extrusion resulted in the small fragments of arabinoxylans that were distributed thought homogeneous starch phase of completely disrupted starch granules (Johansson, Vázquez Gutiérrez, Landberg, Alminger, & Langton, 2018). Increased crystallinity, as in the boiled rye, reduces the rate of starch digestion, or allows for

more resistant starch to be present for fermentation (Singh, Dartois, & Kaur, 2010). Thus, these changes in structure likely led to the increased level of resistant starch. Previously, extrusion processing has been shown to increase the extractability of arabinoxylans, but is less effective at breaking down cellulose (Singkhornart, Lee, & Ryu, 2013).

Consequently, these two processes would have very different carbohydrates structures available for fermentation especially after *in vitro* starch digestion. Boiled wheat would have a larger portion of resistant starch while extruded wheat would have more small fragments of arabinoxylans and other cell wall components available for fermentation. The different carbohydrate structures that are available for fermentation could have promoted the growth of different carbohydrate consumers and SCFA producers in the microbiome. *In vitro*, starch fermentation produces more butyrate than the fermentation of non-starch polysaccharides (Weaver, Wolin, Krause, Miller, & Wol, 1992). This could contribute to the differences in SCFA production based on the processing method.

While the overall process had an effect on the fermentation response, the conditions within the process had minimal effects on the results in this study. Still some of the parameters tested modified MAC and butyrate production. Lower moisture content in boiled wheat did increase MAC in the microbiome of subject 1. This could have been a result of decreased gelatinization at the lower moisture contents. However, there were no significant differences in residual starch indicating these changes are a result of some other modification in the processed wheat. Cuq (2003) saw that gelatinization of wheat starch in an excess of water, as in this experiment, is minimally affected by water content (Cuq, Gonçalves, Mas, Vareille, & Abecassis, 2003). Additionally, higher shear in extrusion resulted in increased butyrate. Increasing screw speed results in increased volumetric expansion index, or a greater porosity in

the extrudate (Robin, Dubois, Pineau, Schuchmann, & Palzer, 2011). This change in structure would allow for increased access to microbes that would be able to produce butyrate.

2.6. CONCLUSION

These results show that people respond differently to processing methods. The microbiome of subject 1 had increase MAC and butyrate production in boiled process due to increased resistant formation and fermentation. Extrusion increased acetate and propionate production due to the increased availability of the NDC in the cell walls of the wheat bran. However, the microbiome of subject 2 did not respond to processing due to the lower amount of complex carbohydrate utilizing taxa present in their microbiome as a result of the habitual low dietary fiber and whole grain diet of the subject. Conditions within the processing effect had limited effects on the *in vitro* fermentation result. Lower moisture content in boiled products resulted additional MAC that could be utilized by the microbiome from subject 1. Increased screw speed resulted in increased butyrate production. Overall, processing and the microbiome have an interactive effect on the fermentability of carbohydrates. This information can be used to better understand how to use food processing for personalized nutrition and the promotion of a healthy microbiome.

2.5. REFERENCES

Anderson, N. E., & Clydesdale, F. M. (1980). Effects of processing on the dietary fiber content of wheat bran, pureed green beans, and carrots. *Journal of Food Science, 45(6)*, 1533-1537.

Aoe, S., Nakaoka, T., Ama, M. Y., Ohta, F., & Ayan, Y. (1990). Availability of dietary fiber in extruded wheat bran and apparent digestibility in rats of coexisting nutrients. *Cereal Chemistry*, 145-148.

Arcila, J. A., Weier, S. A., & Rose, D. J. (2015). Changes in dietary fiber fractions and gut microbial fermentation properties of wheat bran after extrusion and bread making. *Food Research International, 74*, 217-223.

Backhed, F., Ley, R., Sonnenburg, J., Peterson, D., & Gordon, J. (2005). Host-bacterial mutualism in the human intestine. *Science 307*, 1915–1920.

Basumallick, L., & Rohrer, J. (2015). Determination of Carbohydrates in Acid Hydrolysates of Wood.

Biddle, A., Stewart, L., Blanchard, J., & Leschine, S. (2013). Untangling the genetic basis of fibrolytic specialization by Lachnospiraceae and Ruminococcaceae in diverse gut communities. *Diversity, 5(3)*, 627-640.

Bjorck, I., Asp, N. G., & Lundquist, I. (1984). Digestion in vitro and In vivo; I extrusion cooking of wheat flours and starch. *Journal of Cereal Science (2)*, 91-103.

Bjorck, I., Asp, N. G., Birked, A. C., Eliasson, L. B., Shjoberg, L. B., & Lundquist, I. (1984). Effects of processing on starch availability in vitro and in vivo; II drum drying of wheat flour. *Journal of Cereal Science (2)*, 165-178.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., . . . Bai, Y. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology, 37(8)*, 852-857.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J., & Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods, 13(7)*, 581.

Chassard, C., Delmas, E., Robert, C., Lawson, P. A., & Bernalier-Donadille, A. (2012). Ruminococcus champanellensis sp. nov., a cellulose-degrading bacterium from human gut microbiota. *International Journal of Systematic and Evolutionary Microbiology, 62(1)*, 138-143.

Cho, S. S., Qi, L., Fahey, G. C., & Klurfeld, D. M. (2013). Consumption of Cereal Fiber, Mixtures of Whole Grains and Bran, and Whole Grains and Risk Reduction in Type 2 Diabetes, Obesity, and Cardiovascular Disease. *American Journal of Clinical Nutrition , 98*, 594–619.

Cuq, B., Gonçalves, F., Mas, J. F., Vareille, L., & Abecassis, J. (2003). Effects of moisture content and temperature of spaghetti on their mechanical properties. *Journal of Food Engineering, 59(1)*, 51-60.

Daniel, M., Wisker, E., Rave, G., & Feldheim, W. (1997). Fermentation in human subjects of nonstarch polysaccharides in mixed diets, but not in a barley fiber concentrate, could be predicted by in vitro fermentation using human fecal inocula. *Journal of Nutrition 127(10)*, 1981-1988.

De Filippo, C., Cavalieri, D., Di Paola, M., Poullet, J. B., Massart, S., Collini, S., . . . Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and Rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*, 14691–14696.

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., . . . Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology, 72(7)*, 5069-5072.

De Vos, P., Garrity, G. M., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., . . . Whitman, W. B. (2009). *Bergey's manual of systematic bacteriology: volume three the firmicutes.* NY: Springer.

Den Besten, G., Van Eunen, K., Groen, A. K., Venem, A. K., Venema, K., Reijngoud, D. J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research, 54(9)*, 2325-2340.

Doblado-Maldonado, A. F., Flores, R. A., & Rose, D. J. (2013). Low moisture milling of wheat for quality testing of wholegrain flour. *Journal of Cereal Science 58*, 420-423.

Everard, A., Belzer, C., Geurts, L., Ouwerkerk, J. P., Druart, C., Bindels, L. B., & De Vos, W. M. (2013). Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proceedings of the National Academy of Sciences, 110(22)*, 9066-9071.

Foster, Z. S., Sharpton, T. J., & Grünwald, N. J. (2017). Metacoder: An R package for visualization and manipulation of community taxonomic diversity data. *PLoS Computational Biology, 13(2),* e1005404.

Hartzell, A. L., Maldonado-Gómez, M. X., Hutkins, R. W., & Rose, D. J. (2013). Synthesis and in vitro digestion and fermentation of acylated inulin. *Bioactive Carbohydrates and Dietary Fibre 1.1*, 81-88.

Johansson, D. P., Vázquez Gutiérrez, J. L., Landberg, R., Alminger, M., & Langton, M. (2018). Impact of food processing on rye product properties and their in vitro digestion. *European Journal of Nutrition 57*, 1651–1666.

Kahlon, T. S., Berrios, D. J., Smith, G. E., & Pan, J. L. (2006). Extrusion conditions modify hypocholesterolemic properties of wheat bran fed to hamsters. *Cereal Chemistry*, 152-156.

Kasubuchi, M., Hasegawa, S., Hiramatsu, T., Ichimura, A., & Kimura, I. (2015). Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. *Nutrients, 7(4)*, 2839-2849.

Konikoff, T., & Gophna, U. (2016). Oscillospira: a central, enigmatic component of the human gut microbiota. *Trends in Microbiology, 24(7)*, 523-524.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and Environmental Microbiology*, 5112-5120.

Kund, E., & Bach, K. (2015). Microbial Degradation of Whole-Grain Complex Carbohydrates and Impact on Short-Chain Fatty Acids and Health. *Advances in Nutrition, 6(2)*, 206-213.

National Cancer Institute. (2018). Retrieved from Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool: https://epi.grants.gov/asa24

Nyman, M., Asp, N. G., Cummings, J., & Wiggins, H., J., & Wiggins, H. (1986). Fermentation of dietary fibre in the intestinal tract: comparison between man and rat. *British Journal of Nutrition, 55(3)*, 487-496.

Puertollano, E., Kolida, S., & Yaqoob, P. (2014). Biological significance of short-chain fatty acid metabolism by the intestinal microbiome. *Current Opinion in Clinical Nutrition & Metabolic Care, 17(2)*, 139-144.

Ralet, M., Thibault, J., & Della Valle, G. (1990). Influence of extrusion-cooking on the physico-chemical properties of wheat bran. *Journal of Cereal Science*, 249-259.

Robin, F., Dubois, C., Pineau, N., Schuchmann, H., & Palzer, S. (2011). Expansion mechanism of extruded foams supplemented with wheat bran. *Journal of Food Engineering, 107*, 80-89.

Singh, J., Dartois, A., & Kaur, L. (2010). Starch digestibility in food matrix: a review. *Trends in Food Science and Technology, 21(1)*, 168-180.

Singkhornart, S., Lee, S. G., & Ryu, G. H. (2013). Influence of twin-screw extrusion on soluble arabinoxylans and corn fiber gum from corn fiber. *Journal of the Science of Food and Agriculture, 93(12)*, 3046-3054.

Sonnenburg, E. D., & Sonnenburg, J. L. (2014). Starving our microbial self: The deleterious consequences of a diet deficient in microbiota- accessible carbohydrates. *Cell Metabolism, 20(5)*, 779-786.

US Department of Health and Human Services. (2017). Dietary Guidelines for Americans 2015-2020. *Skyhorse Publishing Inc.*.

Wang, D. D., Leung, C. W., Li, Y., Ding, E. L., Chiuve, S. E., Hu, F. B., & Hu, F. B. (2014). Trends in dietary quality among adults in the United States, 1999 through 2010. *JAMA Internal Medicine 174*, 1587–1595.

Weaver, G. A., Wolin, M. J., Krause, J. A., Miller, T. L., & Wol, J. A. (1992). Cornstarch fermentation by the colonic microbial community yields more butyrate than does cabbage fiber fermentation; cornstarch fermentation rates correlate negatively with methanogenesis. *The American Journal of Clinical Nutrition, 55(1)*, 70-77.

Wisker, E., Daniel, M., Rave, G., & Feldheim, W. (1998). Fermentation of non-starch polysaccharides in mixed diets and single fibre sources: comparative studies in human subjects and in vitro. *British Journal of Nutrition, 80(3)*, 253-261.

Wisker, E., Knudsen, K. E., Daniel, M., Eggum, B. O., & Feldheim, W. (1997). Energy values of non-starch polysaccharides: comparative studies in humans and rats. *The Journal of Nutrition, 127(1)*, 108-116.

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y. Y., Keilbaugh, S. A., . . . Sinha, R. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science, 334(6052)*, 105-108.

Yang, J., Keshavarzian, A., & Rose, D. J. (2013). Impact of dietary fiber fermentation from cereal grains on metabolite production by the fecal microbiota from normal weight and obese individuals. *Journal of Medicinal Food, 16(9)*, 862-867.

TABLES AND FIGURES

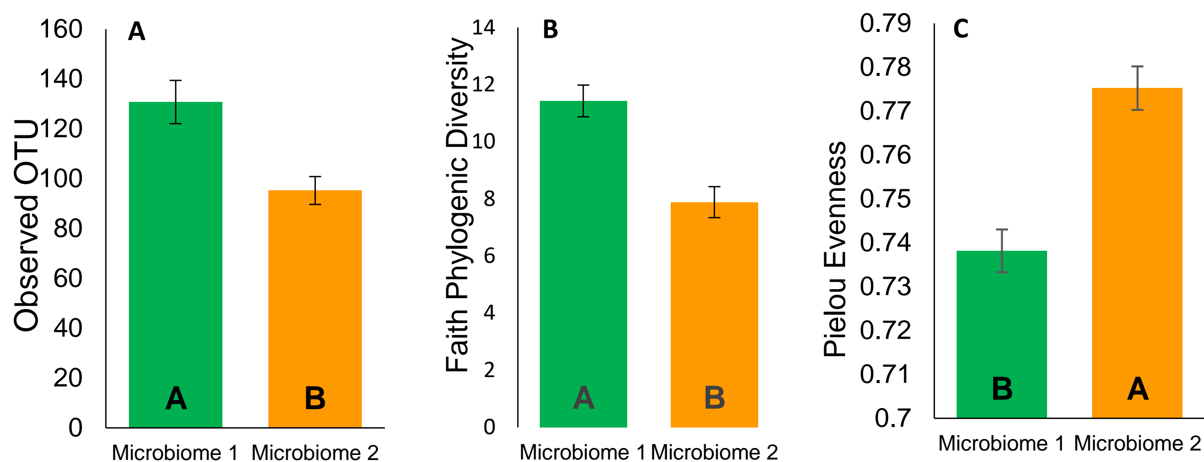

Figure 2.1. α-Diversity of subject fecal samples used for *in vitro* fecal fermentation by (A) observed operational taxonomical units (OTU), (B) Faith's Phylogenic Diversity, and (C) Pielou Evenness.

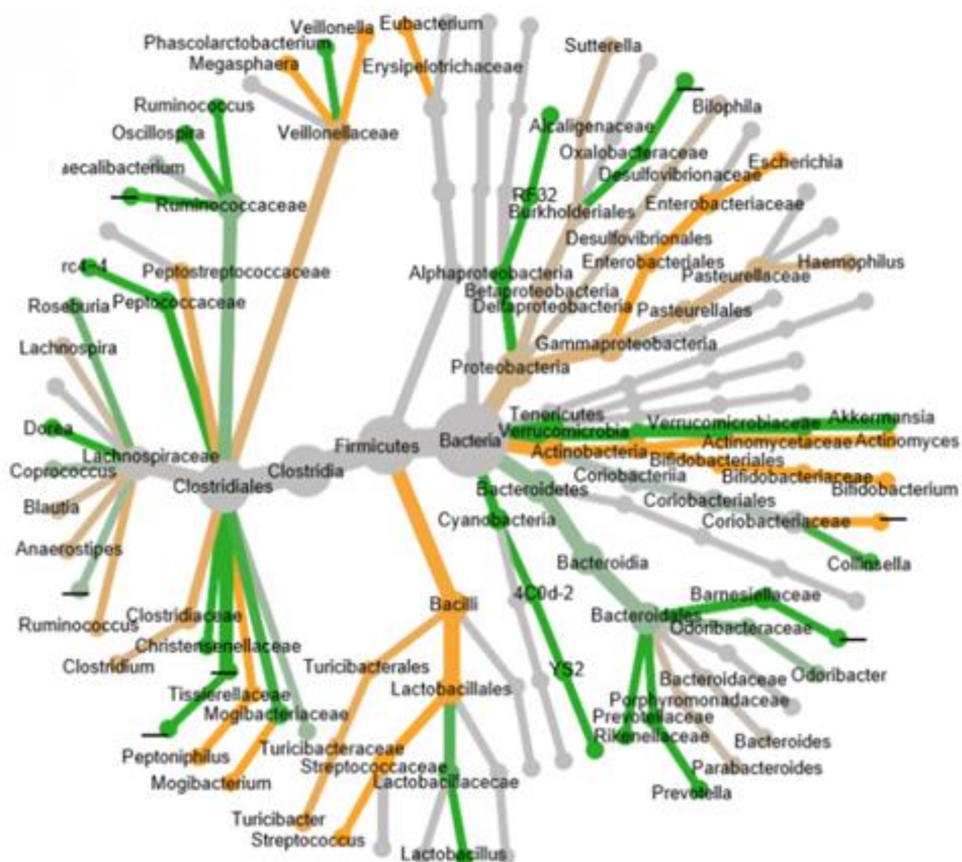

Figure 2.2. Heat Tree depicting taxonomic significant differences between the two microbial communities; green is more abundant in subject 1 while gold is more abundant in subject 2 (Wilcoxon rank sum test, $p < 0.05$).

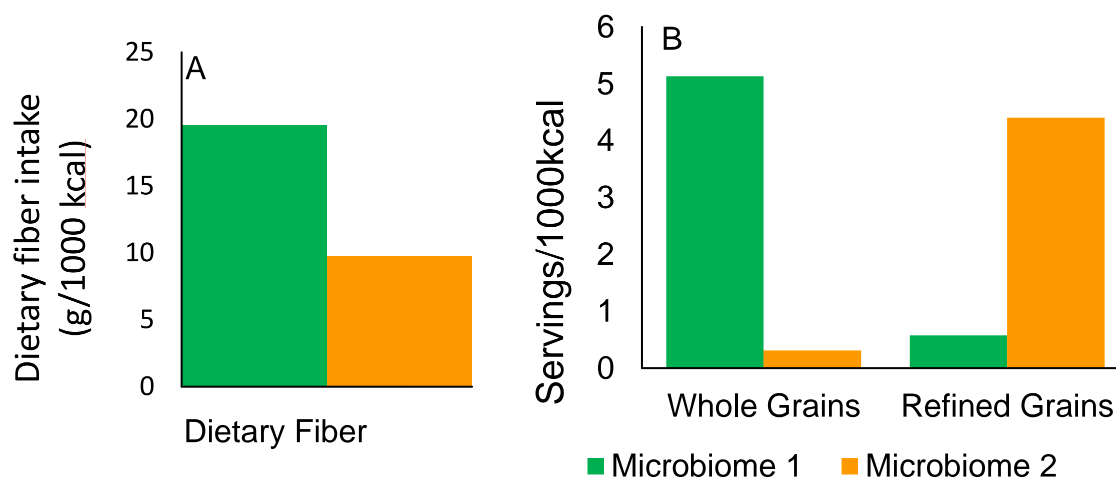

Figure 2.3. Average grams of dietary fiber (A), serving of whole grains, and servings of refined grains (B) expressed per 1000 kcal.

Table 2.1. I Non-digestible carbohydrates (NDC) and residual starch content in processed and digested whole wheat products.

	NDC		Residual Starch	
Boiling	Cooking time[A]		Cooking time[B]	
Moisture[B]	Low	High	Low	High
Low	49.4 ±3.6	58.9 ±3.6	10.23 ±0.8	9.85 ±0.8
High	42.7 ±3.6	42.3 ±3.6	11.43 ±0.8	10.64 ±0.8
Average	49.4 ±0.37		10.5 ± 0.2	
Extrusion	Screw speed[C]		Screw speed[D]	
Moisture[D]	Low	High	Low	High
Low	45.1 ±4.3	35.1 ±4.3	4.9 ±0.5	4.6 ±0.5
High	51.2 ±4.3	45.0 ±4.3	4.9 ±0.5	5.0 ±0.5
Average	45.0 ±4.3		4.9 ± 0.2*	

[A]Low=2.5 min; high=5 min; [B]Low=2.7:1 (water: flour); high=5.30:1; [C]Low=125 rpm; high=250 rpm; [D]Low=20% (dry flour basis); high=30%; *significantly different from boiling average.

Table 2.2. ANOVA results (mean squares) for boiled wheat.

Source	DF	Fermentable NDC	Acetate	Propionate	Butyrate	Total SCFA
Cooking time (CT)	1	85.4	7.03	0.64	3.15	4.87
Moisture content (MC)	1	1945 *	2.32	0.83	14.6	13.2
Subject (S)	1	7119 ***	6975 ***	24.12 ***	5229 ***	174
CT*MC	1	8.94	3.01	0.17	0.01	1.44
CT*S	1	1.64	0.09	0.2	0.01	6.26
MC*S	1	1041 *	2.88	0.08	0.07	27.6
CT*MC*S	1	31.1	2.29	0.19	12.2	26.5
Error	17	0.022	1.79	0.30	0.85	5.07

*$p < 0.05$; ***$p < 0.001$.

Table 2.3. ANOVA results (mean squares) for extruded wheat.

Source	DF	Fermentable NDC	Acetate	Propionate	Butyrate	Total SCFA
Cooking time (CT)	1	85.38	4.25	1.54	26.62 *	18.16
Moisture content (MC)	1	1944.71 *	5.09	0.08	1.47	0.31
Subject (S)	1	8.94	1.41 ***	0.60	0.35	11.12
CT*MC	1	7119.06 ***	2746.94 ***	11.55 *	1966.07 ***	5.87
CT*S	1	1.64	0.03	0.98	4.73	0.87
MC*S	1	1041.24 *	0.04	0.02	1.53	12.53
CT*MC*S	1	31.11	0.25	0.03	1.13	7.14
Error	17	0.073	1.79	0.10	0.85	2.66

*p <0.05; ***p<0.001.

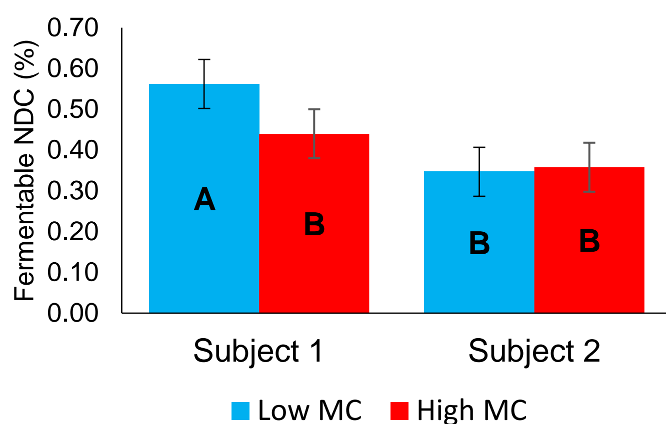

Figure 2.4. Effect of boiling conditions on change in fermentable carbohydrates due to moisture content, low mc (270g:100g) and high mc (530g:100g), and subject after 12 hours of *in vitro* fecal fermentation. Letters denote Tukey honest significant difference at $p < 0.05$ results (mean squares) for boiled wheat.

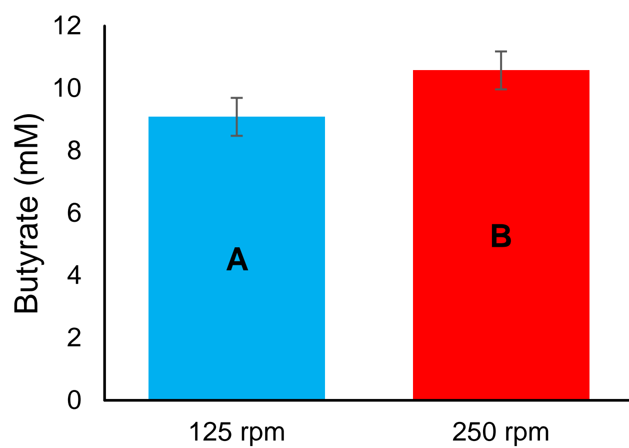

Figure 2.5. Effect of extrusion screw speed revolutions per minute (rpm) on butyrate production after 12 hours of *in vitro* fecal fermentation. Letters significant difference at $p < 0.05$.

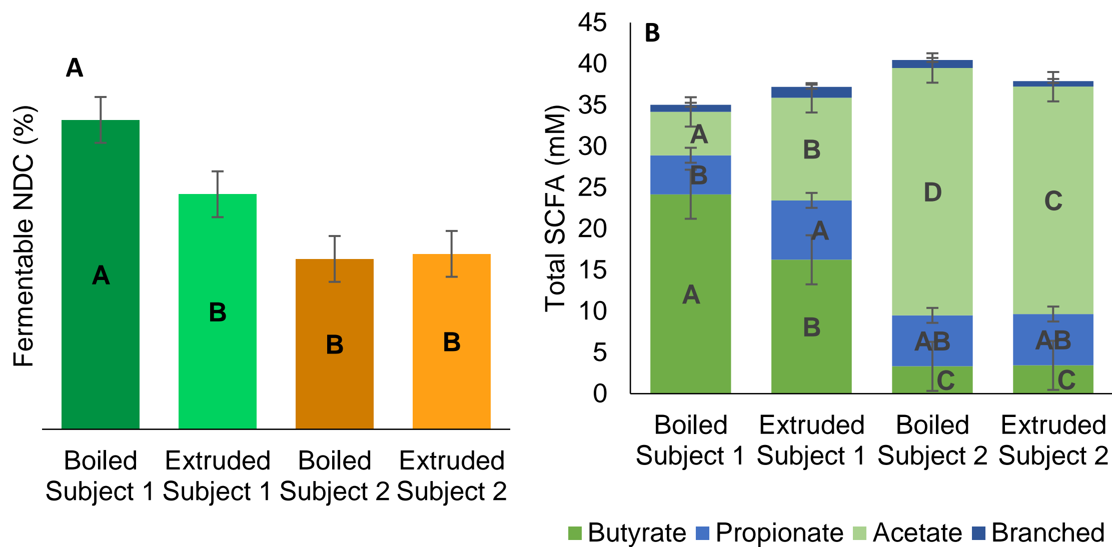

Figure 2.6. Effect of processing technique and subject on (A) fermentable carbohydrates (% of NDC fermented) and (B) short chain fatty acids (SCFA) after 12 hours of *in vitro* fecal fermentation. Letters denote Tukey honest significant difference at $p < 0.05$.

CHAPTER 3 PROCESSING HAS DIFFERENTIAL EFFECTS ON MICROBIOTA ACCESSIBLE CARBOHYDRATES IN WHOLE GRAINS

3.1. ABSTRACT

Whole grains are generally low in non-digestible carbohydrates that are available for fermentation by the gut microbiota, or microbiota accessible carbohydrates (MAC). However, there is potential to increase MAC in whole grains through food processing. Five processing methods: boiling, extrusion, unleavened bread, yeast bread, and sourdough bread, were applied to whole wheat flour and then subjected to *in vitro* digestion followed by fermentation using the fecal microbiota from 10 subjects. Processing led to dichotomous results for MAC, changes in microbiota composition, and short chain fatty acid production, with extrusion and sourdough bread being at opposite ends of the spectrum. Extrusion resulted in high MAC but diminished microbiota α-diversity and butyrate production, while sourdough bread resulted in low MAC but high butyrate and propionate production. The increase in MAC as a result of extrusion was attributed to changes in the microstructure of the cell wall components that increased accessibility of the cell wall polysaccharides to fermentation by fast-growing microbes, such as E*nterococcus species*, that outcompete the consortia of butyrate-producing bacteria that typically break down cell wall polysaccharides. In contrast, the increase in butyrate production as a result of sourdough bread processing was attributed to increased residual resistant starch and maintenance of specialized cell wall degraders, such as members of the Ruminococcaceae and Lachnospiraceae. In conclusion, this study demonstrated that different grain processing operations cause varying shifts in microbiota composition and activity during *in vitro* fermentation. These results will be useful in the development of microbiota directed foods.

3.2. INTRODUCTION

Non-digestible dietary carbohydrates (NDC), mainly from dietary fiber, are the major energy sources for the microbiome (Koropatkin, Cameron, & Martens, 2012; Salyers, West, Vercellotti, & Wilkins, 1977). Fermentation of these carbohydrates by the microbiota results in numerous metabolites, including short chain fatty acids (SCFA), which are absorbed by the host and have pleiotropic local effects on intestinal function as well as systemic roles in insulin secretion, lipid metabolism, and inflammation, among others (Den Besten, et al., 2013; Kasubuchi, Hasegawa, Hiramatsu, Ichimura, & Kimura, 2015; Puertollano, Kolida, & Yaqoob, 2014).

Unfortunately, a large proportion of non-digestible carbohydrates in whole grains are not metabolically available to gut microbes (Daniel , Wisker, Rave, & Feldheim, 1997; Nyman, Asp, Cummings, J., & Wiggins, H., & Wiggins, 1986; Wisker, Knudsen, Daniel, Eggum, & Feldheim, 1997; Wisker, Daniel, Rave, & Feldheim, 1998). Human feeding trials have suggested that only about one-third of the dietary fibers in grains are metabolically available for fermentation by the microbiota compared with 75-90% for fruits and vegetable fibers (Nyman, Asp, Cummings, & Wiggins, H., & Wiggins, 1986). Therefore, although whole grains are rich sources of dietary fiber, they may not be good sources of microbiota accessible carbohydrates (MAC) (Arcila, Weier, & Rose, 2015; Sonnenburg & Sonnenburg, 2014).

Processing has been proposed as a way to manipulate the bioavailability of carbohydrates to the microbiota. Boiling is a minimal form of grain processing that involves moderate temperatures (100 °C), atmospheric pressure, minimal shear, and an abundance of water. During boiling of rye flour, for example, water swelled the starch granules resulting in the leaching of amylose, starch gelatinization, and an amorphous structure (Johansson, Vázquez Gutiérrez,

Landberg, Alminger, & Langton, 2018). Boiling also resulted in swollen cell walls, but the cell walls appeared to remain intact and the structure of the principal dietary fibers, β-glucan and arabinoxylan, appeared unaffected. In wheat, boiling is commonly used as a control process for whole grain and bran experiments (Arcila, Weier, & Rose, 2015; Bjorck, Asp, & Lundquist, 1984; Bjorck, et al., 1984).

Extrusion is a form of processing that uses heat (>120 °C), pressure, and mechanical shear at low moisture contents to process whole grains into an edible food material. This process commonly results in gelatinization and breakdown of the starch, but also affects the cell wall structures (which are comprised of dietary fibers) by increasing surface porosity (Robin, Dubois, Pineau, Schuchmann, & Palzer, 2011). Furthermore, extrusion enables the conversion of some insoluble fibers to soluble fibers; the latter of which are generally highly fermentable (Kahlon, Berrios, Smith, & Pan, 2006; Ralet, Thibault, & Della Valle, 1990; Robin, Dubois, Pineau, Schuchmann, & Palzer, 2011; Wang, Klopfenstein, & Ponte, 1993). In extruded rye, β-glucan and arabinoxylan separated and distributed throughout a starch matrix as small fragments. The starch granules were also completely disrupted, resulting in a homogeneous starch phase, which encapsulated small fragments of cell walls and aleurone layers and a thinner lamella (Johansson, Vázquez Gutiérrez, Landberg, Alminger, & Langton, 2018). Accordingly, extrusion has been shown to increase the metabolism of dietary fibers from grains by gut bacteria both *in vitro* using human fecal microbiota (Arcila, Weier, & Rose, 2015) and *in vivo* in rat feeding studies (Aoe, Nakaoka, Ama, Ohta, & Ayan, 1990). When wheat bran residues were recovered from rat fecal pellets, the cell walls were thinner and rougher and more severely disrupted in extruded wheat bran compared with unprocessed wheat bran (Aoe, Nakaoka, Ama, Ohta, & Ayan, 1990).

Bread making is the most common processing technique for wheat worldwide (Reicks, Jonnalagadda, Albertson, & Joshi, 2014). During the bread making process, a dough is formed through the addition of water and mechanical agitation (kneading), which creates a continuous matrix of protein (gluten) with starch granules and cell wall particles (containing dietary fiber) embedded within. Following dough development, bread making commonly involves fermentation of the dough by either *Saccharomyces cerevisiae* for yeast leavened bread or a use of co-cultures of lactic acid bacteria (LAB) and yeasts that develop from the flour and environment in the case of sourdough bread (Minervini et al., 2014). During fermentation, yeast metabolizes mono- and disaccharides (glucose, galactose, sucrose and when needed maltose) into ethanol and carbon dioxide (among other minor, albeit important, flavor compounds) to leaven the dough. The bacteria in a sourdough culture, which are commonly LAB, have a symbiotic relationship with the yeast. The bacteria break down more complex carbohydrates (larger sugars and for some species starch) to simpler sugars for the yeast to ferment. LAB additionally produce lactic and acetic acid that lower the pH of the co-culture (Corsetti, et al., 1998). During baking, volatilization of carbon dioxide and steam further leaven the bread until the protein structure is set and starch is gelatinized.

The breadmaking process has been shown to increase soluble dietary fiber at the expense of insoluble fiber (Cleemput et al., 1997). Spontaneous fermentation of wheat bran increased solubility of arabinoxylan with a lower average degree of arabinose substitution than native bran, which are thought to be more easily degradable by intestinal microbiota (Brouns, Hemery, Price, & Anson, 2012; Damen, et al., 2011; Grootaert, et al., 2009; Karppinen, Kiiliäinen, Liukkonen, Forssell, & Pouranen, 2001; Manini, et al., 2014). Bread making techniques were compared in whole grain rye bread in yeast-fermented crispbread, unfermented crispbread, and sourdough

bread. In all breads, β-glucan and arabinoxylan were distributed as small fragments throughout the starch matrix, which suggest that fiber was degraded. The yeast-fermented crispbread and unfermented crispbread had highly swollen starch granules where the unfermented crispbread appeared to have a higher degree of starch gelatinization and a thin lamella. Additionally, the unfermented crispbread had larger pieces of bran and intact cell structures compared with the other breads. For sourdough, starch granules were less swollen and surrounded by more leached amylose, which was proposed to act as a protective layer during digestion (Johansson, Vázquez Gutiérrez, Landberg, Alminger, & Langton, 2018). In wheat bran, sourdough resulted in increasing the swelling of starch granules, and more leaching of amylose (Salmenkallio-Marttila M. K., 2001). Furthermore, pre-fermentation of wheat bran with yeast and enzymes in bread making resulted in increased SCFA production and bread was more degraded when visualized with microscopy after the *in vitro* upper gut and colon models (Nordlund, Katina, Aura, & Poutanen, 2013).

Clearly, different processing methods of grains affect the chemical physical structures of dietary fibers in differential ways. While most of these chemical and physical changes would likely improve bio-accessibility of the fibers to gut microbial fermentation, no studies have compared these processing methods to determine which method might result in the greatest improvement in fermentable carbohydrates in whole grain. Thus, the purpose of this study was to evaluate how different common food processing techniques change the fermentability of carbohydrates and shift the microbiome during *in vitro* fermentation.

3.3. MATERIALS AND METHODS

3.3.1. *Whole wheat processing*

Milling. Hard red wheat was obtained from Bay State Milling (Quincy, MA). Wheat kernels were dried at 40°C for 16 h before milling according to Doblado-Maldonado et al. (2013). All milled fractions obtained from the mill were mixed together to obtain whole wheat flour (Doblado-Maldonado, Flores, & Rose, 2013).

Boiling. Water (530 g) was brought to a boil on a gas range. Once boiling, the heat was reduced to a simmer and 1 g of salt and 100 g whole wheat flour were added under rapid manual stirring for 5 min. The boiled wheat porridge was then cooled to room temperature before being frozen at -80°C and then freeze dried. This procedure was performed in triplicate.

Extrusion. Whole wheat flour (1 kg) was mixed for 10 min with 1% salt (w/w) and water to adjust to 20% moisture content (dry weight basis) in a stand mixer (c-100, Hobart, Troy, OH). The mixtures were equilibrated in closed containers at 4 °C overnight. The moisture-adjusted whole wheat flours were then extruded using a benchtop-scale twin-screw extruder equipped with a single stage mixing zone and a 3 mm outlet die at 250 rpm, 3:1 compression ratio, and a 20:1 L/D ratio (CW Brabender Instruments, NJ, USA). The extruder was operated by a direct current drive unit (Intelli-Torque, Pastic Corder Lab-station, C.W. Brabender) with a 5.6 kW motor. The flour was fed into the extruder using a volumetric feeder (FW 40 Plus, C. W. Brabender) set at a constant flow rate of ~50 g/min. Barrel temperatures were set at 60 °C (zone 1; inlet), 70 °C (zone 2), 120 °C (zone 3) , and 120 °C (zone 4; die assembly). Samples were collected from the extruder die once steady state had been reached. Extrudates were then dried in a convection oven overnight at 70 °C to complete the extrusion processing. Although the product contained very low moisture at this stage (~3%), the dried extrudates were subject to freeze drying (3600, Freeze Dry Co., Pine River, MN) because the other processed wheat products were freeze dried following processing. The whole wheat flour was extruded in triplicate.

Bread making. This study employed an in-house method for bread making that involved production of flatbreads. Flatbread-type breads were selected because they are made using any of the three selected fermentation procedures: unleavened, baker's yeast fermentation, or sourdough fermentation. The procedures were planned to unify the essential bread making procedures and only varying the steps that were unique to each fermentation procedure. For the baker's yeast fermentation treatment, instant dry yeast was used (Saf Instant Red, Lesaffre, Milwaukee, WI). For the sourdough fermentation, a type1 spontaneous sourdough starter culture was used. To prepare the culture, 50 g of whole wheat flour and 50 g of water (100% absorption) was incubated at 30°C for 24 h, whereupon 50 g of that mixture was mixed with 50 g of fresh whole wheat flour and 50 g of water and then allowed to ferment for another 24 h at 30°C. This step was repeated for 9 d. The starter culture had 10^7 CFU/g of lactic acid bacteria (Njongmeta, Hall, Ledenbach, & Flowers, 2015) and 10^6 CFU/g yeast (Tournas, Stack, Mislivec, Koch, & Bandler, 2013). The starter culture was used 5 d after measuring due to the length of the test protocol. All breads were prepared on the same day in triplicate 500-g batches with 70% absorption (70 g water/100 g whole wheat flour on a 14% moisture basis). The doughs were prepared by kneading for 10 min in an electric stand mixer with a dough hook at speed 2 (KSM3316PBM, Kitchenaid). The dough was then rounded by hand, covered, and allowed to rest for 20 min (unleavened) or ferment for 90 min (baker's yeast) or 240 min (sourdough). The dough was then divided into 5, 100-g pieces, rounded by hand, covered, and allowed to rest for 20 min. The dough was then sheeted by passing through a dough sheeting device (KSMPRA, KitchenAid) to a thickness of 6 mm. A fork was then used to poke holes in the top of the dough piece uniformly 5 cm apart (docking) to prevent bubbling up during baking. The flatbreads were then baked for 4 min at 232

°C. The baked flatbread was cooled overnight at room temperature inside a plastic bag once the flatbreads were at room temperature and then freeze dried.

3.3.2. *In vitro digestion*

Samples were digested following the procedure of Yang, Keshavarzian, and Rose (2013) with minor modifications. Briefly, freeze-dried processed whole wheat products were broken into small pieces by hand. Twenty-five grams of sample were then stomached with 300 mL of water for 1 min. Then, the pH was adjusted to 2.5 with 1 M HCl followed by the addition of 10 mL of 10% (w/v) pepsin (P7000, Sigma, St Louis, MO) in 50 mM HCl. The slurry was then incubated for 30 min at 37 °C with orbital shaking at 150 rpm. Following pepsinolysis, 50 mL of 0.1 M sodium maleate buffer (pH 6, containing 1 mM $CaCl_2$) was added and the pH was adjusted to 6.9 with 1 M $NaHCO_3$. Next, 50 mL of 12.5% (w/v) pancreatin (P7545, Sigma) in sodium maleate buffer was added followed by 2 mL of amyloglucosidase (3260 U/mL, Megazyme, Bray, Ireland). The slurry was then incubated for 6 h 37 °C with orbital shaking at 150 rpm. Following digestion, the material was transferred to dialysis tubing (molecular weight cutoff, 12,000-14,000) and dialyzed against distilled water for 4 d at 4 °C with a water change at least every 12 h. The retentate from dialysis was collected and freeze dried.

3.3.3. In vitro fermentation

Fresh fecal samples from 10 healthy adults with no history of gastrointestinal abnormalities, no prebiotic or probiotic consumption, or antibiotic consumption within the past 6 months were collected. A fecal slurry was prepared in an anaerobic hood (Bactron X, Sheldon manufacturing, Cornelius, OR USA, containing 5% H2, 5% CO2, and 90% N2) within 2 h of defecation by blending (Sunbeam 2774) each fresh fecal sample separately with sterile phosphate

buffered saline pH 7.0 (1:9 w/v) containing 10% glycerol as a cryoprotectant for 1 min. The slurry was then filtered through 4 layers of sterile cheesecloth and then frozen at -80° until fermentation was performed. Two fecal slurries from fecal samples collected on different days were prepared from each individual.

In vitro batch fecal fermentation was performed as described (Yang, Keshavarzian, and Rose 2013). Briefly, inside of an anaerobic hood (Bactron X, Sheldon manufacturing, Cornelius, OR USA, containing 5% H2, 5% CO2, and 90% N2), 15 mg of the freeze dried material obtained after *in vitro* digestion and dialysis of the processed whole wheat products was suspended in 1 mL of sterile fermentation medium containing (per L): peptone (2 g, Fisher Scientific, Waltham, MA), yeast extract (2 g, Fisher Scientific, Waltham, MA), bile salts (0.5 g, Oxoid, Cheshire, England), $NaHCO_3$ (2 g), NaCl (0.1 g), K_2HPO_4 (0.08 g), $MgSO_4 \cdot 7H_2O$ (0.01 g), $CaCl_2 \cdot 6H_2O$ (0.01 g), L-cysteine hydrochloride (0.5 g, Fisher Scientific, Waltham, MA), hemin (50 mg dissolved in DMSO), Tween 80 (2 mL, Fisher Scientific, Waltham, MA), vitamin K (10 µL, dissolved in ethanol, Alfa Aesar, Haverhill, MA), and 0.025% (w/v) resazurin solution (4 mL, dissolved in water, Alfa Aesar, Haverhill, MA), capped, and hydrated for 1 h. Tubes were then inoculated with 0.1 mL of fecal slurry, capped, and incubated at 37 °C with orbital shaking (140 rpm) for 12 h before being flash frozen in liquid nitrogen. Samples that were immediately flash frozen in liquid nitrogen following inoculation were used as controls (0 h of fermentation). After freezing in liquid nitrogen, tubes were stored at -80 °C until analysis. Because most of the dietary fibers in whole grains are not soluble, separate fermentation tubes were prepared for analysis of carbohydrates versus microbiota composition and SCFA.

3.3.4. Microbiota composition

Changes in microbial population were observed using 16S rRNA sequencing. A kit from Biovet (BioSprint 96 One-For-All Vet Kit, Quebec Canada) was used. Microbiome characterization was performed by amplicon sequencing of the V4 region of the 16S rRNA gene on the Illumina MiSeq platform using the MiSeq Reagent kit v2 (2 X 250 bp) following the protocol of Kozich et al. (2013). Sequences were demultiplexed and barcodes were removed prior to sequence analysis with the QIIME 2 platform (Boylen et al., 2019). Sequence quality control, trimming and denoising was performed with DADA2 (Callahan et al., 2016). Forward and reverse reads were trimmed to maintain sequence qualities above a phred score of 30. Taxonomy was assigned using the Greengenes database (DeSantis et al., 2006). Reads were rarefied to a sampling depth of 9000 prior to analysis.

Diversity of each fermented and fecal sample was determined using QIIME 2. The log 2 fold change in relative abundance was calculated by the log of the quotient of the fermented sample (12 h) by its corresponding unfermented sample (0 h).

3.3.5. Carbohydrate analysis

For analysis of the proportion of non-digestible carbohydrates fermented by the microbiota, a modified version of the procedure used by Basumallick and Rohrer (2015) was followed. The entire contents of the tubes designated for carbohydrate analysis were freeze dried. The freeze-dried samples were then treated with 0.3 mL of 12 M sulfuric acid for 1 h at 30° C with periodic vortex mixing to begin the hydrolysis. One milliliter of a fucose solution (5 mg/mL) was then added as an internal standard and 7.7 mL of water was added to bring the final sulfuric acid concentration to 0.4 M. The samples were then autoclaved at 121°C for 1 h, cooled, and filtered through a 0.45 µm membrane filter. Ten µL was injected into a high performance

anion exchange chromatograph (ICS-5000+ SP, Dionex) equipped with an anion exchange column (CarboPac PA1, Dionex) operating 30 °C with a 1 mL/min flow rate. The eluent was 200 mM NaOH for column cleaning followed by 10 min of equilibration of 3 mM NaOH before injection of the sample. The sugars were detected over 22 min with pulsed amperometry waveform (ICS-5000+ SP, Dionex). Sugar residues were quantified by calculating response factors for each sugar relative to fucose using injections of pure standards. The concentration of each sugar residue was corrected for its weight as it occurs in a polysaccharide (0.88*weight for pentose sugars and 0.9*weight for hexose sugars. The percent change in the amount of carbohydrates measured was calculated to determine MAC. Total carbohydrates were measured with the same procedure using 100 mg of raw, processed, or digested whole wheat. Total starch in the processed whole grain samples and after digestion were measured using a kit according to the manufacturer's directions (K-TSTA, Megazyme, Bray, Ireland). The starch remaining in the processed samples after digestion was considered resistant starch.

3.3.6. Short chain fatty acids

SCFA were extracted and measured by gas chromatography as described (Hartzell 2013). In brief, 0.4 mL of fermented sample, 0.1 mL of 7 mM 2-ethylbutyric acid in 2 M potassium hydroxide, 0.2 mL of 9 M sulfuric acid, and ~0.1 g of sodium chloride was mixed together. Then, 0.5 mL of diethyl ether was added and mixed. The top layer was injected into a gas chromatograph (Clarus 580, PerkinElmer, MA USA) equipped with a capillary column (Nukol, 30 m × 0.25 mm inner diameter × 0.25 μm film thickness, Supelco, Bellefonte, PA) and flame ionization detector. SCFA were quantified by calculating response factors for each SCFA relative to 2-ethylbutyric acid using injections of pure standards.

3.3.7. Statistical analysis

Analysis of variance (ANOVA) was used to determine the effect of subject and process on the experimental parameters using general linear models (SAS version 9.4 Cary, NC USA). When the ANOVA was significant for processing method, tukey honest significant difference was used to determine the effect of process and subject. Pearson correlations of the least squared means of each process and subject combination with process and person as a partial variable were calculated.

3.4. RESULTS

Because grain processing is known to affect the physical and, in some cases, the chemical composition of the non-digestible carbohydrates in wheat, whole wheat flour was processed by five common grain processing methods to determine how these processes may change gut microbiota composition and metabolism, especially regarding carbohydrate metabolism during fermentation. Processing did not significantly affect the total carbohydrate concentration among samples. All samples contained 61.6% (56.5%-66.7%) total carbohydrate, of which 83% was starch and 17% was non-starch polysaccharides. Following processing, whole wheat samples were subject to *in vitro* digestion and dialysis to remove digestible starch, sugars, and protein. After digestion, the freeze-dried, processed wheat samples contained 36.6% (36%-37%) total carbohydrates, with significant differences in resistant starch due to processing (Figure 3.1). Sourdough bread and boiled wheat had more residual starch compared to the other processes.

For *in vitro* fermentation, duplicate fecal samples were obtained from 10 donors. As expected, microbiota composition varied among the 10 fecal donors (Figure 3). The microbiota

from subjects differed in α-diversity from 6.9 to 13.1 Faith's Phylogenetic Diversity Index (Figure 3.2.B).

α-Diversity of the microbiotas decreased during fermentation of the processed wheat samples, but varied significantly depending on fecal donor and wheat processing method (Figure 3.3). Extrusion, a severe processing method, resulted in the greatest loss in α-diversity while the minimal processing methods, boiling, and unleavened bread, maintained the most diversity. Microbiotas from the 10 subjects varied in the magnitude that α-diversity changed during fermentation, but the effect of processing was consistent within a subject. For example, the microbiotas from subjects 1,3 and 9 consistently showed the largest decrease in α-diversity during fermentation, while the microbiotas from subjects 5, 7, and 8 consistently showed the least decrease in α-diversity. While Figure 3.2 shows results for Faith's phylogenetic diversity, other metrics of α-diversity, including Shannon's index, Pielou evenness, and observed OTUs, showed similar results (data not shown).

β-Diversity was assessed as the Bray Curtis distance from the fecal inoculum following fermentation. Extrusion, yeast bread, and sourdough bread resulted in large changes in β-diversity during fermentation, while boiling and unleavened bread resulted in less change. However, in contrast to α-diversity, β-diversity showed that different microbiotas varied in their response to grain processing method (Figure 3.4). For instance, the microbiotas from subjects 4, 9, and 10 had a variable shift in Bray Curtis distance from the fecal inoculum depending on processing method, while microbiotas from subjects 2, 3, and 8 hardly varied.

Significant differences in microbiota composition after fermentation were apparent depending on grain processing method (Figure 3.5). The majority of the significant changes due to processing occurred within the Lactobacillales order. Extrusion and yeast bread resulted in the

greatest increase in *Enterococcus.* Sourdough bread resulted in increases in *Pediococcus, Luconstoc*, and an unclassified member of the Lactobacillales order. Other changes induced by processing occurred where an unclassified member of the *Enterobacteriaceae* family increased more in relative abundance for the boiled sample than the extruded sample and sourdough bread. Additionally, an unidentified member of the Staphylococcaceae family had the opposite result where the extruded sample resulted in greater growth. Extrusion resulted in the greatest decrease in Lachnospiraceae *and Ruminococcus* (Ruminococcaceae), and the greatest increase in *Acinetobacter*.

The proportion of NDC that were fermented by the microbiotas, or MAC, varied by fecal donor and grain processing method (Figure 3.6). The microbiotas had varying capacity to ferment carbohydrates from the processed grains, ranging from 14 to 35%. Extrusion resulted in higher MAC compared to boiled wheat and sourdough bread. The microbiotas from subjects 5, 6, 8, and 10 had high capacity to ferment carbohydrates, while microbiotas from subjects 3, 4, 7, and 9 had a lower capacity.

Microbiotas varied significantly in SCFA production during fermentation depending on processing method and fecal donor (Figure 3.7). Across all the microbiotas from all subjects sourdough bread resulted in more propionate and butyrate than extrusion. Among microbiotas, those from subjects 5, 6, and 9 had higher production of all SCFA, while those from subjects 1 and 9 had lower production. The microbiotas from subjects 1, 2, 3 and 7 generally produced low levels of butyrate, while the microbiotas from subject 5, 6, 8 and 10 produced low levels. Microbiotas from subjects 1, 9, and 10 produced low levels of propionate, while propionate production was high in the microbiotas from subjects 2, 7, and 8.

Across all samples, MAC was associated with increased butyrate and α-diversity after 12 h of *in vitro* fermentation (Figure 3.8). However, among processing methods it appeared that the relationships between MAC, butyrate, and α-diversity after 12 h of *in vitro* fermentation were not consistent. For instance, most data for the extruded samples were below the correlation line while sourdough appeared to have a much sharper slope with points appearing both below and above the trend line.

3.5. DISCUSSION

Due to the effect of processing on the physicochemical composition of the NDC in whole grains, five common processing methods for whole wheat were evaluated for their influence on the gut microbiota composition and functionality (in terms of MAC and SCFA production). Processing method had significant effects on carbohydrate fermentation and microbial metabolite production, but the results varied greatly depending on microbiotas. The microbiotas that were able to harvest more MAC from whole grains, such as those from subjects 5, 6, 8, and 10, had increased α-diversity and were more butyrogenic. Having a diverse microbial community results in a wider range of carbohydrate fermenters that are able to hydrolyze a wider range of glycosidic bonds and carbohydrate configurations (Holscher, 2017).

Despite the varying capacity to harvest MAC from whole grains and produce SCFA among the 10 subjects, the effect of the processing methods was consistent generally across all subjects. The five processing method resulted in a spectrum with sourdough and extrusion being at opposite ends. The other three processing methods had more minute changes on the microbiome and MAC.

Sourdough was one processing method that had a pronounced effect on microbiota composition and fermentation results. Sourdough resulted in relatively lower MAC compared

with the other processing methods, yet resulted in higher butyrate and propionate production and increased growth of beneficial microbes that ferment complex carbohydrates into SCFA such as *Rummniococcus* (Ruminococcaceae) , and an unknown genus from the Lachnospiraceae family (Biddle, Stewart, Blanchard, & Leschine, 2013; Chassard, Delmas, Robert, Lawson, & Bernalier-Donadille, 2012). Sourdough-like fermentation of wheat bran with enzyme mixtures and yeast has been previously shown to promote SCFA production *in vitro* compared to native wheat bran (Nordlund, Katina, Aura, & Poutanen, 2013).

The increase in SCFA production could be a result of the changes that occur in the microstructure of bread during the sourdough process. Sourdough has been shown to increase the leaching of amylose during breadmaking, which is rapidly retrograded during cooling and is associated with resistant starch (Johansson, Vázquez Gutiérrez, Landberg, Alminger, & Langton, 2018; Salmenkallio-Marttila, Katina, & Autio, 2001). Feeding a diet higher in amylose, or resistant starch, has been shown to result in increased butyrate and SCFA production and absorption in mice (Regmi, Van Kempen, Matte, & Zijlstra, 2011). Additionally, certain species of *Ruminococcus* favor resistant starch fermentation (Ze, Duncan, Louis, & Flint, 2012; Umu, et al., 2015). The higher levels of residual starch in sourdough (and in boiled wheat) compared to the other processing methods indicate that sourdough bread (and boiled wheat) had more resistant starch available for fermentation *in vitro*.

Extrusion, being among the most severe grain processing methods, resulted in increased carbohydrates available for fermentation by the microbiota. The changes in MAC and SCFA production due to extrusion may be attributed to changes in the physical structural of the cell wall polysaccharides that occur during processing. During extrusion, whole grains are subjected to high temperatures (>120 °C), pressure, and mechanical shear at a low moisture content. These

conditions lead to modifications in the carbohydrates, such as increased porosity and fineness of cell wall structures and the conversion of a portion of the insoluble fiber to soluble fiber, which is generally considered more fermentable (Kahlon, Berrios, Smith, & Pan, 2006; Ralet, Thibault, & Della Valle, 1990; Robin, Dubois, Pineau, Schuchmann, & Palzer, 2011; Wang, Klopfenstein, & Ponte, 1993). These changes in structure likely increased the availability of carbohydrates to the microbiota by increasing the number and potential types of binding sites for microbes and their enzymes. For example, Aoe (1990) saw more disruption in extruded wheat bran compared to native wheat bran after passage through the colon of rats (Aoe, Nakaoka, Ama, Ohta, & Ayan, 1990).

Extrusion is capable of physically breaking down cell wall components and allows for carbohydrates to be available to microbes that may have not been available before. These changes likely influence the types of microbes that are capable of utilizing cell wall components. In one instance, the disruption of the cell wall matrix by extrusion may change the competition dynamics such that the microbiota is less reliant on specialized complex carbohydrate-degrading microorganisms to initiate cell wall metabolism, i.e., cell wall breakdown is already initiated by the extrusion process. This was demonstrated by a large decrease in α-diversity and in an abundance of *Ruminococcus* (Ruminococcaceae) and a genus in the Lachnospiraceae family, genera known for fermenting complex carbohydrates into SCFA, in particular butyrate. Extrusion also resulted in the largest increase in the abundance of *Enterococcus* and *Streptococcus,* which grow rapidly and prolifically in fermentation media (De Vos, et al., 2009). These fast-growing microbes could have outcompeted other bacteria for the newly available MAC leading to decreased diversity and decreases in butyrate and propionate producers. Thus, with extrusion releasing previously unavailable carbohydrates more microbes are able to degrade

cell wall components. This would then result in the fastest and most competitive microbes having the advantage and proliferating on extruded samples.

As expected, extrusion resulted in increased carbohydrates available for fermentation by the microbiota. However, unexpectedly, extrusion also led to decreased butyrate and propionate production compared with the other processed grain samples. This is different from a previous study conducted by our laboratory group using wheat bran where extrusion resulted in increased carbohydrate consumption during fermentation, but also showed increases in propionate and butyrate production compared to boiled wheat bran (Arcila, Weier, & Rose, 2015). The differences in results could be due to subject differences. As shown here, results for MAC and SCFA production vary substantially among fecal microbiotas. Additionally, these differences could be due to different substrates (whole wheat versus wheat bran). Wheat bran, which primarily consists of non-starch polysaccharides, has an increased concentration of cell wall components. Thus, the effect of extrusion on MAC and SCFA production could have been diminished in the present study due to the dilution of the cell wall components in whole grains compared to wheat bran. In the previous study, the extruded bran was reconstituted to whole wheat flour to make whole grain breads, and there were no significant differences between the whole grain breads in NDC fermentation or SCFA production after 12 h (Arcila, Weier, & Rose, 2015).

MAC was positively correlated to butyrate and α-diversity. Diverse microbial communities are more capable of hydrolyzing a wider range of bonds and carbohydrate configurations (Holscher, 2017) and increased MAC consumption and metabolite production, such as SCFA and butyrate (Macfarlane & Macfarlane, 2003). However, the effect of processing on MAC and diversity are varied. For instance, extrusion resulted in high MAC but low diversity

and butyrate production, while sourdough bread resulted in low MAC and high butyrate production.

Overall, it is hypothesized that different processing methods have different influences on the carbohydrates during digestion and fermentation(Figure 3.9). Extrusion changes the microstructure and solubility of carbohydrates in whole grains by breaking down the cell wall components. These changes in structure result in the release of previously unavailable carbohydrates that allow for increased MAC degradation and remove the many of needs for specialized microbes to degrade complex carbohydrates. This changes the relationships and interactions of the microbes so that the fastest growing taxa, such as *Enterococcus* and *Streptococcus,* are able to grow quickly and outcompete other microbes for these nutrients. This would lead to increased proliferation of select microbes, causing high carbohydrate consumption while decreasing diversity. With decreases in diversity, the production of metabolites such as butyrate and propionate likely decreased due to the diminished number of genera that produce these SCFA. However, other processing methods such as sourdough bread change the microstructure differently. The sourdough bread process leads to increased amylose leaching that leads to more resistant starch with little modification to the cell wall matrix. This creates higher quality MAC that is of a wider variety and contains different carbohydrates that are available for fermentation. These higher quality MAC are better able to maintain the need for specialized cell wall degrading microorganisms. These phenomena lead to a more diverse microbiome that has increased capacity to produce SCFA, especially butyrate. The specific effect and relationship of grain processing on MAC, diversity, and SCFA production is inconsistent based on these structural changes. Still, these variables remain related and interconnected.

3.6. CONCLUSION

Fecal microbiota collected from different individuals had varying capacity to utilize non-digestible carbohydrates in whole grains and produce SCFA. Nevertheless, across all subjects some overarching effects of grain processing on the microbiota composition and functionality were evident. Sourdough bread was able to support a diverse microbial community that was capable of high butyrate and propionate production. In contrast, extrusion led to decreases in diversity and butyrate and propionate production, but enabled increase carbohydrate fermentation. These results are likely a result of changes to the microstructure of the cell wall matrix in the grains. In extrusion, this is likely due to the breakdown of the cell wall components into new MAC that allow for the growth of fast-growing microbes that outcompete butyrate-producing cell wall degraders and drives down diversity. While in sourdough bread the cell wall components largely stay intact, but new carbohydrates, such as resistant starch, are available to support a diverse community and promote butyrate production. Thus, both the quantity and quality of MAC are important considerations in evaluating fiber sources and optimum processing methods. This information can be used to better understand how to use food processing for personalized nutrition and the promotion of a healthy microbiome.

3.7. REFERENCES

Aoe, S., Nakaoka, T., Ama, M. Y., Ohta, F., & Ayan, Y. (1990). Availability of dietary fiber in extruded wheat bran and apparent digestibility in rats of coexisting nutrients. *Cereal Chemistry*, 145-148.

Arcila, J. A., Weier, S. A., & Rose, D. J. (2015). Changes in dietary fiber fractions and gut microbial fermentation properties of wheat bran after extrusion and bread making. *Food Research International, 74*, 217-223.

Basumallick, L., & Rohrer, J. (2015). Determination of Carbohydrates in Acid Hydrolysates of Wood.

Biddle, A., Stewart, L., Blanchard, J., & Leschine, S. (2013). Untangling the genetic basis of fibrolytic specialization by Lachnospiraceae and Ruminococcaceae in diverse gut communities. *Diversity, 5(3)*, 627-640.

Bjorck, I., Asp, N. G., & Lundquist, I. (1984). Digestion in vitro and In vivo; I extrusion cooking of wheat flours and starch. *Journal of Cereal Science, (2)*, 91-103.

Bjorck, I., Asp, N. G., Birked, A. C., Eliasson, L. B., Shjoberg, L. B., & Lundquist, I. (1984). Effects of processing on starch availability in vitro and in vivo; II drum drying of wheat flour. *Journal of Cereal Science, (2)*, 165-178.

Brouns, F., Hemery, Y., Price, R., & Anson, N. M. (2012). Wheat aleurone: separation, composition, health aspects, and potential food use. *Critical Reviews in Food Science and Nutrition, 52(6)*, 553-568.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J., & Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods, 13(7)*, 581.

Chassard, C., Delmas, E., Robert, C., Lawson, P. A., & Bernalier-Donadille, A. (2012). Ruminococcus champanellensis sp. nov., a cellulose-degrading bacterium from human gut microbiota. *International Journal of Systematic and Evolutionary Microbiology, 62(1)*, 138-143.

Cleemput, G., Booij, C., Hessing, M., Gruppen, H., & Dekcour, J. A. (1997). Solubilisation and changes in molecular weight distribution of arabinoxylans and protein in wheat flours during bread-making, and the effects of endogenous arabinoxylan hydrolyzing enzymes. *Journal of Cereal Science, 26(1)*, 55-66.

Corsetti, A., Gobbetti, M., Balestrieri, F., Paoletti, F., Russi, L., & Rossi, J. (1998). Sourdough lactic acid bacteria effects on bread firmness and stalin. *Journal of Food Science, 63(2)*, 347-351.

Damen, B., Verspreet, J., Pollet, A., Broekaert, W. F., Delcour, J. A., & Courtin, C. M. (2011). Prebiotic effects and intestinal fermentation of cereal arabinoxylans and arabinoxylan oligosaccharides in rats depend strongly on their structural properties and joint presence. *Molecular Nutrition & Food Research, 55(12)*, 1862-1874.

Daniel, M., Wisker, E., Rave, G., & Feldheim, W. (1997). Fermentation in human subjects of nonstarch polysaccharides in mixed diets, but not in a barley fiber concentrate, could be predicted by in vitro fermentation using human fecal inocula. *Journal of Nutrition, 127(10)*, 1981-1988.

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., . . . Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology, 72(7)*, 5069-5072.

De Vos, P., Garrity, G. M., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., . . . Whitman, W. B. (2009). *Bergey's manual of systematic bacteriology: volume three the firmicutes.* NY: Springer.

Den Besten, G., Van Eunen, K., Groen, A. K., Venem, A. K., Venema, K., Reijngoud, D. J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research, 54(9),* 2325-2340.

Doblado-Maldonado, A. F., Flores, R. A., & Rose, D. J. (2013). Low moisture milling of wheat for quality testing of wholegrain flour. *Journal of Cereal Science 58*, 420-423.

Grootaert, C., Van den Abbeele, P., Marzorati, M., Broekaert, W. F., Courtin, C. M., Delcour, J. A., . . . Van de Wiele, T. (2009). Comparison of prebiotic effects of arabinoxylan oligosaccharides and inulin in a simulator of the human intestinal microbial ecosystem. *FEMS Microbiology Ecology, 69(2)*, 231-242.

Hartzell, A. L., Maldonado-Gómez, M. X., Hutkins, R. W., & Rose, D. J. (2013). Synthesis and in vitro digestion and fermentation of acylated inulin. *Bioactive Carbohydrates and Dietary Fibre 1.1*, 81-88.

Holscher, H. D. (2017). Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes, 8(2)*, 172-184.

Johansson, D. P., Vázquez Gutiérrez, J. L., Landberg, R., Alminger, M., & Langton, M. (2018). Impact of food processing on rye product properties and their in vitro digestion. *European Journal of Nutrition 57*, 1651–1666.

Kahlon, T. S., Berrios, D. J., Smith, G. E., & Pan, J. L. (2006). Extrusion conditions modify hypocholesterolemic properties of wheat bran fed to hamsters. *Cereal Chemistry*, 152-156.

Karppinen, S., Kiiliäinen, K., Liukkonen, K., Forssell, P., & Pouranen, K. (2001). Extraction and in vitro fermentation of rye bran fractions. *Journal of Cereal Science, 34(3)*, 269-278.

Kasubuchi, M., Hasegawa, S., Hiramatsu, T., Ichimura, A., & Kimura, I. (2015). Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. *Nutrients, 7(4)*, 2839-2849.

Koropatkin, N. M., Cameron, E. A., & Martens, E. C. (2012). How glycan metabolism shapes the human gut microbiota. *Nature Reviews Microbiology, 10(5)*, 323.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and Environmental Microbiology*, 5112-5120.

Macfarlane, S., & Macfarlane, G. T. (2003). Regulation of short-chain fatty acid production. *Proceedings of the Nutrition Society, 62(1)*, 67-72.

Manini, F., Brasca, M., Plumed-Ferrer, C., Morandi, S., Erba, D., & Casiraghi, M. C. (2014). Study of the chemical changes and evolution of microbiota during sourdoughlike fermentation of wheat bran. *Cereal Chemistry, 91(4)*, 342-349.

Minervini, F., De Angelis, M., Di Cagno, R., & Gobbetti, M. (2014). Ecological parameters influencing microbial diversity and stability of traditional sourdough. *International Journal of Food Microbiology, 171*, 136-146.

Njongmeta, N. A., Hall, P. A., Ledenbach, L., & Flowers, R. S. (2015). 19. Acid-Producing Microorganisms. In Y. Salfinger, & M. L. Tortorello (Eds.), C*ompendium of methods for the microbiological examination of foods* (5 ed.).

Nordlund, E., Katina, K., Aura, A. M., & Poutanen, K. (2013). Changes in bran structure by bioprocessing with enzymes and yeast modifies the in vitro digestibility and fermentability of bran protein and dietary fibre complex. *Journal of Cereal Science,58(1)*, 200-208.

Nyman, M., Asp, N. G., Cummings, J., & Wiggins, H., J., & Wiggins, H. (1986). Fermentation of dietary fibre in the intestinal tract: comparison between man and rat. *British Journal of Nutrition, 55(3)*, 487-496.

Puertollano, E., Kolida, S., & Yaqoob, P. (2014). Biological significance of short-chain fatty acid metabolism by the intestinal microbiome. *Current Opinion in Clinical Nutrition & Metabolic Care, 17(2)*, 139-144.

Ralet, M., Thibault, J., & Della Valle, G. (1990). Influence of extrusion-cooking on the physico-chemical properties of wheat bran. *Journal of Cereal Science*, 249-259.

Regmi, P. R., Van Kempen, T. A., Matte, J. J., & Zijlstra, R. T. (2011). Starch with high amylose and low in vitro digestibility increases short-chain fatty acid absorption, reduces peak insulin secretion, and modulates incretin secretion in pigs. *The ISME Journal 6(8)*, 398-405.

Reicks, M., Jonnalagadda, S., Albertson, A. M., & Joshi, N. (2014). Total dietary fiber intakes in the US population are related to whole grain consumption: results from the National Health and Nutrition Examination Survey 2009 to 2010. *Nutrition Research, 34(3)*, 226-234.

Robin, F., Dubois, C., Pineau, N., Schuchmann, H., & Palzer, S. (2011). Expansion mechanism of extruded foams supplemented with wheat bran. *Journal of Food Engineering, 107*, 80-89.

Salmenkallio-Marttila, M., Katina, K., & Autio, K. (2001). Effects of bran fermentation on quality and microstructure of high-fiber wheat bread. *Cereal Chemistry*, 429-435.

Salyers, A. A., West, S. E., Vercellotti, J. R., & Wilkins, T. D. (1977). Fermentation of mucins and plant polysaccharides by anaerobic bacteria from the human colon. *Applied and Environmental Microbiology, 34(5)*, 529-533.

Sonnenburg, E. D., & Sonnenburg, J. L. (2014). Starving our microbial self: The deleterious consequences of a diet deficient in microbiota- accessible carbohydrates. *Cell Metabolism, 20(5)*, 779-786.

Tournas, V., Stack, M. E., Mislivec, P. B., Koch, H. A., & Bandler, R. (2013). Yeasts, Molds and Mycotoxins. In *Bacteriological analytical manual.*

Umu, Ö. C., Frank, J. A., Fangel, J. U., Oostindjer, M., Da Silva, C. S., Bolhuis, E. J., . . . Diep, D. B. (2015). Resistant starch diet induces change in the swine microbiome and a predominance of beneficial bacterial populations. *Microbiome, 3(1)*, 16.

Wang, W. M., Klopfenstein, C. F., & Ponte, J. (1993). Effects of twin-screw extrusion on the physical properties of dietary fiber and other components of whole wheat and wheat bran and on the baking quality of the wheat bran. *Cereal Chemistry*, 707-711.

Wisker, E., Daniel, M., Rave, G., & Feldheim, W. (1998). Fermentation of non-starch polysaccharides in mixed diets and single fibre sources: comparative studies in human subjects and in vitro. *British Journal of Nutrition, 80(3)*, 253-261.

Wisker, E., Knudsen, K. E., Daniel, M., Eggum, B. O., & Feldheim, W. (1997). Energy values of non-starch polysaccharides: comparative studies in humans and rats. *The Journal of Nutrition, 127(1)*, 108-116.

Yang, J., Keshavarzian, A., & Rose, D. J. (2013). Impact of dietary fiber fermentation from cereal grains on metabolite production by the fecal microbiota from normal weight and obese individuals. *Journal of Medicinal Food, 16(9)*, 862-867.

Ze, X., Duncan, S. H., Louis, P., & Flint, H. J. (2012). Ruminococcus bromii is a keystone species for the degradation of resistant starch in the human colon. *The ISME journal, 6(8)*, 1535.

90

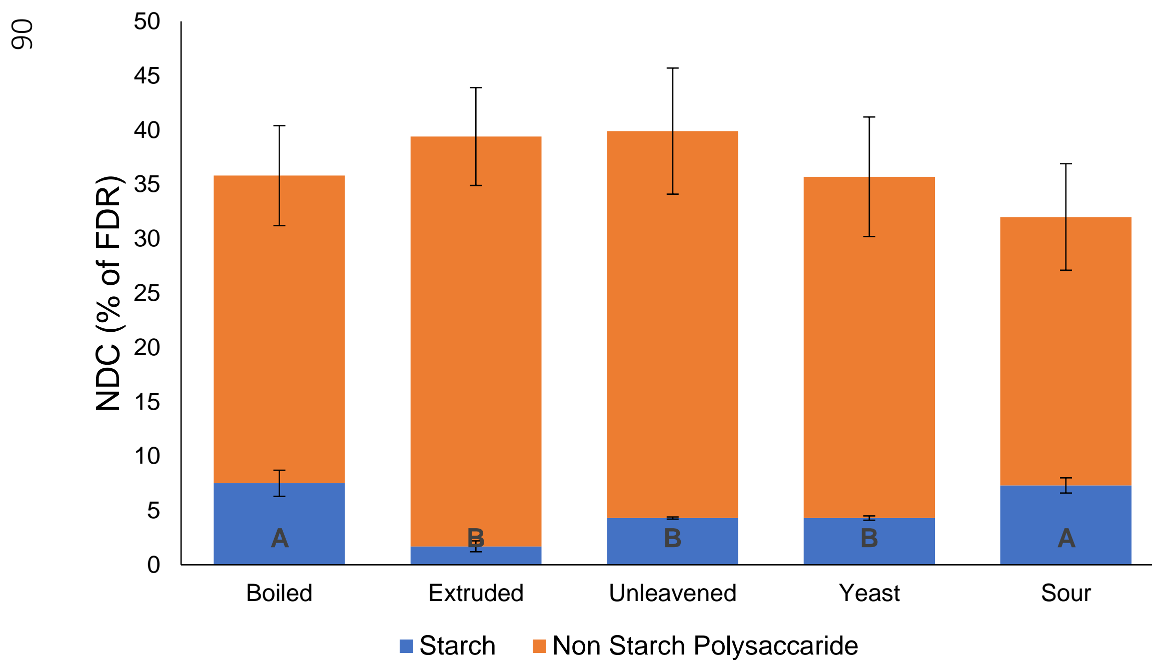

Figure 3.1. NDC (% of FDR) non-digestible carbohydrates (%of freeze dried retentate) in digested and dialyzed processed grains; error bars show standard error; different letters denote a significant difference ($p<0.05$).

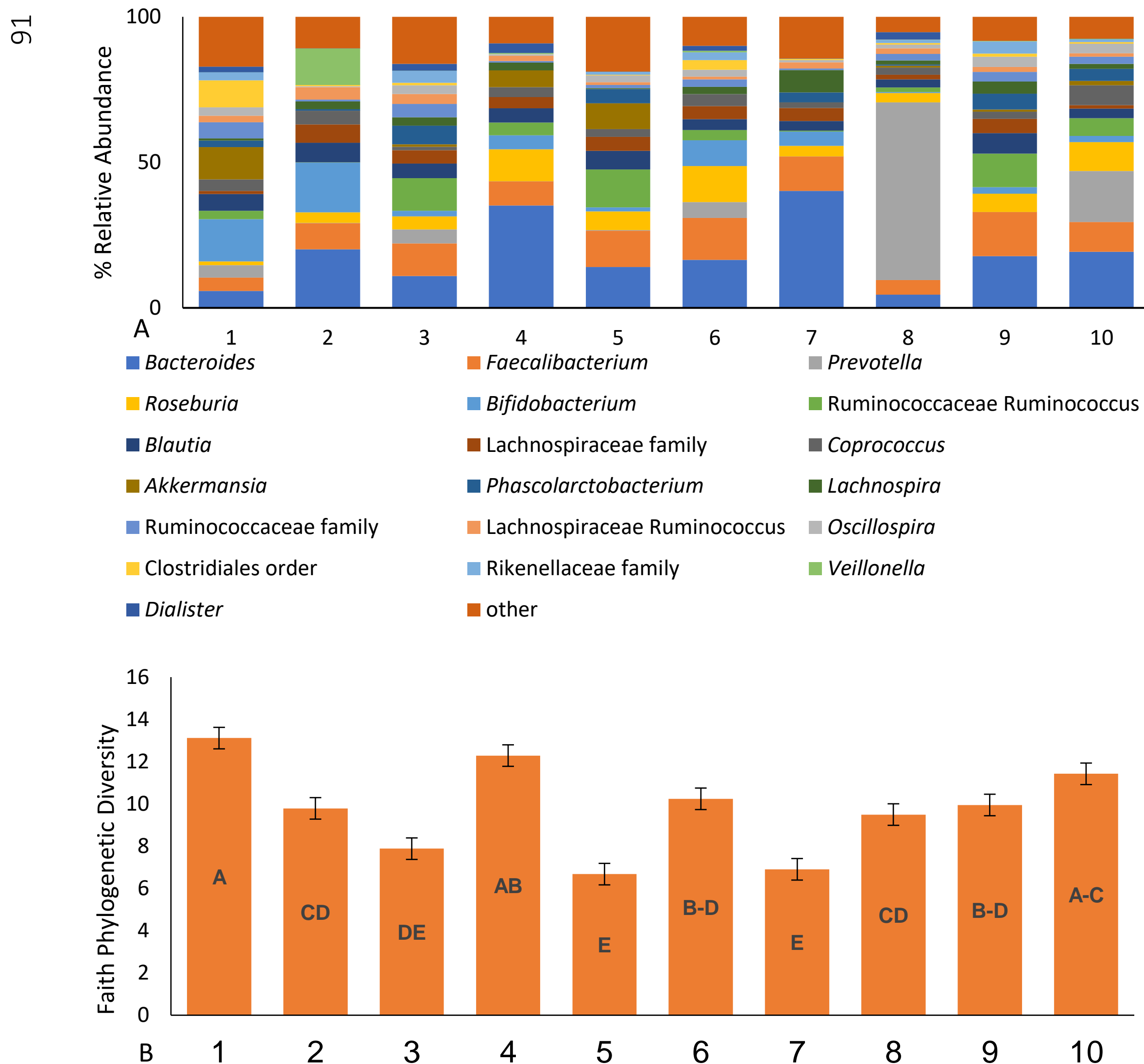

Figure 3.2. Characteristics of subject fecal samples. Genera relative abundance in fecal samples (A) and differences in α- diversity with Faith Phylogenic Diversity (B).

92

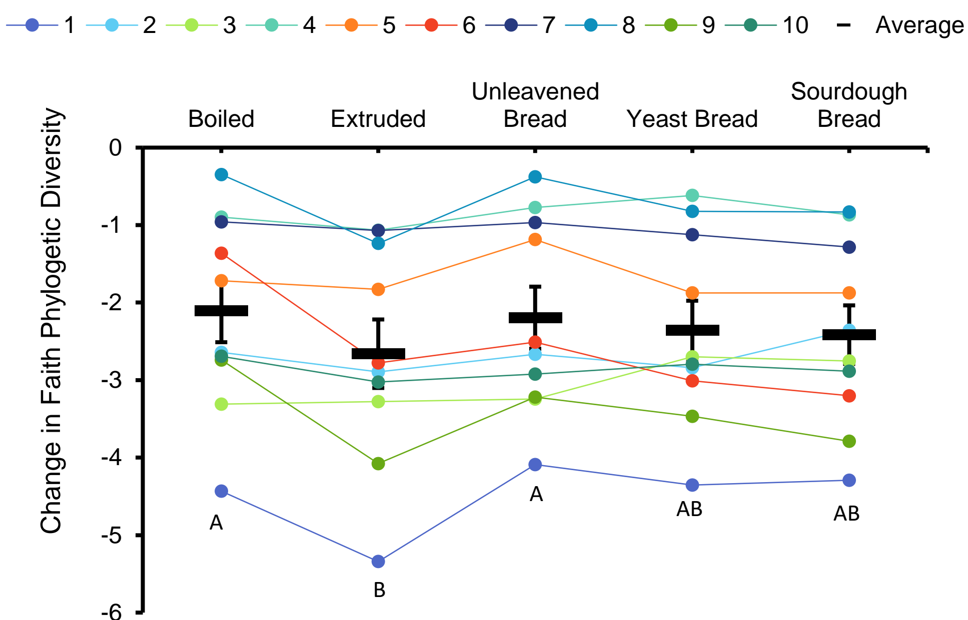

Figure 3.3. Change in microbiota α-diversity during *in vitro* fermentation (12 h - 0 h) of processed wheat samples by subject. Error bars show standard error across all 10 subjects; different letters denote significant differences among overall means ($p<0.05$).

93

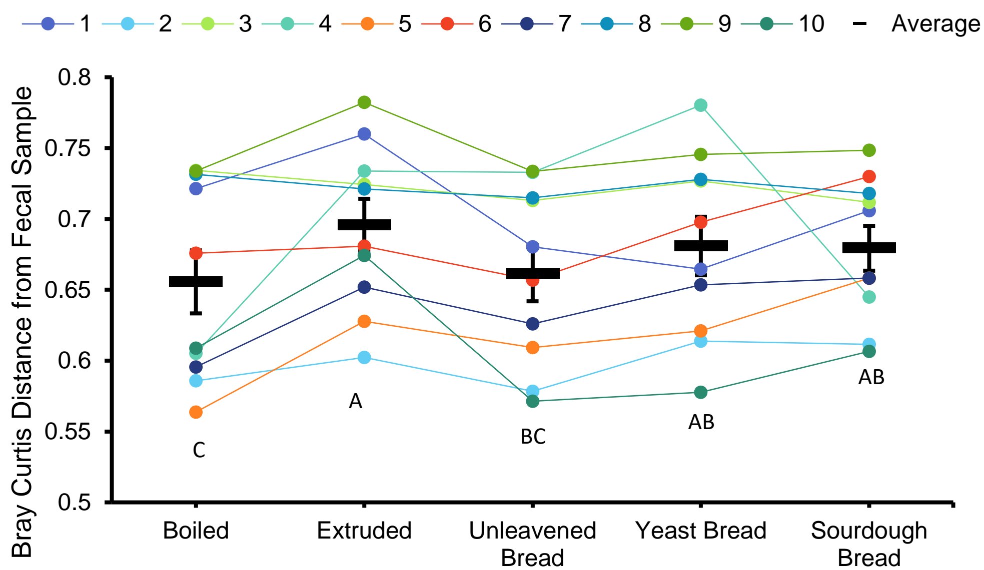

Figure 3.4. Bray Curtis distance from fecal sample after 12 hours of *in vitro* fecal fermentation by subject and process; error bars show standard error; different letters denote significant differences among overall means ($p<0.05$).

94

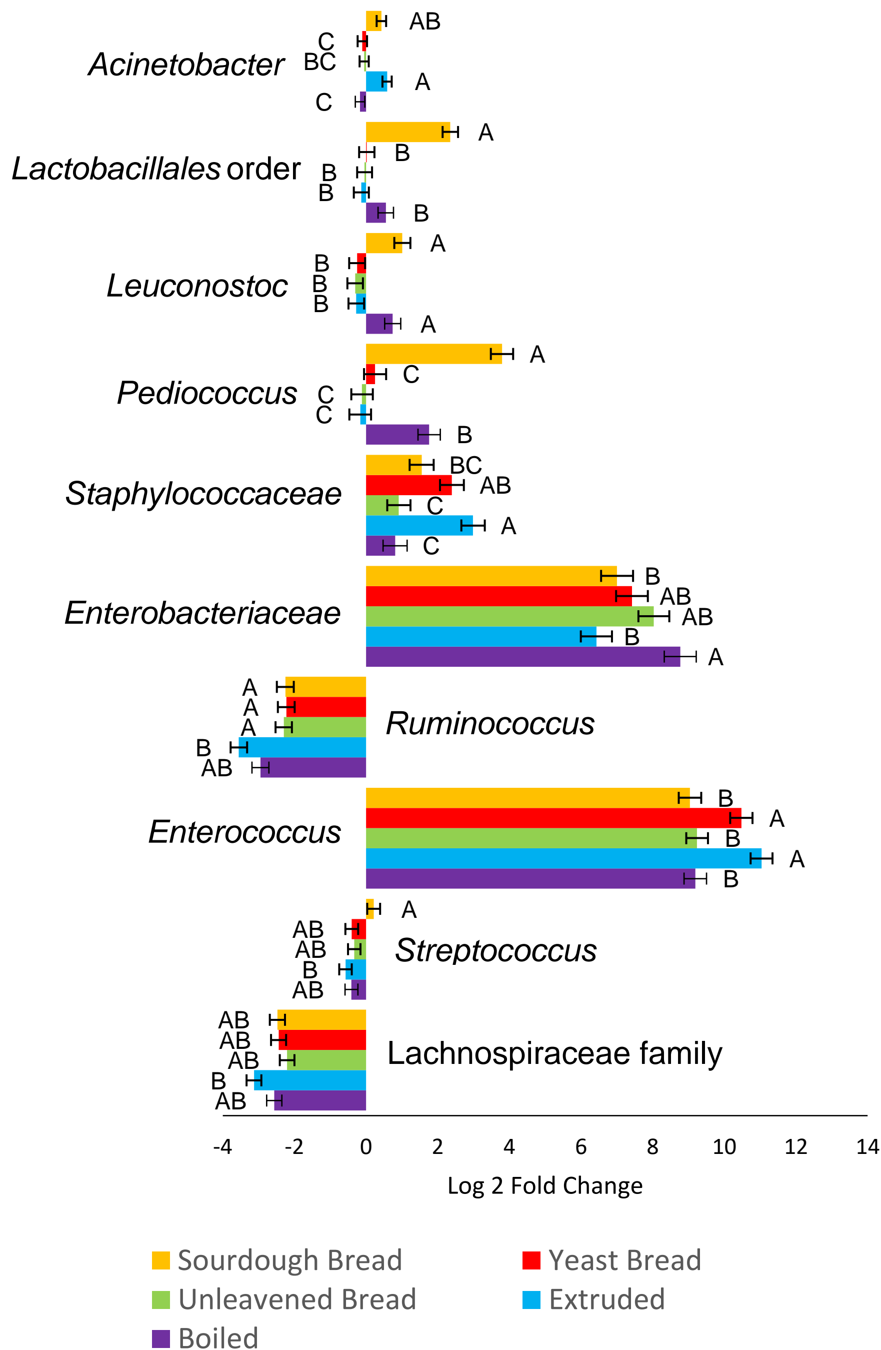

Figure 3.5. Log fold change of relative abundance of genera that significantly varied by processing method after 12 hours of *in vitro* fermentation by processing method; different letters denote a significant difference ($p<0.05$).

95

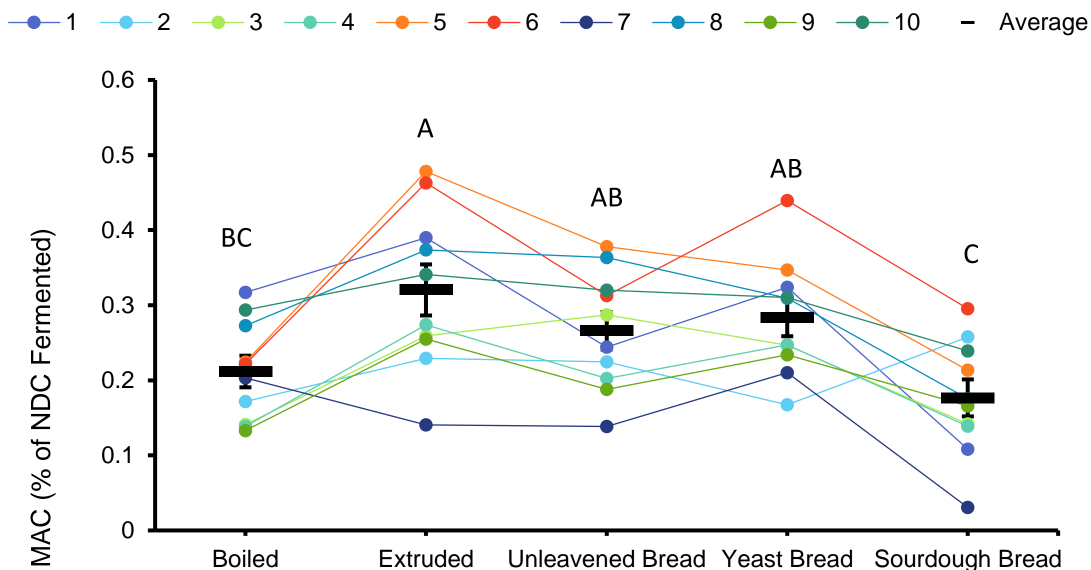

Figure 3.6. Percentage of microbiota accessible carbohydrates (MAC) after 12 hours of *in vitro* human fecal fermentation of whole wheat flour by subject and by processing method compared to after 0h of fermentation; error bars show standard error; different letters denote significant differences among overall means ($p<0.05$).

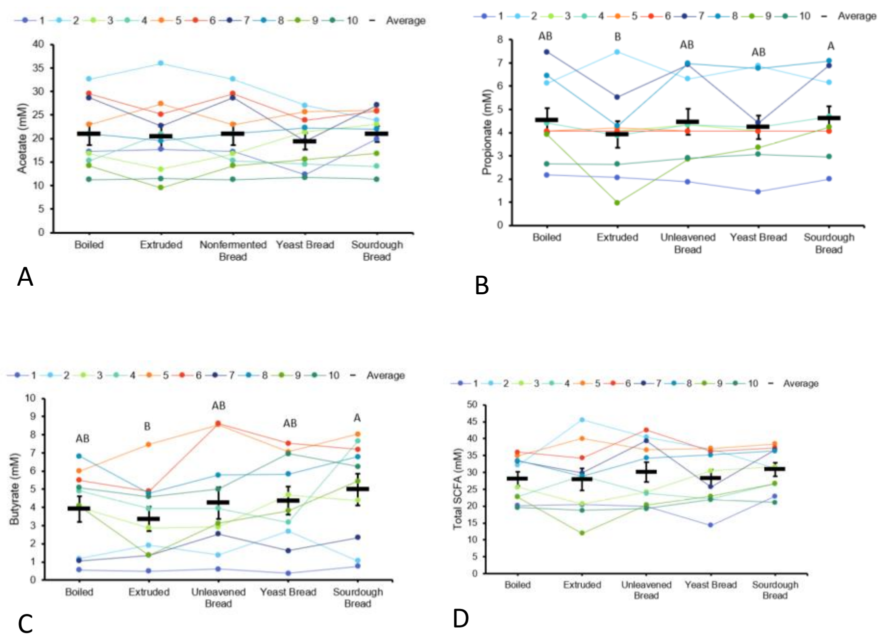

Figure 3.7. Short chain fatty acid (SCFA) production after 12 h of *in vitro* human fecal fermentation of whole wheat flour by subject and process for (A) acetate (B) propionate (C) butyrate and (D) total SCFA; error bars show standard error; different letters denote significant differences among overall means ($p<0.05$).

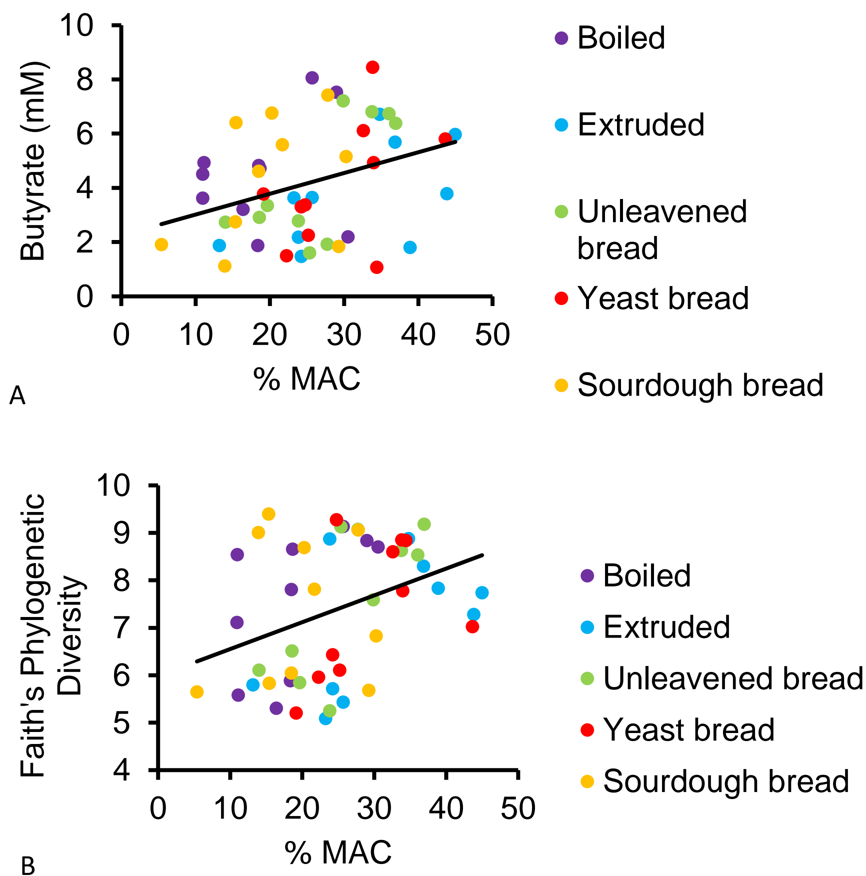

Figure 3.8. (A) significant partial correlations with subject and processing method as partial variables between Butyrate and % carbohydrates fermented (p 0.0174) (B) correlation between Faith's Phylogenetic Diversity and % carbohydrates fermented (p 0.0109).

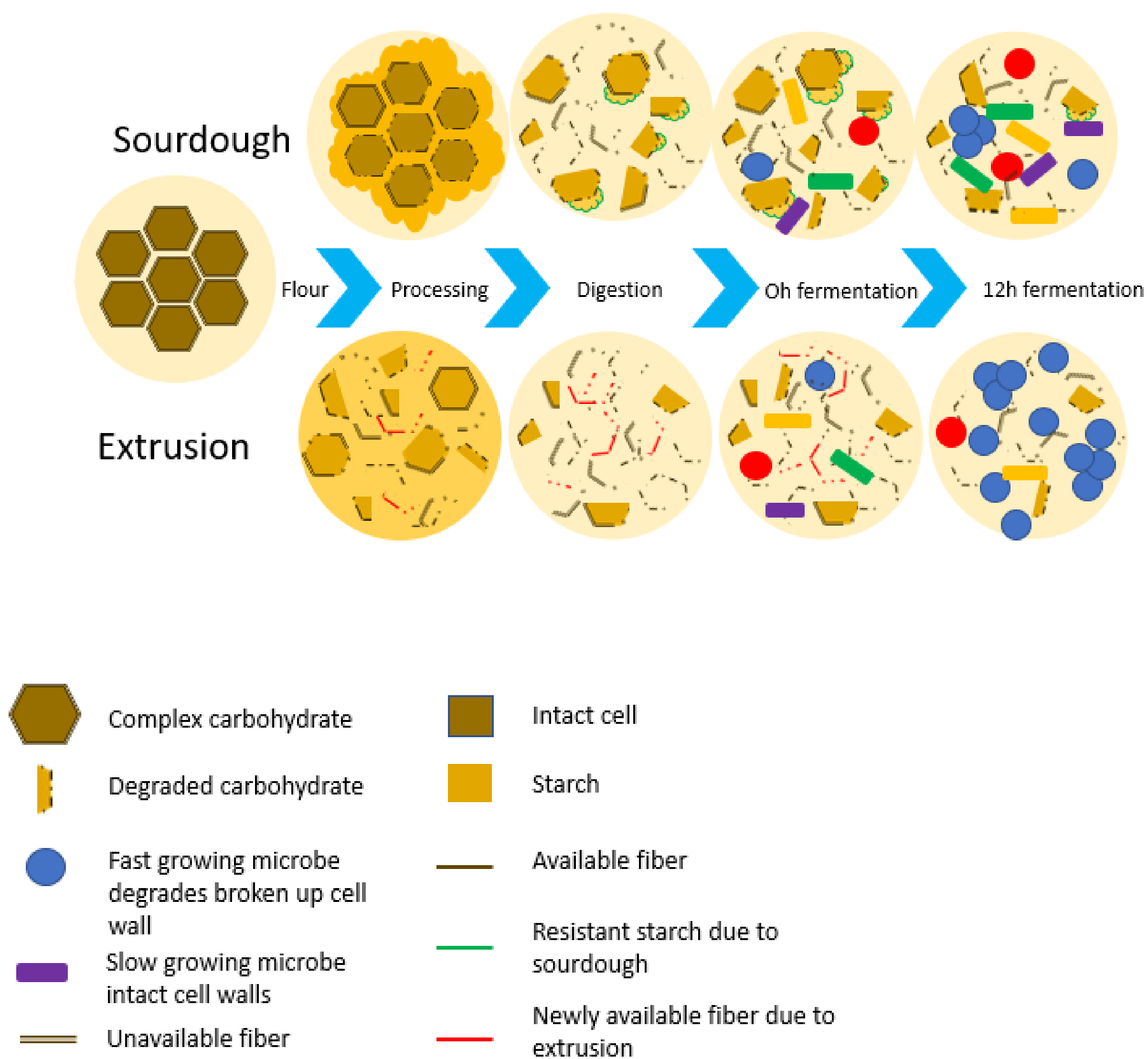

Figure 3.9. Illustration of hypothesis for transformation of carbohydrates though processing, digestion and fermentation.

.

CHAPTER 4 EFFECT OF WHOLE GRAIN TYPE AND PROCESSING METHOD ON HOST HEALTH IN CONTRASTING MICROBIOMES

4.1. ABSTRACT

Whole grains and dietary fibers have been shown to influence microbiota composition and reduce the risk of western disease. Germ free mice were humanized with one of two contrasting microbiomes. The microbiomes were collected from human subjects that habitually consumed high and low levels of whole grains and dietary fiber (HiFi and LoFi, respectively) and varied in diversity and composition. The mice were fed isocaloric Western diets containing equivalent concentrations of dietary fiber from cellulose (control), boiled whole wheat, extruded whole wheat, or extruded brown rice. Mice inoculated with the LoFi microbiome showed significant increases in diversity due to whole grains consumption and the whole grain type and processing method had differential effects. The HiFi microbiome was initially more diverse and was not significantly affected by treatment. Mice inoculated with the HiFi microbiome but not the LoFi microbiota showed significant differences in body fat and body weight gain across dietary treatments, where boiled wheat showed reductions compared to extruded wheat. All mice had reduced glucose tolerance when consuming extruded whole grains compared to the western control. Additionally, extrusion trended towards increased digestibility of the dietary carbohydrates in the diet, which suggests that extrusion increased glycemic load. Thus, both digestion and fermentation are important factors in effect of whole grains and food processing on host health

Keywords: gut microbiome, extrusion, fermentable carbohydrates, whole wheat, brown rice,

4.2. INTRODUCTION

Whole grain intake has been shown to be associated with a reduced risk of type 2 diabetes, cardiovascular disease, cancer, and obesity (Cho, Qi, Fahey, & Klurfeld, 2013). These diseases continue to be critical health concerns in Western countries (Benjamin, Muntner, & Bittencourt, 2019). (Hales, Fryar, Carroll, Freedman, & Ogden, 2018). Whole grains and dietary fibers have been shown to influence microbiota composition. De Filippo (2010) observed that children in rural Africa and South America that ate more carbohydrates and dietary fiber had increased microbiota diversity and higher abundances of *Xylanibacter* and *Prevotella* compared with European children who had low fiber, Western diets. In a 2-arm, 12-week human intervention study, *Bacteroides* and *Prevotella* were shown to increase in abundance when subjects were fed whole grain/fiber-rich rye breads (Lappi, et al., 2013). In another study, whole grain maize was shown to increase *Bifidobacterium* (Carvalho-Wells, et al., 2010) compared to pre-intervention baseline. Whole grain barley and brown rice led to increased diversity and higher abundance of *Blautia* and *Roseburia* and lower abundance of *Bacteroides* (Martínez, et al., 2013).

Westerners do not consume adequate levels of dietary fiber and whole grains despite decades of recommendations and public health initiatives (Albertson, Reicks, Joshi, & Gugger, 2015). Processing has been proposed as a way to manipulate the carbohydrates in food to be more metabolically available to the gut microbiome to influence the health benefits of whole grains. For instance, extrusion is a form of processing that uses pressure, mechanical shear and higher temperatures (>120 °C) to process grains into foods such as breakfast cereals and crispy snacks. The extrusion process results in gelatinization and partial depolymerization of starch together with fragmentation of cell wall structures (Robin, Dubois, Pineau, Schuchmann, &

Palzer, 2011). These cell wall structures contain most of the non-digestible carbohydrates (NDC) in whole grains. In extruded whole grain rye, small, fragmented cell walls and aleurone layers containing β-glucan and arabinoxylan were visualized embedded within a continuous, amorphous starch (Johansson, Vázquez Gutiérrez, Landberg, Alminger, & Langton, 2018). Extrusion has been shown to convert insoluble fiber to soluble fiber, which is generally considered more fermentable, in different cereals (Kahlon, Berrios, Smith, & Pan, 2006; Ralet, Thibault, & Della Valle, 1990; Robin, Dubois, Pineau, Schuchmann, & Palzer, 2011; Wang, Klopfenstein, & Ponte, 1993).

In contrast, boiling is a minimal form of grain processing that involves lower temperatures (100 °C), atmospheric pressure, minimal shear, and an abundance of water. During boiling, water swells the starch granules resulting in the leaching of amylose, starch gelatinization, and an amorphous structure. In whole grain rye flour, boiling has been shown to have minimal influences on the NDC, β-glucan and arabinoxylan. Boiling did result in swollen cell walls, but the cell walls appeared to remain intact (Johansson, Vázquez Gutiérrez, Landberg, Alminger, & Langton, 2018). Boiling of whole wheat flour or wheat bran has commonly been used as a control process for whole grain experiments (Arcila, Weier, & Rose, 2015; Bjorck, Asp, & Lundquist, 1984; Bjorck, et al., 1984).

Changes in NDC structure that happen as a result of processing have been shown to change the fermentability of carbohydrates by the microbiota. Arcila, Weier, & Rose (2015) studied the *in vitro* fermentability of extruded and boiled wheat bran. Extrusion resulted in a 114% increase in fermentable NDC and a significant increase in short chain fatty acids (SCFA) production compared to boiled wheat bran. Extruded wheat bran was more available to the gut microbes in rats than native bran based on the neutral detergent fiber. Furthermore, the cell walls

of the extruded bran were more disrupted after passage (Aoe, Nakaoka, Tama, Ohta, & Ayan, 1990).

Differences in dietary fiber composition has been shown to change their fermentation by the gut microbes and microbial composition. Dietary fiber from different botanical sources, such as fruits and cereals, vary in fermentability and SCFA production *in vitro* (Casterline, Oles, & Ku, 1997). Yang (2013), studied five cereal grains, wheat rye, maize, rice, and oats, with *in vitro* digestion and human fecal fermentation. The studied grains varied in SCFA production where rye had highest butyrate production and lowest propionate production (Yang, Keshavarzian, & Rose, 2013). Different fibers have been shown to be favored by different microbes, such as xylooligosaccharides favoring increased growth of *Bifidobacterium* species. Moreover, SCFA production has been shown to favor different levels of SCFA production, for instance beta-glucan favoring the propionate production (Carlson, Erickson, Hess, Gould, & Slavin, 2017; Poeker, et al., 2018).

There is clear evidence that processing, fiber source, and the microbiome influence the health benefits of whole grains. However, understanding of these elements, how they interact, and how they influence host health has not been previously studied to our knowledge. Thus, in this study two whole grains, brown rice and whole wheat, and two processing methods, boiling and extrusion, were studied on humanized mice from two subjects with different microbiome and dietary characteristics in an obesity model. Based on previous studies, it is hypothesized that whole grains will increase diversity and improve the health of the humanized mice. Furthermore, different whole grain types will have a different response due to differences in fiber present. Additionally, it is hypothesized that processing changes the fermentability of NDC and will

influence host health. Furthermore, it is expected that the differences seen will be host dependent and determined by the habitual diet of subjects.

4.3. MATERIALS AND METHODS

4.3.1. Whole grain processing

Hard red wheat was obtained from Bay State Milling (Quincy, MA). Wheat kernels were dried at 40°C for 16 h before milling according to Doblado-Maldonado et al. (2013). All milled fractions obtained from the mill were mixed together to obtain whole wheat flour. Brown Rice flour was obtained from Bay State Milling (Quincy, MA).

For boiled wheat, water (530 g) was brought to a boil on a gas range. Once boiling, the heat was reduced to a simmer and 1 g of salt and 100 g whole wheat flour were added under rapid manual stirring for 5 min. This process was repeated for 30 batches. The boiled wheat porridge was then cooled to room temperature before being frozen at -80°C and then freeze dried. The freeze dried product was then coarsely ground and milled with a pin mill twice to create a flour (Buhler, Uzwil, Switzerland). All batches were mixed together to create a consistent flour.

For extrusion, whole grain flour (wheat or brown rice) (1 kg) was mixed for 10 min with 1% salt (w/w) and water to adjust to 20% moisture content (dry weight basis) in a stand mixer (c-100, Hobart, Troy, OH). The mixtures were equilibrated in closed containers at 4 °C overnight. The moisture-adjusted whole grain flours were then extruded using a benchtop-scale twin-screw extruder equipped with a single stage mixing zone and a 3 mm outlet die at 250 rpm, 3:1 compression ratio, and a 20:1 L/D ratio (CW Brabender Instruments, NJ, USA). The extruder was operated by a direct current drive unit (Intelli-Torque, Pastic Corder Lab-station, C.W. Brabender) with a 5.6 kW motor. The flour was fed into the extruder using a volumetric feeder

(FW 40 Plus, C. W. Brabender) set at a constant flow rate of ~50 g/min. Barrel temperatures were set at 60 °C (zone 1; inlet), 70 °C (zone 2), 120 °C (zone 3) , and 120 °C (zone 4; die assembly). Samples were collected from the extruder die once steady state had been reached. Extrudates were then dried in a convection oven overnight at 70 °C to complete the extrusion processing. Although the product contained very low moisture at this stage (~3%), the dried extrudates were subject to freeze drying (3600, Freeze Dry Co., Pine River, MN) because the boiled wheat was freeze dried following processing. The freeze dried material was then milled into flour with a pin mill twice (mill). Protein (AACC International, 2018), fat (AACC International, 2018), total starch (Megazyme), moisture (AACC International, 2018) and integrated total dietary fiber (Megazyme) were measured on the processed flours.

4.3.2. Experimental diets

The diets used in the study were prepared by a commercial provider (Research Diets, New Brunswick, NJ USA) and included: 1) control diet (C); 2) western diet (WD); 3) western diet with boiled whole wheat (WD + BWW); 4) western diet with extruded whole wheat (WD + EWW); and 5) western diet with extruded brown rice (WD + EBR). All western diets were isocaloric and contained equivalent concentrations of dietary fiber (Table 4.1). The level of whole grains included in the diet were equivalent to the recommendations in the 2015 USDA dietary guidelines of 3 servings a day (48g/2000 kcal). The C diet was included to confirm metabolic aberrations induced by the WD diet.

4.3.3. Fecal samples

Two microbiomes were selected from 10 microbiomes analyzed in a previous *in vitro* study (Chapter 3). The selection was based on contrasting microbiota composition, capacity to

ferment non-digestible carbohydrates, and butyrate production. The two replication fecal samples were mixed together on the day of humanization and stored on ice until use.

4.3.4.Dietary patterns of fecal donors

The selected subjects completed a 24h diet recall survey for 3 days in a 2 week period. Dietary intake data were collected and analyzed using the Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool (National Cancer Institute, 2018). The average grams of dietary fiber, serving of whole grains, and servings of refined grains were expressed per 1000 kcal consumed.

4.3.5.Mouse Experiment

The Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln approved all procedures involving animals (Project ID: 1700). The study was conducted in two arms due to the number of mice being studied. Germ free C57BL/6 male mice were humanized with one of two microbiomes (for each arm) from human feces selected at an average age of 7 weeks (±11 d). Animals were maintained in an environment with a 14 h light/10h dark cycle and controlled temperature and humidity. Mice were maintained on autoclaved bedding, and fed autoclaved water. While acclimating to their environment for two weeks, all mice were fed a regular autoclaved chow diet (Purina Lab Diets, St. Louis, MO). Mice were randomly assigned to one of the five dietary treatments (n = 10-11 mice/group), and housed 2 to 3 in individually ventilated cages, before starting the dietary interventions the next day, which were provided for 12 weeks. Diet replacement and recording of both food intake and body weights were performed weekly. Body composition was measured bi-weekly (Minispec LF50, Bruker, Billerica, MA).

Two mice died during the course of the study from reasons unrelated to the study, due to stress from body composition analysis.

Feces were collected from individual mice four times during the experiment (weeks 0, 1, 7 and 11) and stored at -80°C until further analysis. After 12 weeks of experimental diet feeding, mice were euthanized via CO2 asphyxiation. Blood was harvested by cardiac puncture and plasma was collected by centrifugation at 13,000 × g for 3 min at 4°C. Adipose tissues, liver tissue, spleen tissue, full ceca, and empty cecal tissues were weighed at the time of collection. All biological samples were snap frozen in liquid nitrogen and then stored at -80°C until analysis.

4.3.6.Intraperitoneal glucose tolerance test

An intraperitoneal glucose tolerance test (ipGTT) was performed after 11 weeks of dietary intervention. Mice were fasted in clean cages for 6 h prior to the test. Thirty minutes prior to the test, blood was collected from the tip of the tail to measure fasting glucose concentration using a glucose meter (ACCU-CHEK, Aviva Plus system, Indianapolis, IN, USA). An aliquot of blood was also saved. At time zero, a glucose solution (20 g/100 mL) was injected into the peritoneal cavity (1 g glucose/kg body weight) (De Vadder, et al., 2014). Blood glucose was then measured at 0, 30, 60, 90, 120 and 150 min after glucose injection.

4.3.7.Cecal short chain fatty acids

SCFA were extracted from the cecum collected at necropsy. SCFA were measured by gas chromatography as described (Hartzell, Maldonado-Gómez, Hutkins, & Rose, 2013) with 7 mM 2-ethylbutyric acid the internal standard.

4.3.8.Characterization of the fecal microbiota composition

The fecal samples were analyzed for differences in the microbiome using 16 sRNA sequencing. A kit from Biovet (BioSprint 96 One-For-All Vet Kit, Quebec Canada) was used. Microbiome characterization was performed by amplicon sequencing of the V4 region of the 16S rRNA gene on the Illumina MiSeq platform using the MiSeq Reagent kit v2 (2 X 250 bp) following the protocol of Kozich et al. (2013). Sequences were demultiplexed and barcodes were removed prior to sequence analysis with the QIIME 2 platform (Bolyen, et al., 2019). Sequence quality control, trimming and denoising was performed with DADA2 (Callahan, et al., 2016). Forward and reverse reads were trimmed to maintain sequence qualities above a phred score of 30. Taxonomy was assigned using the Greengenes database (DeSantis, et al., 2006). Reads were rarefied to a sampling depth of 10,000 prior to analysis.

Diversity of each fermented and fecal sample was determined using Qiime 2. Change in diversity was calculated by the difference of diversity after 1 and 11 weeks of dietary treatment minus diversity before dietary intervention. Subject's fecal microbiome characteristics were determined using Microbiome analyst platform (https://www.microbiomeanalyst.ca). Through this platform, taxa with <2 reads, <10% in prevalence of sequencing read counts, and <10% variance based on the inner quartile range were removed leaving 98 taxa. The data was rarified to the minimum library size and scaled using total sum squaring. Heat tree analysis was conducted to depict taxonomic differences between microbial communities to show which taxa are more abundant for each person using median abundance and non-parametric Wilcoxon Rank Sum test (p cutoff of 0.05) (Foster, Sharpton, & Grünwald, 2017). Linear Discriminant Analysis Effect Size (LEfSe), which uses non-parametric factorial Kruskal-Wallis (KW) rank-sum test and Linear Discriminant Analysis to detect features with significant differential abundance and

the effect size of those features, was used to determine different defining genera in treatment groups (Segata, et al., 2011).

4.3.9.In vitro digestibility of diets

In vitro digestibility of the diets was determined following the protocol of (Mkandawire, et al., 2013). Briefly, the diets were ground into flour with a food processor and 1 g was dispersed in 2 mL of water in a 15-mL tube. Then, 4 mL of 3.6% w/v pepsin (3802 U/mg protein; Sigma, St. Louis, MO, USA) containing 0.5% w/v guar gum (TIC Gums, Belcamp, MD, USA) in 50 mM hydrochloric acid was added to each tube, vortex mixed, and incubated for 30 min at 37 °C. Subsequently, six glass beads (6-mm diameter) were added to the tubes followed by 2 mL of sodium acetate buffer (0.5 M, pH 5.2, containing 5 mM calcium chloride). Starch digestion was initiated by adding 2.05 mL of an enzyme mixture prepared by dispersing pancreatin (P-7545; SigmaAldrich) in water (15% w/v) on a magnetic stirrer for 10 min and then centrifuging for 10 min at 4000g, whereupon 20 µL of amyloglucosidase (3260 U/mL; Megazyme) and 13 mg of invertase (10600 U/g; S-75136; Fisher Science Education, Hanover, IL, USA) were added per mL of recovered pancreatin supernatant. Starch was digested over 2 h at 37 °C with horizontal shaking at 160 rpm. After exactly 20 and 120 min of digestion, 50 µL of slurry was removed from the tube and mixed with 0.95 mL of 90% aqueous ethanol. The sampled mixtures were kept refrigerated overnight and then centrifuged at 8161g for 5 min. The glucose content was measured in the supernatant using the glucose oxidase-peroxidase method (Megazyme) and converted to starch by multiplying by a factor of 0.9. Results were expressed as rapidly available glucose (RAG), defined as the glucose present in the first 20 min of *in vitro* starch digestion; slowly available glucose (SAG) defined as the difference of glucose between 20 and 120 min; and resistant starch (RS), the quantity of starch that was not converted to glucose

after 120 min, which was determined as the difference between total starch and the starch hydrolyzed at 120 min (Mkandawire, et al., 2013).

4.3.10.Statistical analysis

Statistics for the two microbiomes were conducted separately using analysis of variance (ANOVA) using dietary treatment as a factor (SAS version 9.4 proc glm). When the ANOVA was significant, Duncan's Multiple Range Test was used to compare differences between the treatments. For LEFSe, an FDR adjusted p value of <0.05 was used.

4.4. RESULTS AND DISCUSSION

4.4.1.Fecal microbiome characteristics and dietary patterns

The microbiome from subjects A and B were selected from 10 microbiomes from a previous study (Chapter 3, subjects 10 and 2 respectively). The two microbiomes were selected based on their varying capacity to utilize non-digestible carbohydrates (Figure 3.6), produce butyrate (Figure 3.7), and their large differences in composition (Figure 3.2). Microbiome A had higher microbiota accessible carbohydrates (MAC) in the previous experiment and higher butyrate levels. Microbiome A was characterized by genera including *Rumminococcus* (Ruminococcaceae), *Dorea, Lactobacillus, Prevotella,* and *Akkermansia* (Figure 4.1), while microbiome B was characterized by *Streptococcus, Tuicibacter, Mogibacterium, Bifidobacterium, Actinomyces, Escherichia,* and *Veillonella*. The two microbiomes additionally varied in α-diversity (Figure 4.2). Microbiome A was more phylogenetically diverse and rich while microbiome B was more even. Additionally, the two microbiomes came from subjects with large differences in dietary patterns. Microbiome A came from a subject with unusually high dietary fiber and whole grain intake (Figure 4.3), while microbiome B came from a subject

with low dietary fiber and whole grain intake that was typical of a western diet (Cordain, et al., 2005; US Department of Health and Human Services, 2017).

The differences between microbiome A and B are expected based on subject diets. Microbiome A was from a subject with a habitual diet high in dietary fiber and whole grains. This diet likely led to the microbiome containing high levels of genera commonly associated with the fermentation of complex carbohydrates such as *Prevotella* (De Filippo, et al., 2010; Wu, et al., 2011), and *Ruminococcus (*Ruminococcaceae) (Biddle, Stewart, Blanchard, & Leschine, 2013; Chassard, Delmas, Robert, Lawson, & Bernalier-Donadille, 2012). The abundant supply of dietary fiber for microbiome A supported a more phylogenetically diverse and rich microbial community. In contrast, microbiome B was from a subject with a diet deficient in dietary fiber and whole grains, as in the typical western diet (Cordain, et al., 2005; US Department of Health and Human Services, 2017). Thus, the microbiome was more abundant in less beneficial microbes that range in potential host effects such as *Streptococcus* and *Escherichia* (De Vos, et al., 2009; Quraishi, et al., 2017).

4.4.2.Changes in the microbiome

For both microbiomes, there was a decrease in diversity upon transfer into mice; however, the relative differences between microbiome A and B were maintained (Figure 4.4). Microbiome A mice had a microbiome that is more phylogenetically diverse and rich while microbiome B mice have a more even microbiome. There were changes in the microbial communities present after colonization in the mice compared to the human fecal sample and demonstrated by a large shift in β-diversity (Figure 4.5). However, the two microbiomes remained distinctly different from each other in the humanized mice. The decreases in diversity

and changes in the microbial communities are likely due to microbes not surviving cryogenic storage and not being able to colonize in the mice (Turnbaugh, et al., 2009).

After 1 and 11 weeks of treatment, changes in the diversity of the microbiome were evident. β-Diversity analysis showed that mice clustered together based on the microbiome and whatever dietary intervention had occurred (Figure 4.5). Mice with microbiome B had a larger shift in β-diversity due to dietary intervention than microbiome A mice. The larger shift seen for microbiome B mice after dietary intervention is likely due to a greater change in the fermentable material from the subjects' habitual diet. NDC, which is primary from dietary fiber and whole grains, are the major energy sources for the microbiome (Koropatkin, Cameron, & Martens, 2012; Salyers, West, Vercellotti, & Wilkins, 1977) The higher quantity of whole grains and dietary fiber in the experimental diets was likely a small change in NDC for microbiome A as it came from a subject with unusually high dietary fiber and whole grains intake (Figure 4.3). However, the experimental diets likely supplied additional NDC to microbiome B as it came from a subject with low dietary fiber and whole grain intake that was typical of a western diet (Cordain, et al., 2005; US Department of Health and Human Services, 2017).

Within a given week, such as week 11, there were two distinct clusters, for microbiome B based on dietary treatments, but microbiome A had no clustering due to treatment group (Figure 4.6). For microbiome B, the whole wheat treatments formed a separate cluster from the control diets and WD + EBR.

α-Diversity increased across the dietary treatments for both microbiomes but to a different magnitude. When studying within the given time points and microbiomes, differences based on treatment can be observed based on the microbiome. Microbiome B saw increases in phylogenetic diversity and richness with the whole grain diets compared to WD after 11 weeks

(Figure 4.7). These differences were additionally seen after only 1 week on the diets to a lesser extent. Whole wheats also increased in evenness compared to WD + EBR and C and these increases were also seen after 1 week on the experimental diets.

In vivo, whole grains have been shown to increase diversity compared with refined grains in rye (Ounnas, et al., 2016), barley (Zhong, Nyman, & Fåk, 2015) and baseline (Martínez, et al., 2013). However, these effects can be dimished based on the habitual diet of the subjects. Lappi (2013) conducted a human intervention study of whole grain bread and refined grain bread. In this study, despite the large differences in dietary fiber and whole grain consumption, the microbiota composition did not significantly differ between the groups. However, when the within group effects were examined, the microbiome changed for the refined white bread group but not the whole grain group. This result was partly attributed to the higher whole grain diets of the Finnish population studied suggesting the importance of habitual diets on the effectiveness of a whole grain intervention (Lappi, et al., 2013). Community studies have demonstrated the effects of differences in habitual diet on the composition and diversity of the gut microbe (De Filippo, et al., 2010; Wu, et al., 2011). Thus, in this study, the dietary intervention of whole grains for microbiome B resulted in differences in diversity and defining genera due to the change in dietary intervention. Meanwhile, microbiome A, which came from a subject with high habitual fiber and whole grain intake, did not have changes in the microbiome.

After 11 weeks, there were specific genera that were characteristic of the different treatment groups (Figure 4.8). Microbiome B had more differences due to dietary treatment than microbiome A. The defining genera were primarily split between the three whole grain diets for microbiome B (Figure 4.8). WD + BWW was defined by the Turicibacter order, WD + EWW was defined by the Clostridiale order, and WD + EBR was defined by the Lactobacilla order.

The defining genera for the treatments were varied across the different treatments, but the processed whole grains treatment groups had more defining genera than the two refined control diets. Coupled with the increased α-diversity observed with whole grains, it appears that whole grains were better able to support the growth of more taxa and especially some specific taxa than the control diets. Complex carbohydrate degrading microbes are split between the different whole grain treatments for microbiome B such as *Prevotella* with WD + EBR, *Coprococcus* with WD + EWW, and *Bifidobacterium* with WD + BWW.

After 11 weeks, microbiome A did have some defining genera among treatments. *Clostridium* was characteristic for WD + EBR and *Turicibacter* was characteristic for WD + BWW. This was also the case for microbiome B. In contrast, *Sutterella* was characteristic of WD + EBR and *Coprococcus* was characteristic of WD + BWW; however, in microbiome B *Sutterella* and *Coprococcus* were characteristic for WD + EWW. Microbiome A additionally had *Fecalibacterium* as a defining genus for WD + BWW that did not show up in microbiome A (Figure 4.8).

While microbiome B, had many different defining genera across all treatments, especially across the whole grains, microbiome A had fewer genera defining only WD +BWW and WD +EBR. Some taxa were consistent across both microbiomes while others varied suggesting the individuality of these results. The microbiome of individuals is highly variable in the communities present due to a myriad of factors that influence which of the thousands of different microbes inhabit a host (Conlon, 2015).

4.4.3.Diffrences in short chain fatty acids

The two microbiomes had large differences in cecal SCFA concentrations (Figure 4.9). Microbiome A had more SCFA in the cecum with more butyrate and propionate than microbiome B. There were no significant differences between the dietary treatment groups with either microbiome. The SCFA quantity and profile of the mice are reminiscent of their *in vitro* SCFA results (Figure 3.7).

NDC fermentation by the microbiota results in the production of SCFA that have numerous health benefits. SCFA act as an energy source for colonic cells, inhibit the growth of pathogens, and help maintain tissue integrity. SCFA are additionally absorbed by the host and have a role in insulin secretion, lipid metabolism, and inflammation, among others (Den Besten, et al., 2013; Kasubuchi, Hasegawa, Hiramatsu, Ichimura, & Kimura, 2015; Puertollano, Kolida, & Yaqoob, 2014).

Higher levels of SCFA production by Microbiome A imply increased fermentation of carbohydrates. Previous studies have shown that whole grains increase cecal SCFA levels, especially butyrate and propionate (Garciamazcorro, Ivanov, Mills, & Noratto, 2016; Ounnas, et al., 2016; Zhong, Marungruang, & Nyman, 2015; Zhong, Nyman, & Fåk, 2015). However, these studies were not conducted on humanized mice and there is a role of the microbiome source in these results.

4.4.4.Changes in physiology

As expected, mice on WD had significantly more body fat and weight gain than C for both microbiomes. WD + BWW had reduced body fat compared to WD + EWW for microbiome A. There was no significant difference in percent body fat between WD and any of the whole grain treatments or between WD + EWW and WD + EBR for Microbiome A (Figure 4.10).

Microbiome B mice did not have any significant differences between WD and WD treatments in percent body fat (Figure 4.10). Body weight gain showed similar results to percent body fat. WD + BWW had reduced body weight gain compared to WD + EWW for microbiome A. There was no significant difference in weight gain between WD and any of the WD treatments or between WD + EWW and WD + EBR for Microbiome A (Figure 4.11). Microbiome B mice did not have any significant differences between WD and the whole grain treatments in body weight gain (Figure 4.11). There was no significant difference in feed intake between the treatment groups.

In previous studies where processing has been studied in animal models, there have not been significant differences in body weight or body composition. However, these studies have been relatively short in time and in non-humanized animals. Still, these studies did see differences in some health markers and microbiome characteristics. Bird (2000) studied the difference in parboiled brown rice and boiled white rice substituted with heat-stabilized rice bran to equate the amount of fiber in pig diets for two weeks. While there was no difference in body weight, the pigs fed parboiled brown rice had significantly more fecal output in the first week, greater digesta mass, and higher total and individual SCFA in the feces and the colon (Bird, et al., 2000). Kraler (2015) studied native, fermented and extruded wheat bran in piglets for 6 weeks. There were no significant differences in performance parameters such as body weight gain and feed intake, but the amount of goblet cells in the ileum was higher when pigs were on the native and extruded wheat bran, compared to fermented bran. Zhong (2015) studied the effect of malting barley in high fat diets of rats for 4 weeks. Rats consuming malted barley (and the cellulose control) had higher microbiota α-diversity than the rats consuming unmalted barley. Moreover, there was a clear separation in the microbiota communities based on diet and malting also increased butyric acid (Zhong, Nyman, & Fåk, 2015). Thus, these studies may not have been

conducted for long enough periods of time to observe body composition and body weight differences.

Both mice from microbiome A and B differed in the glucose tolerance after 11 weeks of treatment as seen in Figure 4.12. As expected WD mice had lower glucose tolerance than C. In both microbiome groups, WD + BWW showed improved glucose tolerance compared with the extruded treatments, except only in microbiome A was this difference significant (Figure 4.12). While the effect of whole grain type and processing method on glucose tolerance has not been studied, the glycemic response of both has been previous studied. It is suggested that a diet with a high glycemic index or glycemic load results in a higher postprandial blood glucose and insulin concentrations that lead to decreases in glucose tolerance and risk of eventual type 2 diabetes (Jenkins, et al., 1981).

Different cereals have been shown to have different glycemic indexes (Atkinson, Foster-Powell, & Brand-Miller, 2008; Jenkins, Wolever, & Jenkins, 1988). However, the glycemic index of a given cereal can be widely variable based on the cultivar, food form, and processing method (Miller, Pang, & Bramall, 1992). Processing has been shown to have an influence on the glycemic index and load of cereals and whole grains (Atkinson, Foster-Powell, & Brand-Miller, 2008; Jenkins, Wolever, & Jenkins, 1988; Ross, Brand, Thorburn, & Truswell, 1987). For instance, the processing of whole wheat into bread (GI=74), roti (62), and spaghetti (48) can elicit different glycemic indexes (Atkinson, Foster-Powell, & Brand-Miller, 2008). Moreover, extrusion of whole grain wheat, under extreme conditions, has been shown to increase plasma glucose after a gastric load (Bjorck, Asp, & Lundquist, 1984). Other processing methods, such as popping and expanding, have been shown to result in foods with high glycemic indexes, 105 and 109 respectively. Moreover, the change in glycemic index with the process was much greater

than the difference between grains (wheat and rice) processed in the same method, further suggesting the vast influence of processing (Shobana, Usha Kumari, Malleshi, & Ali, 2007). Comparison of glycemic index across multiple studies shows that grain type can have a large influence. For instance, when comparing the glycemic indexes of different porridges, glycemic index can vary for millet (GI=67), rice (78), instant oat (79), rolled oat (55), porridges when looking at similar preparation methods. However, this range is still considerably smaller (55-79) than seen with different processes of the same grain(48-74) (Atkinson, Foster-Powell, & Brand-Miller, 2008).

The two microbiomes from subjects with different levels of dietary fiber and whole grain consumption had different changes in the gut microbiome and physiology. The microbiome B mice, from the subject with a diet lower in whole grains and dietary fiber, had increased diversity and beneficial microbes after treatment. However, microbiome A mice, from a subject with a diet high in dietary fiber and whole grains, had minimal changes in the microbiome. These differences are likely due to the small degree to which the dietary intervention was different from the habitual diet of the subjects. However, these changes in the microbiome were not consistent with the changes in host physiology. Microbiome A mice had significantly less increase in body fat and body weight gain with WD + BWW compared to WD + EWW. However, microbiome B mice had no significant difference in body weight gain or body composition of the western diets. However, both sets of mice had increased glucose intolerance with the extruded grains. These differences in glucose intolerance are thought to be due to potential differences in the glycemic indexes and loads of the diets. In order to test this hypothesis, the *in vitro* starch digestibility was measured.

4.4.5.Diffrences in diet in vitro digestibility

The experimental diets differed in the *in vitro* digestibility of the total glucose present within the diet as seen in Figure 4.13. Extrusion appeared to increase the digestibility of the diets, where WD + EWW increased RAG while SAG decreased compared to WD and WD + BWW. Furthermore, WD + EBR had increased SAG at the expense of RS (Figure 4.13).

The starch digestibility profiles have been related to glycemic index, fat mass and other physiological markers. The digestibility profile of carbohydrates has been shown to be significantly correlated to *in vivo* glycemic index (Garsetti, et al., 2005; Ross, Brand, Thorburn, & Truswell, 1987). Rapidly digestible glucose, after correcting for subject variability, explained 70% of the variance in glycemic response (Englyst, Veenstra, & Hudson, 1996). Furthermore. RS has been shown to decrease postprandial glucose response, insulin, and body fat mass when it is substituted for rapidly digestible starch and insulin response when it replace digestible starch (Higgins, 2004; Pawlak, Kushner, & Ludwig, 2004).

Thus, in this study the increased glucose intolerance of the extruded diets is likely a result of the trend towards increased digestibility of the carbohydrates *in vitro* as the difference in glucose intolerance was seen with both microbiomes. Extrusion increased starch digestibility in the ilium of pigs (Fadel, Newman, Newman, & Graham, 1988). Sun (2006) compared raw and extruded barley, pea, and potato starch wheat bran *in vitro* and in pigs. Extrusion increased the proportion of rapidly digestible starch, and reduced the proportion of slowly digestible starch and RS *in vitro*. In the raw material, barley was more rapidly digested, but there were no significant differences between the extruded cereals. When fed to pigs, extrusion increased the digestibility of starch, but this was dependent upon cereal source as barley was not significantly affected by

extrusion(Sun, Lærke, Jørgensen, & Knudsen, 2006). Thus, the lack of difference between the grain types in this study is likely due to the overarching effect of extrusion on starch digestibility.

The differently processed grains resulted in differences in the body fat in the mice with microbiome A. In a study comparing rapidly absorbed carbohydrates and slowly absorbed carbohydrates in mice, mice fed rapidly absorbed carbohydrates accumulated more body fat, which agrees with the result of this study. However, there was no significant difference in body weight. Differences in body fat in the present study were not seen in both of the microbiome groups in the present study. It is hypothesized that in both microbiomes, extrusion increased the glycemic response which led to increased fat mass gain and weight gain. However, in microbiome B, there was a more varied influence of the microbiome due to the dietary treatments. Thus, it is hypothesized that extrusion led to increased digestibility and weight gain, but with microbiome B mice extrusion had positive influences of the microbiome such as increased diversity. Thus, the microbial communities could have been better able to mitigate some of physiological effects and acted to cancel out the change in digestibility so there were no differences in weight or body composition for these mice.

4.5. CONCLUSIONS

The microbiome from two subjects with different levels of dietary fiber and whole grain consumption were studied in humanized mice in an obesity model for 12 weeks. During the study, the mice harboring microbiome B, from the subject with a diet lower in whole grains and dietary fiber, had increased diversity and beneficial microbes after treatment. These changes began in as little as one week after treatment. Moreover, whole wheat and extrusion had a greater influence on the microbiome in these mice. However, microbiome A mice, from a subject with a diet high in dietary fiber and whole grains, had minimal changes in the microbiome. These

differences are likely due to the small degree to which the dietary intervention was different from the habitual diet of the subjects. After 12 weeks of the study, the microbiome A mice had significantly less body weight gain and increase in percent body fat with WD + BWW compared to WD + EWW. However, there was no significant difference between the treatments and control or between the different whole grains. Additionally, microbiome B mice had no significant difference in body weight gain or body composition of the western diets. However, both sets of mice had increased glucose intolerance with the extruded grains, likely due to the increased starch digestibility that occurred in the extruded grains. This difference in glycemic likely resulted in excess energy that led to increased obesity in the mice. However, microbiome B mice, who had improvements in the microbiome with whole grains, did not see these changes due to the influence of the microbiome. Thus, both digestion and fermentation are important factors in effect of whole grains and food processing on host health.

4.6. REFERENCES

AACC International. (2018). Method 30-25.01 Crude Fat in Wheat, Corn, and Soy Flour, Feeds, and Mixed Feeds. In *Approved Methods of Analysis* (11 ed.). St. Paul, MN, USA.

AACC International. (2018). Method 44-15.02 Moisture-Air Oven Methods. St. Paul, MN, USA.

AACC International. (2018). Method 46-30.01 Crude Protein- Combustion Method. In *Approved Methods of Analysis* (11 ed.). St Paul, MN, USA.

Albertson, A. M., Reicks, M., Joshi, N., & Gugger, C. K. (2015). Whole grain consumption trends and associations with body weight measures in the United States: results from the cross sectional National Health and Nutrition Examination Survey 2001–2012. *Nutrition Journal, 15(1)*, 8.

Aoe, S., Nakaoka, T., Ama, M. Y., Ohta, F., & Ayan, Y. (1990). Availability of dietary fiber in extruded wheat bran and apparent digestibility in rats of coexisting nutrients. *Cereal Chemistry*, 145-148.

Arcila, J. A., Weier, S. A., & Rose, D. J. (2015). Changes in dietary fiber fractions and gut microbial fermentation properties of wheat bran after extrusion and bread making. *Food Research International, 74*, 217-223.

Atkinson, F. S., Foster-Powell, K., & Brand-Miller, J. C. (2008). International tables of glycemic index and glycemic load values: 2008. *Diabetes Care, 31(12)*, 2281-2283.

Benjamin, E. J., Muntner, P., & Bittencourt, M. S. (2019). Heart Disease and Stroke Statistics—2019 Update: A Report From the American Heart Association. *Circulation, 139(10),* e56-e528.

Biddle, A., Stewart, L., Blanchard, J., & Leschine, S. (2013). Untangling the genetic basis of fibrolytic specialization by Lachnospiraceae and Ruminococcaceae in diverse gut communities. *Diversity, 5(3)*, 627-640.

Bird, A. R., Hayakawa, T., Marsono, Y., Gooden, J. M., Record, I. R., Correll, R. L., & Topping, D. L. (2000). Coarse Brown Rice Increases Fecal and Large Bowel Short-Chain Fatty Acids and Starch but Lowers Calcium in the Large Bowel of Pigs. *The Journal of Nutrition 130 (7)*, 1780-1787.

Bjorck, I., Asp, N. G., & Lundquist, I. (1984). Digestion in vitro and In vivo; I extrusion cooking of wheat flours and starch. *Journal of Cereal Science (2)*, 91-103.

Bjorck, I., Asp, N. G., Birked, A. C., Eliasson, L. B., Shjoberg, L. B., & Lundquist, I. (1984). Effects of processing on starch availability in vitro and in vivo; II drum drying of wheat flour. *Journal of Cereal Science (2)*, 165-178.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., . . . Bai, Y. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology, 37(8)*, 852-857.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J., & Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods, 13(7)*, 581.

Carlson, J., Erickson, J., Hess, J., Gould, T., & Slavin, J. (2017). Prebiotic dietary fiber and gut health: comparing the in vitro fermentations of beta-glucan, Inulin and Xylooligosaccharide. *Nutrients, 9(12)*, 1361.

Carvalho-Wells, A. L., Helmolz, K., Nodet, C., Molzer, C., Leonard, C., McKevith, B., . . . Tuohy, K. M. (2010). Determination of the in vivo prebiotic potential of a maize-based whole grain breakfast cereal: a human feeding study. *British Journal of Nutrition, 104(9)*, 1353-1356.

Casterline, J. L., Oles, C. J., & Ku, Y. (1997). In vitro fermentation of various food fiber fractions. *Journal of Agricultural and Food Chemistry, 45(7)*, 2463-2467.

Chassard, C., Delmas, E., Robert, C., Lawson, P. A., & Bernalier-Donadille, A. (2012). Ruminococcus champanellensis sp. nov., a cellulose-degrading bacterium from human gut microbiota. *International journal of systematic and evolutionary microbiology, 62(1)*, 138-143.

Cho, S. S., Qi, L., Fahey, G. C., & Klurfeld, D. M. (2013). Consumption of Cereal Fiber, Mixtures of Whole Grains and Bran, and Whole Grains and Risk Reduction in Type 2 Diabetes, Obesity, and Cardiovascular Disease. *American Journal of Clinical Nutrition, 98*, 594–619.

Colon, M., & Bird, A. (2015). The impact of diet and lifestyle on gut microbiota and human health. *Nutrients,* 7(1), 17-44.

Cordain, L., Eaton , S. B., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B. A., . . . Brand-Miller, J. (2005). Origins and evolution of the Western diet: health implications for the 21st century. *The American Journal of Clinical Nutrition, 81(2)*, 341-343.

De Filippo, C., Cavalieri, D., Di Paola, M., Poullet, J. B., Massart, S., Collini, S., . . . Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in

children from Europe and Rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*, 14691–14696.

De Vadder, F., Kovatcheva-Datchary, P., Goncalves, D., Vinera, J., Zitoun, C., Duchampt, A., . . . Mithieux, G. (2014). Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell, 156(1-2)*, 84-96.

De Vos, P., Garrity, G. M., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., . . . Whitman, W. B. (2009). *Bergey's Manual of Systematic Bacteriology: Volume Three The Firmicutes.* NY: Springer.

Den Besten, G., Van Eunen, K., Groen, A. K., Venem, A. K., Venema, K., Reijngoud, D. J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research, 54(9)*, 2325-2340.

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., . . . Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology, 72(7)*, 5069-5072.

Doblado-Maldonado, A. F., Flores, R. A., & Rose, D. J. (2013). Low moisture milling of wheat for quality testing of wholegrain flour. *Journal of Cereal Science 58*, 420-423.

Englyst, H. N., Veenstra, J., & Hudson, G. J. (1996). Measurement of rapidly available glucose (RAG) in plant foods: a potential in vitro predictor of the glycemic response. *British Journal of Nutrition, 75(3)*, 327-337.

Fadel, J. G., Newman, C. W., Newman , R. K., & Graham, H. (1988). Effects of extrusion cooking of barley on ileal and fecal digestibilities of dietary components in pigs. *Canadian Journal of Animal Science, 68(3)*, 891-897.

Foster, Z. S., Sharpton, T. J., & Grünwald, N. J. (2017). Metacoder: An R package for visualization and manipulation of community taxonomic diversity data. *PLoS Computational Biology, 13(2)*, e1005404.

Garciamazcorro, J. F., Ivanov, I., Mills, D. A., & Noratto, G. (2016). Influence of wholewheat consumption on fecal microbial community structure of obese diabetic mice. *PeerJ, 4(3).*

Garsetti, M., Vinoy, S., Lang, V., Holt, S., Loyer, S., & Brand-Miller, J. C. (2005). The glycemic and insulinemic index of plain sweet biscuits: relationships to in vitro starch digestibility. *Journal of the American College of Nutrition, 24(6)*, 441-447.

Hales, C. M., Fryar, C. D., Carroll, M. D., Freedman, D. S., & Ogden, C. L. (2018). Trends in obesity and severe obesity prevalence in US youth and adults by sex and age, 2007–2008 to 2015–2016. *JAMA 319*, 1723-1725.

Hartzell, A. L., Maldonado-Gómez, M. X., Hutkins, R. W., & Rose, D. J. (2013). Synthesis and in vitro digestion and fermentation of acylated inulin. *Bioactive Carbohydrates and Dietary Fibre 1.1*, 81-88.

Higgins, J. A. (2004). Resistant starch: metabolic effects and potential health benefits. *Journal of AOAC International, 87(3)*, 761-768.

Jenkins, D. J., Wolever, T. M., & Jenkins, A. L. (1988). Starchy foods and glycemic index. *Diabetes Care, 11(2)*, 149-159.

Jenkins, D. J., Wolever, T. M., Taylor, R. H., Barker, H., Fielden, H., Baldwin, J. M., . . . Goff, D. V. (1981). Glycemic index of foods: a physiological basis for carbohydrate exchange. *The American Journal of Clinical Nutrition, 34(3)*, 362-366.

Johansson, D. P., Vázquez Gutiérrez, J. L., Landberg, R., Alminger, M., & Langton, M. (2018). Impact of food processing on rye product properties and their in vitro digestion. *European Journal of Nutrition 57*, 1651–1666.

Kahlon, T. S., Berrios, D. J., Smith, G. E., & Pan, J. L. (2006). Extrusion conditions modify hypocholesterolemic properties of wheat bran fed to hamsters. *Cereal Chemistry*, 152-156.

Kasubuchi, M., Hasegawa, S., Hiramatsu, T., Ichimura, A., & Kimura, I. (2015). Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. *Nutrients, 7(4)*, 2839-2849.

Koropatkin, N. M., Cameron, E. A., & Martens, E. C. (2012). How glycan metabolism shapes the human gut microbiota. *Nature Reviews Microbiology, 10(5)*, 323.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and Environmental Microbiology*, 5112-5120.

Kraler, M., Schedle, K., Schwarz, C., Domig, K. J., Pichler, M., Oppender, A., . . . Somoza, A. (2015). Fermented and extruded wheat bran in piglet diets: impact on performance, intestinal morphology, microbial metabolites in chyme and blood lipid radicals *Animal Feed Science and Technology* 197, 121-129.

Lappi, J., Salojärvi, J., Kolehmainen, M., Mykkänen, H., Poutanen, K., De Vos, W. M., & Salonen, A. (2013). Intake of whole-grain and fiber-rich rye bread versus refined wheat bread does not differentiate intestinal microbiota composition in Finnish adults with metabolic syndrome. *The Journal of Nutrition 143(5)*, 648-655.

Martínez, I., Lattimer, J. M., Hubach, K. L., Case, J. A., Yang, J., Weber, C. G., . . . Haub, M. D. (2013). Gut microbiome composition is linked to whole grain-induced immunological improvements. *The ISME Journal, 7(2)*.

Miller, J. B., Pang, E., & Bramall, L. (1992). Rice: a high or low glycemic index food? *The American Journal of Clinical Nutrition, 56(6)*, 1034-1036.

Mkandawire, N. L., Mkandawire, N. L., Kaufman, R. C., Bean, S. R., Weller, C. L., Jackson, D. S., & Rose, D. J. (2013). Effects of sorghum (Sorghum bicolor (L.) Moench) tannins on α-amylase activity and in vitro digestibility of starch in raw and processed flours. J*ournal of Agricultural and Food Chemistry, 61(18)*, 4448-4454.

National Cancer Institute. (2018). Retrieved from Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool: https://epi.grants.gov/asa24

Ounnas, F., Privé, F., Salen, P., Gaci, N., Tottey, W., & Calani, L. (2016). Whole rye consumption improves blood and liver n-3 fatty acid profile and gut microbiota composition in rats. *PLoS One, 11(2).*

Pawlak, D. B., Kushner , J. A., & Ludwig, D. S. (2004). Effects of dietary glycemic index on adiposity, glucose homoeostasis, and plasma lipids in animals. *The Lancet, 364(9436)*, 778-785.

Poeker, S. A., Geirnaert, A., Berchtold, L., Greppi, A., Krych, L., Steinert, R. E., . . . Lacroix, C. (2018). Understanding the prebiotic potential of different dietary fibers using an in vitro continuous adult fermentation model (PolyFermS). *Scientific Reports 8(1)*, 4318.

Puertollano, E., Kolida, S., & Yaqoob, P. (2014). Biological significance of short-chain fatty acid metabolism by the intestinal microbiome. *Current Opinion in Clinical Nutrition & Metabolic Care, 17(2)*, 139-144.

Quraishi, M. N., Sergeant, M., Kay, G., Iqbal, T., Chan, J., Constantidou, C., . . . Hirschfield, G. M. (2017). The gut-adherent microbiota of PSC–IBD is distinct to that of IBD. *Gut, 66(2)*, 386-388.

Ralet, M., Thibault, J., & Della Valle, G. (1990). Influence of extrusion-cooking on the physico-chemical properties of wheat bran. *Journal of Cereal Science*, 249-259.

Robin, F., Dubois, C., Pineau, N., Schuchmann, H., & Palzer, S. (2011). Expansion mechanism of extruded foams supplemented with wheat bran. *Journal of Food Engineering, 107*, 80-89.

Ross, S. W., Brand, J. C., Thorburn, A. W., & Truswell, A. S. (1987). Glycemic index of processed wheat products. *The American Journal of Clinical Nutrition, 46(4)*, 631-635.

Salyers, A. A., West, S. E., Vercellotti, J. R., & Wilkins, T. D. (1977). Fermentation of mucins and plant polysaccharides by anaerobic bacteria from the human colon. *Applied and Environmental Microbiology, 34(5)*, 529-533.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology, 12(6)*, R60.

Shobana, S., Usha Kumari, S. R., Malleshi, N. G., & Ali, S. Z. (2007). Glycemic response of rice, wheat and finger millet based diabetic food formulations in normoglycemic subjects. *International Journal of Food Sciences and Nutrition, 58(5)*, 363-372.

Sun, T., Lærke , H. N., Jørgensen, H., & Knudsen, K. E. (2006). The Effect of Extrusion Cooking of Different Starch Sources on the In Vitro and In Vivo Digestibility in Growing Pigs. *Animal Feed Science and Technology, 131(1-2)*, 67-86.

Turnbaugh, P. J., Ridaura, V. K., Faith, J. J., Rey, F. E., Knight, R., & Gordon, J. L. (2009). The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Science Translational Medicine, 1(6)*.

US Department of Health and Human Services. (2017). Dietary Guidelines for Americans 2015-2020. *Skyhorse Publishing Inc.*

Wang, W. M., Klopfenstein, C. F., & Ponte, J. (1993). Effects of Twin-Screw Extrusion on the Physical Properties of Dietary Fiber and Other Components of Whole Wheat and Wheat Bran and on the Baking Quality of the Wheat Bran. *Cereal Chemistry*, 707-711.

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y. Y., Keilbaugh, S. A., . . . Sinha, R. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science, 334(6052)*, 105-108.

Yang, J., Keshavarzian, A., & Rose, D. J. (2013). Impact of dietary fiber fermentation from cereal grains on metabolite production by the fecal microbiota from normal weight and obese individuals. *Journal of Medicinal Food, 16(9)*, 862-867.

Zhong, Y., Marungruang, N., & Nyman, F. (2015). Effects of two whole-grain barley varieties on caecal SCFA, gut microbiota and plasma inflammatory markers in rats consuming low- and high-fat diets. *British Journal of Nutrition, 113(10)*, 1558-1570.

Zhong, Y., Nyman, M., & Fåk, F. (2015). Modulation of gut microbiota in rats fed high-fat diets by processing whole-grain barley to barley malt. *Molecular Nutrition & Food Research, 59(10)*, 2066-2076.

FIGURES AND TABLES

Table 4.1. Formulation and composition of experimental diets (g).

Ingredient	C	WD	WD + BWW	WD + EWW	WD + EBR
Casein	200	200	175.8	175.8	186.5
L-Cystine	3	3	3	3	3
Corn Starch	550	72.8	0	0	0
Maltodextrin 10	150	100	67	72.3	60.1
Sucrose	0	172.8	172.8	172.8	172.8
Boiled Whole Wheat	0	0	161.3	0	0
Extruded Whole Wheat	0	0	0	163.2	0
Extruded Brown Rice	0	0	0	0	165.3
Cellulose	50	50	34.2	36.8	47.4
Lard	20	177.5	177.5	177.5	177.5
Soybean Oil	25	25	23.5	24	24.1
Mineral Mix S10026	10	10	10	10	10
Dicalcium Phosphate	13	13	13	13	13
Calcium Carbonate	5.5	5.5	5.5	5.5	5.5
Potassium Citrate	16.5	16.5	16.5	16.5	16.5
Vitamin Mix V10001	10	10	10	10	10
Choline Bitartrate	2	2	2	2	2
Red Dye #40 FD&C	0.025	0.05	0	0	0.025
Blue Dye #1 FD&C	0.025	0	0.05	0.025	0
Yellow Dye #5 FD&C	0	0	0	0.025	0.025
Total	1055.05	858.15	872.15	882.15	893.75
Protein	179	179	179	179	179
Carbohydrate	710	355.6	355.6	355.6	355.6
Fat	47.4	204.9	204.9	204.9	204.9
Fiber	50	50	50	50	50
Boiled Whole Wheat	0	0	161.3	0	0
Extruded Whole Wheat	0	0	0	163.2	0
Extruded Brown Rice	0	0	0	0	165.3

132

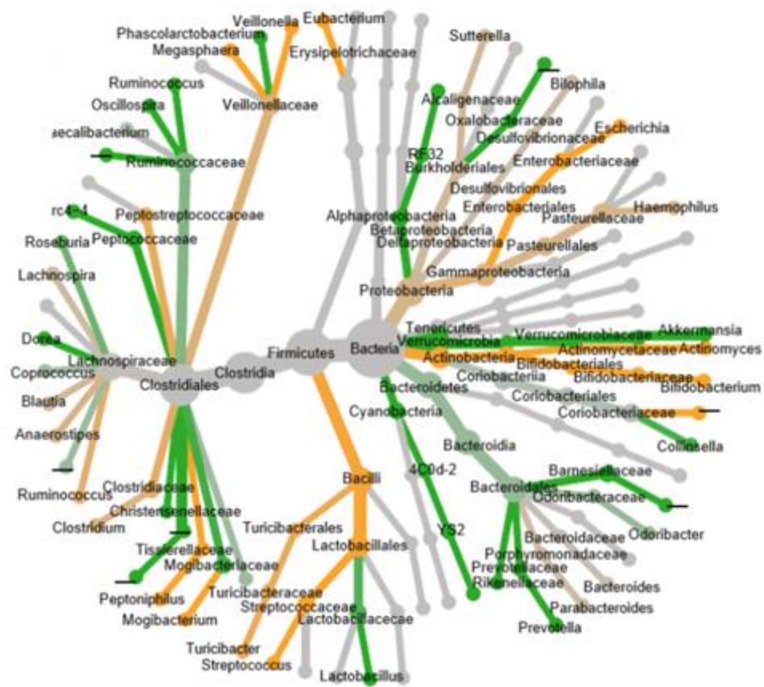

Figure 4.1. Heat Tree depicting taxonomic significant differences between the two microbial communities; green is more abundant in microbiome A while gold is more abundant in microbiome B (Wilcoxon rank sum test, $p< 0.05$.

133

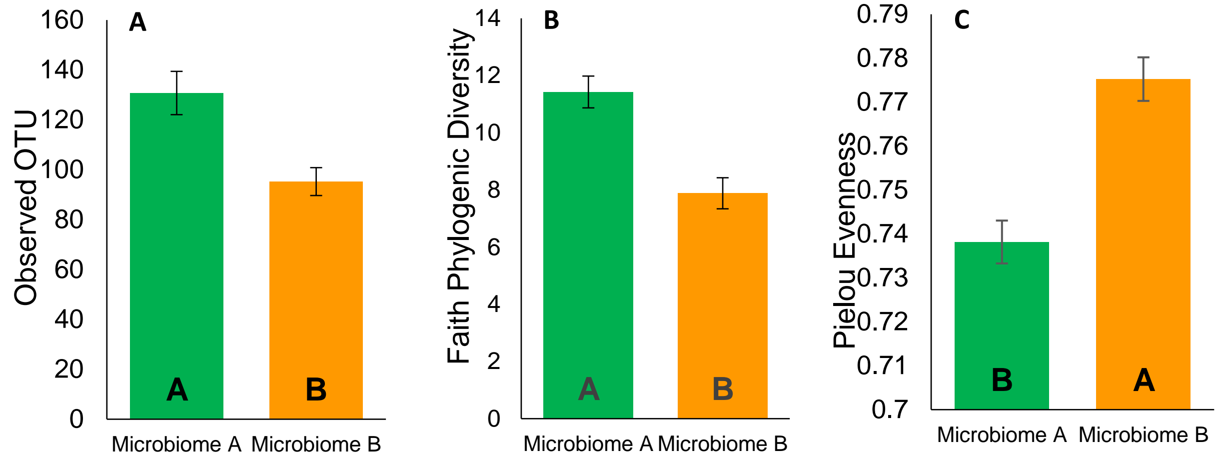

Figure 4.2. α-Diversity of subject fecal samples by (A) observed operational taxonomical units (OTU), (B) Faith's Phylogenic Diversity, and (C) Pielou Evenness.

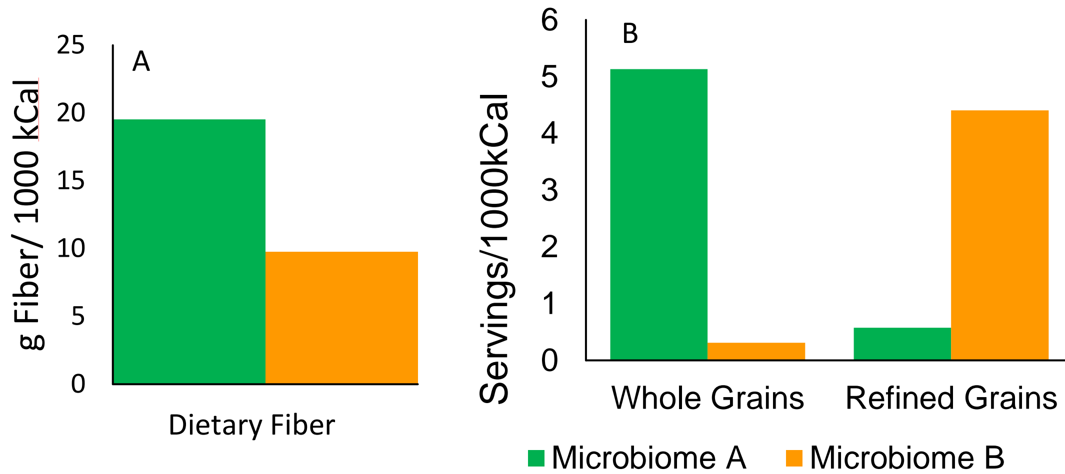

Table 4.3. Dietary patterns of subjects adjusted for total caloric intake for (A) grams of dietary fiber per 1000 kCal, and (B) servings of whole grains and refined grains per 1000 kCal. of variance (mean squares) of measured variables on whole wheat flour.

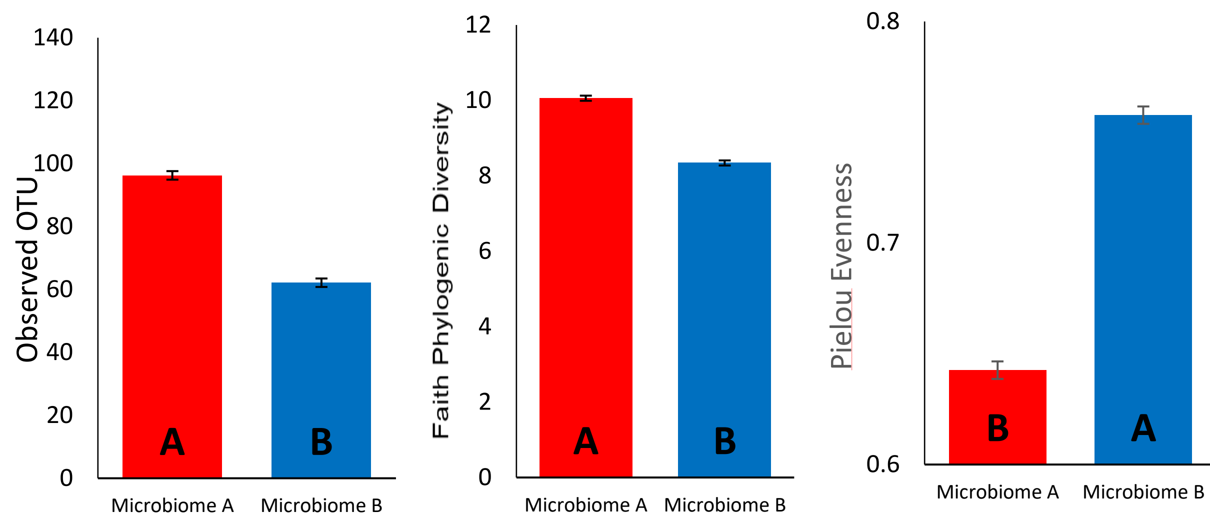

Figure 4.4. α-Diversity of humanized mice by (A) observed operational taxonomical units (OTU), (B) Faith's Phylogenic Diversity, and (C) Pielou Evenness.

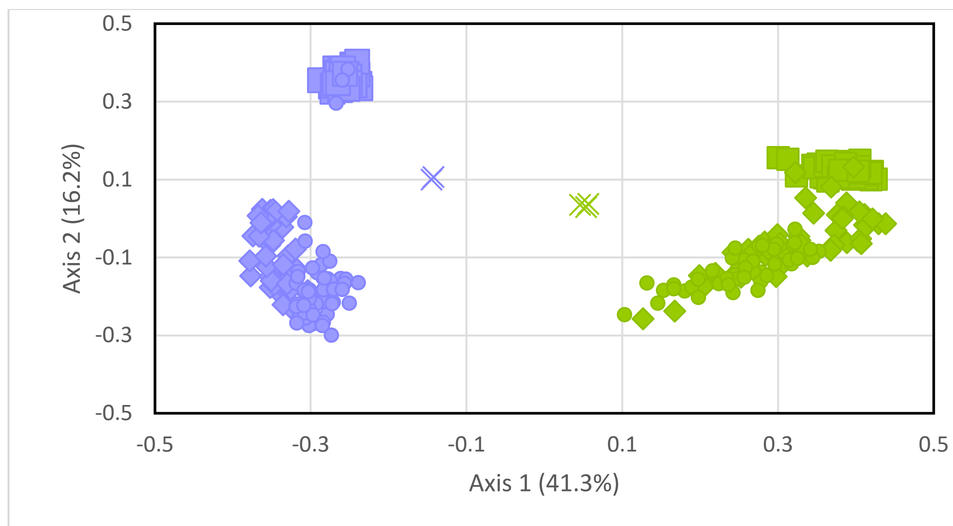

Figure 4.5. β-Diversity of human fecal samples and humanized mice over the course of the study. Purple denotes microbiome A and green denotes microbiome B. Cross denotes fecal microbiome, square denotes humanized mice microbiome before dietary intervention, diamond denotes microbiome after 1 week of dietary intervention, and sphere denotes microbiome after 11 week of dietary intervention using Bray Curtis diversity metric.

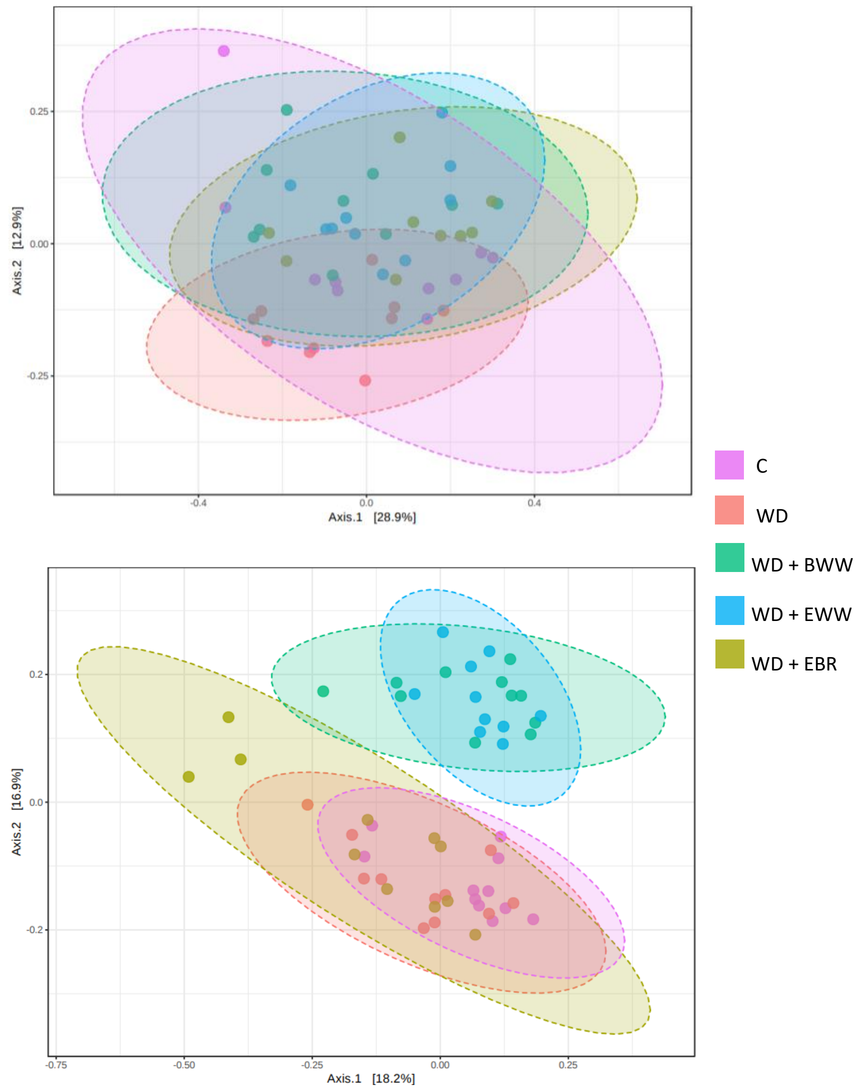

Figure 4.6. β-Diversity after 11 weeks of dietary intervention for (A) microbiome A and (B) microbiome B using Bray Curtis diversity metric.

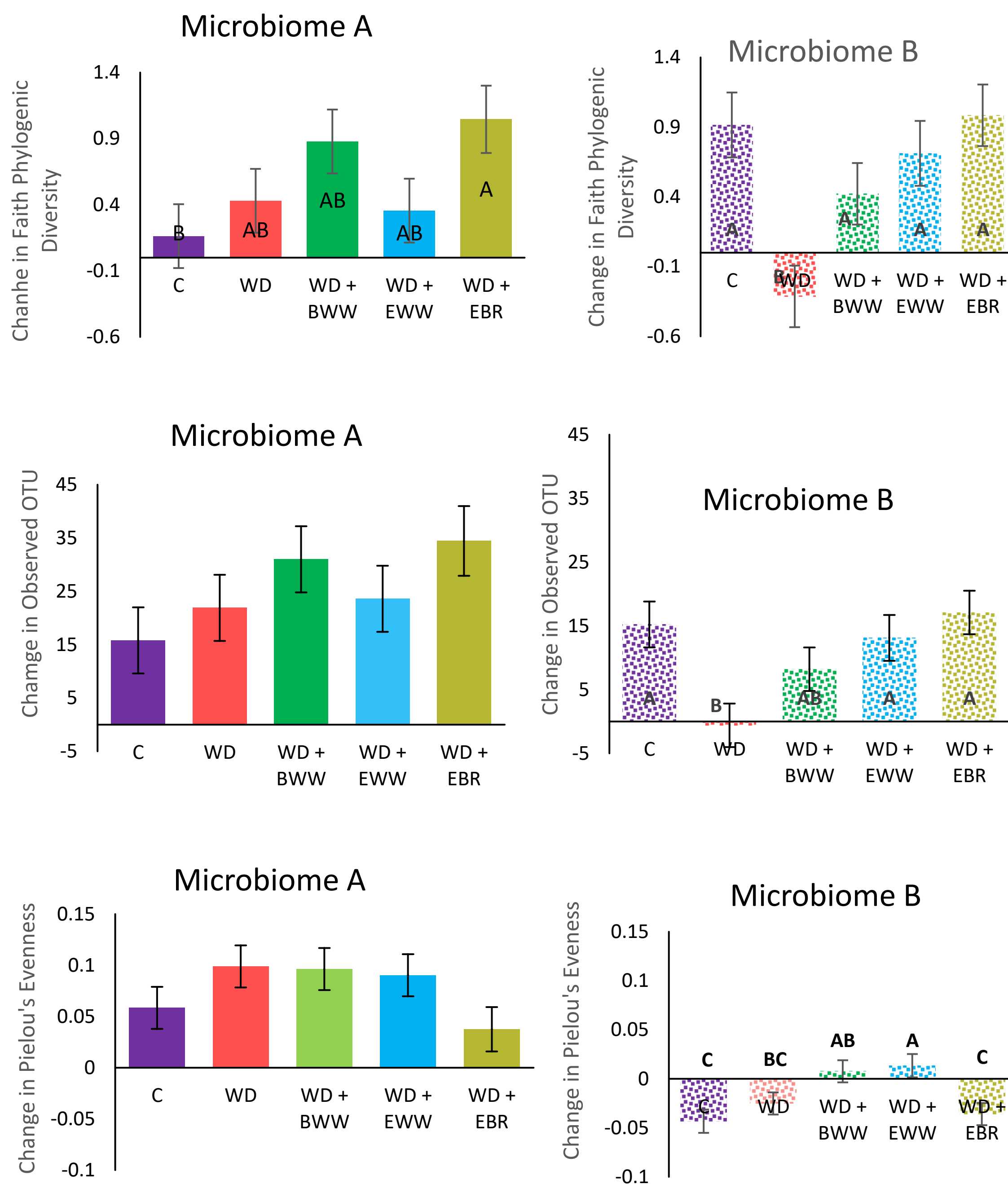

Figure 4.7. Change in α-diversity after 11 weeks of dietary intervention for microbiome A and B using (A) Faith's Phylogenic Diversity (B) observed operational taxonomical units (OTU), and (C) Pielou Evenness.

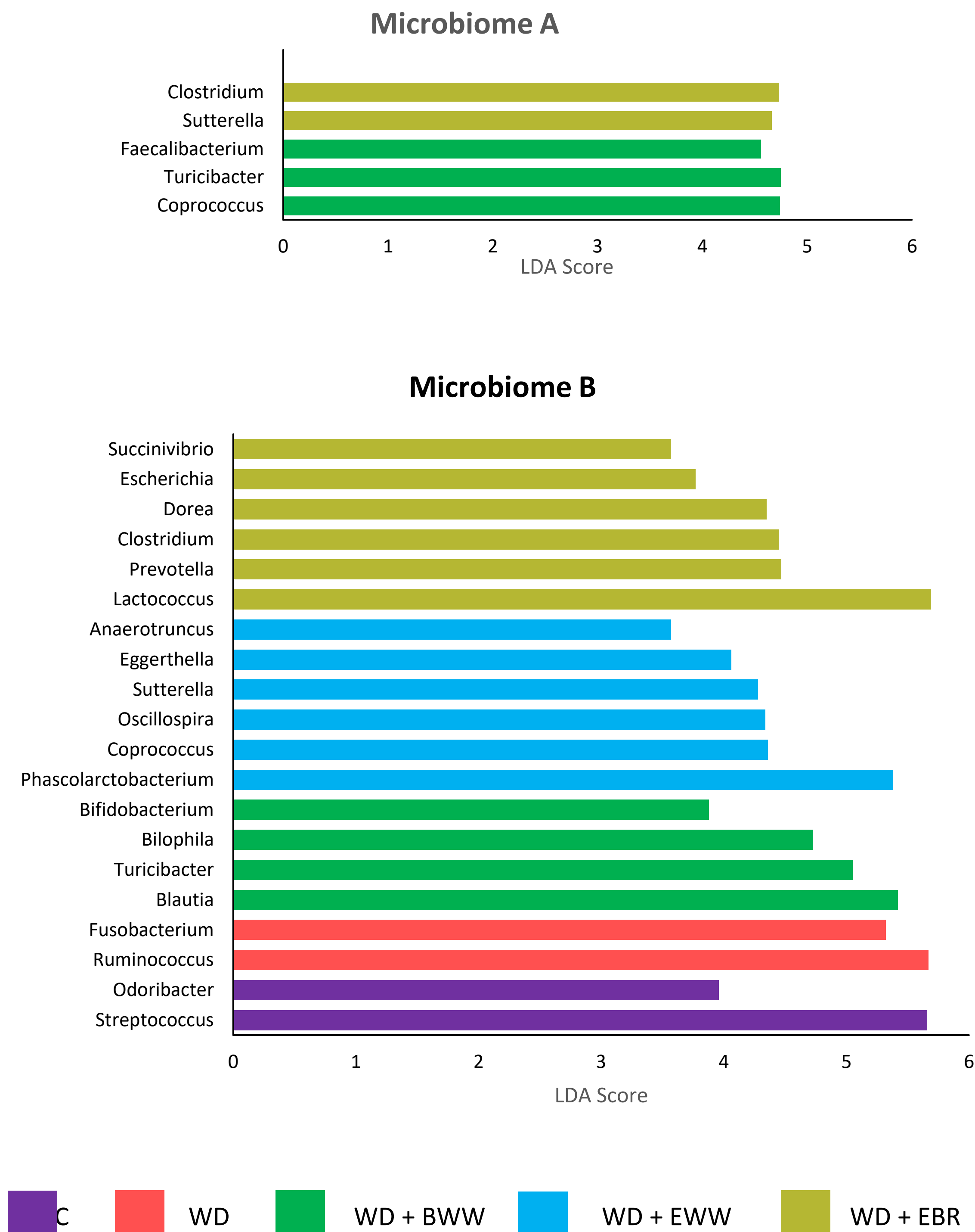

Figure 4.8. Linear Discriminant Analysis Effective Size (LEfSe) for the differentially abundant genera, using Kruskal-Wallis (KW) sum-rank test with an FDR adjusted p value of 0.05 for both microbiome mice based on dietary treatment C (control), WD (western diet), WD + BWW (western diet + boiled whole wheat), WD + EWW (western diet + extruded whole wheat), WD + EBR (western diet + extruded brown rice).

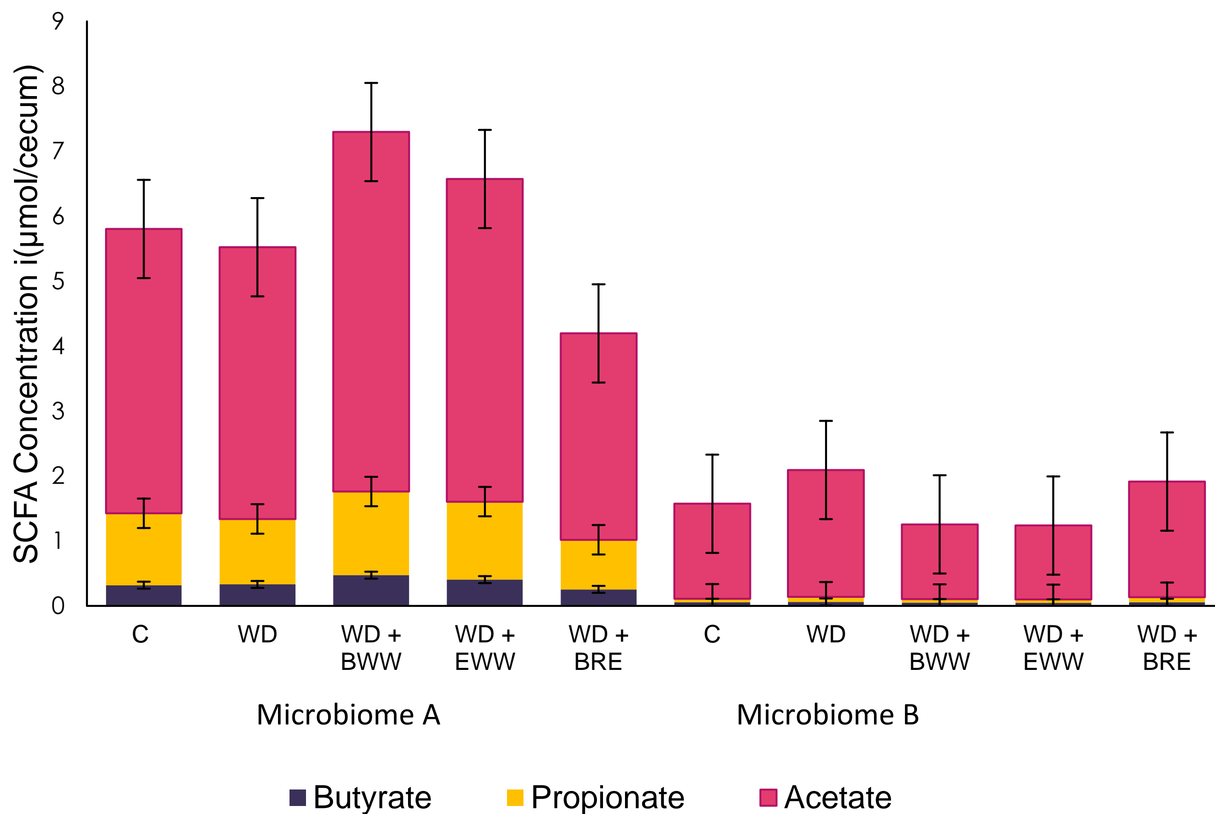

Figure 4.9. Cecal short chain fatty acids (SCFA) based on mouse microbiome and dietary treatment C (control), WD (western diet), WD + BWW (western diet + boiled whole wheat), WD + EWW (western diet + extruded whole wheat), WD + EBR (western diet + extruded brown rice). Error bars show standard error.

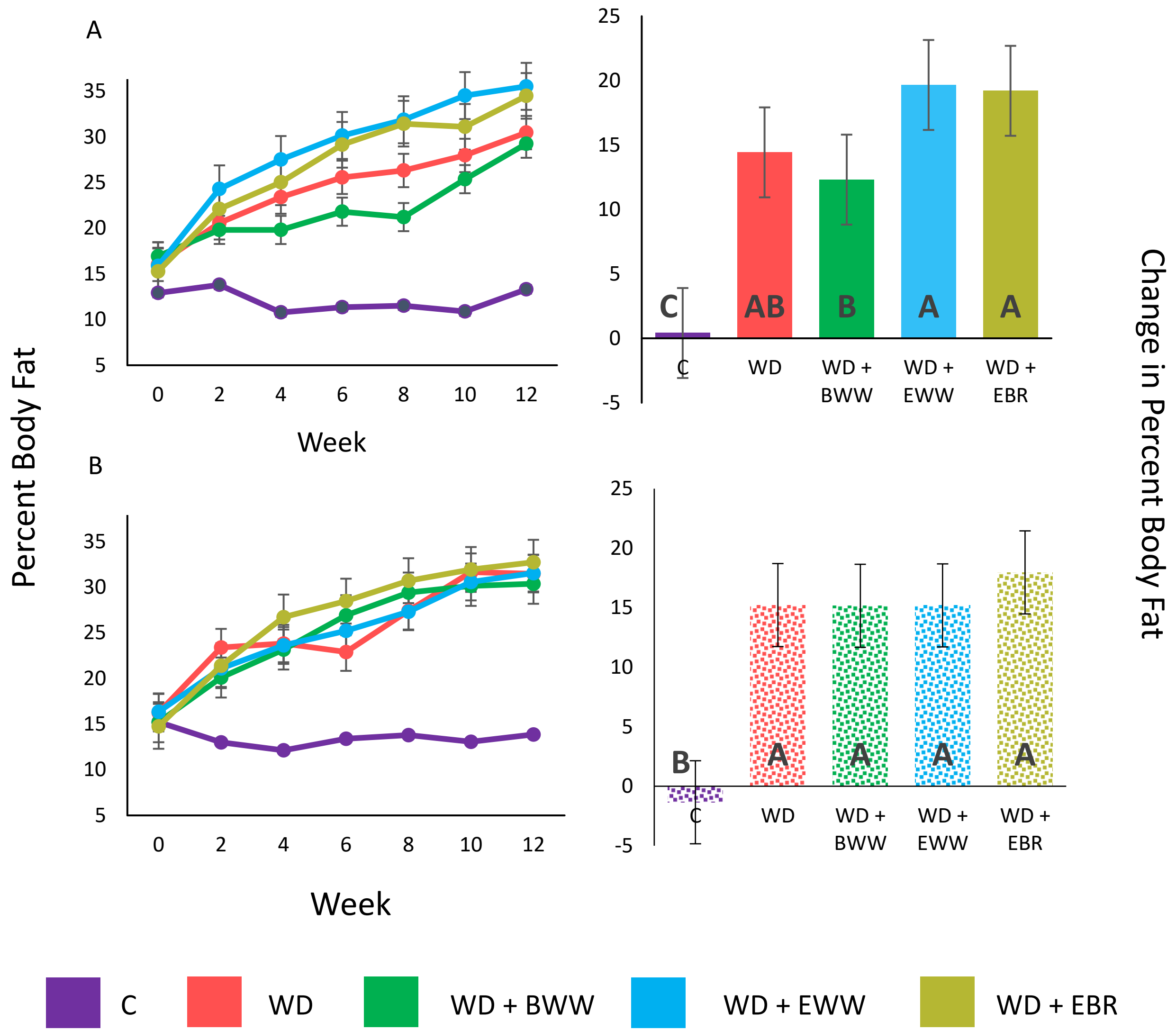

Figure 4.10. Change in body composition based on percent body fat due to change in percent body fat for microbiome A (A) and microbiome B (B) due to dietary treatment C (control), WD (western diet), WD + BWW (western diet + boiled whole wheat), WD + EWW (western diet + extruded whole wheat), WD + EBR (western diet + extruded brown rice). Error bars express standard error.

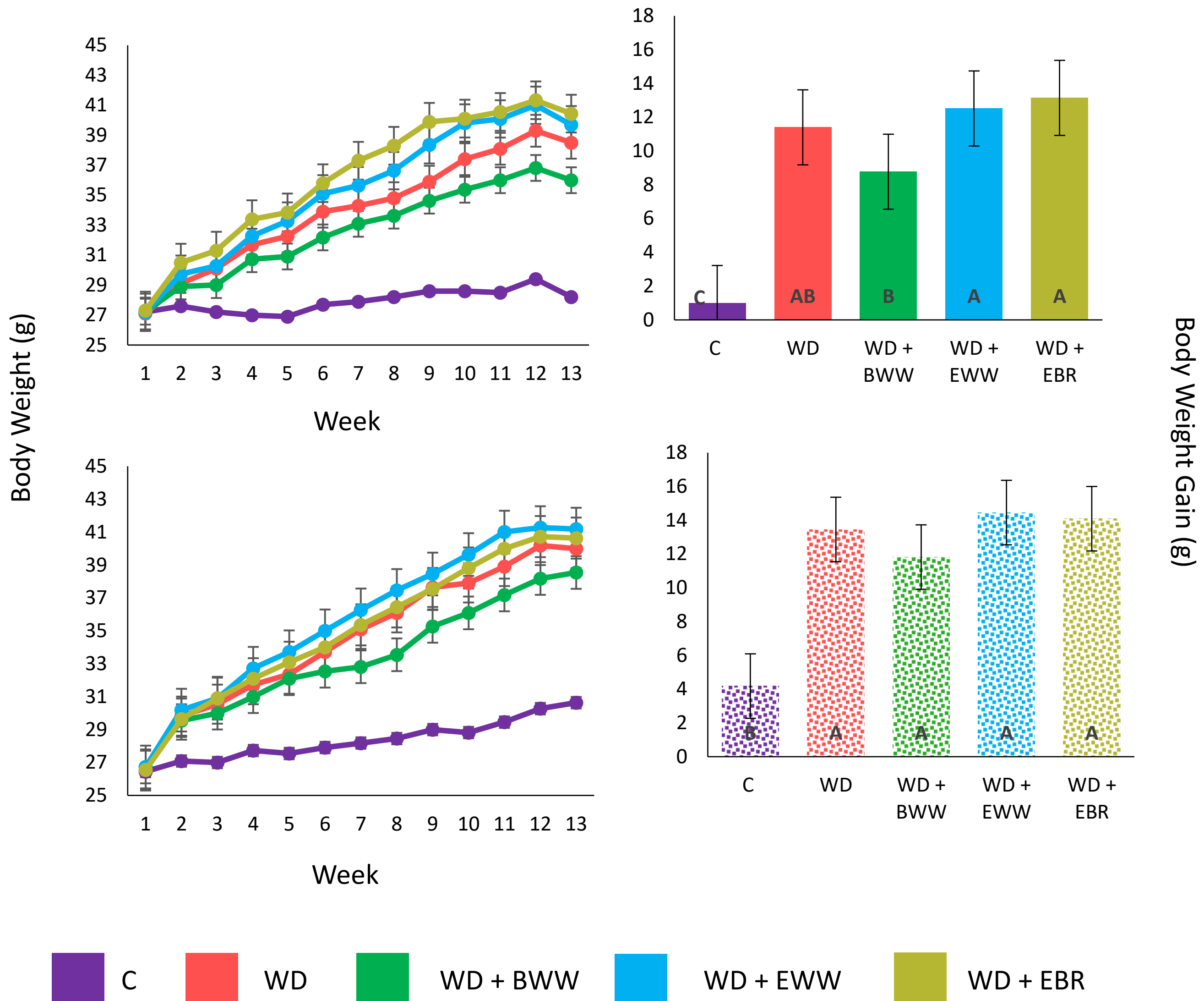

Figure 4.11. Change in body weight for microbiome A (A) and microbiome B (B) due to dietary treatment C (control), WD (western diet), WD + BWW (western diet + boiled whole wheat), WD + EWW (western diet + extruded whole wheat), WD + EBR (western diet + extruded brown rice). Error bars express standard error.

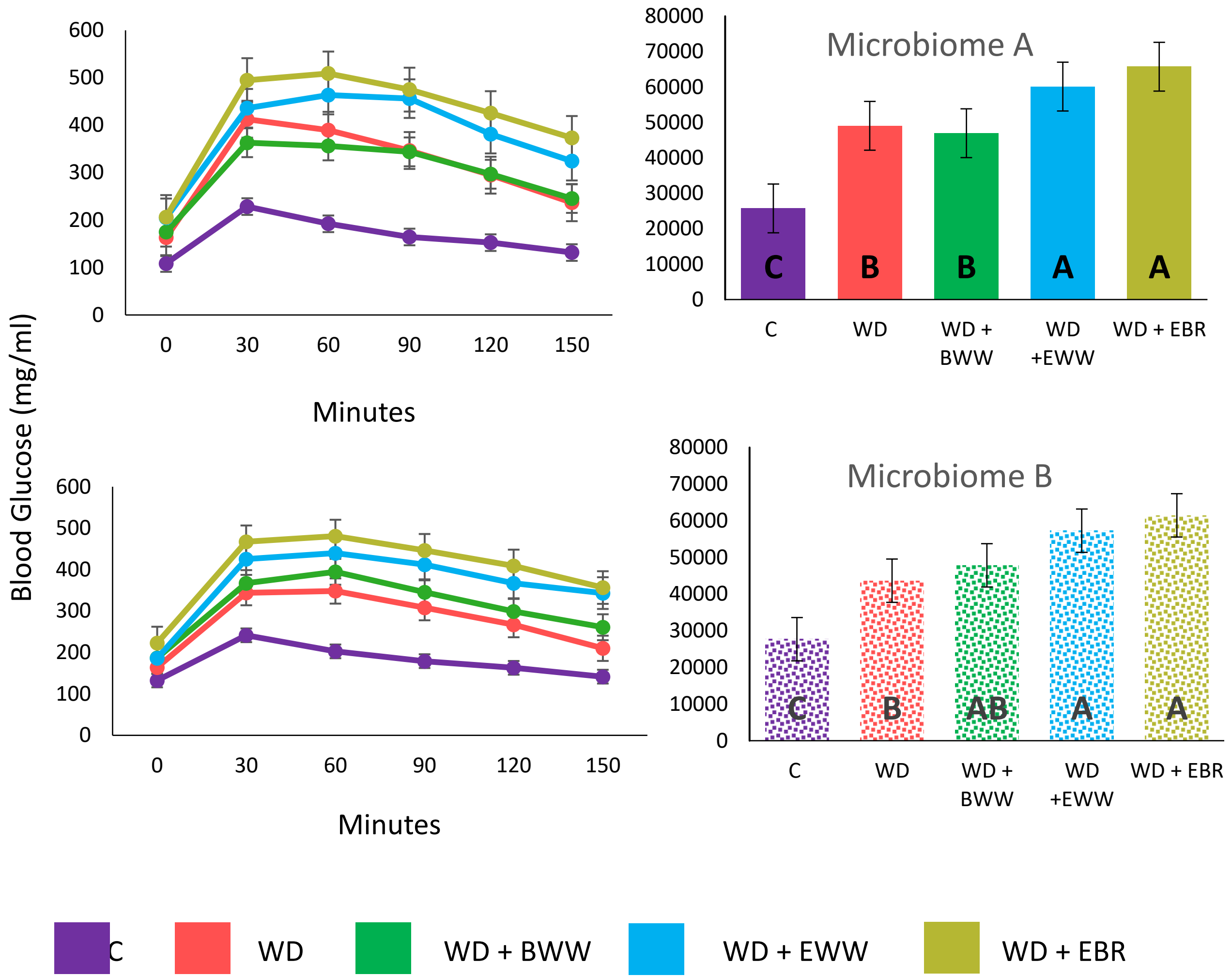

Figure 4.12. Glucose intolerance for microbiome A (A) and microbiome B (B) due to dietary treatment C (control), WD (western diet), WD + BWW (western diet + boiled whole wheat), WD + EWW (western diet + extruded whole wheat), WD + EBR (western diet + extruded brown rice). Area under the curve of glucose intolerance response for microbiome A (C) ad microbiome B (D). Error bars express standard error.

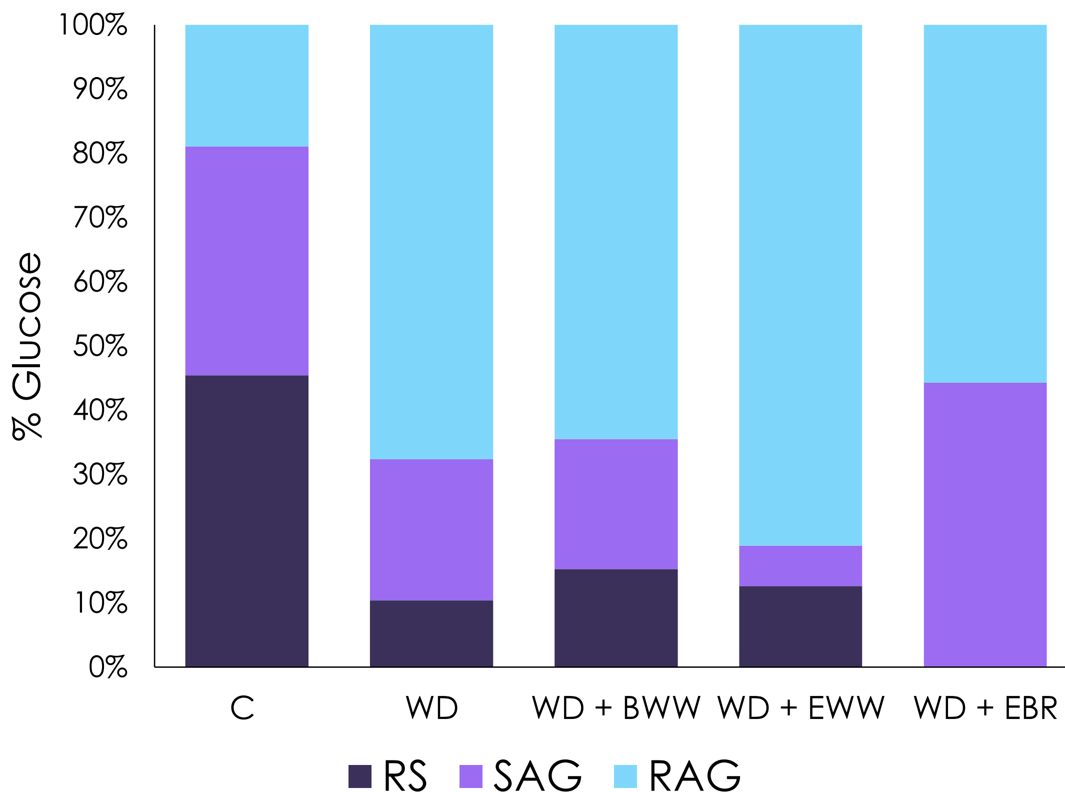

Figure 4.13. *In vitro* starch digestibility profile of experiment diets C (control), WD (western diet), WD + BWW (western diet + boiled whole wheat), WD + EWW (western diet + extruded whole wheat), WD + EBR (western diet + extruded brown rice). Rapidly available glucose (RAG), the glucose available after 20 minutes, slowly available glucose (SAG), the glucose available after 120 minutes, and resistant starch (RS), the starch not digested within 120 minutes.