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The effects of fermented vegetable consumption on the composition of the intestinal microflora and levels of inflammatory markers in women: A pilot and feasibility study

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THE EFFECTS OF FERMENTED VEGETABLE CONSUMPTION ON THE
COMPOSITION OF THE INTESTINAL MICROFLORA AND LEVELS OF
INFLAMMATORY MARKERS IN WOMEN: A PILOT AND FEASIBILITY STUDY

by

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A dissertation submitted to the Department of Nutrition and Dietetics

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Dedication and Acknowledgements

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Abbreviations

CRP C-reactive protein, d day, CVD cardiovascular disease, g grams, GI gastrointestinal, HMP Human Microbiome Project, IBD inflammatory bowel disease, IBS irritable bowel syndrome, IL interleukin, ISAPP International Scientific Association for Probiotics and Prebiotics, LBP lipopolysaccharide-binding protein, LPS Lipopolysaccharide, NF- κ B nuclear factor-kappa beta, RA relative abundance, rRNA ribosomal RNA, SCFAs short-chain fatty acids, T2D type 2 diabetes, TGF transforming growth factor, TNF tumor necrosis factor, UC ulcerative colitis

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Abstract

Background: The gut microbiome plays a key role in metabolic disease development. Diet is a modifiable factor that significantly influences gut microbial composition, and fermented foods are a reliable source of probiotic microorganisms that can contribute to gut homeostasis. The primary objective of this study was to explore the feasibility of fermented vegetable consumption for six weeks on markers of inflammation and gut microflora profiles in women.

Methods: Thirty-one women consumed 100 g/day of fermented vegetables (group A), non-fermented vegetables (group B), or no vegetables (group C) for six weeks. Dietary intake was assessed twice during the intervention by a food frequency questionnaire. Participants provided fasting blood samples and stool samples before and after the intervention. Next-generation sequencing of the V4 region of the 16S rRNA gene was performed on the Illumina MiSeq 500 platform. Nonparametric tests were used to analyze the data.

Results: Participants' ages ranged between 18 and 69 years. Compliance with vegetable consumption was 82% and 87% in groups A and B, respectively. We found 28 significant Pearson's correlation coefficients between diversity and diet and metabolic biomarkers. There were no significant changes in levels of inflammatory markers among groups. At timepoint 2, Group A showed an increase in *Faecalibacterium prausnitzii* ($P=.022$), a decrease in *Ruminococcus torques* ($P<.05$), and an upward trend in alpha diversity measured by the Shannon index ($P=.074$).

Conclusions: This suggests that regular consumption of fermented vegetables may shift gut microbiota towards a more beneficial composition. Further feeding trials test the role of regular consumption of fermented vegetables on metabolic markers and the gut microbiome are needed to determine whether consumption of fermented vegetables is an effective strategy against gut dysbiosis.

Chapter One: Literature Review

1. Introduction

1.1. The Gut Microbiome

The gut microbiome may be referred to as the collective entity of genes, microbes, and their metabolites present in the gut, whereas gut microbiota is the group of microscopic organisms present in the microbiome.^{1,2} We now know microbiome homeostasis relies on the symbiotic relationship between the host and bacteria, archaea, viruses, protozoans, fungi, and metabolites such as vitamins, amino acids, short-chain fatty acids (SCFAs) and that imbalances in gut homeostasis may lead to inflammation and disease.³ The gut microbiome is fundamental to human health and a deep understanding of interplay between microbes and host is of significant medical importance to understand disease development and treatment options.

1.1.1. Composition

In 2008, the Human Microbiome Project (HMP) was funded by the National Institutes of Health to begin a well-organized, comprehensive catalogue of reference genomes of the intestinal microbiota and their communities through metagenomic analysis to identify taxonomic and functional information for disease intervention.⁴⁻⁷ Various metagenomic analysis strategies, such as analysis of the bacterial 16S ribosomal RNA (rRNA) gene, are used to identify and sequence gut microbiota. The 16S rRNA gene is present in all bacteria, and analysis of its subregions provides estimates of microbiota composition and diversity.² Over 100 trillion (10^{14}) microorganisms, containing approximately 2172 bacteria species, over 200 genera, and 12 phyla have been found to inhabit the human gastrointestinal (GI) tract. Out of the 12 phyla that make up the human microbiota, Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria are the dominate phyla with Bacteroidetes and Firmicutes comprising about 90%.^{1,2,8,9} Table 1 shows examples of the taxonomic classification of different bacteria.

Table 1: Examples of Taxonomic Gut Microbiota Composition of the Predominate Four Phyla.^{10,11}

Phylum	Bacteroidetes	Firmicutes	Proteobacteria	Actinobacteria
Class	Sphingobacteriia, Bacteroidia	Clostridia, Bacilli	Gamma proteobacteria, Epsilon proteobacteria	Actinobacteria, Coriobacteria
Order	Sphingobacteriales, Bacteroidales	Clostridiales, Lactobacillales	Enterobacteriales, Campylobacteriales	Actinomycetales, Bifidobacteriales, Coriobacteriales
Family	Sphingobacteriaceae, Bacteroidaceae	Clostridiaceae, Lactobacillaceae, Staphylococcaceae	Enterobacteriaceae, Helicobacteraceae	Corynebacteriaceae, Bifidobacteriaceae, Coriobacteriaceae
Genus	Sphingobacterium, Bacteroides	Clostridium, Lactobacillus, Enterococcus	Escherichia, Helicobacter	Corynebacterium, Bifidobacterium, Atopobium
Species	<i>Bacteroides fragilis</i> , <i>Prevotella</i> spp.	<i>Clostridium</i> spp., <i>Roseburia intestinalis</i> , <i>Lactobacillus reuteri</i>	<i>Escherichia coli</i> , <i>Helicobacter pylori</i>	<i>Bifidobacterium longum</i> , <i>Bifidobacterium bifidum</i>

The intestinal microbial composition not only varies throughout different GI tract areas, but also changes throughout the lifespan of an individual with mode of delivery, genetics, disease, age, lifestyle, antibiotic/medication use, and diet as the main influencing factors.^{11–22} Intestinal microbiota begin to develop before birth likely from a combination of maternal diet, mother's pre-pregnancy weight and weight gain during pregnancy, gestational age, genetics, and environmental factors.²³ Mode of delivery (vaginal vs C-section) also influences infant gut microbiota with vaginal delivery resulting in an infant's microbiota that most closely resembles its mother (72% vs 41%). Rapid colonization of the gut begins after birth.² A neonate demonstrates an intestinal microflora of low diversity that is dominated by Proteobacteria and Actinobacteria. During infancy, microbial diversity increases and shifts resulting in Firmicutes and Bacteroidetes as the dominant phyla. Diet and environment continue to influence major microbial shifts until about 3 years of age when the child's microbial composition more closely resembles an adult-like profile.²⁴ Major changes in gut microbiota continue throughout life

with common gut microbiota profiles occurring among four different age groups; young (22-48 years), elderly (65-75 years), centenarian (99-104 years), and semi-supercentenarian (105-109 years).²⁵ The elderly population have increased amounts of Bacteroidetes and *Clostridium cluster IV* as compared to the young adult population, and the centenarians show decreased diversity with an abundance of facultative anaerobes (such as *Escherichia coli*) and shift in butyrate producers (such as a decrease in *Faecalibacterium prausnitzii*).² As the body ages, physiological differences occur such as changes in acid secretion by the gut mucosa and a greater gut permeability that have been linked to increased circulation of antibodies to the intestinal microflora that result in alterations of gut microbiota composition.²⁶ A small number of studies have demonstrated a gender difference in the gut microbial composition of men and women; however, these findings are inconsistent.²⁷⁻³²

1.1.2. Functions

Also known as a “superorganism”, the gut microbiome has a significant influence on health.¹¹ Research suggests that high gut microbial diversity helps achieve and/or maintain gut homeostasis by improving gut microbiome functions that reduce risk of disease.^{13,33,34} The overall functions include metabolic, protective (immune and barrier), and trophic.^{2,35}

1.1.2.1. Metabolic functions

1. Energy production from nutrient biotransformation. The gut microbiota extract energy from the fermentation of nondigestible polysaccharides and oligosaccharides into short-chain fatty acids (SCFAs).³⁵ SCFAs are the primary energy source for colonic cells and provide approximately 500-1200 calories per day for the human host³⁶ with the most of the calories getting utilized by the microbiota.³⁷ SCFAs are known influencers of energy metabolism and intake.^{38,39}

2. Conversion of nitrogenous compounds into microbial protein such as the conversion of L-histidine to histamine and glutamate to γ -amino butyric acid (GABA).³⁵
3. Breakdown of various inactive dietary polyphenols into active compounds that the body can use. For example, “the conversion of inactive isoflavones to the aglycon equol, which has antiandrogenic and hypolipidemic effects”.³⁵
4. Synthesis of vitamins B and K to extrapolate their immunomodulatory properties.^{35,40}
5. Xenobiotic and drug metabolism, which may significantly influence disease therapy. For example, *Eggerthella lenta* from the Actinobacteria phyla inactivates digoxin.³⁵
6. Positive influences on lipid metabolism via modulation of lipogenesis and fatty acid oxidation.³⁵

1.1.2.2. Protective functions

In pre-clinical and clinical research, *Akkermansia muciniphila* supplementation has demonstrated a reduction in lipopolysaccharides (LPS), improved glucose metabolism, and improved hepatic inflammatory markers; thus, contributing to reduced inflammation and improved gut barrier function.^{41–45} Commensal gut bacteria have been observed to promote the migration and function of neutrophils as well as the differentiation of the T cell population into the various types of T helper cells that help control inflammation and promote immune homeostasis through modulation of cytokines such as interleukin-22 (IL)-22, IL-17A, and IL-17F.¹⁸ Several bacterial species *Clostridium ramosum*, *Eggerthella lenta*, *Coprococcus eutactus*, *Lactobacillus casei*, *Leuconostoc mesenteroides*, and *Bacteroides uniformis*, and are associated with the expression of proinflammatory immunoglobins such as IgA and IgE that increase production of cytokines⁴⁶ such as IL-10, IL-4, transforming growth factor beta (TGF- β), and IL-6.⁴⁷ *Akkermansia muciniphila* has demonstrated that gut microflora exert antimicrobial functions by stimulating the release of various lectins with antimicrobial properties such as RegIIIc, α -defensin, and angiogenins that are secreted in response to

potential bacterial infections. These lectins function by decreasing the secretion of IL-17 that plays an important role in the onset of irritable bowel disease (IBD).⁴⁸ Also, the synthesis of SCFAs reduces the pH of the intestinal lumen that provides inhibition against intestinal pathogens,⁴⁹ prevention of pro-inflammatory bacterial translocation, and maintenance of gut epithelial barrier protection.³⁹ SCFAs such as butyrate have been observed to bind to G-protein coupled receptor 4 (GPR4) inhibiting the activation of the nuclear factor-kappa beta (NF-κB) pathway that is typically activated during inflammation. The binding of SCFA to GPR43 is one of the pathways that has been observed to regulate immune responses in the gut.⁵⁰ The production of extracellular polysaccharides by bacterial strains has immunomodulatory effects by shielding the intestinal lining from inflammatory factors such as the antimicrobial peptide LL-37 and IL-17; thereby, preventing the occurrence of inflammatory processes that might be caused by antigens from foreign bacterial particles.^{51,52} The production of polysaccharide A provides significant anti-inflammatory effects in the gut through the activation of regulatory T cells.⁵⁰ Gut homeostasis provides structural integrity of the gut barrier that protects against colonization of pathogens into the GI tract and translocation of microbiota into circulation; thus, preventing a systemic infection.³⁵ The compartmentalization of the gut microbiota is performed by lamina propria macrophages. The macrophages perform this function by phagocytosing commensal gut microorganisms that penetrate the intestinal epithelial cell barrier. Bacteria that succeed in penetrating the intestinal epithelial cell barrier are engulfed by dendritic cells that reside in the intestinal mucosa.⁵³

1.1.2.3. Trophic functions

Many trophic functions that contribute to the development of the immune system occur as a result of cross-talk between the gut microbiota and its host's immune system. Gut microbiota can modulate epithelial cell proliferation and differentiation, intestinal motor activity, induction and homeostatic regulation of adaptive immunity, and neuroendocrine pathways.

Cross-talk can induce regulatory T cells that increase the host's tolerance to gut antigens; thus, preventing inflammation.⁵⁴ Specifically, *B. lactis* has demonstrated an increase of peripheral blood leukocytes and natural killer cells that play a prominent role in the recognition and destruction of pathogenic viruses, bacteria, and tumor cells.⁵⁵

1.1.3. Short Chain Fatty Acids

As metabolites of species such as *Bacteroides*, *Bifidobacterium*, *Streptococcus*, *Coprococcus catus*, *Anaerostipes*, *Roseburia*, *Salmonella*, *Dalister*, *Ruminococcus*, and *Faecalibacterium prausnitzii*,⁵⁶ SCFAs (butyrate, acetate, and propionate) are key players in microbiome health due to their role in metabolic (i.e. glucose, lipid, appetite, and pancreatic) and immune regulation.^{39,57,58} Mostly, SCFAs have positive correlation with health benefits; however, overproduction of SCFAs has promoted adverse health reactions.⁵⁷⁻⁶¹ For example, butyrate is essential in the pathogenesis of IBD due to its anti-inflammatory effects,²¹ but in high concentrations, it has been positively correlated to obesity and insulin resistance. Perhaps these correlations are related to various concentrations of SCFAs and/or their absorption capability in the gut.^{57,59,60}

1.1.4. Dysbiosis

Dysbiosis refers to an imbalance of gut microflora⁶² that is associated with diseases such as obesity,^{63,64} IBD (colitis and Crohn's disease)⁶⁵⁻⁶⁸ irritable bowel syndrome (IBS),^{69,70} celiac disease, metabolic disease, cardiovascular diseases,^{50,71} and fatty liver disease.^{74,75} Initially thought to arise from an increased Firmicutes to Bacteroidetes ratio, it is now known that dysbiosis can occur as a result of changes in other gut microbiota species as well.^{8,77,78} Koliada and colleagues⁷⁹ demonstrated that obesity is positively correlated with higher levels of Firmicutes and lower levels of Bacteroidetes as compared to those of normal weight. Furthermore, obese individuals have elevated levels of pro-inflammatory cytokines and a less

diverse microbiota as compared to healthy subjects.^{80,81} Million et al⁸¹ reported an increase in *Lactobacillus reuteri* and a decrease in *Bifidobacterium animalis* in obese subjects relative to healthy volunteers.⁸¹ A study by Farup et al⁸² that aimed to highlight the gut-brain axis, examined psychobiological disorders in morbidly obese adolescents and found that approximately 62% of the study subjects had dysbiosis along with significant abdominal complaints such as food intolerance that were scored using the IBS Severity Score system.⁸² However, new research by Magne and colleagues⁶⁴ proposed that the influx of studies reporting that a higher Firmicutes to Bacteroidetes ratio is a marker of obesity-related dysbiosis may be inaccurate. Discrepancies in supporting research may be explained by, interpretative bias, methodological differences, poor characterization of recruited subjects, and/or lifestyle variables known to affect microbiota composition. The highly variable relative abundances (RA) of Firmicutes and Bacteroidetes across participants is likely related to the multitude of microflora-related diet and lifestyle factors and the inadequate stratification of patients in subgroups that makes this obesity biomarker less convincing.⁶⁴

A study conducted on type 1 diabetic children showed a reduction in gut microbiota diversity with an unfavorable Firmicutes/Bacteroidetes ratio in the participants' stool samples.⁸³ Studies conducted by de Goffau⁸⁴ demonstrated that pre-diabetic children with antibodies against β -cells showed an increased number of Bacteroides with a decrease in lactate and butyrate producing bacteria being observed in the study subjects compared to healthy controls.⁶⁰

Several studies have correlated microbiota with type 2 diabetes.^{85–88} A recent study by Zhao et al⁸⁸ compared the gut microflora of 65 type 2 diabetic (T2D) patients, 49 with diabetic complications and 16 without complications, and 35 healthy controls. Through 16S mRNA analysis of fecal samples, these authors found the RA of Proteobacteria and the Firmicutes/Bacteroidetes ratio was higher among the T2D patients as compared to the control

subjects. Furthermore, the T2D patients also showed significant disorders in SCFAs, bile acids, and lipids when compared to the control subjects. For example, the abundances of the SCFA producers, Lachnospiraceae and Ruminococcaceae were significantly increased among the T2D patients suggesting that these bacterium families have other functions than just SCFA production. The genera *Bacteroides* and *Prevotella* were significantly lower in the control group, but *Prevotella* species were increased in obesity and hypertension subjects. Altogether, 44 microbes of various taxa were identified to have significant correlations with the metabolic traits of body mass index (BMI), blood glucose levels, blood pressure, blood cholesterol, fecal bile acids, and fecal lipids.⁸⁸ Moreover, *Bifidobacteria* have a potential role in improving the maintenance and remission of mild-to-moderate ulcerative colitis (UC) along with the prevention of pouchitis relapse, IBS, constipation, antibiotic-associated diarrhea, necrotizing enterocolitis (NEC), colorectal cancer, and non-alcoholic steatohepatitis (NASH) among several other extra-intestinal diseases such as cardiovascular disease and psychiatric disorders.⁷⁶

1.2. The Gut Microbiome and Inflammation

Through a symbiotic relationship, gut microbiota play a fundamental role in the induction and function of the innate and adaptive immune system.⁸⁹ Inflammation begins when cell surface receptors react to adverse stimuli that trigger inflammatory pathways.⁹⁰ Dysbiosis stimulates inflammation due to an imbalance of commensal and pathogenic bacteria in addition to the production of microbial antigens and metabolites that activate tissue-resident macrophages contributing to metabolic diseases such as diabetes, obesity, and metabolic syndrome.³ Macrophages 1 (M1) and 2 (M2) are two major inflammatory phenotypes. M1 is signaled by LPS and T helper 1 (proinflammatory) cytokines, whereas M2 phenotype is triggered by T helper 2 (allergic) and anti-inflammatory cytokines.³ Some inflammatory biomarkers such as cytokines, LPS, lipopolysaccharide-binding protein (LBP), tumor necrosis factor alpha (TNF-

α) and C-reactive protein (CRP) have been used in the research of dysbiosis-related disease.^{91–}

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1.2.1. Cytokines

During inflammation, macrophages and adipocytes secrete inflammatory cytokines.⁹⁸ Cytokines are pleiotropic signaling proteins that regulate many biological functions such as inflammation, immunity, hematopoiesis, and cellular proliferation and differentiation. Extensive research has highlighted cytokine's involvement in disease pathogenesis and immune homeostasis.⁹⁹ Cytokines act by binding to a receptor that sends a signal to the recipient cell causing a change in function. Types of cytokines include interferons (IFN), interleukins (IL), adipokines, transforming growth factors (TGF), and tumor necrosis factors (TNF). Cytokines are classified according to the cell type that secretes them. Lymphokines such as IL-17, IL-17F, IL-22, IL-10, IFN- γ , TGF- β , IL-1, IL-2, and, TNF- α are secreted from lymphocytes such as T cells that regulate immune responses to antigens. Growth factors such as TGF- β promote cell survival. Chemokines such as CCL2, CCL9, CCL10, CCL11, IL-8 are chemotactic for inflammatory cells. Proinflammatory cytokines such as IL-1, IL-6, IL-8, TNF- α , TNF- β , IFN- γ amplify the inflammatory process, whereas anti-inflammatory cytokines such as IL-10, IL-11, IL-13, granulocyte-macrophage colony stimulating factor (GM-CSF), and TGF- β attenuate the inflammatory response. However, some cytokines such as IL-4, IFN- γ , and GM-CSF are bi-functional and show stimulatory and inhibitory effects. Research shows a significant association between dysbiosis and increased levels of TNF- α and IL-6.⁹³ A comprehensive study by Schirmer and colleagues⁹¹ demonstrated that gut microbiota composition is linked to cytokine production.

1.2.2. Tumor Necrosis Factor-Alpha

Gut microbiota also play an important role in TNF- α response.⁹² TNF- α , a pro-inflammatory cytokine mainly produced by adipocytes and macrophages,¹⁰⁰ regulates cell survival through

cell proliferation, differentiation, and apoptosis.¹⁰¹ Known for its role in initiating an inflammatory cytokine cascade, TNF- α signals through the two transmembrane receptors TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2). While both receptors are important for cytotoxicity and NF- κ B activation, TNFR1 is more responsible for cell growth and TNFR2 is targeted towards lymphoid cell proliferation. Increased TNF- α production and TNF receptor signaling are correlated to the pathogenesis of many diseases such as Crohn's disease, diabetes, and obesity.¹⁰¹

1.2.3. Lipopolysaccharide (LPS) and Lipopolysaccharide-Binding Protein (LBP)

The endotoxin LPS is a component of the outer membrane of many gram-negative bacteria found in the gut and is a crucial inducer of inflammatory responses.^{100,102,103} Chronically elevated LPS levels can cause metabolic endotoxemia that is a condition characterized by low grade inflammation, insulin insensitivity, and an increased prevalence of cardiovascular disease (CVD) and obesity.^{103–109} LBP, a biomarker of endotoxemia,⁹⁷ is an acute phase protein that is mainly produced by the liver and helps mediate the biological actions of LPS. Research suggests that LBP may be a better inflammatory marker than LPS and may fill a "diagnostic gap" between other inflammatory markers.^{110–114} The LPS recognition by Toll Like Receptor 4 (TLR4) is a critical pathway of the innate immune system. This pathogen-associated molecular pattern uses the key accessory proteins, LBP, CD14, and MD-2, for LPS to be recognized by TLR4 and result in a pro-inflammatory signaling cascade. First, LBP is needed to dissociate the LPS monomer from LPS aggregates such as micelles and carry the LPS monomer to a CD14 molecule. Then the CD14 molecule dissociates the LPS monomer from the LBP and facilitates the transfer of LPS to the TLR4/MD-2 receptor complex which leads to homodimerization of TLR4. This process causes dimerization of the cytoplasmic TIR-domain (Toll-interleukin-1 receptor) that provides a binding site for MyD88 (myeloid differentiation primary response gene 88). Finally, the transcription factor NF- κ B and MAP

(mitogen-activated protein) Kinase pathways are activated resulting in expression of pro-inflammatory cytokines.¹¹⁵ Pro-inflammatory cytokines such as IL-6 and TNF- α are secreted from adipocytes; thus, showing how the LPS-mediated inflammatory response correlates to obesity.⁹⁴ Research has found increased LPS and LBP levels among overweight and obese individuals as compared to normal-weight individuals,^{94,97,116–118} and that positive changes in gut microbiota can reduce these levels.^{97,117,119}

1.2.4. C-Reactive Protein

Dysbiosis is significantly associated with increased levels of CRP.⁹³ CRP, an acute-phase protein that is synthesized by hepatocytes, is positively correlated with chronic inflammation and related diseases^{120,121} and is associated with the gut microbiota.¹²² A recent review by Munckhof et al¹²² concluded that the abundance of gut bacteria such as *Bifidobacterium*, *Faecalibacterium*, *Ruminococcus*, and *Prevotella* was inversely related to the inflammatory markers CRP and IL-6; thus, demonstrating the importance of bacterial changes in the microbiome for the modulation of systemic inflammation. Obesity and dysbiosis are correlated to increased CRP and LPS levels^{94,103,123} among individuals with and without diabetes.^{38,108,124,125} In a study conducted by Visser et al,¹²³ increased CRP levels were found in overweight and obese subjects likely as a result of the release of IL-6 from adipocytes that mediate the synthesis and release of the CRP.

1.3. The Gut Microbiome and Diet

Diet significantly affects gut microbiota throughout the lifespan of an individual.^{21,126–128} An infant's diet of breast milk will tend to favor a simple intestinal microbiota with *Bifidobacteria* being the predominant bacteria while formula fed infants have enriched amounts of *Bifidobacteria* and *Clostridia*.^{126,129} Fiber has a significant impact on gut microbiome. Various types of plants have different chemical compositions, physicochemical properties, and fibers.

Plant-based foods contribute several types of fiber including prebiotic fibers such as β -glucan and pectins; thus, supporting a more diverse microbiota composition.¹³⁰ Prebiotic fibers found in fermented and non-fermented vegetables contribute to the growth of probiotic bacteria as they move through the GI tract untouched until fermented by the colon.^{131,132} The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus panel proposed the most recent definition of prebiotic as, “A substrate that is selectively utilized by host/commensal microorganisms conferring a health benefit.”¹³³ Moreover, this symbiotic relationship of pro- and prebiotics may be classified as a functional food.¹³⁴ For example, research demonstrated that prebiotics may also reduce body fat through altering intestinal microflora among obese and overweight children.¹³⁵ Another study noted that improved IBS symptoms after sauerkraut consumption were likely related to prebiotics rather than lactic acid bacteria strains.¹³⁶ Salazar and colleagues³⁸ reported that inulin-type fructan prebiotic promoted alterations in gut microbiota composition by selectively modulating *Bifidobacterium* spp. and decreasing fecal SCFA concentration in obese women. Thus, prebiotic consumption may decrease metabolic risk factors associated with higher fecal SCFA concentration in obese individuals.³⁸ There exist striking differences in the microbiota of children living in rural Africa than their counterparts from Western Europe. Children from Burkina Faso, whose diet was principally composed of dietary fiber, had a high prevalence of *Bacteroides* that are specifically known to contain genes that encode for molecules that play a role in the hydrolysis of polysaccharides found in dietary fiber. Two other gut bacteria genera, *Prevotella* and *Xylanibacter*, were observed to be more prevalent in the African population than in the Western European population. *Prevotella* and *Xylanibacter* synthesize enzymes necessary for the hydrolysis of cellulose and xylan. Moreover, European children had significantly less SCFAs in their fecal matter than those in the African cohort.⁵⁰ A polysaccharide-rich diet such as a low-fat/high-fiber diet is correlated with an increased amount of Actinobacteria and

Bacteroidetes and a decreased amount of Firmicutes, while the high-fat/low-fiber diet rich in sugar and animal protein is correlated with a significantly lower amount of Actinobacteria and Bacteroidetes.^{16,137} Vegans, vegetarians, and omnivores will have completely different microbiome profiles.^{20,21,138,139} The total amount of *Bacteroides* spp., *Bifidobacterium* spp., *Escherichia coli*, and *Enterobacteriaceae* spp. was lower in vegan fecal samples than in the control subjects. Maintaining a vegetarian diet results in a complete shift in microbiota diversity towards microbial compositions associated with health benefits.^{20,138,139} An 8-week randomized control trial of 82 healthy, overweight/obese subjects with metabolic disease risk factors such as habitually low fruit and vegetable intakes and sedentary lifestyles aimed to explore the effects of an isocaloric Mediterranean diet (MD) on the gut microbiome. Forty-three participants consumed a MD diet with energy intake tailored to their typical dietary intake, and 39 participants functioned as the control group and consumed their typical dietary patterns. The MD group demonstrated decreased low-density lipoprotein, plasma cholesterol, inflammation, *Ruminococcus gnavus* (proinflammatory species) abundance, urinary carnitine levels, insulin resistance, and increased *Faecalibacterium prausnitzii* (SCFA-producing species) abundance, butyrate metabolism, urinary urolithins, and fecal bile acid degradation.¹⁴⁰ A Western-type diet that is high in animal protein and fat, and low in fiber, led to a decreased microbial diversity. Specifically, a decrease in the beneficial *Bifidobacterium* and *Eubacterium* species was noted.^{137,141} Furthermore, a preclinical study by Sonnenburg et al¹⁴² reported that a low fiber diet such as the Western diet causes a reduced gut microbiota diversity and that after many generations this may lead to extinction of some gut microbiota compounds. Humans who consume a large proportion of dietary fiber, including those who live in developing countries and rural areas, have lower incidences of inflammatory diseases such as IBD, colitis, and diabetes due to positive changes in gut microbiota.^{21,84,139,143,144} An animal-based diet was shown to increase bile tolerant microorganisms such as *Alistipes*, *Bilophila*, and *Bacteroides*,

and decrease *Roseburia*, *Eubacterium rectale*, and *Ruminococcus bromii* that metabolize plant polysaccharides.^{145,146} Furthermore, due to the high sulfur content of animal-derived protein and the H₂S (hydrogen sulfide) toxin hypothesis, an omnivore's diet may play a significant role in the pathogenesis of ulcerative colitis (UC). Consumption of high sulfur-containing compounds such as amino acids methionine, cysteine, and taurine largely found in animal proteins¹⁴⁷ increases H₂S production, increases sulfate-reducing bacteria such as *Desulfovibrio* spp., and alters the microbial community that may lead to an increased risk of IBD.⁶⁶ Additionally, the intestinal microbiota of omnivores was shown to synthesize significantly more trimethylamine-N-oxide (TMAO) from choline and L-carnitine, whereas vegetarians show a diminished capacity to produce TMAO as a microbial metabolite. Choline and carnitine are precursors of TMAO and are primarily found in foods of animal origin. High levels of TMAO is a risk factor for atherosclerosis and IBD.^{148–150}

In addition to long-term dietary patterns having longitudinal stability on gut microbiome composition, short-term dietary intake can cause rapid changes in composition.¹⁴⁶ For example, in a preclinical review, Delzenne et al¹⁵¹ reported that a high caloric intake promoted a rapid change in gut microbiota with an increase in Firmicutes and a decrease in Bacteroidetes within 24 hours. Zarrinpar and colleagues¹⁵² demonstrated daily cyclical fluctuations in composition related to the daily feeding/fasting cycle. For example, time-restricted feeding (TRF) of nocturnal feeds resulted in Firmicutes as the most abundant bacterial species, while light-time fasting resulted in the lowest concentration of Firmicutes. Moreover, diet-induced obese mice showed a decrease in α -diversity of the gut microbiome, which can be protective against obesity, suggesting that diet-induced obesity hinders the daily feeding/fasting rhythm and according cyclical functions. Nocturnal TRF was shown to improve cyclical fluctuations and protect against metabolic diseases such as obesity. The fundamental mechanisms of the relationship between the feeding/fasting cycle and the gut microbiome are

unknown, however, nocturnal TRF was shown to increase gut microflora that influence host metabolism demonstrating that timely feeding patterns in addition to diet are important contributors to host metabolism.¹⁵²

1.3.1. Fermented Foods

Originally intended to preserve food, fermentation dates back thousands of years ago. The identification of microorganisms involved in fermentation was done by Van Leuwenhoek and Hooks around 1665.^{153,154} Circa 1877 Sir John Lister demonstrated the lactic acid bacteria, *Lactococcus lactis*, as the predominant species responsible for milk fermentation and consequently yogurt formation.^{154,155} Later, Louis Pasteur defined fermentation from the Latin word “Feverē” that means life without air. The discoveries regarding fermented vegetables coincided with Europe’s industrial revolution era that led to a population exodus from the rural to the urban areas. As a consequence, food production was involved in large scale preparations.^{154,156} As the production of fermented products improved to modern-day, large-scale productions, the use of well-defined starter cultures became popular as the use of the undefined strains used in ancient times became less popular.^{154,157} However, several drawbacks exist regarding this large-scale fermentation process. For example, nisin, a product of lactic acid bacteria, has been observed to inhibit the growth of other bacterial cultures needed for the fermentation process.^{154,158} Today, fermentation may be defined as, "Those foods or beverages made through controlled microbial growth and enzymatic conversions of major and minor food components."¹⁵⁹

The main components involved in the fermentation process include the following: microorganisms such as yeast and bacteria, organic material that needs to be fermented, a solution whereby fermentation will take place, and several tools that will be used to monitor and control the fermentation process.¹⁶⁰ The fermentation process of vegetables is as follows: the vegetables are harvested, washed, and disinfected. Salt is added to the vegetables to create

a brine solution. Then, the vegetables are soaked in the brine solution and followed by the fermentation process that is carried out between 5-30 days at 25-30°C. Drying and pressing are then carried out with the resulting fermented products being either pasteurized or packaged dry.¹⁶¹ When vegetables are sealed in a jar and undergo the fermentation process in a brine solution, the live microorganisms created increase their nutritional value¹⁶² and help restore gut microbial communities.¹⁶³ For example, the lactic acid bacteria present in fermented vegetables release enzymes and vitamins that lower intestinal pH levels through lactic acid and folic acid.¹⁶⁴ This not only results in increased bioavailability of folate, riboflavin, vitamin B12,¹⁶⁵ and iron as the lactic acid bacteria help change iron into its more absorbable form (Fe^{3+}),¹⁶⁶ but also an increased calcium ion pool¹⁶⁷ and amino acid synthesis.¹⁶⁵ Specifically, pickled vegetables and fermented soybean (tempeh) have increased vitamin B levels,¹⁶⁵ while “Tarhana” contains high concentrations of vitamins C, B₃, B₅, and B₉.^{168,169} Sauerkraut not only has increased levels of vitamins C and B, but also increased levels of minerals such as calcium, iron, potassium, and phosphorous.^{169,170} Moreover, probiotic lactic acid bacteria contain high levels of lactase that when released into the intestinal lumen aids in digestion of ingested lactose, thereby relieving the symptoms of lactose intolerance.¹⁷¹ Fermented food consumption has demonstrated significant shifts in gut microbiota towards microbial compositions related to health-promoting functions.^{172–174}

Due to different fermentation processes, confusion exists over which fermented products actually contain live microorganisms (probiotics). Some commercial foods such as pickles and olives are not fermented at all, but instead placed in a brine solution under conditions that do not lead to fermentation. Additionally, processes such as thermal processing create an inhospitable environment for microbial populations. For example, sauerkraut is often cooked after fermentation causing the live organisms to be inactivated.¹⁷⁵ However, even though some fermented vegetables no longer contain live bacteria, they remain great sources

of prebiotic fiber.^{131,176} Table 2 describes the methods of production, microorganism composition, and health benefits of four popular fermented vegetables.

Table 2: Methods of production, microorganism composition, and health benefits of popular fermented vegetables.

Type of fermented vegetables	Method of fermentation	Microorganism composition	Health benefits
Olives	Spontaneous fermentation in 8 to 10% NaCl brine solution. ¹⁷⁷	<i>Lactobacillus brevis</i> , <i>Lactobacillus coryniformis</i> , <i>Enterobacteria</i> spp., <i>Lactobacillus paracasei</i> , <i>Lactobacillus plantanum</i> ¹⁷⁸	Prevention of enteric infections such as listeria; reduction of cholesterol levels; good sources of vitamins A, B, and E. ¹⁷⁹
Kimchi	Shredded cabbage is brined in 8 to 10% salt solution for 2 to 7 hours. Addition of other vegetables such as onions and green pepper is carried with dry salt, placed in a fermentation vessel for 1 to 3 weeks at low temperatures of 2 to 10°C. ^{180,181}	<i>Lactobacillus acidophilus</i> , ¹⁸² <i>Lactobacillus plantanum</i> , <i>Lactobacillus brevis</i> , and <i>Lactobacillus mesenteroides</i> ¹⁸³	Contain high levels of vitamin B, β -carotene, dietary fiber, sodium, potassium, and calcium; β -sisosterol has demonstrated anti-cancer and anti-obesity activity; ^{182,184} Kimchi's primary nutrients include vitamins A, vitamins B1, vitamins C, vitamins K, calcium, and niacin. ¹⁸²
Sauerkraut	Freshly shredded cabbage is brined in 0.7 to 2.5% sodium chloride solution. After salting, the cabbage is placed in a fermentation vessel that is tightly sealed in order to exclude air. The fermentation process occurs usually between 1 week to several months at room temperature. ¹⁸⁵	<i>Lactobacillus brevis</i> , <i>Lactobacillus plantarum</i> , and <i>Leuconostoc mesenteroides</i> ¹⁸⁶	Components in sauerkraut such as ascorbic acid and ascorbigen are known to decrease DNA damage and cell mutation rates in patients with cancer. ¹⁸⁷ Sauerkraut is additionally rich in lactic acid bacteria that have been shown to aid in preventing lactose intolerance by producing lactase that aids in the digestion of lactose. ¹⁵⁸ Sauerkraut increases the immune system's ability to fight off pathogenic bacteria through its

			metabolite D-phenyllactic acid. ¹⁸⁸
Pickles	Pickled vegetables are submerged in a brine solution containing 3.5% of sodium chloride solution. The pickling process is usually performed at low temperatures for up to two weeks' time. Fermentation occurs in a loosely closed fermentation vessel in order to allow for the escape of gases produced during the fermentation process. ¹⁸⁹	<i>Lactobacillus brevis</i> , <i>Lactobacillus plantarum</i> and <i>Leuconostoc mesenteroides</i> ¹⁹⁰	Pickles mainly consist of vitamins such as vitamin A, riboflavin, niacin, and thiamin that help prevent anemia while lactic acid bacteria may help reduce diarrhea caused by microorganisms such as <i>E.coli</i> . ¹⁹¹ Fermented pickles harbor many lactic acid bacteria strains with conjugated linoleic acid-producing ability ¹⁹² may possess anti-carcinogenic, anti-obesity, anti-cardiovascular and anti-diabetic activities. ¹⁹³

1.4. Fermented Vegetables and Disease

While increasing in numbers and popularity, there still exists limited human studies that have evaluated the effects of fermented vegetable consumption on gut microbial composition and inflammation. Additionally, most research has been conducted in Asian countries that primarily studied the effects of kimchi, miso, and natto.¹⁹⁴ In addition to the research reported in Table 2, further discussion on the health benefits of soy, kimchi, and sauerkraut is presented below as there has been more research on these foods compared to other fermented vegetables.

1.4.1. Soy

Consumption of fermented soy products such as fermented tofu, tempeh, miso, and natto has been shown to increase gut microflora diversity and demonstrate anti-obesity, anti-diabetic, and anti-inflammatory effects.^{194,195} In a cross-sectional study that used a validated food frequency questionnaire, Wu et al¹⁹⁶ found that regular consumption of fermented soy was associated with reduced inflammatory markers such as IL-6, TNF- α , and soluble TNF receptors 1 and 2 among Chinese women. Similarly, another cross-sectional study among Japanese

workers (men and women) found that fermented soy intake was associated with decreased IL-6 concentrations in Japanese men.¹⁹⁷ Stephanie and colleagues¹⁹⁸ demonstrated that 100 mg of steamed tempeh led to increased concentrations of *Akkermansia muciniphila* and IgA among 16 healthy participants (8 women and 8 men); thus, demonstrating tempeh's potential role in immune function. A 2-week consumption of natto-containing miso soup among eight healthy participants improved gut microbiota compositions via increased abundances of Bacilli and Bifidobacteria, and decreased abundances of *Clostridium perfringens* and Enterobacteriaceae.¹⁹⁹ However, non-fermented soy products have also demonstrated these properties. Research attributes soy's health benefits to several factors such as the prebiotics, probiotics, and isoflavones (genistein and daidzein) contained in soy products. More research is needed to decipher the impact of fermented soy versus non-fermented soy on health and the mechanisms involved.^{194,195,200} For example, a reduced risk of type 2 diabetes was associated with increased consumption of fermented soy foods that were rich in phytoestrogens and bioactive peptides. In combination with estrogen receptors, the isoflavonoids and proteins contained in fermented soybeans help alleviate some of the symptoms commonly associated with type 2 diabetes such as insulin resistance. Phytoestradiol found in soybeans is similar in structure to estradiol that binds to insulin receptors found at the surface of β cells; thus, they might play an important role in the regulation of insulin synthesis and secretion by the β cells²⁰¹

1.4.2. Kimchi

Kimchi is a traditional fermented food from Korea that consists mostly of the Napa cabbage variety. A cross-over study by Kim et al²⁰² that included 22 overweight and/or obese subjects, assessed metabolic outcomes after consumption of 300 g per d (100 g/meal) of fresh or fermented kimchi for four weeks. Both groups showed a decrease in body weight. The fermented kimchi group, as compared to the fresh kimchi group, showed significant improvements in waist-to-hip ratio, fasting glucose levels, percent body fat, blood pressure,

and total cholesterol. Additionally, leptin, that is usually synthesized by genes associated with obesity, was elevated in obese subjects and positively correlated to insulin resistance. Fermented kimchi consumption was shown to have a net effect on the decrease in serum leptin levels in the study cohort. Circulating adhesion molecules such as vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM)-1 were tested due to their reported positive correlation with increased cholesterol levels in obese individuals with type 2 diabetes. As such, activation of oxidative stress occurs leading to the attraction of inflammatory cells that in turn overexpress inflammatory factors that are commonly associated with the onset of type 2 diabetes. The effect of kimchi and its ability to decrease body weight was primarily correlated to a decrease in total cholesterol, leptin, and monocyte chemoattractant protein (MCP)-1 levels. However, there was no significant differences in the levels of proinflammatory cytokines such as CRP, TNF- α , and IL-6 after consumption of fermented kimchi in the study subjects.²⁰² These results were hypothesized to be from the increased proportion of lactic acid bacteria strains in the fermented kimchi as compared to fresh kimchi. ²⁰² A randomized controlled study conducted by Han et al²⁰³ reported the contrasting effects of fresh versus fermented kimchi on the gut microbiota and gene expression related to the prevalence of metabolic syndrome in obese Korean women. Consumption of 180 g/d (60 g/meal) of fermented kimchi as compared to fresh kimchi led to improvements in gene expression profiles with regards to metabolism, immunity, and digestion. A decrease in Firmicutes and an increase in Bacteroidetes was observed after consumption of fermented kimchi. A direct correlation between increased Firmicutes and decreased Bacteroidetes among obese subjects as compared to their lean counterparts has been found by prior research. *Bifidobacterium longum* is one of the bacteria species found in fermented kimchi that is known to possess anti-obesity properties such as improvement of body weight, fasting glucose levels, and insulin sensitivity. *Bifidobacterium longum* levels increased with decreases in body weight and waist

circumference among the fermented kimchi group. Moreover, the Acyl-CoA synthetase long-chain family member 1 (*ASCL1*) gene, known for its involvement in lipid metabolism and promotion of fatty acid degradation via AMP (adenosine monophosphate)-activated protein kinase metabolic pathway, and the aminopeptidase N (*ANPEP*) gene that functions in inflammation, apoptosis, and angiogenesis were significantly elevated in subjects that consumed fermented kimchi. Specifically, these authors hypothesize that the subjects with improvements in waist-to-hip ratio, blood pressure, fasting blood glucose and insulin, and total cholesterol were correlated to the increased expression in these two genes.²⁰³ The ability of kimchi to improve the serum lipid profile in healthy young adults was also probed. Choi¹⁸³ demonstrated that total cholesterol, blood glucose levels, and low-density lipoprotein significantly decreased after seven days of intake of 15 g per d of fermented kimchi. *Lactobacillus acidophilus* that is predominantly found in fermented kimchi has been shown to exert its cholesterol lowering properties by binding to cholesterol in their cell wall leading to decomposition for assimilation in the gut. Consumption of fermented kimchi was thus observed to have an effect in regulating the metabolic profile of human subjects.¹⁸³

1.4.3. Sauerkraut

A six-week, randomized, double-blinded study by Nielsen and colleagues²⁰⁴ investigated the effects of daily lacto-fermented sauerkraut on IBS symptoms of 34 Norwegian patients (15 consumed a pasteurized sauerkraut supplement and 19 consumed unpasteurized sauerkraut supplement). Gut microbiota composition was analyzed using 16S rRNA gene amplicon sequencing of fecal samples and IBS symptoms were assessed through the IBS-Symptom Severity Score (IBS-SSS) questionnaire. IBS symptoms significantly improved in both groups without significant differences between groups. Both groups also showed significant changes in gut microbiota composition with *Lactobacillus plantarum* and *Lactobacillus brevis* significantly elevated in the unpasteurized sauerkraut group. Due to significant improvements

in the measured outcomes of both groups, the authors highlight the potential effects of the prebiotics rather than lactic acid bacteria.²⁰⁴

Several lactic acid bacteria strains possess conjugated linoleic acid (CLA)-producing ability.²⁰⁵ CLA has demonstrated positive influences on gut microbiota in addition to efficacy against cancer, obesity, atherosclerosis, and diabetes.^{193,206} Lactic acid bacteria found in sauerkraut has proven effective in the reduction of pouchitis in patients with UC. Mechanisms of action of these probiotics might be through the interaction of these microorganisms with regulatory T cells and cytokine transcription factor regulation in response to disease-causing bacteria.²⁰⁷ Specifically, *Lactobacillus acidophilus* found in sauerkraut was observed to reduce the activation of the NF- κ B signaling pathway leading to a reduction of IL-8 and TNF- α in the lamina propria mononuclear cells; thus reducing inflammation in individuals with ulcerative colitis.²⁰⁸ Moreover, sauerkraut reduced the incidence of diarrhea in patients receiving pelvic irradiation and prevented urogenital infections caused by *E. coli* and other bacterial pathogens related to the presence of *Lactobacillus acidophilus*.²⁰⁹

1.5. Significance of the Problem

“Chronic diseases are the leading causes of death and disability worldwide.”²¹⁰ As of 2016, more than 1.9 billion adults and over 340 million children and adolescents worldwide were classified as overweight or obese.²¹¹ The prevalence of obesity in America has increased from 30.5% in 1999-2000 to 42.4% in 2017-2018.²¹² In 2008, the estimated annual medical cost of obesity was \$147 billion, or \$1,429 higher than those of normal weight.²¹³ By 2010, obesity-related medical care costs totaled \$315.8 billion or \$3,508 per obese individual among US adults.²¹⁴ The US diabetes prevalence is projected to increase from 9.3% in 2012 to 33% by 2050²¹⁵ with related medical costs of approximately \$245 billion per year.²¹⁶ In 2018, the prevalence of diabetes in the US was about 34.2 million Americans with approximately 210,000 of those under the age of 20.²¹⁷ CVD is the leading cause of death in the US, and by

2030, 40.5% of the US population is projected to have some form of CVD with an according increase of direct medical cost from \$273 billion in 2010 to \$818 billion in 2030.²¹⁸ In the past decade, IBD has become a major public health challenge with an estimated annual rise of 0.3% worldwide.^{143,144} In North America and Europe alone, approximately three million adults suffer from IBD.²¹⁹

Consumption of fermented foods might be a realistic strategy to decrease chronic disease. While fermented food consumption in the US is rising, a more consistent intake is needed to improve gut health and reduce disease. In addition to increasing kimchi consumption, experts suggest that Americans substitute fermented pickles and fermented sauerkraut for non-fermented pickles and cabbage as strategies to improve fermented food intake.^{220,221}

1.6. Problem Statement

There exists a high prevalence of dysbiosis-related diseases worldwide that are correlated to poor dietary habits.^{19,83,172,222} Several strategies have been proposed to tackle the accumulating disease rates. Till date, strategies used to treat these underlying conditions involve the use of surgery (inflammatory bowel diseases and obesity), insulin (to lower blood glucose levels), dietary changes and exercise, and medication.^{223–226} There is limited evidence of the effect of consuming fermented vegetables on changes in the gut microbiome and disease.^{173,182,227,228} Feeding trials are needed to determine whether consumption of fermented vegetables is an effective strategy to prevent and treat disorders associated with inflammation. Consuming fermented vegetables for the prevention and treatment of disease might prove to be a least expensive option.

1.7. Research Questions and Hypothesis

1.7.1. Research questions

1. What is the impact of regular consumption of fermented vegetables for six weeks on metabolic markers of adult women?
2. What is the impact of regular consumption of fermented vegetables for six weeks on the intestinal microbiome of adult women?

1.7.2. Study aims and hypothesis

Study aim 1: To examine the effects of regular consumption of fermented vegetables for six weeks on markers of metabolic syndrome and inflammation in women.

Hypothesis 1: Regular consumption of 100 g of fermented vegetables for six weeks will improve obesity-related markers such as blood pressure, insulin, adiposity, and inflammatory markers such as C-reactive protein and lipopolysaccharide in women.

Study aim 2: To examine the effects of regular consumption of fermented vegetables for six weeks on the profile of the gut microflora

Hypothesis 2: Regular consumption of 100 g of fermented vegetables will lead to a shift in microbial communities towards an increase in communities associated with health benefits, such as Bifidobacteria and Lactobacilli.

Chapter Two: Methods

2. Study Design

This was a six-week, parallel arm, pilot and feasibility trial aimed at testing the effects of regular consumption of fermented vegetables on inflammation and the composition of the gut bacteria in women (clinical trial registration: NTC03407794). Participants were randomly assigned to one of three treatment groups: Group A (fermented vegetable group), Group B (non-fermented vegetable group), or Group C (control group). Over the course of six weeks, participants in the vegetable groups were asked to consume 100 g of vegetables per day in addition to following their regular diet. All vegetables were provided by the study. Group C was asked to follow their regular diet. Feces, urine, and blood samples were collected at two time points, baseline prior to randomization and at completion of the intervention. Group B served as a positive control, as subjects randomized into this group were instructed to consume the same vegetables as Group A, but without the presence of live bacteria.

All fermented vegetables were provided weekly by a local producer. All non-fermented vegetables were obtained at a local grocery store. The non-fermented vegetables were comprised of shelf-stable pickles and sauerkraut. The study staff portioned out the vegetables using food safety precautions and delivered the vegetables to participants every two weeks. The microbial compositions of all four vegetable types; fermented sauerkraut, fermented pickles, non-fermented sauerkraut, and non-fermented pickles are shown in supplementary Figures S1-S5.

2.1. Study Participants

Due to funding specifications, this pilot study aimed at recruiting 35 to 40 female participants. Inclusion criteria were:

- Non-smoker
- No previous diagnosis of cancer

- No thyroid disease
- No diabetes
- Willing to consume one half cup of vegetables per day for six weeks
- Not on weight loss medication
- Not on a weight loss diet
- Not taking antibiotics over the past three months
- Not consuming fermented vegetables on a regular basis
- No history of autoimmune disease, including gastrointestinal disease
- Those aged 18-70 years
- No history of a psychiatric disorder
- A signed informed consent

Exclusion criteria:

- Smoker
- Taking medications that affect appetite or body weight
- Uncontrolled hypertension
- Not willing to consume one half cup of vegetables daily for six weeks
- Not willing to show up at two appointments
- Following a fad diet
- Using antibiotics frequently
- Diagnosed with autoimmune disease such as psoriasis, rheumatoid arthritis, thyroid disease, and colitis
- Regular consumption of fermented vegetables or probiotics

- Having diminished capacity to consent (i.e. limited decision-making capacity, difficulty hearing, cognitive impairment that may impact understanding or compliance with nutritional counseling)
- Non-English speaking or other language barrier
- Having chronic kidney disease
- Having any form of cancer that impacts nutritional status or undergoing radiation or chemo
- Being treated for a psychiatric disorder or taking monoamine oxidase inhibitors
- Not willing to provide informed consent

This project was approved by the University of North Florida Institutional Review Board (IRB) and all participants provided informed consent prior to starting the study.

2.2. Study Procedures

2.2.1. Recruitment

This study recruited only women due to their high accessibility and desire to limit significant variability of sex among participants. Flyers were posted at the UNF Women's Center and Jacksonville Women's clinics, and newspaper advertisements were also used to recruit participants. In addition, a recruitment email was sent to a random sample of females affiliated with the University of North Florida. Willing participants were instructed to contact the staff via phone or email using the contact information provided in the recruitment material. Once contacted by a potential participant, the study staff performed a screening interview to further confirm eligibility criteria. The flow of participants through the study is shown in Figure 1.

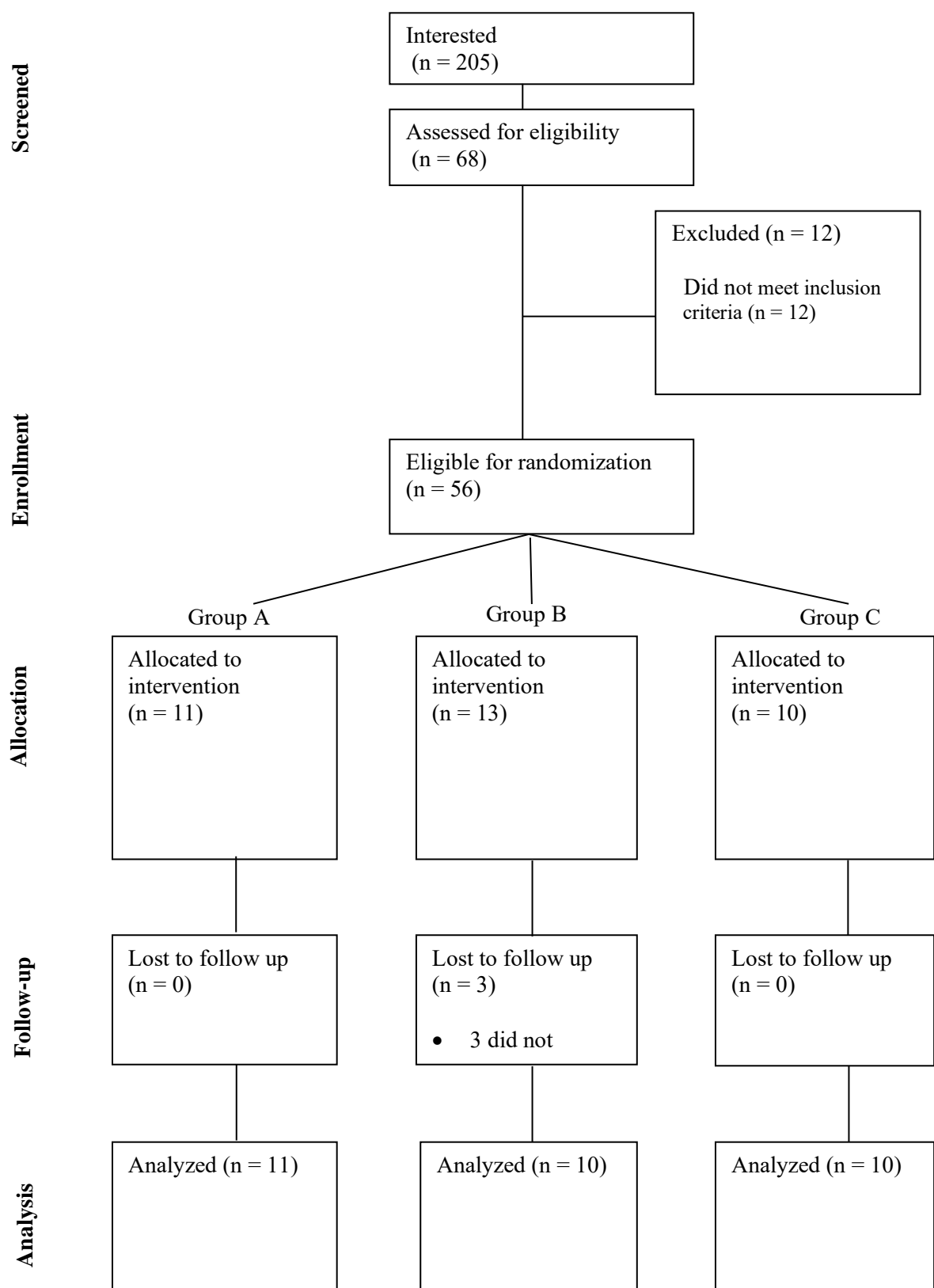


Figure 1. Flow of participants

2.3. Study Visits

Those who were eligible completed an in-person orientation session where the study procedures were explained in detail. At the orientation session, the consent form was reviewed and a baseline clinic visit was scheduled for those who signed the consent form. All participants who scheduled a baseline clinic visit at the orientation sessions received a urine and stool collection kit. Participants were instructed to collect a stool sample within 24 hours of their baseline clinic visit and the first-morning urine on the day of the baseline clinic visit. Coolers and ice packs were provided to help participants maintain the urine and stool samples cold until the morning of the clinic visit. At the baseline clinic visit, participants provided their urine, stool, and blood samples, and randomization into one of the three groups took place. Those randomized into one of the vegetable groups, made arrangements for the pick-up and delivery of the vegetables. Similarly, prior to the follow-up clinic visit, participants were provided with more supplies to collect urine and stool samples.

2.4. Processing of biological samples

Once received at clinic visits, all biological samples were stored at -70C until analysis. Blood was collected in two 8-mL red top tubes and left at room temperature for 30 minutes before centrifugation at room temperature for 10 minutes at 1400 rpm. Both serum and urine were transferred to 1.5 mL cryogenic tubes in 1-mL aliquots. Stool samples were transferred to 1.0 mL cryogenic tubes in 150 mg-aliquots.

2.5. Data Collection

2.5.1. Surveys

Participants completed online surveys to assess food intake, demographics, and prescription medication intake. The DHQ-3,²²⁹ a 135-item food frequency questionnaire designed by the National Cancer Institute, was used to assess the participant's diet intake at baseline and

follow-up. As shown in Figure S6, participants were given a log to record their daily vegetable intake, gastrointestinal function (frequency of defecation and consistency of stools), and side effects (bloating, diarrhea, constipation, and headache).

2.5.2. Compliance

Participants reported daily intake of vegetables by filling out a log sheet (see Figure S6).

2.5.3. Clinical Data

Study staff members obtained participants' height, weight, and body composition at each clinic visit. A Detecto 439 Eye Level Beam Physician Scale 400lb x 4oz with Height Rod was used to measure height in centimeters to the nearest 0.1 cm. Weight and percent body fat were measured by multifrequency bioelectrical impedance (InBody 570, Cerritos, CA.) Blood pressure was measured twice by a nurse using a sphygmomanometer.

2.6. Measurement of Biomarkers

C-Reactive Protein (CRP) and Tumor Necrosis Factor (TNF) alpha were measured by commercial ELISA kits (Cat#DCRP00 for CRP and Cat#DTA00D for TNF alpha, R&D Systems, Minneapolis, MN). Lipopolysaccharide Binding Protein (LBP) was measured by a Pierce LAL chromogenic endotoxin quantitation kit (Cat#88282, ThermoFisher Scientific, Waltham, MA) and a sandwich enzyme immunoassay (catalog# DINS00, R&D Systems, Minneapolis, MN) was used to measure insulin levels. All analyses were conducted in the laboratory of Dr. Arikawa at the University of North Florida.

2.7. Microbial Data Analysis

DNA was extracted from the frozen stool samples with the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Germantown, MD, USA) per manufacturer's protocol. A NanoDrop One (Thermo Fisher Scientific, Madison, WI, USA) was used to measure DNA concentration and diluted to 10 ng/μL. DNA extraction and next-generation sequencing of the V4 region of the

16S rRNA gene were performed. Amplicon PCR was performed on the V4 region of 16S rRNA using the forward (5'-GTGCCAGCMGCCGCGGTAA-3') and reverse (5'-GGACTACHVGGGTWTCTAAT-3') primers. PCR (polymerase chain reaction) amplicons were barcoded and pooled in equal concentrations using the SequalPrep Normalization Plate Kit (Invitrogen, Carlsbad, CA, USA). qPCR (quantitative PCR) was used to quantify consolidate libraries using the Kappa Library Quantification Kit (Roche, Indianapolis, IN, USA), and the quality of the library was determined by an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). Sequencing was performed in a pair-end modality on the Illumina MiSeq 500 platform rendering 2 x 150 bp paired-end sequences (Illumina, San Diego, CA, USA)). Sequencing reads were analyzed using mothur v1.39.1²³⁰ following the MiSeq SOP, including steps for quality-filtering, alignment against a 16S reference database (SILVA v132), and clustering into operational taxonomic units (OTUs) with a pairwise 97% identity threshold. The OTUs were then classified using the Ribosomal Database Project database.²³¹ Mothur v1.39.1. was used to calculate alpha diversity (microbial diversity within each sample) and beta diversity (microbial diversity between samples).²³² For alpha diversity, we used observed operational taxonomic units (OTUs) to measure microbial richness (number of species present),²³³ Chao1 to measure species abundance,^{234,235} and the Shannon index to measure species richness and evenness (distribution).^{236,237} Three indices were also used to measure beta diversity. A principal component analysis (PCoA) was used to discover the percent of variability and potential associations among the groups represented by the Bray-Curtis (measure of differences in taxa abundance between communities) and Jaccard index (taxa presence/absence). Associations were computed between frequencies of the components and the two PCoA axes. An analysis of similarity (ANOSIM) was used to evaluate whether gut microbiota and diet composition were significantly different among the groups.³⁴ Third, linear discriminant analysis (LDA) effect size (LEfSe) was used to identify

specific bacterial features that were enriched between conditions and diet patterns in each group or subgroup at the OTU level.³⁴ All microbial analyses were conducted in the laboratory of Dr. Jiangchao Zhao from the Department of Animal Science at the University of Arkansas.

2.8. Statistical Data Analysis

The primary goal of the data analysis was to assess the feasibility of the study and obtain data on variability of the measures for the design of future adequately-powered studies. Given that there are no studies conducted in the United States that have investigated the effects of fermented vegetables on the gut bacteria and inflammatory markers, this study employed basic statistical tests for comparisons between groups and within group. Wilcoxon tests were used to compare pre- and post-data within the treatment groups for all study outcomes. Pearson's correlation coefficients were calculated between all outcomes and alterations in the intestinal microflora. The Kruskal-Wallis test was used to compare medians across the three treatment groups at baseline, follow-up, and change (treatment effect). Change was calculated by subtracting follow-up data from baseline data.

All analyses were performed using IBM SPSS (Statistical Package for the Social Sciences), version 26. A *P*-value lower than 0.05 was considered statistically significant.

Chapter Three: Results

3.1. Characteristics of Study Participants

Out of the 205 women who showed interest in the study, 34 were randomized into either Group A (fermented vegetable), Group B (non-fermented vegetable), or Group C (control), and 31 participants completed the study. Participants' ages ranged from 18 to 69 years, weights ranged from 47.5 kg to 114.1 kg, and BMIs ranged from 18.5 to 42.3 kg/m². Participant's baseline characteristics by treatment group are shown in Table 3.

Table 3. Baseline characteristics of study participants

Characteristic ^a	Group A (n=10)	Group B (n=11)	Group C (n=10)	P-value ^b
Age (years)	37 (19-63)	44 (18-69)	27.5 (21-50)	.575
BMI (kg/m ²)	23 (19-44)	26 (18-37)	23 (21-34)	.352
Race (number, %)				
White	8 (80)	9 (81.8)	6 (60)	.477
Black	1 (10)	1 (9.1)	1 (10)	
Asian	0 (0)	0 (0)	1 (10)	
More than 1	1 (10)	1 (9.1)	2 (20)	
Ethnicity (number, %)				
Non-Hispanic	8 (80)	9 (81.8)	8 (80)	.978
Hispanic	1 (10)	2 (18.2)	2 (2)	
Did not disclose	1 (10)	0 (0)	0 (0)	
Education (number, %)				
Some college	2 (20)	2 (18.2)	2 (20)	.775
College degree	3 (30)	4 (36.4)	5 (50)	
Graduate degree	5 (50)	5 (45.4)	3 (30)	

^aData are medians (min-max) or number (%).

^bP-values represent between group comparisons among all three treatment groups using the Kruskal-Wallis test.

Group A (fermented vegetable group), Group B (non-fermented vegetable group), Group C (control group)

3.2. Regular Dietary Intake of Participants

Participant's dietary characteristics were tracked throughout the study. Within and between group comparisons were made for timepoint 1, timepoint 2, and change values (Table 4). Group A showed a significant decrease ($P=.043$) in alcohol intake at the end of the study. Group C showed a 468 calorie decrease at timepoint 2 ($P=.043$) that was likely driven by the significant decrease in total protein intake from 72 g at timepoint 1 to 57 g at timepoint 2 ($P=.043$), along with a decrease in animal protein ($P=.043$), total fat ($P=.043$), cholesterol ($P=.043$), MUFA ($P=.043$), PUFA ($P=.043$), vegetable ($P=.043$), and vitamin E ($P=.043$) intakes. Between group comparisons show significant differences for animal protein ($P=.009$) and cholesterol ($P=.015$) intakes at timepoint 1, and cholesterol intake at timepoint 2 cholesterol ($P=.023$).

Table 4. Dietary characteristics^a

Variable	Timepoint 1	Timepoint 2	Change	P-value ^b
Energy (kcal/d)				
Group A	1264 (628)	1284 (793)	116 (837)	.859
Group B	1413 (816)	1185 (1069)	-276 (952)	.735
Group C	1586 (451)	1118 (816)	-418 (645)	.043
P-value ^c	.398	.929	.275	
Total Fat (g/d)				
Group A	42 (37)	44 (43)	11 (36)	.767
Group B	46 (38)	37 (54)	-7 (52)	.866
Group C	54 (29)	54 (32)	-7 (11)	.043
P-value ^c	.304	.874	.592	
Total Carbohydrate (g/d)				
Group A	168 (90)	181 (132)	-4 (152)	.678
Group B	191 (120)	187 (95)	-28 (114)	.866
Group C	207 (46)	121 (105)	-86 (136)	.080
P-value ^c	.573	.356	.365	
Total Protein (g/d)				
Group A	49 (27)	43 (51)	10 (23)	.260
Group B	51 (70)	60 (46)	-10 (31)	.398
Group C	72 (22)	57 (14)	-20 (20)	.043
P-value ^c	.085	.849	.068	
Animal Protein (g/d)				

Group A	23 (11)	31 (32)	2 (23)	.314
Group B	32 (13)	39 (39)	-3 (34)	.866
Group C	48 (26)	42 (13)	-13 (17)	.043
<i>P</i> -value ^c	.009	.676	.096	
Cholesterol (mg/d)				
Group A	108 (125)	133 (156)	25 (66)	.066
Group B	152 (202)	253 (272)	30 (189)	.612
Group C	269 (250)	205 (171)	-152 (230)	.043
<i>P</i> -value ^c	.015	.514	.023	
Total SFA (g/d)				
Group A	123 (9)	12 (24)	4 (17)	.214
Group B	15 (4)	14 (21)	1 (20)	.612
Group C	18 (7)	18 (11)	-3 (6)	.138
<i>P</i> -value ^c	.309	.900	.280	
Total MUFA (g/d)				
Group A	17 (15)	10 (15)	3 (15)	.859
Group B	18 (11)	13 (14)	-2 (19)	.866
Group C	22 (11)	20 (14)	-5 (6)	.043
<i>P</i> -value ^c	.304	.976	.454	
Total PUFA (g/d)				
Group A	10 (8)	15 (12)	3 (7)	.139
Group B	12 (12)	8 (13)	-1 (14)	.398
Group C	12 (15)	10 (8)	-1 (1)	.043
<i>P</i> -value ^c	.652	.973	.158	
Starch Intake (g/d)				
Group A	53 (24)	69 (55)	17 (46)	.139
Group B	48 (43)	47 (39)	-14 (36)	.398
Group C	48 (24)	42 (34)	-11 (27)	.225
<i>P</i> -value ^c	.980	.609	.125	
Fiber Intake (g/d)				
Group A	20 (18)	21 (15)	1 (9)	.477
Group B	21 (29)	24 (17)	-2 (22)	.398
Group C	18 (9)	11 (13)	-5 (7)	.080
<i>P</i> -value ^c	.598	.224	.063	
Sugar Intake (g/d)				
Group A	101 (80)	72 (91)	-6 (77)	.767
Group B	103 (44)	115 (42)	11 (61)	.310
Group C	112 (59)	60 (47)	-52 (90)	.080
<i>P</i> -value ^c	.910	.054	.059	
Glycemic Load (total/d)				
Group A	145 (74)	123 (110)	4 (114)	.678
Group B	144 (69)	152 (74)	-4 (95)	.499

Group C	164 (33)	88 (77)	-65 (114)	.080
<i>P</i> -value ^c	.724	.373	.165	
Alcohol Intake (g/d)				
Group A	5 (10)	0 (2)	-5 (9)	.043
Group B	1 (10)	1 (1)	1 (4)	.917
Group C	3 (9)	5 (7)	-1 (14)	.500
<i>P</i> -value ^c	.790	.110	.218	
Vegetable Intake (cups/d)				
Group A	1 (3)	2 (2)	1 (1)	.110
Group B	2 (2)	2 (3)	0 (1)	.866
Group C	1 (1)	1 (1)	0 (1)	.043
<i>P</i> -value ^c	.936	.391	.070	
Vitamin A Activity (RE/d)				
Group A	982 (765)	774 (743)	165 (595)	.260
Group B	1254 (1533)	1388 (1518)	-154 (546)	.499
Group C	976 (924)	981 (1726)	-207 (434)	.080
<i>P</i> -value ^c	.620	.835	.403	
Vitamin E Intake (IU/d)				
Group A	13 (11)	11 (12)	-0 (7)	.767
Group B	14 (11)	14 (7)	-1 (14)	.310
Group C	13 (8)	10 (7)	-2 (4)	.043
<i>P</i> -value ^c	.740	.427	.260	
Vitamin C Intake (mg/d)				
Group A	75 (112)	107 (62)	16 (68)	.441
Group B	102 (112)	111 (78)	6 (35)	.735
Group C	113 (93)	81 (90)	-70 (102)	.080
<i>P</i> -value ^c	.823	.427	.065	
HEI 2015 Score (total/d)				
Group A	70 (15)	68 (18)	-3 (14)	.260
Group B	71 (19)	68 (8)	-4 (10)	.237
Group C	67 (23)	63 (13)	2 (9)	.686
<i>P</i> -value ^c	.945	.250	.507	

^aValues are reported as median (IQR).

^b*P*-values represent within group comparisons between timepoint 1 and timepoint 2 obtained using the Wilcoxon Signed Ranks Test.

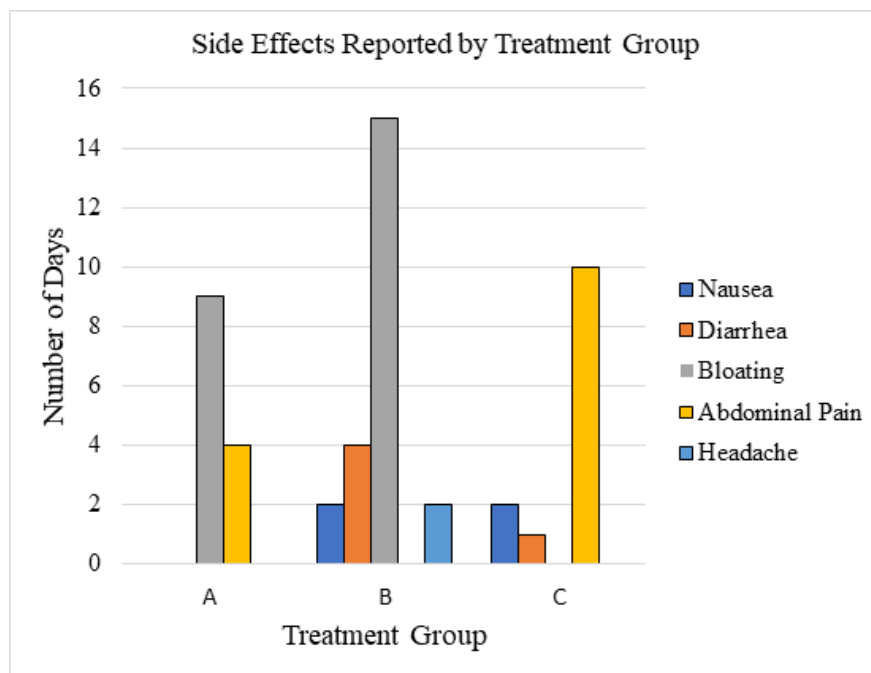
^c*P*-values represent between group comparisons among all three groups for timepoint 1, timepoint 2, and change obtained using the Kruskal-Wallis test.

Group A (fermented vegetable group), Group B (non-fermented vegetable group), Group C (control group), SFA (saturated fatty acid), MUFA (monounsaturated fatty acid), PUFA (polyunsaturated fatty acid), HEI (healthy eating index)

3.3. Vegetable Consumption of Study Participants

Group A consumed an average of 91 g/d for 32 days (82% compliance) and Group B consumed an average of 91 g/d for 36 days (87% compliance).

3.3.1. Side Effects of Vegetable Consumption



Percent of participants who reported side effects:

- Group A (fermented vegetable): 22.4%
- Group B (non-fermented vegetable): 32.8%
- Group C (control): 17.5%

3.4. Metabolic Biomarkers

Participants' clinical parameters are shown in Table 5. Significant changes between groups were found for body fat mass (BFM) ($P=.048$) and percent body fat PBF ($P=.015$). Group C showed the largest reductions in BFM, PBF, weight, and systolic blood pressure (SBP).

Table 5. Clinical parameter changes after six weeks of intervention

Clinical parameter ^a	Group A (n=11)	Group B (n=10)	Group C (n=10)	P-value ^b
BMI (kg/m ²)				
Timepoint 1	22.7 (7.3)	26.1 (4.4)	22.9 (6.0)	0.594
Timepoint 2	23.3 (7.0)	26.7 (4.0)	22.8 (5.0)	0.317
Change	0.3 (0.5)	0.1 (1.1)	-0.1 (0.8)	0.255
P-value ^c	0.058	0.964	0.443	
BFM (kg)				
Timepoint 1	18.8 (19.3)	26.2 (10.4)	18.0 (11.3)	.493
Timepoint 2	20.0 (17.7)	27.4 (9.1)	17.3 (10.0)	.239
Change	1.0 (2.0)	-0.02 (1.8)	-0.6 (1.0)	.024
P-value ^c	0.131	0.894	0.008	
PBF (%)				
Timepoint 1	30.4 (22.6)	36.7 (5.6)	32.4 (12.8)	.769
Timepoint 2	31.4 (21.0)	36.8 (6.0)	31.1 (12.0)	.478
Change	0.6 (2.3)	0.0 (1.2)	-0.9 (1.9)	.019
P-value ^c	0.247	0.859	0.011	
DBP (mmHg)				
Timepoint 1	81.0 (14.0)	75.5 (16.0)	75.0 (16.0)	.599
Timepoint 2	75.0 (17.0)	72.5 (10.0)	70.0 (13.0)	.241
Change	-4.0 (9.5)	-4.5 (10.5)	-5.0 (20.0)	.720
P-value ^c	0.476	0.389	0.374	
SBP (mmHg)				
Timepoint 1	118.0 (18.0)	110.5 (15.0)	114.0 (23.0)	.804
Timepoint 2	121.0 (19.0)	107.0 (18.0)	104.0 (14.0)	.093
Change	2.0 (6.5)	1.0 (10.5)	-11.0 (21.0)	.091
P-value ^c	0.858	0.866	0.037	
TNF (pg/mL)				
Timepoint 1	2.8 (4.0)	4.5 (2.0)	3.7 (3.0)	.378
Timepoint 2	2.6 (6.0)	4.4 (2.0)	3.1 (6.0)	.651
Change	-0.16 (0.6)	-0.20 (1.5)	0.14 (3.3)	.764
P-value ^c	0.314	0.374	0.575	
CRP (ng/mL)				
Timepoint 1	129.2 (308)	209.2 (229)	251.9 (1370)	.268
Timepoint 2	173.4 (375)	211.4 (228)	160.7 (746)	.772
Change	24.6 (198)	-39.1 (103)	-34.6 (214)	.101
P-value ^c	0.214	0.086	0.139	
LBP (µg/mL)				
Timepoint 1	13.3 (4.0)	14.8 (6.0)	12.8 (2.0)	.232
Timepoint 2	13.0 (5.0)	12.7 (5.0)	12.7 (7.0)	.621
Change	2.1 (6.8)	-2.4 (2.4)	0.3 (6.3)	.893
P-value ^c	0.508	0.066	0.721	

^aData are shown as median (IQR) or number (%).^bP-values represent within group comparisons using the Wilcoxon Signed Rank test.^cP-values represent between group comparisons among all three groups using the Kruskal-Wallis test.

TNF (tumor necrosis factor), CRP (C-reactive protein), BMI (body mass index), Wt (weight), BFM (body fat mass), LBF (lean body mass), PBF (percent body fat), DBP (diastolic blood pressure), SBP (systolic blood pressure), LBP (lipopolysaccharide binding protein) Group A (fermented vegetable group), Group B (non-fermented vegetable group), Group C (control group), TP1 (timepoint 1), TP2 (timepoint 2), TNF (tumor necrosis factor), CRP (C-reactive protein), BMI (body mass index), Wt (weight), BFM (body fat mass), LBF (lean body mass), PBF (percent body fat), DBP (diastolic blood pressure), SBP (systolic blood pressure), LBP (lipopolysaccharide binding protein)

3.5. Alpha Diversity

Microbial alpha diversity (within sample) can be analyzed on all taxa levels from phyla to subspecies and can be compared across groups. Although more indices exist, alpha diversity is typically measured using three indices; richness (number of species present such as observed OTUs²³³), species abundance (concentrations of the same species such as the Shannon^{236,237} and Chao1 index^{234,235}), and evenness (distribution of species such as the Shannon index^{236,237}).^{34,238} Alpha diversity measures such as taxa abundance and taxa level, have correlated specific bacteria to disease phenotypes.^{239,240}

3.5.1. Alpha Diversity of Vegetables

Alpha diversity of the fermented and non-fermented vegetables is represented via taxa abundance, Shannon index, and observed OTUs as shown in Figures S1-S5. Figure S1 shows Firmicutes as the predominant phyla. Figure S2 shows Bacillales as the main genus. As represented by the Shannon index in Figure S3 and the observed OTUs in Figure S4, the non-fermented sauerkraut contained the most alpha diversity among the four diet groups (non-fermented pickles, non-fermented sauerkraut, fermented pickles, and fermented sauerkraut). Lastly, Figure S5 ranks the top 20 observed OTUs per relative abundance.

3.5.2. Alpha Diversity of Stool

Alpha diversity measures of participants' stool samples were also computed for taxa abundance, Shannon index, and observed OTUs. Firmicutes, Actinobacteria, and Bacteroidetes

represented the three predominant phyla among group and individual abundances. When stratified by treatment group and timepoint, Firmicutes remained the predominant phyla across all treatment groups (Figure 2). No significant abundance differences were found for within or between group comparisons using the Wilcoxon signed-rank and Kruskal-Wallis test, respectively. When stratified per individual, Firmicutes was the main phylum among most participants, but not all participants as shown in Figure 3.

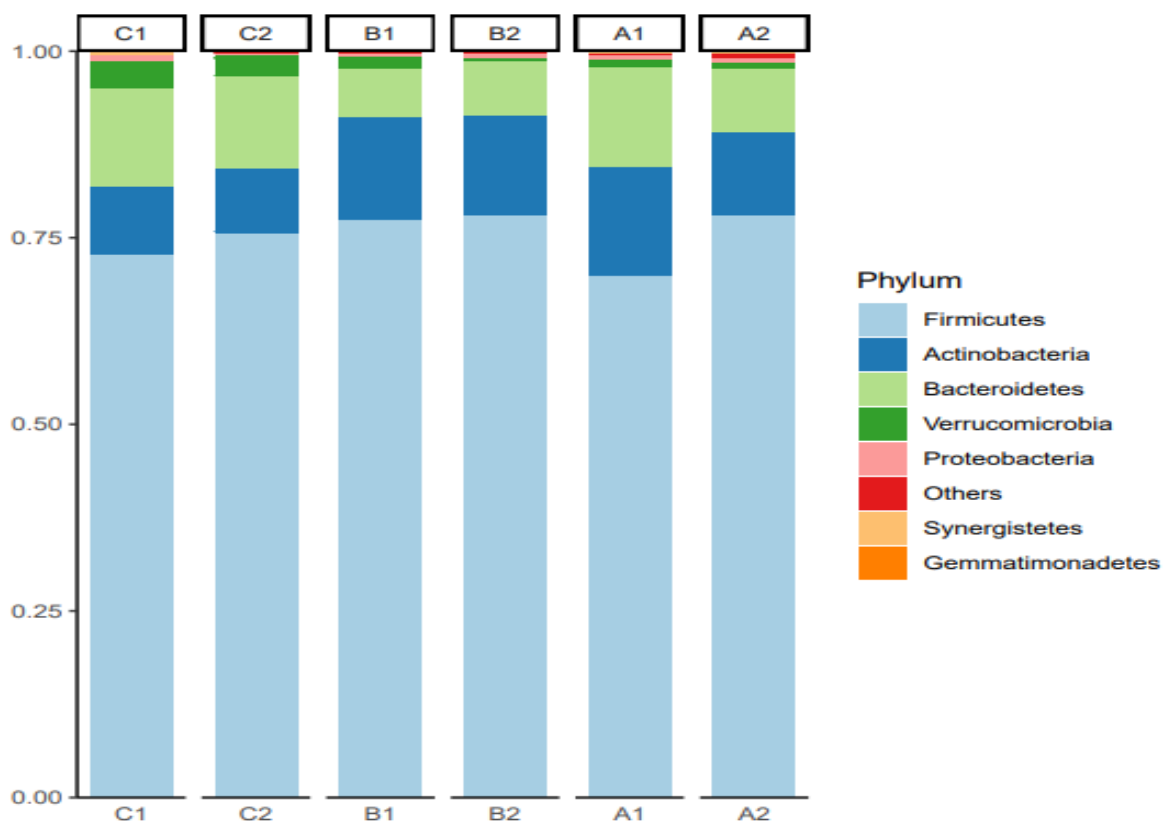


Figure 2. Taxonomy of phyla ranked by relative abundance and stratified by treatment group. Firmicutes' shows the highest abundance range of 70%-78% among all groups. A1 and A2 (fermented vegetable group timepoint 1 and timepoint 2), B1 and B2 (non-fermented vegetable group timepoint 1 and timepoint 2), and C1 and C2 (control group timepoint 1 and timepoint 2)

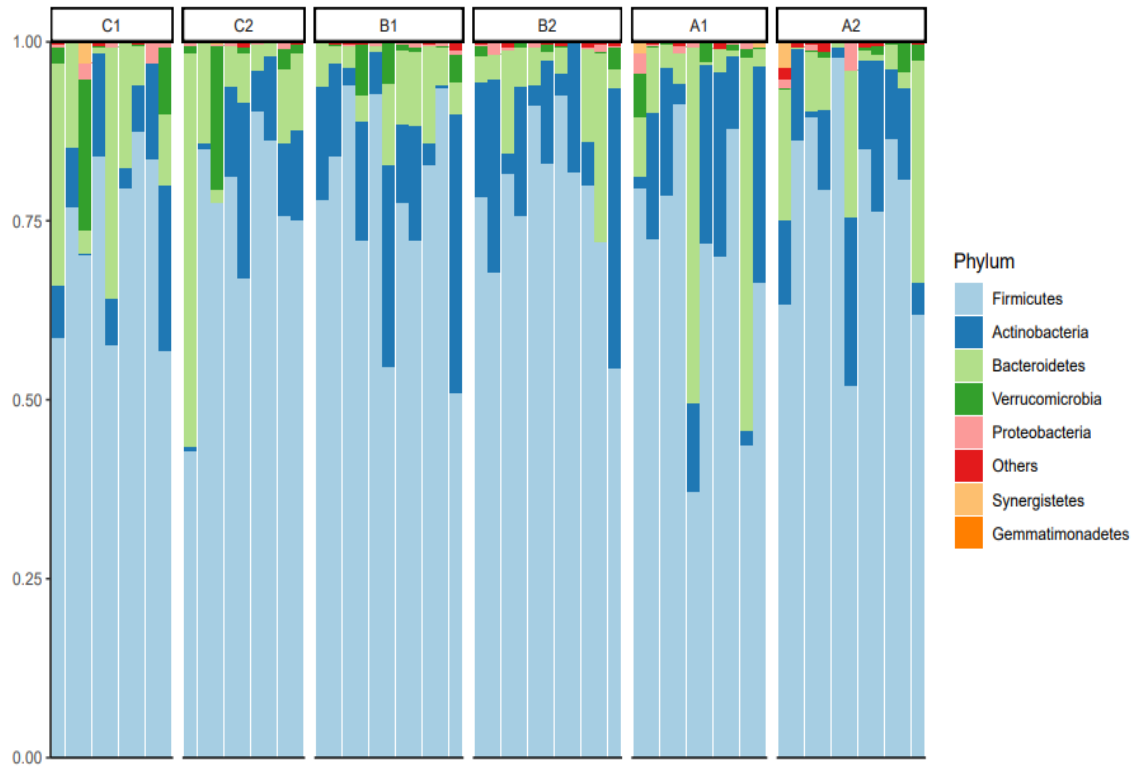


Figure 3. Taxonomy of phyla ranked by relative abundance stratified by participant. A1 and A2 (fermented vegetable group timepoint 1 and timepoint 2), B1 and B2 (non-fermented vegetable group timepoint 1 and timepoint 2), and C1 and C2 (control group timepoint 1 and timepoint 2)

Relative abundance on the genus level shows *Blautia* as the predominant genus across treatment groups with a relative abundance of 17-24% (Figure 4). No significant differences were found for within group comparisons using the Wilcoxon signed-rank test. Between group comparisons (Kruskal-Wallis test) showed significant diversity for Pasteurellaceae ($P=.045$) and *Antinomyces* ($P=.035$) genera; however, due to their low overall abundances, these genera are not shown in Figure 4. When stratified by individuals, *Blautia* remained the predominant genus among most, but not all individuals as shown in Figure 5.

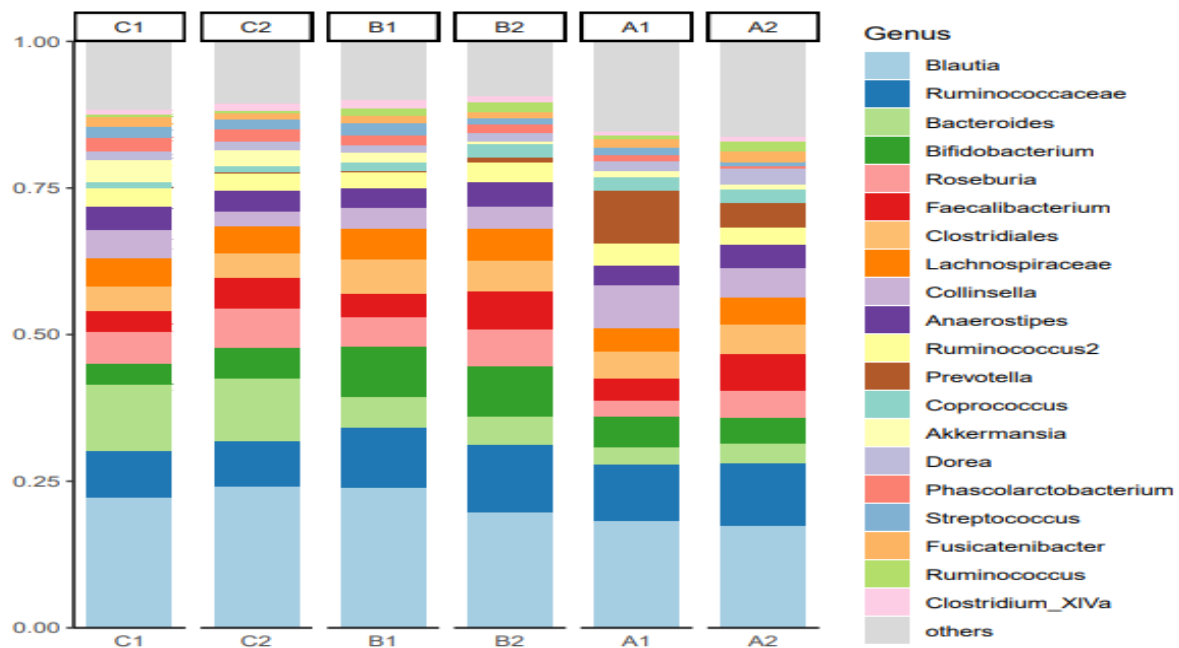


Figure 4. Genus taxonomy ranked by relative abundance stratified by group. A1 and A2 (fermented vegetable group timepoint 1 and timepoint 2), B1 and B2 (non-fermented vegetable group timepoint 1 and timepoint 2), and C1 and C2 (control group timepoint 1 and timepoint 2)

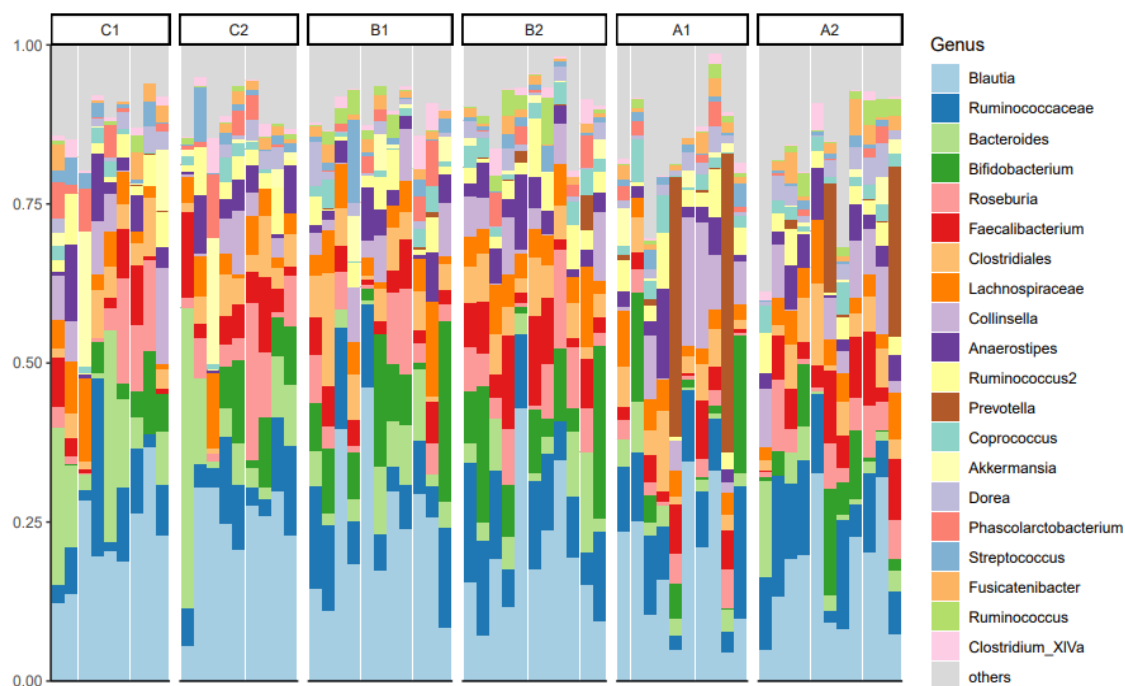


Figure 5. Genus and order taxonomy ranked by relative abundance stratified by participant. A1 and A2 (fermented vegetable group timepoint 1 and timepoint 2), B1 and B2 (non-fermented vegetable group timepoint 1 and timepoint 2), and C1 and C2 (control group timepoint 1 and timepoint 2)

Figure 6 shows significant differences in the Shannon index (measure of species richness and evenness)²³⁸ between group C and A at timepoint 2 ($P=.037$). Also, there was a trend towards an increase in alpha diversity measured by the Shannon in Group A. The observed OTUs (measure of species richness)²³⁸ showed significant differences between groups A and C and between Group B and C for timepoint 1 ($P=.012$ and $P=.031$, respectively). These differences were no longer significant between Groups B and C, or between Groups A and C.

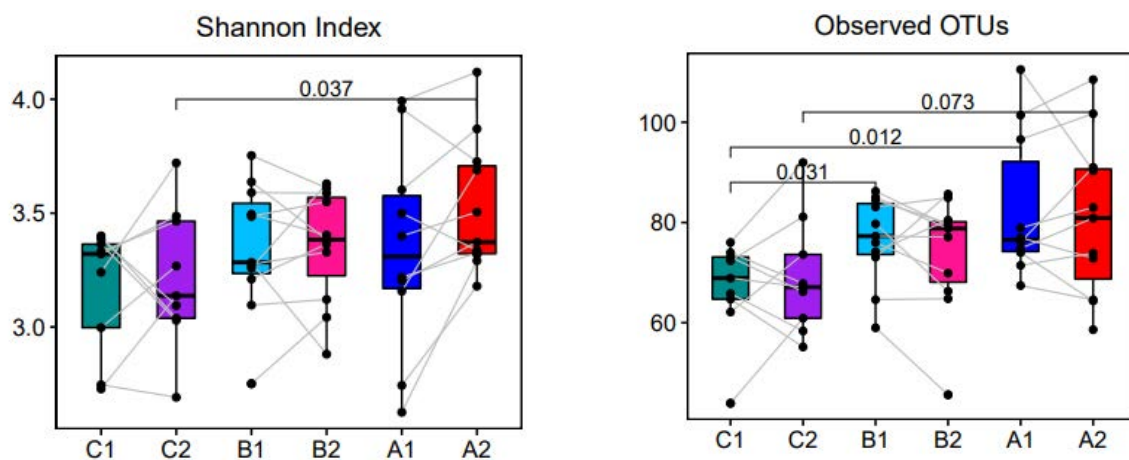


Figure 6. The Shannon index and observed OTUs (operational taxonomic units) represented through box-and-whisker plots. The whiskers show minimum and maximum values, the box is the 25th-75th percentile, and the line within the box is the median.

A1 and A2 (fermented vegetable group timepoint 1 and timepoint 2), B1 and B2 (non-fermented vegetable group timepoint 1 and timepoint 2), C1 and C2 (control group timepoint 1 and timepoint 2).

Figure 7 ranks the 20 most abundant OTUs found in participants' stool samples. The top OTUs on the species level for each treatment group follow; Group A timepoint 1 *Prevotella copri* and *Collinsella aerofaciens*; Group A timepoint 2 *Faecalibacterium prausnitzii* and *Blautia lut*; Group B timepoint 1 *Blautia wexlerae* and *Bifidobacterium longum*; Group B timepoint 2 *Blautia wexlerae* and *Bifidobacterium longum*; Group C timepoint 1 *Blautia wexlerae* and *Roseburia faecis*; and Group C timepoint 2 *Blautia wexlerae*, and *Roseburia faecis*. We found significant between group difference for OTU 17 (*Gemmiger formicilis*). *Faecalibacterium prausnitzii* was significantly ($P=.022$) enriched in Group A at timepoint 2.

Moreover, as shown in Table 6, the alpha diversity results for S_{obs} ($P=.014$) and Chao1 ($P=.009$) show significant differences for Group C (control group) at baseline.

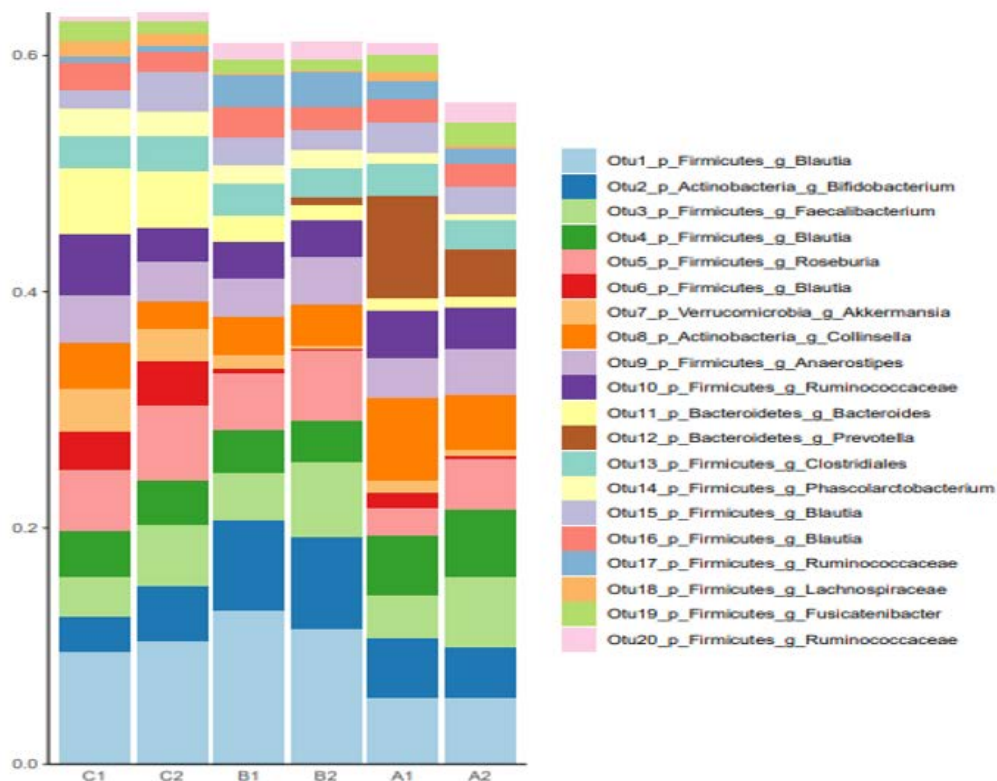


Figure 7. Top 20 OTUs (operational taxonomic units). OTUs are classified at the subgenus level and by relative abundance. The top 10 OTUs (species) follow; OTU 1 (*Blautia wexlerae*), OTU 2 *Bifidobacterium* (*Bifidobacterium longum*), OTU 3 *Faecalibacterium* (*Faecalibacterium prausnitzii*), OTU 4 *Blautia* (*Blautia lut*), OTU 5 *Roseburia* (*Roseburia faecis*), OTU 6 *Blautia* (*Blautia glucerasea*), OTU 7 (*Akkermansia muciniphila*), OTU 8 (*Collinsella aerofaciens*), OTU 9 (*Anaerostipes hadrus*), OTU 10 (*Ruminococcus bromii*). A1 and A2 (fermented vegetable group timepoint 1 and timepoint 2), B1 and B2 (non-fermented vegetable group timepoint 1 and timepoint 2), and C1 and C2 (control group timepoint 1 and timepoint 2)

Table 6. Alpha Diversity between Groups

Variable ^a	Timepoint 1	Timepoint 2	Change	P-value ^b
Shannon Index				
Group A	3.45 (0.67)	3.34 (0.44)	0.12 (0.13)	.386
Group B	3.28 (0.38)	3.38 (0.47)	0.02 (0.30)	.859
Group C	3.32 (0.50)	3.14 (0.44)	0.135 (0.65)	.401
P-value ^c	.307	.341	.583	
S_{obs}				
Group A	77.94 (24.43)	76.12 (25.74)	-0.20 (16.32)	.575
	77.28 (11.39)	78.80 (14.19)	-4.64 (8.71)	.286

Group B	68.90 (10.14)	67.10 (17.74)	-1.80 (10.91)	.327
Group C	.014	.368	.500	
<i>P</i> -value ^c				
Chao1				
Group A	109.35 (36.27)	108.12 (40.85)	-7.67 (16.94)	.059
Group B	107.76 (15.92)	106.97 (23.00)	-3.51 (20.17)	.213
Group C	98.71 (17.30)	91.65 (31.41)	-1.62 (31.64)	.327
<i>P</i> -value ^c	.009	.475	.310	

Table 6. Alpha diversity measures across the study.

^aValues are reported as median (IQR).

^b*P*-values represent within group comparisons between timepoint 1 and timepoint 2 calculated via the Wilcoxon Signed Ranks Test.

^c*P*-values represent between group comparisons among group A, B, and C for timepoint 1, timepoint 2, and change values calculated via the Kruskal-Wallis test. Group A (fermented vegetable group), Group B (non-fermented vegetable group), Group C (control group), S_{obs} (observed subsample species richness)

Correlations between alpha diversity, dietary intake, and clinical parameter values were computed for groups and individuals for timepoint 1, timepoint 2, and change values using Pearson's correlations (Table 7). We found 13 medium ($r=0.3-0.5$, $P<.05$) correlations. The OTUs used in Table 7 reflect species level and were chosen because they demonstrated significant alpha or beta diversity measures.

Table 7. Diet and clinical parameters correlated with alpha diversity measures

Variable	Shannon	SOBS	Chao1	OTU3 <i>Faecalibacterium prausnitzii</i>	OTU5 <i>Roseburia faecis</i>	OTU8 <i>Collinsella aerofaciens</i>	OTU12 <i>Prevotella copri</i>	OTU32 <i>Ruminococcus torques</i>
Dietary Parameters								
Energy	.361 (.011)	.227 (.117)	.190 (.191)	.072 (.624)	.219 (.130)	-.037 (.802)	-.230 (.111)	-.020 (.893)
Total Fat	.096 (.513)	.102 (.487)	.117 (.424)	-.173 (.235)	.138 (.343)	.122 (.402)	-.230 (.112)	-.079 (.587)
Total	.098 (.505)	.140 (.338)	.120 (.410)	-.110 (.452)	.145 (.321)	.136 (.351)	-.041 (.777)	-.122 (.404)
Carbohydrate								
Total Protein	.169 (.245)	.057 (.699)	.062 (.671)	-.073 (.618)	.244 (.091)	-.114 (.437)	-.339 (.017)	-.106 (.465)
Animal Protein	.214 (.140)	.012 (.932)	.001 (.992)	.007 (.962)	.123 (.399)	-.271 (.060)	-.339 (.017)	.100 (.495)
Cholesterol	-.055 (.708)	-.037 (.802)	.051 (.728)	-.263 (.068)	.031 (.831)	-.162 (.266)	-.361 (.011)	-.073 (.617)
SFA	.254 (.078)	.170 (.242)	.171 (.241)	-.032 (.825)	.057 (.700)	-.078 (.594)	-.321 (.025)	.061 (.678)
MUFA	.109 (.455)	.039 (.792)	.040 (.783)	-.106 (.470)	.171 (.241)	.118 (.420)	-.275 (.056)	-.016 (.912)
PUFA	.265 (.066)	.096 (.511)	.054 (.711)	.309 (.031)	.036 (.807)	-.171 (.240)	-.004 (.977)	.080 (.587)
Starch Intake	.276 (.055)	.214 (.140)	.166 (.255)	-.047 (.749)	.192 (.187)	.106 (.467)	-.197 (.174)	-.093 (.524)
Fiber Intake	.163 (.264)	.162 (.265)	.150 (.304)	-.009 (.951)	.138 (.343)	.077 (.597)	-.069 (.637)	-.089 (.544)
Sugar Intake	.219 (.130)	.282 (.045)	.257 (.075)	.078 (.594)	.162 (.266)	-.048 (.744)	.133 (.362)	-.028 (.851)
Glycemic Load	.291 (.042)	.260 (.072)	.218 (.132)	.109 (.457)	.186 (.201)	.087 (.551)	.029 (.841)	-.022 (.882)
Alcohol Intake	.109 (.457)	.035 (.810)	.014 (.926)	-.173 (.235)	-.016 (.911)	.067 (.649)	-.202 (.164)	.162 (.268)
Vegetable Intake	.202 (.165)	.165 (.257)	.148 (.311)	.092 (.527)	.140 (.338)	.048 (.744)	-.181 (.214)	.310 (.030)

Yogurt	.255 (.077)	.299 (.037)	.329 (.021)	-.125 (.392)	.035 (.812)	-.261 (.070)	-.137 (.348)	-.034 (.815)
Vitamin B1	.305 (.033)	.105 (.471)	.080 (.587)	.185 (.203)	.328 (.021)	.014 (.927)	-.240 (.096)	-.126 (.389)
Vitamin B2	.203 (.161)	.210 (.147)	.216 (.135)	-.046 (.752)	.027 (.854)	.060 (.680)	-.201 (.166)	-.092 (.528)
Vitamin B12	.060 (.683)	.093 (.523)	.122 (.404)	-.092 (.529)	.058 (.692)	-.007 (.964)	-.191 (.188)	-.152 (.297)
Vitamin A	.221 (.128)	.108 (.459)	.115 (.432)	.141 (.333)	.135 (.356)	.203 (.162)	-.165 (.256)	-.014 (.925)
Activity								
Vitamin E	.182 (.210)	.007 (.964)	-.025 (.866)	.250 (.083)	.271 (.060)	.042 (.777)	-.066 (.650)	-.083 (.571)
Intake								
Vitamin C	.126 (.389)	-.008 (.955)	-.012 (.934)	.324 (.023)	.358 (.011)	.071 (.629)	-.065 (.657)	-.005 (.972)
Intake								
HEI 2015 Score	.263 (.068)	.212 (.144)	.111 (.447)	.104 (.478)	.046 (.753)	-.110 (.453)	.022 (.881)	.078 (.593)
Clinical Parameters								
Age	.254 (.052)	.265 (.042)	.180 (.172)	-.224 (.088)	-.133 (.314)	-.244 (.062)	-.095 (.476)	.226 (.085)
Wt	-.135 (.310)	-.199 (.131)	-.220 (.107)	.109 (.409)	.114 (.392)	.166 (.209)	-.080 (.546)	.001 (.996)
BMI	-.176 (.181)	-.175 (.186)	-.192 (.145)	.153 (.247)	.115 (.385)	.122 (.357)	.075 (.572)	.014 (.917)
LBM	.047 (.726)	-.075 (.574)	-.149 (.261)	.003 (.982)	.056 (.672)	.137 (.302)	-.156 (.239)	-.091 (.494)
BFM	-.194 (.140)	-.221 (.093)	-.212 (.107)	.139 (.294)	.118 (.372)	.148 (.262)	-.029 (.825)	.043 (.744)
PBF	-.237 (.071)	-.206 (.118)	-.168 (.204)	.130 (.327)	.080 (.545)	.134 (.313)	-.010 (.938)	.108 (.414)
SBP	.023 (.871)	.047 (.738)	.101 (.468)	-.302 (.026)	-.067 (.632)	.138 (.319)	-.125 (.367)	.072 (.607)
DBP	.015 (.917)	-.082 (.556)	-.112 (.421)	-.109 (.434)	-.079 (.568)	.087 (.534)	-.010 (.938)	.023 (.866)
TNF	.004 (.977)	-.093 (.508)	-.097 (.491)	-.085 (.546)	.200 (.152)	-.055 (.694)	-.078 (.577)	.297 (.031)
CRP	-.033 (.813)	-.182 (.191)	-.235 (.090)	.041 (.773)	.121 (.388)	-.089 (.525)	.072 (.607)	-.011 (.937)

LBP	-.033 (.815)	-.180 (.197)	-.237 (.088)	-.042 (.767)	.199 (.153)	.023 (.869)	-.096 (.494)	.132 (.346)
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Values are reported as Pearson's coefficient (*P*-value). SFA (saturated fatty acid), MUFA (monounsaturated fatty acid), PUFA (polyunsaturated fatty acid), HEI (healthy eating index), Wt (weight), BMI (body mass index), LBM (lean body mass), BFM (body fat mass), PBF (percent body fat), SBP (systolic blood pressure), DBP (diastolic blood pressure), TNF (tumor necrosis factor), CRP (C-reactive protein), LBP (lipopolysaccharide binding protein)

Correlations between specific genera, dietary intake, and clinical parameter values were computed among groups and individuals for timepoint 1, timepoint 2, and change values using Pearson's correlations (Table 8). We found one high ($r > 0.5$, $P < .05$) and 14 medium ($r = 0.3-0.5$, $P < .05$) correlations. The genera in Table 8 were chosen based on significant diversity values and/or publications that report significant correlations with the gut microbiome.

Table 8. Diet and clinical parameters correlated with genus

Variable	Blautia	Ruminococ caceae	Bacteroides	Bifidobact erium	Roseburia	Faecalibac terium	Clostridial es	Lachnosp iraceae	Prevotella	Lactobacil lus
Dietary Parameters										
Energy	-.058 (.694)	.119 (.417)	.098 (.501)	.090 (.537)	.214 (.140)	.060 (.681)	.209 (.150)	-.065 (.657)	-.226 (.119)	.133 (.364)
Total Fat	.043 (.769)	.055 (.707)	.138 (.343)	.051 (.727)	.139 (.341)	-.220 (.129)	.131 (.369)	-.070 (.633)	-.226 (.118)	-.052 (.725)
Total Carbohydrate	-.031 (.830)	-.064 (.661)	.179 (.217)	.081 (.581)	.147 (.313)	-.128 (.379)	.052 (.723)	-.122 (.403)	-.040 (.787)	-.094 (.519)
Total Protein	.119 (.414)	.002 (.988)	.098 (.501)	-.001 (.995)	.236 (.102)	-.105 (.475)	.278 (.053)	.046 (.754)	-.332 (.020)	.027 (.854)
Animal Protein	.146 (.317)	.015 (.921)	-.047 (.747)	-.050 (.733)	.105 (.472)	.004 (.978)	.126 (.389)	.139 (.339)	-.330 (.021)	.266 (.065)
Cholesterol	.278 (.053)	-.076 (.606)	-.059 (.685)	-.082 (.574)	.019 (.895)	-.285 (.047)	.115 (.430)	.169 (.247)	-.345 (.015)	.122 (.404)
SFA	.113 (.438)	.096 (.511)	-.038 (.795)	.063 (.668)	.050 (.735)	-.070 (.633)	.068 (.641)	.038 (.794)	-.315 (.028)	.291 (.042)
MUFA	.033 (.821)	.078 (.594)	.170 (.243)	.058 (.691)	.166 (.253)	-.162 (.267)	.112 (.445)	-.052 (.723)	-.274 (.057)	.068 (.643)
PUFA	-.129 (.375)	.129 (.376)	-.076 (.606)	.047 (.751)	.031 (.833)	.344 (.015)	.046 (.756)	.031 (.831)	-.006 (.969)	.223 (.123)
Starch Intake	-.067 (.647)	.040 (.783)	.182 (.211)	.048 (.745)	.192 (.187)	-.080 (.587)	.230 (.112)	-.038 (.798)	-.197 (.174)	-.032 (.827)
Fiber Intake	-.070 (.635)	-.004 (.976)	.100 (.494)	.050 (.734)	.141 (.334)	-.051 (.730)	.303 (.035)	-.052 (.724)	-.066 (.654)	-.151 (.301)
Sugar Intake	-.205 (.158)	-.009 (.954)	.155 (.289)	.053 (.719)	.166 (.256)	.129 (.377)	-.035 (.811)	-.046 (.753)	.137 (.346)	-.062 (.670)
Glycemic Load	-.193 (.185)	.003 (.984)	.178 (.221)	.067 (.646)	.187 (.197)	.141 (.334)	-.028 (.847)	-.103 (.482)	.029 (.842)	-.054 (.715)
Alcohol Intake	.059 (.689)	.312 (.029)	-.030 (.836)	-.077 (.599)	-.022 (.881)	-.196 (.176)	.163 (.264)	-.167 (.250)	-.208 (.152)	-.035 (.814)
Vegetable Intake	-.010 (.947)	.200 (.168)	.054 (.715)	.133 (.362)	.145 (.319)	.023 (.876)	.214 (.140)	-.126 (.389)	-.176 (.226)	-.001 (.993)
Yogurt	.059 (.686)	-.064 (.662)	.251 (.082)	-.141 (.333)	.024 (.870)	-.110 (.453)	.124 (.396)	.096 (.513)	-.119 (.414)	.191 (.188)
Vitamin B1	-.035 (.804)	-.011 (.939)	.193 (.183)	.170 (.243)	.319 (.025)	.181 (.214)	.243 (.093)	.003 (.985)	-.244 (.091)	.102 (.485)
Vitamin B2	-.172 (.236)	.155 (.289)	.064 (.663)	.429 (.002)	.019 (.896)	-.032 (.826)	.067 (.646)	-.033 (.822)	-.201 (.166)	.011 (.938)
Vitamin B12	-.073 (.616)	.122 (.404)	.009 (.953)	.398 (.005)	.049 (.739)	-.083 (.571)	.107 (.464)	-.087 (.553)	-.192 (.185)	.013 (.927)
Vitamin A Activity	-.051 (.727)	.239 (.098)	-.005 (.974)	.171 (.239)	.137 (.348)	.085 (.561)	.192 (.185)	-.230 (.112)	-.170 (.242)	.018 (.901)
Vitamin E Intake	-.122 (.402)	-.038 (.796)	.215 (.137)	.205 (.158)	.269 (.061)	.213 (.142)	.115 (.431)	-.110 (.451)	-.069 (.637)	.010 (.944)
Vitamin C Intake	-.091 (.533)	-.011 (.941)	.038 (.796)	.189 (.194)	.362 (.011)	.299 (.037)	.231 (.110)	-.190 (.192)	-.066 (.651)	.006 (.965)
HEI 2015 Score	-.248 (.085)	-.011 (.942)	.152 (.297)	.048 (.741)	.044 (.764)	.125 (.390)	.117 (.423)	.035 (.813)	.020 (.893)	.088 (.548)
Clinical Parameters										
Age	-.047 (.724)	.055 (.680)	.086 (.519)	-.389 (.002)	-.138 (.298)	-.176 (.183)	-.171 (.196)	.532 (.000)	-.095 (.475)	.085 (.524)
Wt	-.202 (.124)	-.105 (.453)	-.296 (.023)	.296 (.023)	.115 (.387)	.124 (.350)	.157 (.236)	.055 (.680)	-.101 (.447)	-.011 (.933)
BMI	-.278 (.033)	.050 (.708)	-.241 (.066)	.274 (.036)	.123 (.352)	.158 (.233)	.069 (.605)	.007 (.958)	.054 (.684)	-.072 (.587)
LBM	-.143 (.281)	.095 (.475)	-.290 (.026)	.277 (.034)	.047 (.726)	.046 (.727)	.351 (.006)	.116 (.380)	-.164 (.215)	.119 (.371)

BFM	-.193 (.142)	.056 (.673)	-.241 (.066)	.250 (.056)	.124 (.378)	.138 (.299)	.033 (.806)	.013 (.920)	-.052 (.698)	-.073 (.584)
PBF	-.132 (.318)	.005 (.967)	-.170 (.198)	.197 (.135)	.090 (.499)	.109 (.409)	-.063 (.633)	-.032 (.810)	-.039 (.772)	-.109 (.410)
SBP	-.056 (.634)	.138 (.321)	-.204 (.138)	.077 (.578)	-.073 (.601)	-.235 (.087)	.111 (.423)	-.071 (.609)	-.127 (.361)	-.231 (.092)
DBP	-.055 (.691)	-.021 (.882)	-.214 (.120)	.031 (.824)	-.087 (.532)	-.065 (.642)	.152 (.273)	.089 (.521)	-.059 (.671)	.252 (.066)
TNF	-.092 (.511)	-.105 (.453)	.117 (.404)	.156 (.266)	.192 (.169)	-.042 (.768)	-.275 (.046)	.020 (.888)	-.082 (.558)	-.227 (.103)
CRP	.061 (.665)	-.121 (.389)	.230 (.098)	-.076 (.587)	.124 (.378)	.028 (.840)	-.078 (.579)	.071 (.612)	.053 (.706)	-.119 (.396)
LBP	.091 (.517)	.039 (.782)	-.163 (.245)	.102 (.468)	.198 (.155)	-.075 (.592)	.142 (.311)	-.054 (.703)	-.116 (.408)	.133 (.343)

Values are reported as Pearson's coefficient (*P*-value).

SFA (saturated fatty acid), MUFA (monounsaturated fatty acid), PUFA (polyunsaturated fatty acid), HEI (healthy eating index), Wt (weight), BMI (body mass index), LBM (lean body mass), BFM (body fat mass), PBF (percent body fat), SBP (systolic blood pressure), DBP (diastolic blood pressure), TNF (tumor necrosis factor), CRP (C-reactive protein), LBP (lipopolysaccharide binding protein)

3.6. Beta Diversity

Beta diversity compares diversity between samples by calculating microbial dissimilarity that is shown in a distance matrix such as the Bray-Curtis and Jaccard index.²⁴¹ The Bray-Curtis is a quantitative measure of taxa abundance,²³² while the Jaccard distance is a qualitative measurement that represents feature presence/absence rather than relative abundances.²⁴² In terms of beta diversity (between samples), no significant within group plot variations or patterns were found for longitudinal comparisons of PCoA plots for Bray-Curtis (Figure 9) or Jaccard (Figure 10) distances.

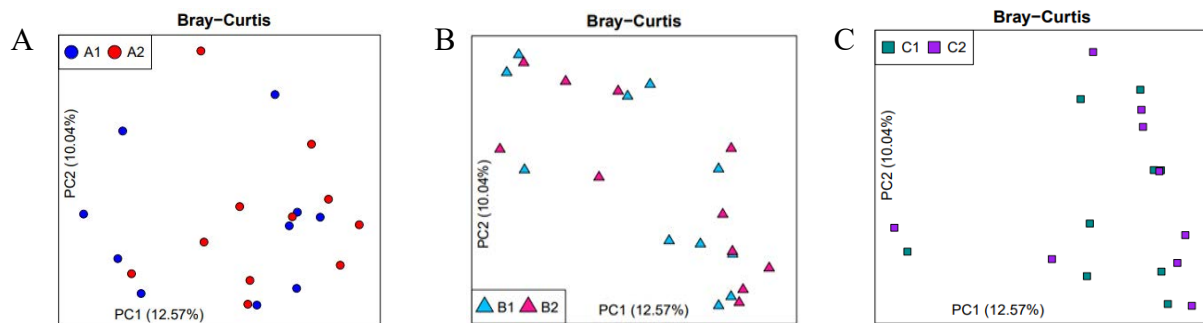


Figure 9. PCoA plots showing Bray-Curtis distances for within group (Group A, B, and C), longitudinal (timepoint 1 to timepoint 2) measures. (A) fermented vegetable group, (B) non-fermented vegetable group, (C) control group

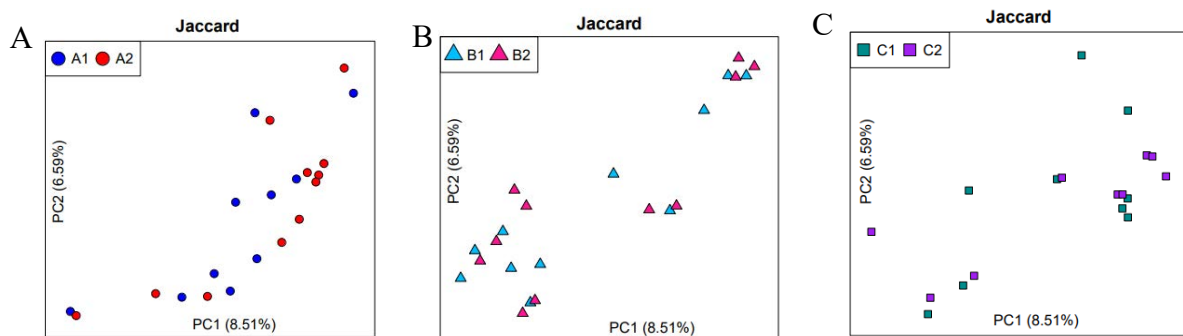


Figure 10. PCoA plots showing Jaccard distances for within group (Group A, B, and C), longitudinal (timepoint 1 to timepoint 2) measures. (A) fermented vegetable group, (B) non-fermented vegetable group, (C) control group

Further microbial analysis used ANOSIM based on Bray-Curtis and Jaccard distances to compared beta diversity within and between all groups (Groups A, B, and C) and timepoints

(timepoint 1 and 2) (Figure 11). No strong dissimilarities were found for the Bray-Curtis or Jaccard distances.

Bray-Curtis			Jaccard		
Comparison	<i>r</i> -value	<i>P</i> -value	Comparison	<i>r</i> -value	<i>P</i> -value
A1-A2	-.046	.745	A1-A2	-.062	.857
B1-B2	-.100	.991	B1-B2	-.097	.987
C1-C2	-.121	.994	C1-C2	-.130	.994
A1-B1	.048	.171	A1-B1	.020	.281
A1-B2	.082	.092	A1-B2	.010	.337
A1-C1	.038	.243	A1-C1	.141	.035
A1-C2	.033	.232	A1-C2	.058	.130
A2-B1	.012	.340	A2-B1	.030	.208
A2-B2	-.015	.574	A2-B2	-.006	.471
A2-C1	.102	.063	A2-C1	.243	.005
A2-C2	.042	.198	A2-C2	.139	.024
B1-C1	-.001	.425	B1-C1	-.010	.522
B1-C2	-.071	.931	B1-C2	-.078	.947
B2-C1	.035	.266	B2-C1	.005	.417
B2-C2	-.046	.796	B2-C2	-.042	.743

Figure 11. ANOSIM (analysis of similarity) via Bray-Curtis and Jaccard distances. A1 fermented vegetable group at timepoint 1), A2 (fermented vegetable group at timepoint 2), B1 (non-fermented vegetable group at timepoint 1), B2 (non-fermented group at timepoint 2), C1 (control group at timepoint 1), C2 (control group at timepoint 2)

Longitudinal LEfSe results identified several enriched OTUs. LDA scores greater than 2 are considered significant ($P > 0.05$). The LEfSe results for Group A (fermented vegetable group) show OTU 32 (*Ruminococcus torques*) significantly more enriched at timepoint 1 than timepoint 2 as represented by a LDA score of 3.6 in Figure 12A, and a side-by-side comparison of relative abundance for timepoint 1 and timepoint 2 in Figure 12B.

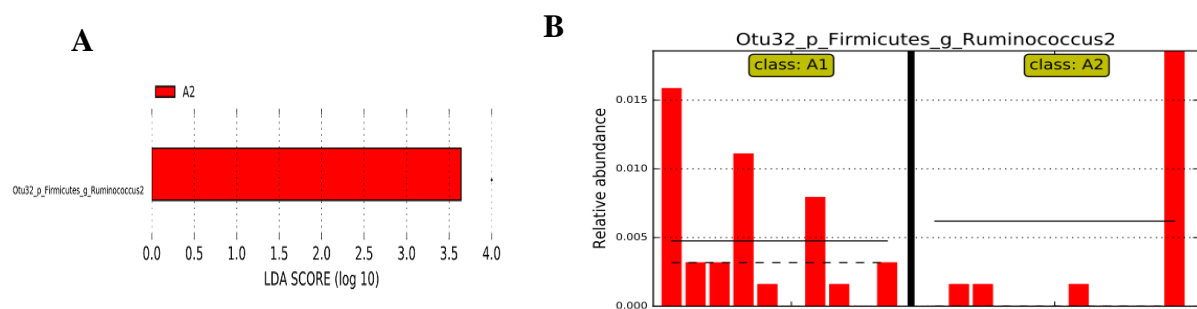


Figure 12. (A) Longitudinal linear discriminant analysis (LDA) effect size (LEfSe) for Group A (fermented vegetable group) at timepoint 2 (B) Relative abundance of OTU 32 (*Ruminococcus torques*) stratified by study participant and timepoint (A1 = timepoint 1, A2=timepoint 2).

The LEfSe results for Group B (non-fermented vegetable group) show OTU 206 (*Negativibacillus massiliensis*) significantly more enriched at timepoint 2 than at timepoint 1 as represented by a LDA score of 3.0 in Figure 13A, and a side-by-side comparison of relative abundance for timepoint 1 and timepoint 2 in Figure 13B.

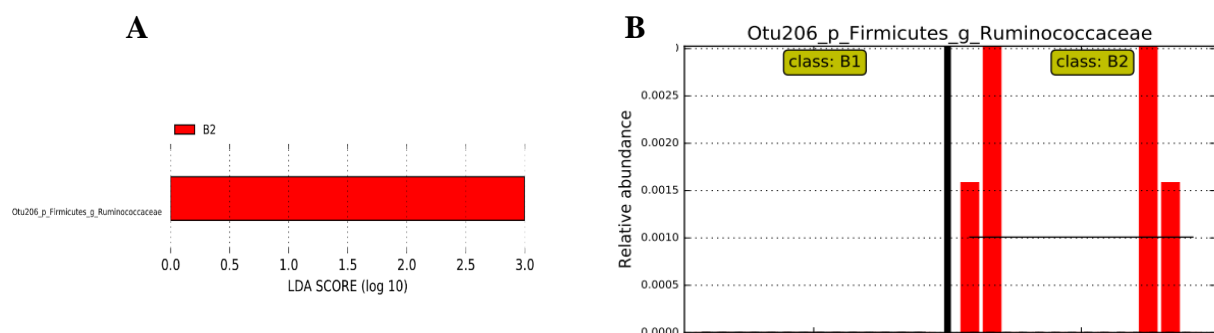


Figure 13. (A) Longitudinal linear discriminant analysis (LDA) effect size (LEfSe) for Group B (non-fermented vegetable group) at timepoint 2 (B) Relative abundance of OTU 206 (*Negativibacillus massiliensis*) stratified by study participant and timepoint (B1 = timepoint 1, B2 = timepoint 2).

The LEfSe results for Group C (control group) show OTU 163 (*Mediterraneibacter glycyrrhizinilyticus*) significantly more enriched at timepoint 2 than at timepoint 1 as represented by a LDA score in Figure 14A, and a side-by-side comparison of relative abundance for timepoint 1 and timepoint 2 in Figure 14B.

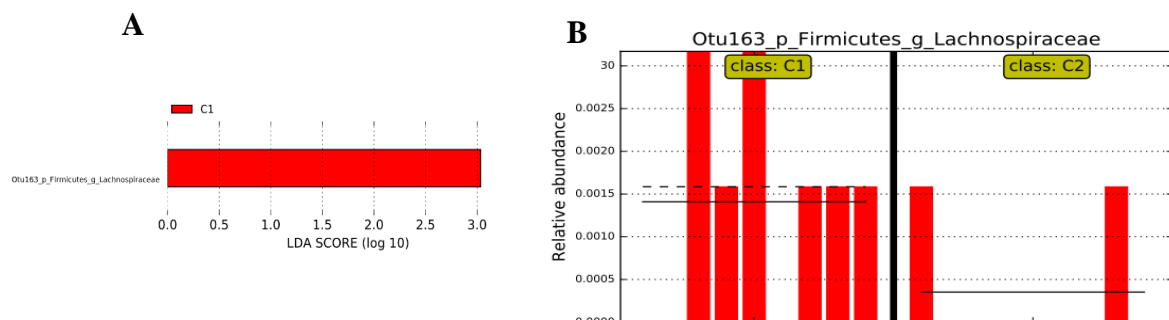


Figure 14. (A) Longitudinal linear discriminant analysis (LDA) effect size (LEfSe) for Group C (control group) at timepoint 2 (B) Relative abundance of OTU 163 (*Mediterraneibacter glycyrrhizinilyticus*) stratified by study participant and timepoint (C1 = timepoint 1, C2=timepoint 2).

Some species within the same phylum, genus, and other taxa have various functions and benefits. There is tremendous diversity in the activities of bacteria. To highlight the various functions of species noted in this study, Table 9 lists the 10 most abundant species and four species with significant diversity measures along with their reported functions and benefits.

Table 9: Reported function(s) and Association(s) of significant species

OTU	Subgenera	Species	Reported Function(s) and Association(s)
1	Blautia	<i>Blautia wexlerae</i>	Intestinal immune homeostasis, glucose homeostasis, anti-obesogenic ²⁴³
2	Bifidobacterium	<i>Bifidobacterium longum</i>	Produces SCFAs, conjugated linoleic acid, and bacteriocins that protect against infection; ²⁴⁴ decreased in CF, CD, and IBD; increased in diabetes ²⁴⁵
3	Faecalibacterium	<i>Faecalibacterium prausnitzii</i>	Butyrate-producing bacteria with anti-inflammatory effects ^{246–248} through blockage of the NF-κB pathway; ²⁴⁹ decreased in IBD ²⁵⁰ with potential role as an IBD biomarker; ²⁴⁹ decreased in CF, CD, hepatitis B cirrhosis, gastroenteritis; increased in obesity ²⁴⁵
4	Blautia	<i>Blautia luti</i>	Intestinal immune homeostasis, glucose homeostasis, anti-obesogenic; ²⁴³ decreased in graft-versus-host disease ²⁴⁵
5	Roseburia	<i>Roseburia faecis</i>	Decreased in IBD ²⁴⁵
6	Blautia	<i>Blautia glucerasea</i>	Decreased in Parkinson's disease ²⁴⁵
7	Akkermansia	<i>Akkermansia muciniphila</i>	Increased in gastrointestinal helminths infection, colorectal cancer; decreased in nonalcoholic liver disease, CD, UC, obesity ²⁴⁵
8	Collinsella	<i>Collinsella aerofaciens</i>	Decreased in CF, IBS, UC, CD; increased in metabolic syndrome, CAD, colorectal cancer ²⁵¹
9	Anaerostipes	<i>Anaerostipes hadrus</i>	Decreased in primary sclerosing cholangitis, obesity, MS, CD, UC, CF ²⁵¹
10	Ruminococcaceae	<i>Ruminococcus bromii</i>	Decreased in CD, increased in IBS ²⁴⁵

12	Prevotella	<i>Prevotella copri</i>	Increased in peritoneal dialysis; decreased in Parkinson's disease ²⁴⁵
32	Ruminococcus	<i>Ruminococcus torques</i>	Increased in CD, UC, IBS ²⁴⁵
163	Lachnospiraceae	<i>Mediterraneibacter glycyrrhizinilyticus</i>	No information found
206	Ruminococcaceae	<i>Negativibacillus massiliensis</i>	No information found

CAD (coronary artery disease), CD (Crohn's disease), CF (cystic fibrosis), IBS (irritable bowel syndrome), IBD (inflammatory bowel disease), MS (multiple sclerosis), UC (ulcerative colitis)

Chapter Four: Discussion and Conclusions

4. Discussion

This parallel arm, pilot and feasibility study, explored the effects of fermented vegetable consumption for six weeks on markers of inflammation and gut microflora profiles of women. Findings included, a significant increase in *Faecalibacterium prausnitzii* among Group A (fermented vegetable group) at timepoint 2, an upward trend in Shannon index among Group A at timepoint 2, and 28 moderate to strong correlations between alpha diversity and dietary and clinical parameters.

Most research on this topic has been conducted in Asian countries where fermented vegetables are widely consumed and in much larger quantities as compared to the typical consumption of fermented vegetables in the United States.^{184,194,200,227} We believe that this is the only study conducted in the United States that examined the effects of regular consumption of fermented vegetables for six weeks on markers of metabolic syndrome and inflammation, and gut microflora profiles in women. Moreover, these studies of Asian origin used kimchi as the main source of the fermented vegetables as compared to our study that used fermented sauerkraut and pickles that contain different bacteria strains and amounts. The lack of research on this topic, particularly in the United States, leaves a huge gap in the knowledge about the role of fermented vegetables in Western cultures. This study assessed the feasibility and the effects of regular consumption of fermented vegetables in a group of women living in Florida. The amount and duration of fermented vegetable consumption that our participants were asked to consume were selected based on what the researchers considered a realistic amount for our population to consume, given that fermented vegetables are not widely common in the Western diet.

4.1. Vegetable Consumption

Vegetable intake compliance was similar between Group A and Group B, which helped contribute to a more accurate analyses of between group comparisons for outcomes measured; however, vegetable consumption is at risk for inaccuracies due to the nature of self-reported intakes. It appears that 100 g of vegetable consumption for 6 weeks was well-tolerated. The groups did well with vegetable compliance and tolerance, although some participants commented that compliance was difficult towards the end mainly due to the taste that fermentation gave the vegetables and redundancy of consuming the same vegetables. Bloating was the main side effect reported in Groups A and B with Group B reporting the most bloating. Bloating was expected due to the nature of sauerkraut being a cruciferous vegetable. Abdominal pain was the main side effect among Group C perhaps due to the placebo effect.

4.2. Measured Outcomes

4.2.1. Baseline Parameters

Baseline characteristics such as age, BMI, race, ethnicity, and education were not significantly different between the three groups; thus, limiting bias contributions to study results.

4.2.2. Dietary and Clinical Parameters

Group A had a significant within group reduction for alcohol intake likely related to personal choices rather than fermented vegetable intake, and Group B did not show any significant within group changes. Even with the high sodium content of fermented vegetables due to the brine solution, no related, significant, increases in blood pressure were found as salt sensitivity does not occur in most people.²⁵² Surprisingly, Group C demonstrated the most within group changes in dietary intake as represented by eight significant reductions; energy, total protein, animal protein, total fat, cholesterol, MUFA, PUFA, vegetable, and vitamin E. It is unclear why Group C displayed the most changes (reductions) from timepoint 1 to timepoint 2. The 468 calorie decrease between timepoints in Group C was likely due to the total protein and

total fat intake reductions, which are further connected to the other reductions in cholesterol, MUFA, PUFA, vegetable, and vitamin E intakes. Group C's overall intake dropped significantly more than Group A and B. In addition to a small sample size, intentional desire of some participants to improve dietary intake and/or physical activity level, or unintentional changes in dietary intake such as loss of appetite related to stress or sickness may have also played a factor. Inaccurate self-reporting on the FFQ is not a likely cause for changes in Group C's dietary intake changes because the reductions in the group's metabolic markers (BFM, PBF, weight, SBP) support the dietary changes. Group C demonstrated the significant reductions in BFM, PBF, weight, and SBP as compared to Groups A and B. None of the groups demonstrated significant changes in inflammatory markers (TNF, CRP, LBP). It was hypothesized that Group A would demonstrate at least one metabolic or inflammatory marker; however, this did not occur most likely due to the study's small population size and insufficient probiotic/fermented vegetable intake. Standardized prebiotic and probiotic definitions are a major contributor to developmental research, but further research is needed to develop recommended dietary intakes and their according food labels that clearly show abundance measures to better know how much is needed to create desired change.¹³³

4.2.3. Alpha Diversity

4.2.3.1. Vegetables

Alpha diversity measures of the fermented and non-fermented vegetables showed higher diversity in the non-fermented vegetables compared with the fermented vegetables. However, the fermented sauerkraut and fermented pickles showed a greater enrichment of the genera *Lactobacillus* and *Leuconostoc* as compared to their non-fermented counterparts, which is in agreement with literature findings that report *Lactobacillus* and *Leuconostoc* are among the main bacteria in fermented sauerkraut and fermented pickles.^{186,190} *Bacillales* is the predominant order among the non-fermented sauerkraut and non-fermented pickles. Fresh

vegetables are known to harbor bacteria such as Bacillales^{253–255} at any time from the field to consumption.^{255,256} In addition to sauerkraut's cruciferous benefits, the differences in diversity between the pickles and sauerkraut, likely contributed to participants' microbial outcomes.

4.2.3.2. Fecal

The fecal alpha diversity analysis did not show any significant differences in the RA for within or between group comparisons for any phyla, but there were significant between group differences for the family Pasteurellaceae and genera Antinomyces; however, due to their very low abundances, these genera were not reported in the figure. The main five phyla identified in stool samples were Firmicutes (RA 75%), Actinobacteria (RA 12%), Bacteroidetes (RA 10%), Verrucomicrobia (RA 1.8%), and Proteobacteria (RA 0.5%). These results are similar to other research that report Firmicutes is typically the predominant phyla followed by Bacteroidetes followed by Actinobacteria, Proteobacteria, and Verrucomicrobia.^{11,257} King and colleagues²⁵⁸ created a healthy human gut microbiota profile model (GutFeelingKB) based on taxa RA that can be used as a healthy control for dysbiosis-related research, and a standardized Fecal Biome Population Report (FecalBiome) for reporting individual microbiota profiles. GutFeelingKB is a compilation of data collected from a "healthy" people cohort at George Washington University and "healthy" HMP subjects. The healthy people cohort participants were deemed "healthy" according to analysis (by the Nutrition Data System for Research) of their seven-day food journals and were free of disease throughout the study. Forty-eight stool samples from the healthy people cohort along with 50 stool samples from the healthy HMP subjects were ran through CensuScope, a taxonomic profiling software, that calculated abundance quantification. Those organisms with the highest abundance measures were then manually evaluated per four major criteria (inspection of the match count, confirmation of a justifiable taxonomy assignment, completeness of sequence in the GutFeelingKB, and organism verification) that resulted in 157 organisms (8 phyla, 18 classes, 23 orders, 38

families, 59 genera and 109 species) added to the GutFeelingKB database for genome mapping. If closely related proteomes are considered, then the list can be expanded to include 863 organisms. Different than our results, the GutFeelingKB phylum profile suggests a composition with Bacteroidetes (RA $73.13 \pm 22.16\%$) as the dominant phyla followed by Firmicutes (RA $22.2 \pm 18.66\%$), Proteobacteria (RA $2.15 \pm 10.39\%$), and Actinobacteria (RA $1.82 \pm 3\%$),²⁵⁸ which is in accordance with similar research that reports better health and dietary patterns are present when Bacteroidetes is the dominant phyla followed by Firmicutes.^{259,260} Furthermore, this study showed that, on average, Firmicutes was 10.6 times greater than Bacteroidetes and Actinobacteria was 20 times greater than Proteobacteria, while the GutFeelingKB suggests with Bacteroidetes to be approximately 3.3 times the amount of Firmicutes, and Proteobacteria about 1.2 times the RA of Actinobacteria.²⁵⁸ The RA of Proteobacteria and Actinobacteria remain similar among research as phylum minorities; however, even though these phyla are considered minor, they play pivotal roles in gut microbial homeostasis through their metabolic and immune influence. More research is needed to decipher if and how much the Proteobacteria/Actinobacteria ratio is correlated with optimal health, and their optimal RA for use as disease biomarkers.^{11,76,257,258,260–262}

As shown in Figure 4, the top genus bacteria included some order and family taxa; thus, when referring to this study's genus bacteria some order and family taxa are included. When averaged among all participants, our top ten genera (RA) were Blautia (21%), Ruminococcaceae (9.6%), Bacteroides (6.4%), Bifidobacterium (5.9%), Roseburia (5.1%), Faecalibacterium (4.9%), Clostridiales (4.9%), Lachnospiraceae (4.7%), Collinsella (4.5%), and Anaerostipes (3.7%). By combining 22 sequenced fecal metagenomes of individuals from four countries with previously published data sets and two published larger cohorts, Arumugam and colleagues⁸ reported the top ten genus bacteria found in the human gut are Bacteroides, Faecalibacterium, Bifidobacterium, Lachnospiraceae, Roseburia, Alistripes, Collinsella,

Blautia, Coprococcus, and Ruminococcus,^{10,263} This study's findings matched seven of the top ten bacteria, but in a different order. The GutFeelingKB ranked the top genus (RA) are Bacteroides (65.6%), Lachnospiraceae (6.2%), Ruminococcus (4.1%), Faecalibacterium (3.5%), Alistripes (3%), Parabacteroides (2.3%), Clostridiales (2.1%), Escherichia (2%), Roseburia (1.8%), Bifidobacterium (1.7%), Blautia (1.5%), and Akkermansia (0.7%).²⁵⁸ This study's results also matched seven top genera of the GutFeelingKB, but with different RA. Both the reported top genus^{10,263} and the GutFeelingKB genus²⁵⁸ show Bacteroides as the predominant genera rather than Blautia as found in this study. Genus abundance profiles show greater variability than phylum abundance profiles, which likely accounts for the variation shown here between common,²⁶⁴ recommended,²⁵⁸ and our genus profile results. Of note, a substantial number of bacteria were classified as "others" rather than a known taxa. Current technology limits taxonomic identification of new bacteria as well as highly polyphyletic and phylogenetic bacteria; thus, classifying them as "others". Future advances in taxonomy looks promising for increased classification of bacteria.⁹

We found significant differences in alpha diversity between groups at baseline; therefore, rather than focusing on between group comparisons we mainly focused on within group comparisons. We did not find any significant changes for within group comparisons based on the Shannon index or the observed OTUs, but did find significant diversity differences for between group comparisons likely due to the significant diversity differences between groups at baseline; thus, making within group comparisons a more accurate representation of treatment effect for this study.

Faecalibacterium prausnitzii was the predominant species among Group A at timepoint 2, which could be related to fermented vegetable intake. *Faecalibacterium prausnitzii* is one of the most abundant gut bacterium involved in gut homeostasis^{264,265} and it has been described as the "gatekeeper of the gut."²⁶⁶ *Faecalibacterium prausnitzii* is an anti-inflammatory

bacterium that improves intestinal barrier protection, insulin sensitivity, oxidative stress tolerance, and visceral sensitivity.²⁶⁶ *Faecalibacterium prausnitzii* is a butyrate-producers and IL-10 stimulator that can block the IL-1 β -induced NF- κ B signaling pathway.^{267–269} Clemete et al²⁷⁰ reported that *Faecalibacterium prausnitzii* inhibits pathogenic bacteria and increases colonization of nonpathogenic bacteria in the human gut. Group B and C retained the same predominant species for each group from timepoint 1 to timepoint 2.

4.2.3.3. Correlations

Among Pearson's correlation-coefficients that measured alpha diversity indices (Shannon index, Sobs, Chao1, species, and genera) with dietary intake and clinical parameters, we found one high ($r > 0.5$, $P < .05$) correlation and 27 medium ($r = 0.3-0.5$, $P < .05$) correlations. The positive correlation between age and Lachnospiraceae ($r = .532$, $P = .000$) is consistent with previous research reporting that Lachnospiraceae increases from infancy to about 50 years old, then decreases in extreme aging.²⁷¹ Lachnospiraceae has been correlated with disease prevention and progression due to the bacteria's high phylogenetical taxonomy with both pro- and anti-inflammatory contributions.²⁷² We found a negative correlation between age and Bifidobacterium ($r = -.389$, $P = .002$), which is supported by much documentation that reports Bifidobacterium decreases with age,²⁴⁴ We found a positive correlation between Shannon index and energy ($r = .361$, $P = .011$); however, similar studies only found strong correlations between Shannon index and overall diet quality, rather than energy intake.^{273,274} Our correlation findings for Bifidobacterium and vitamin B2 ($r = .429$, $P = .002$) and B12 ($r = .398$, $P = .005$) are supported by much research regarding gut microbiota and vitamin intake.^{275–278} Many Bifidobacterium species can de novo synthesize and supply vitamins such as vitamin B2 (riboflavin) and B12 (cobalamin) to the human body. This is important because like most vitamins, vitamins B2 and B12 cannot be synthesized by humans and must be obtained from other sources such as food or intestinal microbiota. Vitamin-producing microorganisms

provide the host with an ongoing supply of micronutrients.^{40,279,280} Vitamins B2 and B12 are involved in many essential functions of the human body such as cell metabolism and respiration, amino acid synthesis, energy production, cognitive function, immune support, and/or red blood cell production. Deficiencies in these vitamins have caused developmental defects, impair cognitive function, immune dysfunction, and abnormal blood production. Historically, nutritional deficiencies have been treated with supplementation that have either reversed the deficiency or caused vitamin toxicity leading to immune dysfunction, cancer, and increased mortality.^{40,281,282} The correlations between vitamin B1 and *Roseburia* ($r=.319$, $P=.025$) and *Roseburia faecis* ($r=.328$, $P=.021$) are in line with research regarding *Roseburia* and thiamine biosynthesis.²⁸³ We found positive correlations between vitamin C and *Roseburia* ($r=.362$, $P=.011$) and *Roseburia faecis* ($r=.358$, $P=.011$). Research regarding correlations between vitamin C and *Roseburia* or *Roseburia faecis* is lacking. A supplementation study by Pharm and colleagues²⁷⁵ reported a slight but consistent increase in *Roseburia* abundance after vitamin C supplementation of 500 mg of ascorbic acid per day for four weeks among 12 participants. In agreement with similar studies, our results show negative correlations between *Prevotella* and total protein ($r=-.332$, $P=.020$), animal protein ($r=-.330$, $P=.021$), cholesterol ($r=-.345$, $P=.015$), and SFA ($r=-.315$, $P=.028$), and negative correlations between *Prevotella copri* and total protein ($r=-.339$, $P=.017$), animal protein ($r=-.339$, $P=.017$), cholesterol ($r=-.361$, $P=.011$), and SFA ($r=-.321$, $P=.025$). *Prevotella* strains are associated with a plant-based diet characterized by high fiber and low protein.^{284,285} Interestingly, it has been suggested that *Prevotella* is not only associated with beneficial effects, but it is also linked to chronic inflammatory conditions, such as arthritis.^{286,287} Future studies are required to further explore the role of *Prevotella* in health and disease. The genus *Faecalibacterium* and species *Faecalibacterium prausnitzii* were moderately correlated with PUFA intake ($r=.344$, $P=.015$) and ($r=.309$, $P=.031$), respectively. *Faecalibacterium prausnitzii* is a butyrate-producing

bacterium with anti-inflammatory effects^{246–248} that is well-known for its inverse relationship with IBD.²⁵⁰ In accordance with our results, research has demonstrated a correlation between *Faecalibacterium prausnitzii* and PUFA intake; however, results are inconsistent.^{288–290} Mokkalā et al²⁹¹ demonstrated a significantly higher abundance of *Faecalibacterium prausnitzii* among pregnant women after supplementation with PUFAs. We found a correlation between Clostridiales and fiber intake ($r=.303$, $P=.035$), which is comparable to other gut microbiota research. Clostridiales taxa is reported as the most active microbial components in the gut of healthy adults through their role in colonic fermentation of dietary fiber to SCFAs.^{292–295} Clostridiales play a vital role on butyrate modification that may prove to be an effective probiotic treatment for intestinal homeostasis.^{294,296} We found a correlation between Clostridiales and LBM ($r=.351$, $P=.006$). Several studies also reported a correlation between Clostridiales and obesity.⁶³ Zhang et al²⁹⁷ compared the human gut microbiota of nine individuals who were evenly distributed to one of three groups; normal weight, morbidly obese, or post-gastric-bypass. Clostridium, the genus taxa under the order Clostridiales was proportionally reduced among the post-gastric bypass group as compared to the normal weight and morbidly obese groups. The authors hypothesized that due to the bypass of the upper small intestine, these local bacteria relocated to the large intestine; thus, modifying microbiota composition and related outcomes.²⁹⁷ In a Japanese study, researchers extracted DNA from the stool of 20 participants; 10 lean and 10 obese and used 16S rRNA sequencing to detect microflora. Results showed higher Clostridiales levels in the obese as compared to the lean participants.²⁹⁸

4.2.3.4. Beta Diversity

While there were no significant differences for within group beta diversity for Bray-Curtis and Jaccard PCoA distances, the ANOSIM showed a significant difference in the Jaccard results for A2-C2 and A1-C1. The significant difference in A2-C2 is likely influenced by the diversity

differences at baseline and possibly from Group A's fermented vegetable intake. We found strong between group dissimilarities for the Jaccard distance based on ANOSIM. Due to high variations in microbial diversity between groups at baseline, it was more appropriate to focus on within group comparison rather than between group comparisons. Within group LEfSe results for Group A showed significant differences for *Ruminococcus torques*, which was significantly decreased at timepoint 2. Research suggests that *Ruminococcus torques* is correlated with increased inflammation. Research regarding *Ruminococcus torques* and fermented vegetable consumption is very limited.^{140,299–302} Meslier et al¹⁴⁰ found *Ruminococcus torques* to be reduced after an 8-week intervention of a Mediterranean diet as compared to the control group. Due to findings that low microbial richness is found among those with metabolic disease such as IBD^{5,303–305} and obesity,³⁰⁶ Chatelier and colleagues³⁰⁵ further compared microbial richness and metabolic disease and report *Ruminococcus torques* as a "potentially pro-inflammatory" species. Brahe and colleagues³⁰⁷ report findings that *Ruminococcus torques* is positively correlated with insulin resistance and labeled it as a metabolic marker in postmenopausal women with obesity. Lastly, Odenwald and colleagues³⁰⁸ also found *Ruminococcus torques* to be positively associated with insulin resistance due to its adverse effects the gut barrier that contribute to metabolic endotoxemia.

Overall, we did not find significant associations between fermented vegetable consumption and Bifidobacteria or Lactobacilli as hypothesized, but we did find an upward trend in the Shannon index and a significant increase in the anti-inflammatory bacteria, *Faecalibacterium prausnitzii*, among Group A at timepoint 2, which could be related to fermented vegetable intake. The lack of our findings is likely related to a small sample size, lack of comparable studies there conducted in America, comparisons of drastically different dietary patterns over geographical provenances, the use of fermented sauerkraut and fermented pickles rather than fermented kimchi, the significantly higher amounts of fermented vegetable

consumption. In an eight-week controlled clinical trial that compared 180 g per day of fermented kimchi consumption to 180 g per day of fresh kimchi consumption there was a significant increase in *Bifidobacteria* spp. among the fermented kimchi group.²⁰³ A six-week, randomized, double-blinded intervention was conducted with 34 Norwegian IBS patients ($n=19$ fermented sauerkraut, $n=15$ non-fermented sauerkraut) to compared the effects of fermentation on GI symptoms and microflora. After a six-week supplementation with either 75 g per day fermented or fresh sauerkraut, there was a significant increase in *Lactobacillus plantarum* and *Lactobacillus brevis* among the fermented sauerkraut group as compared to fresh sauerkraut group.¹³⁶

Overall, we did not demonstrate significant correlations between fermented vegetable intake and metabolic markers, but we did find 28 significant correlations among microbiota and vitamin B levels, obesity, and age that is supported by comparable research. As compared to similar research, it is possible that we did not find any significant patterns between fermented vegetable intake and metabolic markers as compared to other studies, not only because of a small sample size, but also because most of the comparable studies originated from Korea and are influenced by different dietary habits and geographical provenances that this study's population.²⁶⁰ Perhaps, the drastic differences in a lifelong and generation long dietary pattern contributes to different metabolic reactions between the two populations. Maybe it takes more fermented vegetable consumption and/or for a longer period of time to modulate the gut microflora after years of a Western diet consumption. Perhaps, the wide variations of kimchi that are found in Korea contribute to kimchi's repeated positive effects on metabolic markers. In addition to napa cabbage, Korean kimchi may contain garlic, red chili, seaweed, green leek, ginger, leaf mustard, sweet potato, radish, dropwort, wild grasses, lettuce, cucumber, eggplant, pumpkin, and/or burdock to name a few.³⁰⁹ Additionally, the Korean study interventions used between 180 to 300 g of fermented kimchi as compared to 100 g that recommended in our

study.^{183,203,310,311} Moreover, two out of three kimchi studies used intervention diets planned by a dietitian rather than having the participants follow their baseline diet as we did in this study, which may have contributed to positive results from the effects of both fresh and fermented kimchi.^{183,310,311} For example, Choi et al¹⁸³ investigated the effects fermented kimchi on 100 participants; 50 participants consumed 210 g per day and 50 participants consumed 15 g per day for seven days, while participants from both groups consumed the same diets that were created by a dietitian. Both groups showed improved fasting blood glucose, total glucose, total cholesterol, low density lipoprotein cholesterol, serum lipid levels, and total antioxidant levels; however, the effects were more profound among the high kimchi group. A four-week crossover trial by Kim et al,²⁰² compared the effects of fresh versus fermented kimchi consumption on 44 participants who were randomly assigned to either 300 g per day of fresh kimchi ($n=22$) or 300 g per day of fermented kimchi ($n=22$). Both groups were asked to follow the same diet that was created by a dietitian. Both groups demonstrated significant improvements in body weight, BMI, and body fat; however, the fermented kimchi group also demonstrated significant improvements in waist-to-hip ratio, fasting blood glucose, and fasting insulin levels.

4.3. Challenges

Recruitment of participants was a major challenge. This was mostly due to availability of research assistants who were able to commit time to communicate with potential study participants. Adequate staff was needed for duties such as help with recruitment and on-campus participant visits. Furthermore, potential participants who met the study criteria did not participate because they did not like needles, were not willing to drive to UNF three times in six weeks, and/or were challenged with compliance of consuming the recommended amount of vegetables. The fact that participants could only receive up to \$30 compensation for completing the study, may have also caused compliance and recruitment to be more challenging.

During the review process, there were a limited number of publications that contained information on fermented vegetables, associated metabolic pathways, and their effect on inflammation and metabolic disease. Studies that were conducted to establish the health benefits of these live microorganisms were mostly performed in mice and murine cell lines, while human subject studies were often limited to small sample sizes and frequently produces inconsistent results. Thus, it is difficult to adequately report the effects of fermented vegetables consumption on microbiota composition, inflammation, and mechanisms of actions on disease pathogenesis. Furthermore, most research on this topic has been conducted in Asian countries where fermented vegetables are widely consumed and in much larger quantities as compared to the typical consumption of fermented vegetables in the United States. Also, the studies of Asian origin used kimchi as the main source of the fermented vegetables rather than other fermented vegetables such as sauerkraut and pickles of different origins that contain different bacteria strains; thus, have different effects. The lack of research on this topic, particularly in the United States, leaves high variability to determine a study's needed population size, vegetable type, and vegetable amount required to show a significant effect. Capturing the complexity of pathways between diet, the microbiome, and disease pathogenesis remains a challenge to be tackled, particularly when so many additional co-founding factors exists. While increased microbial diversity remains a hallmark for optimal health, further research is needed to define the best microbiota composition and how to achieve that profile.

4.4. Strengths

Due to the high, world-wide, prevalence of diseases associated with poor dietary habits, dysbiosis, and inflammation,^{19,20,50,71,172} further research is needed to find effective strategies to combat their prevalence rates, and regular consumption of fermented vegetables may prove to be a helpful strategy. Not only was this study unique among the limited amount of comparable research, but it was also innovative because it included positive control group to

help differentiate effects of probiotic consumption from other vegetable benefits such as fiber, vitamin, and prebiotic delivery. Moreover, this study tested the feasibility of regular consumption of fermented vegetables and tried to be realistic with the amount of fermented vegetables provided to study participants, considering that fermented vegetables are commonly present in the American diet. This study included a comprehensive array of variables measured based off similar microbiota research that reported their correlation to diet and inflammation. This pilot and feasibility study contributes to the limited body of knowledge related to the role of fermented vegetables on health outcomes of Western cultures.

4.5. Limitations

Limitation of our pilot and feasibility study include a small sample size, using only women participants who were mostly of Caucasian decent, and the exclusion of those with bowel disease are a few limitations. Specifically, our convenience sample obtained in Jacksonville Florida does not accurately represent cultural diversity. The subjects would have been compared to themselves using their baseline and end results; therefore, it would be possible to include more variety of subjects. As mentioned in the literature review, the diversity of the gut microbiota increases from birth to about age 12 whereby it remains stable through adulthood, and then decreases with older age.¹³ This relationship between age and microbiome diversity presents a limitation when comparing results due to the wide age range (18-69 years) of the participants. Also, this study used normal weight, overweight, and obese participants with BMIs ranging from 18-44, which may have introduced more variability in the findings, given that normal weight women have been reported to have a more favorable gut microbiota compositions at baseline as compared to their overweight/obese counterparts;^{63,312} however, the Western-type diet may override the effects of weight on gut microflora profiles.³¹³ The use of food and gastrointestinal surveys is yet another limitation due the nature of their subjectivity. Moreover, some participants may have incorporated

additional confounding variables because they decided to start eating significantly healthier and/or living a healthier lifestyle such as adding a significant amount of physical activity; thus, affecting the intestinal microbiome.

4.6. Conclusion

The human gut houses trillions of microorganisms that compose a dynamic ecosystem unique to everyone. Diet, genetics, environment, lifestyle, and antibiotic use significantly shape the gut microbial composition. Dysbiosis is correlated to increased inflammation and metabolic disease. Recently, consumption of fermented vegetables has emerged as a possible strategy to help reduce dysbiosis. Fermented foods were among the first processed food products that humans consumed. In addition to preservation, fermentation increases the food's ability to synthesize vitamins and enzymes while enhancing the flavor, texture, nutritional quality, and functionality of the food that contribute to the host's well-being. *Lactobacillus* strains are among the predominant bacteria found in fermented vegetables and play a pivotal role in gut homeostasis. A proposed mechanism of action through which the live microorganisms in fermented vegetables act is through a reduction of inflammatory processes in the gut via a decrease in the signaling associated with the NF- κ B signaling pathway as well as the attenuation of the release of pro-inflammatory cytokines. The health benefits of consuming fermented vegetables have been demonstrated in a limited number of studies that used human subjects; nonetheless, they are perceived as good sources of beneficial, functional organisms that have a significant impact on health and disease; therefore, it is recommended to feed the microbiome accordingly. It is imperative to carry out more studies not only on the effects of consuming fermented vegetables on gut composition and disease, but also to identify the metabolic pathways and biomarkers associated with diet and disease. Modulation of gut microbiota is considered the first target to establish probiotic efficacy in a healthy population. Understanding of the relationship of diet and the gut microbiome is vital for the development

of personalized medicine, food products, eating patterns, and other therapeutic strategies to help combat the global burden of non-communicable diseases. More randomized control trials and large cohort are needed to better understand the interactions between the microbiome and diet, environment, genetics, and lifestyle to discover evidence of the effects of fermented vegetable consumption on inflammation and gut microbiota composition.

Some this study's strengths include a positive control group, randomized study design, and comprehensive array of variables based on research that reported correlations between these variables and inflammation and/or gut microflora. Limitations of this study were a small sample size, use of a FFQ, use of only women participants who were mostly of Caucasian decent and obtained in Jacksonville Florida, a wide age range, and a wide weight range. However, the primary objective of this pilot and feasibility study was to explore the feasibility of fermented vegetable consumption for six weeks on markers of inflammation and gut microflora profiles in women. Indeed, we found one high and 27 medium significant correlations that are in agreement with similar studies that included a positive correlation between age and Lachnospiraceae, age and Bifidobacterium, vitamins B2 and B12 and Bifidobacterium; vitamin B1 and Roseburia, fiber and Clostridiales, and negative correlations between Prevotella and total protein, animal protein, cholesterol, and SFAs. Moreover, we found an upward trend in the Shannon index, a significant increase in the anti-inflammatory bacteria, *Faecalibacterium prausnitzii*, and a decrease in pro-inflammatory bacteria, *Ruminococcus torques*, at timepoint 2 among Group A at timepoint 2 that may be related to fermented vegetable consumption. Future larger randomized controlled trials are needed to determine the precise effects of fermented vegetable consumption on metabolic markers and gut microflora. Our next step will be to obtain three stool samples per participant per timepoint to reduce the limitations of having a small sample size. It is also important to obtain an optimal

level of fermented vegetable consumption that will modulate metabolic markers and gut microflora.

Chapter Five: Implications for Practice

5.1. Nutritional implications

The nutritional implications of the consumption of fermented vegetables on the composition of the intestinal microflora are diverse. It is important to note that whether fermented or not, many vegetables contain prebiotics that promote increased probiotic growth; thus, increasing nutritional benefits.³¹⁴ Microorganisms present in fermented vegetables release various enzymes and produce vitamins such as B and K vitamins in the intestinal gut.³¹⁵ Microorganisms present in fermented vegetables have been known to increase the expression of the main calcium ion transporter in intestinal epithelial cells thereby increasing the calcium pools in the gut resulting in strong teeth and bones. Microorganisms in fermented vegetables have been shown to also increase the availability of vitamin D in the enterocytes of the gut.¹⁶⁷ Hydrolysis of these microorganisms may enhance protein and fat bioavailability as well as induce the production of free amino acids, SCFAs, lactic acid, propionic acid, and butyric acid that aid in increasing the energy pool of the host individual.³¹⁶ They also have been known to improve the digestion of certain food components. *Lactobacillus acidophilus* has been known to release lactase that aids in the digestion of lactose, thereby reducing the symptoms of lactose indigestion in lactose-intolerant individuals.³¹⁷ The potential of fermented vegetable consumption on the prevention and improvement of many health concerns is significant. As more studies confirm the health benefits of fermented vegetables, a need for nutrition education will increase.

Fermented vegetables pose a food-drug interaction with monoamine oxidase inhibitors (MAOIs) due to the high levels of tyramine in fermented foods. Consumption of foods high in tyramine while taking an MAOI can result in dangerous levels of tyramine and can cause increased blood pressure that may require emergency treatment. In addition, the high levels of sodium in the brine solution that is required for fermentation to occur may also increase blood

pressure among those who are salt sensitive. A low tyramine may be needed.³¹⁸ Per the Academy of Nutrition and Dietetics (AND),³¹⁹ a tyramine-restricted diet recommends to avoid fermented and aged foods. No specific tyramine amount was given, but the AND did recommend foods to eat and not to eat. The recommended foods are grains; fresh, frozen, or canned fruit and vegetables; pasteurized dairy; and fresh meats and fish. Foods to limit are alcohol and caffeinated beverages such as coffee and cola. Foods to avoid are fermented vegetables such as sauerkraut and kimchi; decomposed or spoiled fruit and vegetables; aged cheese such as cheddar and gouda; fermented meats such as corned beef and chorizo; wine and beer; and fermented soy products such as soy sauce and soybean curd. Also, it was recommended not to eat food that was left in the fridge for more than 24-48 hours.³¹⁹

5.2. Dietetic Implications

Nutrition is becoming recognized as a necessity of treatment that highlights food as medicine. Due to the nutritional implications of fermented vegetables, there is great opportunity for dietitians to get involved in research that will unravel the specific benefits of fermented vegetables and move registered dietitians further into the forefront of medicine. RDNs could develop diets that are high in the desired bacteria to provide personalized nutritional counseling.³²⁰ For example, some strains of Lactobacilli and Bifidobacteria produce high levels of folate,^{321,322} that could someday be obtained through fermented vegetable consumption³²³ and reduce the risk of B vitamin deficiencies.³²⁴

B vitamin food fortification has drastically reduced deficiencies and conditions such as pellagra, beriberi,³²⁵ neural tube defects,³²⁶ and anemia.³²⁷ However, food fortification does not meet many populations' needs such as those with alcohol dependence, gastrointestinal diseases, and HIV/AIDS, in addition to the elderly, reproductive-aged women, pregnant and postpartum women, young children, female adolescents in low income countries, women and children in low-income countries such as southeast Asia and Africa, vegans, those who cannot drink milk

due to lactose intolerance, children in low-income countries where gastrointestinal infections are prevalent such as Africans and Asians, those with enhanced riboflavin excretion due to diabetes mellitus, trauma, stress, and oral contraceptive use remain at increased risk of morbidity due to B vitamin deficiencies.^{324,327} Perhaps, fermented foods such as fermented vegetables could help bridge the gap of fortification programs and help many at risk populations obtain adequate B vitamin intake.^{323,328–330}

5.3. Policy implications

The policy implications with regards to the health benefits of consuming fermented vegetables are not clearly defined. Probiotics or live microorganisms present in fermented vegetables are overlapping between conventional and regulatory definitions of what constitutes a food and what constitutes a drug.³³¹ Regulatory agencies such as the Food and Drug Administration and the Federal Food, Drug, and Cosmetic Act³³² that oversee food and pharmaceutical manufacturing consider live microorganisms in fermented vegetables as either whole food, enriched, or fortified foods that might have a potential beneficial effect on the health of an individual when consumed regularly. With regards to the study of these microorganisms and their implications on the treatment of specific diseases, these microorganisms in fermented vegetables might be viewed as medicinal food and thus subjected to regulation by the FDA.³³² Live microorganisms present in fermented vegetables might also be viewed as pharmaceutical drugs and regulated by the US regulatory code.

In terms of the guidelines required for the proper use of probiotics found in fermented vegetables, the following dietitian guidelines should be followed:

1. Individuals with impaired immune functions are generally required to seek specific advice from a licensed physician with regards to the use of these live microorganisms to alleviate gut disorder symptoms.³³³

2. Patients with gastrointestinal disorders such as IBS and IBD are recommended by registered dietitians/nutritionists to consume fermented foods with live microorganisms for a trial period of about four weeks in order to relieve symptoms commonly associated with these disorders such as diarrhea and bloating.³³³
3. Patients on antibiotics are at a high risk of being contaminated with the *Clostridium difficile* bacteria that causes diarrhea in these patients. It is often recommended as a preventive measure to ingest probiotics which might prevent the proliferation of this bacteria in the gut of these patients.³³⁴

The identification of cytological and molecular biomarkers may be utilized as screening tools to predict and prevent many diseases such as obesity, diabetes, and cancer. Discovering genetic information related to the consumption of fermented vegetables and host metabolism and disease development could prove vital in the understanding of the effect of probiotics, nutrients, and dietary factors at the molecular level.³³⁵ As the population ages and diseases multiply, it is imperative that various stakeholders such as the government, non-governmental organizations, policy makers, health providers, and dietitians take advantage of this opportunity to perform research in hopes of reducing inflammatory and metabolic disease prevalence. The development of a consensus definitions for prebiotics and probiotics by the ISAPP¹³³ is beneficial for many stakeholders. These consensus definitions help reduce misinformation and misinterpretation among consumers and healthcare providers. This will help facilitate standard guidelines for scientific research, consumer-friendly and informative product labels, accurate marketing messages, safe product manufacturing, defined product regulations, and accurate information provided by healthcare professionals.³³⁶

5.4. Ethical implications

The ethical implications with regards to the role of probiotics present in fermented vegetables and their health benefits have not been widely studied. Till date, little is known about the wider

public's view on the therapeutic and health benefits of live microorganisms found in fermented vegetables. Individuals with gastrointestinal disorders are the target populations that can best answer the questions with regards to the potential benefits of this food in alleviating their symptoms associated with these disorders. Their opinions may provide insight into key issues with regards to the regulation of such foods for therapeutic purposes as well as the use of these foods in clinical trial research, patient care, and the need for this target population to participate in the informed decision-making process. Patients with gastrointestinal disorders likely expect more rigorous regulation of these food products in terms of low costs and low involvement of pharmaceutical companies that might want to market these foods specifically for this target population.³³⁷

Studies should be carried out in human subjects in order to determine the health benefits of consuming fermented vegetables in terms of the immune response and the growth composition of the intestinal microflora. It is important to properly ascertain the strain of bacteria that is responsible for promoting immune regulation observed upon ingestion of these microorganisms present in fermented vegetables. Additionally, there exist several risks associated with the ingestion of bacterial probiotics specifically in immune-compromised individuals such as pregnant women, babies, and the elderly.³³⁸ The interaction of probiotic microorganisms present in fermented vegetables with gut commensal bacteria might have direct implications on the health of the host. Clinical studies are required in order to understand how microorganisms from fermented vegetables interact with the host gut microbiota and their role in the promotion of immune defense mechanisms.³³⁹ Several microorganisms in fermented vegetables particularly enterococci may confer and transmit antibiotic resistance to bacteria of the *Bacillus cereus* group that are known to produce enterotoxins in the gut.³⁴⁰ Probiotics may also cause systemic infections, overstimulation of the immune system, and impaired metabolic activities in vulnerable individuals.³⁴¹

Controlling the microbial ecosystem from fermented food products offers great therapeutic alternatives for the prevention and treatment of diseases. The fact that the ingestion of probiotic microorganisms has great implications in improving the health of individuals and preventing certain diseases is gaining traction. However, the term probiotic has been loosely used and has led to the overpromotion of the health benefits of these microorganisms without reliable data in human subjects to prove such findings.

5.5. Future Directions

Continued development of full shotgun metagenomics sequencing of the genomes of untargeted cells in a community provides community composition and function that allows for greater taxonomy identification and profiling; thus, increasing human understanding of the mechanisms and cross-talk between the gut microbiome and disease. Currently, this method is limited due to the high cost and the technology needed to host DNA interference. 16S mRNA amplicon sequencing identifies microbes but does not provide microbial function. Next generation sequencing such as, shotgun metagenomics, provides gene composition in addition to microbial identity. Shotgun metagenomics reveals genes that are encoded by certain bacteria providing a deeper understanding of the mechanisms between bacteria and disease.³⁴²

Food-grade cloning vectors that genetically modify food-grade probiotics could become industrialized.^{343–345} Also, genetic modification of probiotics as delivery vehicles for bioactive compounds or antigens could provide targeted, disease-specific, delivery of therapeutic molecules.³⁴⁶ Perhaps probiotics that are genetically created could be added to many common, non-fermented foods that allows humans to safely and easily consume desired levels of various health-promoting probiotics. People in developed and developing countries could benefit from probiotic products and foods to help fight against disease. Future research paves the way for biotechnology companies to market personalized microbiome testing and could lead to microbiome-based health screenings. Dietary supplementation of specific

bacteria in therapeutic doses may prove to be a preferred method for targeted, health therapies, as compared with consumption of adequate bacteria through food intake. Standardization of future guidelines for product development and food labeling will be essential.

Vitamin-producing bacteria provide a new perspective and hope of a more consumer-friendly vitamin fortification process than synthesized vitamins; however, more research is needed to determine dosing, absorption, and production of vitamin-producing bacteria. The use of vitamin-producing bacteria may provide an organic, marketable solution that adds nutrition value to fermented products for people obtain vitamin Recommended Daily Intake values. This could help people save money on synthesized vitamins in addition to reducing the risk of vitamin toxicity. Precision medicine is an attractive approach for disease therapy, but further understanding of the interplay between genes, phenotypes, and the microbiome is needed. Lastly, ideas for future studies include:

- A follow-up article that examines case presentations may discover further findings by analyzing specific variables unique to the individual. The results of this study were reported for averaged outcomes per group. It would be interesting to see participant results on the individual level.
- US conducted feeding trials that compare fermented kimchi to other fermented vegetables to obtain feasible amounts needed of various fermented vegetables for effective gut microbiota modulation among people in the US. Kimchi may be a more potent fermented vegetable as compared to fermented pickles and fermented sauerkraut and may be more feasible for many Americans to consume if smaller amounts are needed for microbiota change.
- Feeding trials that examine the effects of fermented vegetable consumption of dysbiosis and disease.

- Studies that obtain three stool samples per participant per timepoint to increase the data pool and to discover fluctuations among individual stool microflora.
- Studies that investigate vitamin B and K uptake and absorption from fermented vegetable consumption to decipher if fermented vegetable consumption is a feasible method to help high risk populations that suffer from these vitamin deficiencies.
- Fermented vegetable feeding trials that exclude salt sensitive subjects to control for blood pressure increases due to the high sodium content of the brine used for fermentation.

Appendix

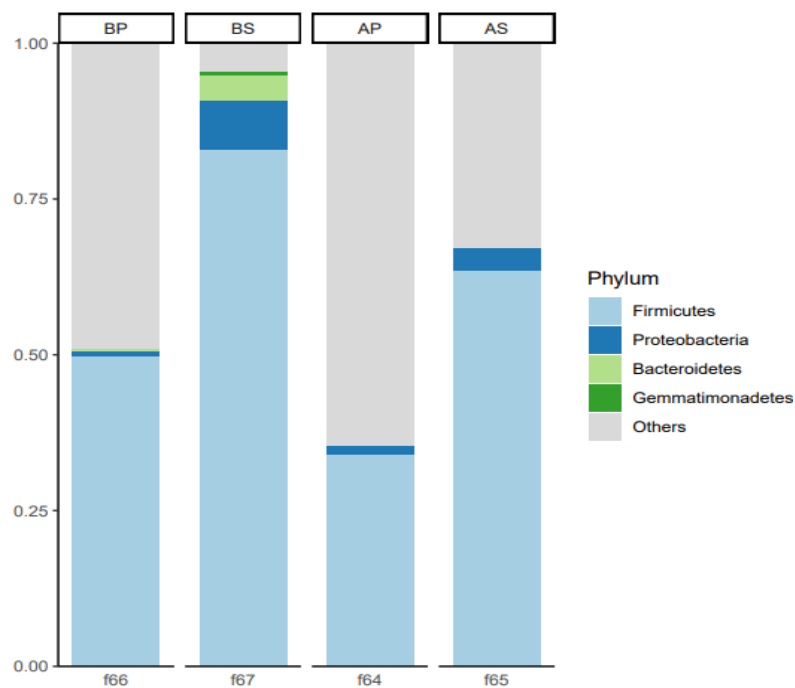


Figure S1: Phylum-Diet: Firmicutes is the predominant phyla across all vegetable types, with the non-fermented sauerkraut having the greatest relative abundance at 82.9% followed by fermented sauerkraut (relative abundance 63.5%), non-fermented pickles (relative abundance 49.8%), and fermented pickles (relative abundance 34.1%). BP (non-fermented pickles), BS (non-fermented sauerkraut), AP (fermented pickles), AS (fermented sauerkraut).

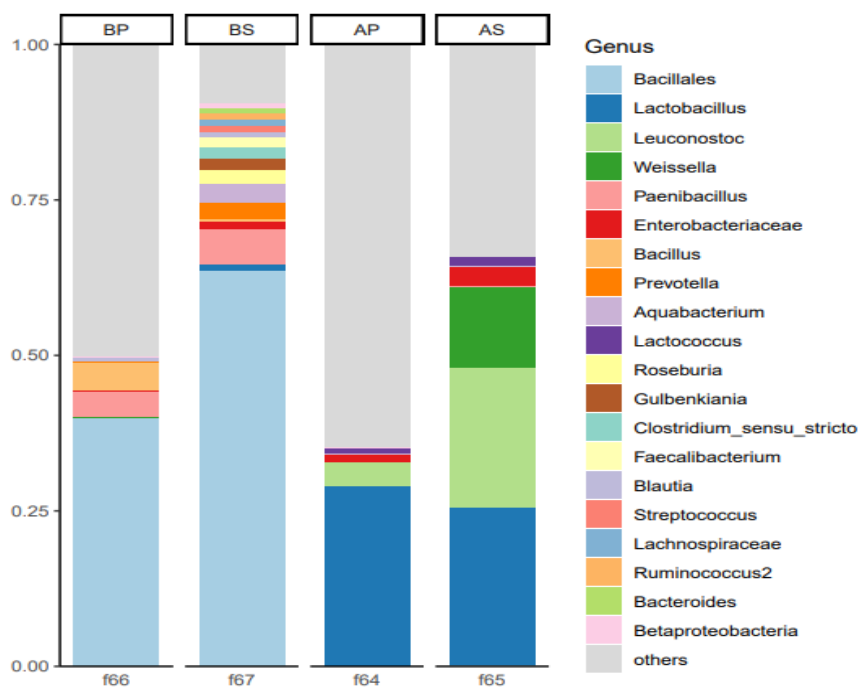


Figure S2: Genus-Diet: Bacillales is the predominant genus for the BP (relative abundance 40%) and BS (relative abundance 63.7%). Lactobacillus is the predominant genus for the AP (relative abundance 28.8%) and AS (relative abundance 25.4%). BP (non-fermented pickles), BS (non-fermented sauerkraut), AP (fermented pickles), AS (fermented sauerkraut).

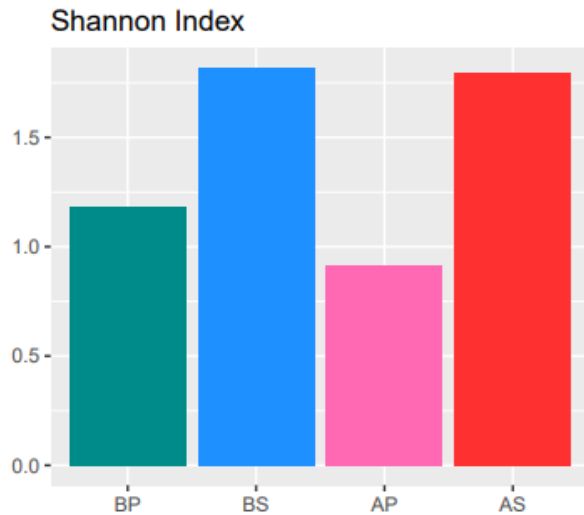


Figure S3. Shannon Index-Diet
BP (non-fermented pickles), BS (non-fermented sauerkraut), AP (fermented pickles), AS (fermented sauerkraut)

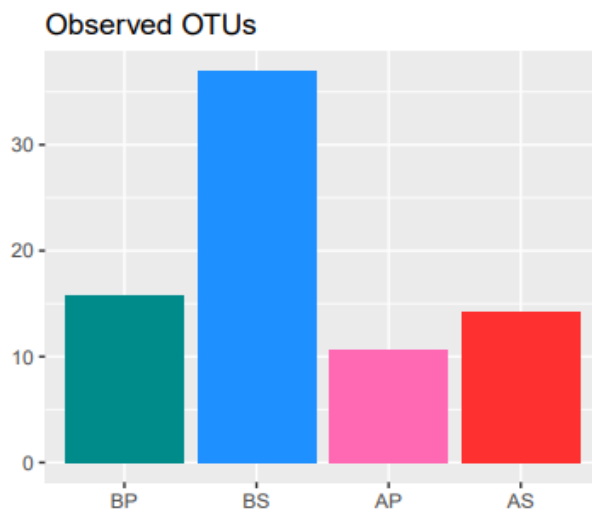
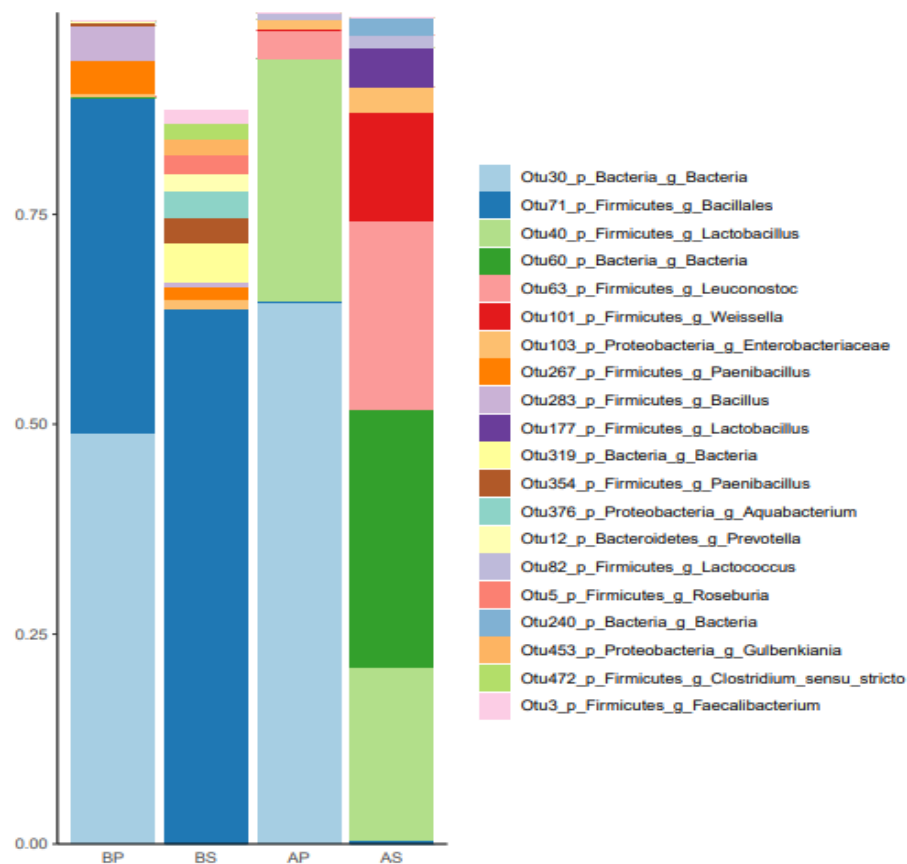


Figure S4. Observed OTUs-Diet
BP (non-fermented pickles), BS (non-fermented sauerkraut), AP (fermented pickles), AS (fermented sauerkraut)

Figure S5: Top OTUs-Diet



BP (non-fermented pickles), BS (non-fermented sauerkraut), AP (fermented pickles), AS (fermented sauerkraut)

Figure S6: Gastrointestinal Function Log

Days	Vegetable Eaten	Amount Eaten	Frequency of Bowel Movements			Stool Consistency				Side Effects				
	C = carrots with sauerkraut P = pickles	Mark 1, 3/4, 1/2, 1/4, or 0 cups	0	Once	Twice or more	Lumpy and hard to pass	Smooth and soft	Fluffy pieces, mushy	Watery, no hard pieces	Nausea/Vomiting	Diarrhea	Bloating/Gas	Abdominal pain	Other*
1														
2														

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