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# Impact of Different Plant-Based Foods Consumption on the Composition and Diversity of Gut Microbiota

Ayoub Al Othaim
*University of Arkansas, Fayetteville*

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by

Ayoub Al Othaim
Qassim University
Bachelor of Medical Microbiology, 2009
Quinnipiac University
Master of Heath Science, Medical Laboratory Sciences, 2014

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University of Arkansas

This dissertation is approved for recommendation to the Graduate Council.

_____
Franck Carbonero, Ph.D.
Dissertation Director

_____
Luke R. Howard, Ph.D.
Committee Member

_____
Jiangchao Zhao, Ph.D.
Committee Member

_____
Charles Rosenkrans, Ph.D.
Committee Member

**ABSTRACT**

Plant-based diets are primarily derived from plant sources, including fruits and vegetables. Such patterns are associated with health benefits by promoting general health and preventing many diseases and metabolic disorders**.** Fruits and vegetables are key sources of high-quality nutrients such as dietary fibers and polyphenols. Since they are rich in dietary fibers and polyphenols, whole fruits and vegetables represent unique and somewhat understudied modulators of the gut microbiota and its associated functions. This study aims to (i) investigate the impact of varying dilutions of cherry juices concentrate on the composition of the murine gut microbiota, (ii) investigate the impact of cranberry juice consumption on the bacterial composition of gut and vaginal microbiota in postmenopausal women, and (iii) investigate the impact of the amounts and botanical diversity of fruits and vegetables on gut microbiota profiles in healthy adults. Fecal samples were collected from mice consuming increasing tart cherry juice concentrations in their drinking water. A randomized, crossover controlled dietary intervention was conducted with cranberry juice and a matched control, and postmenopausal female volunteers provided stool and vaginal swabs for microbiota analyses. Two pilot studies looking at the impact of low and adequate intake of fruits and vegetables (SMART-Diet), as well as low versus high botanical diversity (SMART-Valid), were conducted at Colorado State University; stool samples were collected for gut microbiota analyses and short-chain fatty acids measurements. Microbial analyses were performed using Illumina following a dual-indexing 16S rRNA gene amplicon. Each dietary intervention resulted in distinct microbiota modulations and effects. Cherry juices consumption significantly increased the relative abundance of *Akkermansia* and *Barnesiella* and decreased the relative abundance of *Bacteroides*. Consumption of cranberry juice modulated the gut microbiota composition by elevating the relative abundance

of *Bifidobacterium*, *Clostridium* XIVa members, *Eggerthella*, and *Prevotella*. Cranberry juice consumption also resulted in a beneficial impact on vaginal microbiota by decreasing the relative abundance of *Streptococcus* compared to a placebo. Finally, the SMART-Diet study showed that fruit and vegetable consumption resulted in a reduction of the Firmicutes/Bacteroidetes ratio whereas the SMART-Valid study showed an increase in the Firmicutes/Bacteroidetes ratio after fruit and vegetable consumption, and a significant increase in production of beneficial short-chain fatty acids. Our findings suggest that consumption of cherry juices, cranberry juice, and fruit and vegetable inclusive diets all have a beneficial impact on the composition of gut microbiota. This body of work is consistent with the emerging concept that whole foods should be considered rather than extracts when it comes to gut microbiota and microbiome studies. Human dietary studies with larger sample size are warranted by these suggested beneficial modulations.

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**DEDICATION**

This study is dedicated to my beloved parents, Abdulaziz Al Othaim and Helah Al Othaim, who have been my source of inspiration and have given me endless love and encouragement to complete my studies. My dream of completing this research would not have come true without their support, sacrifice, and prayers. I also dedicate this work to my wife, soulmate, and best friend, Dr. Sarah Alajaji, with my heartfelt thanks for her mental strength, support, encouragement, and patience; and to my dear little boy, Feras, the most precious gift in my life, to whom I express my deep love. Though I did not deliberately neglect him, he has missed out on some of my love, care, and affection during this busy period. I also extend my gratitude to my siblings, Huda, Wafa, Sumayyah, Mohammed, Ibraheem, Younes, Yousef, Motaz, Adeeb, and Jory, whose continued support has motivated me to pursue my education and complete this accomplishment. Last but not least, I express my sincere thanks to my parents-in-law, Mansour and Zainab, for their blessings, affection, and support during my studies.

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## GENERAL INTRODUCTION

Diet plays a crucial role not only on the host's general health but also on modulating the gut microbiota ecosystem. This connecting role of gut microbiota has been illustrated by their impact on many aspects of human homeostasis including immune system modulation and metabolic and physiologic effects. The imbalance of the gut microbiota, often termed dysbiosis, has been associated with several diseases and metabolic disorders. With either beneficial or deleterious consequences, specific dietary patterns can impact the gut microbiota composition and its impact on health. A growing body of studies has shown the beneficial impact of plant-based diet on general health by preventing diseases and disorders as well as improving the gut microbiota ecosystem.

Plant-based diets typically consist of foods that are derived from plant sources, mainly fruits and vegetables. Fruits and vegetables are rich in bioactive compounds such as dietary fibers and polyphenols, as well as other essential nutrients, including vitamins and minerals. The impact of dietary fibers and polyphenols on the gut microbiota composition and diversity has been receiving attention in recent years. Gut microbes use dietary fibers as the primary source of energy and produce bioactive metabolites, such as short-chain fatty acids which are the main fermentation end-products (acetate, propionate, and butyrate). Some metabolites have been shown to exert beneficial impact on various physiological processes. Additionally, gut microbiota plays a vital role in polyphenols bioavailability. Polyphenols with high molecular weights cannot be absorbed in the small intestine; however, gut microbiota in the large intestine converts unabsorbed polyphenols to smaller bioactive metabolites which make them available to the host. These bioactive metabolites might be responsible for potential health benefits rather than the original native polyphenols.

The vast majority of published papers in the human nutrition and gut microbiome field have focused on studying the influence of extracted food compounds on gut microbiota ecosystem instead of whole foods. Therefore, this present dissertation brings attention to the impact of whole foods — including cherry juices, cranberries juice, and the amount and botanical diversity of fruit and vegetable diets — on the gut microbiota composition.

**CHAPTER 1:**

# Impact of Fruit and Vegetable Inclusive Diets on Gut Microbiota Composition and Diversity

Ayoub Al Othaim[1,2] and Franck Carbonero[1,3,4*]

[1] Cell and Molecular Biology Program; University of Arkansas, Fayetteville, AR, 72701 USA; franck.carbonero@wsu.edu
[2]Department of Medical Laboratories, College of Applied Medical Sciences, Majmaah University, Al-Majmaah, 11952, Saudi Arabia
[3]Department of Food Science; University of Arkansas, Fayetteville, AR, 72704 USA
[4]Department of Nutrition and Exercise Physiology, Elson Floyd School of Medicine, Washington State University-Spokane, 412 East Spokane Falls Boulevard, Spokane, WA, 99202 USA
* Correspondence: franck.carbonero@wsu.edu
[†] The work is a part of Ayoub Al Othaim's doctoral thesis

## Abstract

Fruits and vegetables are a fundamental source of dietary fibers, polyphenols, and essential nutrients, including vitamins and minerals. Nutritional recommendations emphasize fruit and vegetable consumption due to their health benefits on the host. Fruits and vegetables rich in dietary fibers and polyphenols can impact the gut microbiota composition and associated functions, whereas gut microbiota can metabolize components in fruits and vegetables into bioactive metabolites. Gut microbiota and a diet-rich in fruits and vegetables play a significant role in promoting the host's general health and preventing multiple diseases and metabolic disorders. This review recapitulates the latest findings on the impact of whole fruit and vegetable consumption on gut microbiota composition as well as the role of gut microbiota and a diet-rich in fruits and vegetables in disease and disorder prevention. This body of literature, while still limited especially in terms of randomized human dietary intervention, strongly suggests overall

beneficial modulation of the gut microbiome by common and less common fruits, and possibly vegetables.

**Keywords:** Fruits, Vegetables, Gut microbiota, Polyphenols, Dietary fibers.

## 1.1 Introduction

Since the emergence of human nutrition as a scientific discipline and the development of nutritional guidelines, what constitutes a healthful diet is still intensely debated. Recently, the debate has become even more complicated with emerging evidence of the complex modulation of nutrients because of overlooked metabolic processes and their influence on health and disease. The mutualistic relationship between diet and gut microbiota is an example of exponential scientific interest. Diet is the most effective factor for shaping gut microbiota composition and consequently metabolites production and absorption with potential impacts on the host's general health.

Gut microbiota refers to the ecological communities that live in the gastrointestinal tract. There are trillions of microbes with thousands of life bacterial species in the human gut. The gut microbiota profile is not constant among humans, and is strongly individualized. This variation occurs due to several factors including host genetics, diet, age, mode of birth, and antibiotics. Disruption of the gut microbiota composition can alter gut homeostasis and thereby cause a variety of diseases. Therefore, the maintenance of the gut microbiota equilibrium is important to maintain a healthy status.

Diet is one of the most important factors that impact the shape, composition, and diversity of gut microbiota. This is to be expected, since microorganisms use undigested foods as fuel for their essential biological processes. Thus, changes in dietary patterns alter the bacterial

metabolism, which in turn influence the abundance of different types of bacteria in the gut. Specific dietary patterns strongly alter gut microbiota and can affect its impact on health, with either beneficial or deleterious consequences.

Western diet is typically characterized by high total energy due to saturated fat (butter, red meat) and sugar with an associated reduced consumption of fruits and vegetables. The high intake of energy-dense and processed foods is a risk factor for many chronic diseases including obesity, diabetes, inflammatory bowel diseases (IBD), and cardiovascular diseases. Inflammation is one of the underlying mechanisms linked with Western diet. There is a growing recognition for the influence of gut microbiota to the complexity of these disorders.

For decades, many studies have sought to substantiate the purported beneficial effects of fruits and vegetables-based diet. Epidemiological and dietary intervention studies have consistently indicated that plant-based diets provide a beneficial impact on human health and biomarkers. The present review discusses the importance of plant-based diets in shaping the gut microbiota and the potential health effects of these diet-microbiota interactions.

### 1.2 Plant-based dietary patterns

A plant-based diet is a diet that is mostly or completely derived from plant sources. It can be obtained by increasing the intake of plant based foods such as fruits, vegetables, beans, seeds, pulses, herbs, whole grains, and nuts and reducing or excluding processed foods and animal products, including red meat, poultry, fish, eggs, and dairy products (Ostfeld, 2017; Tuso, Ismail, Ha, & Bartolotto, 2013). Plant-based dietary patterns have exploded in popularity over the past several decades. In the United States, the percentage of adults who chose a plant-based diet was approximately 3.3% in 2016 ("How Many Adults in the U.S are Vegetarian and Vegan?," 2016).

More recently, the department of agricultural economics at Oklahoma State University developed a project to track consumer's food preferences. This project was a series of monthly surveys called "Food Demand Survey (FooDS)" and was conducted between 2013 and 2018. The project data showed an increase in the number of self-identified vegetarians in the United States, around 4.1% between 2013 and 2018 ("Food demand Survey," 2018). Furthermore, the interest in the potential benefits of plant-based diets has increased in the last few decades. This not only underscores the increased interest in plant-based diets among consumers and food industries but also indicates the improved awareness and understanding of how plant-based diets impact human health.

### 1.2.1 Health benefits of plant-based diets

Plant-based dietary patterns are typically rich in dietary fiber and a variety of phytonutrients, antioxidants, vitamins, and minerals and lower in fat, particularly saturated fat (Hever & Cronise, 2017). Plant-based diets have become a topic of formal scientific inquiry. A substantial number of studies have been conducted to confirm the beneficial effect of plant-based nutrition on the host. Plant-based foods have been associated with a reduced risk of many diseases and metabolic disorders, including obesity, diabetes, cardiovascular diseases, and cancer.

#### *1.2.1.1 Lower risk of obesity*

Obesity is a serious pandemic worldwide. The World Health Organization (WHO) estimated that more than 1.9 billion people are obese or overweight in 2016 ("Obesity and overweight," 2020). According to the Centers for Disease Control and Prevention (CDC), the prevalence of obesity in the United States among adults was 42.4% in 2017-2018 (Hales, Carroll,

Fryar, & Ogden, 2020). Obesity is associated with severe health conditions and triggers obesity-related diseases, including type 2 diabetes, cardiovascular diseases, and cancers ("Obesity and overweight," 2020; Wright, Wilson, Smith, Duncan, & McHugh, 2017).

Poor diet plays a key role in the genesis of obesity. In contrast, plant-based diets show evidence to achieve and maintain healthy body weight (Melina, Craig, & Levin, 2016). A growing body of studies has examined the use of plant-based dietary patterns for weight loss and obesity prevention. Berkow and Barnard reviewed numerous published articles containing information on vegetarians and plant-based diets. While the systematic review indicated the impact of plant-derived foods in weight loss compared with other diets, there was also a clear pattern for subjects who follow plant-based diets to be at a lower risk of obesity. The observational studies showed a lower average body weight in vegetarian individuals compared to non-vegetarians, approximately 3% to 20%. In addition, vegetarians have a consistently lower obesity prevalence rate, 0% to 6%, compared to non-vegetarians, 5% to 45% (Berkow & Barnard, 2006).The Oxford component of the European Prospective Investigation into Cancer and Nutrition (EPIC) compared changes in weight and body mass index (BMI) over a five-year period in approximately 22,000 meat-eating, fish-eating, vegetarian, and vegan participants in the United Kingdom. The study results showed that the lowest weight gain was associated with individuals who followed a vegan diet and those who had changed to a diet containing less animal products. The result also showed a significant difference in BMI between the four diet groups, with the meat-eaters having the highest BMI and the vegans had the lowest BMI (Rosell, Appleby, Spencer, & Key, 2006). In 2016, a meta-analysis of randomized controlled trials was performed to compare the influence of vegetarian diets and non-vegetarian diets on weight loss among individuals. This review included twelve randomized controlled studies with

approximately 1,150 subjects. The results showed a significant weight reduction among individuals assigned to vegetarian diets compared to individuals assigned to non-vegetarian diets (Huang, Huang, Hu, & Chavarro, 2016). A randomized controlled trial study was performed to investigate the impact of a whole food plant-based diet on either obese or overweight individuals with a diagnosis of either type 2 diabetes, ischaemic heart disease, or the cardiovascular risk factors of hypertension or hypercholesterolaemia. At 6 and 12 months, the whole food plant-based diet reduced the BMI to 4.4 and 4.2 kg $m^{-2,}$ respectively, compared with the subjects' typical diet. Furthermore, the total cholesterol tended to decrease after the implementation of whole food plant-based diet in both intervention periods (Wright et al., 2017). Data from the Adventist Health Study-2 showed that obesity and abdominal adiposity were significantly lower among non-Hispanic white adults who followed a vegetarian diet (Matsumoto et al., 2019).

#### *1.2.1.2 Lower risk of diabetes*

Diabetes is one of the most significant global health crises of this century ("Diabetes," 2020). Globally, the number of people diagnosed with diabetes in 2017 was approximately 425 million (Cho et al., 2018). In the United States, around 13% of all US adults over 18 years of age had been diagnosed diabetes in 2018 (Centers for Disease Control and, 2020). Lifestyle changes, mainly dietary changes, can play a crucial role in preventing, treating, or promoting type 2 diabetes (McMacken & Shah, 2017). Unlike calorie-dense foods, such as diets high in saturated fat and sugar, plant-based diets may effectively prevent or treat type 2 diabetes (McMacken & Shah, 2017; Papier et al., 2019).

The association between dietary patterns and the incidence of diabetes was investigated using data from the Adventist Health Study-2. The results showed that vegans, lacto ovo-

vegetarians, and semi-vegetarians were less likely to develop diabetes (Tonstad et al., 2013). A meta-analysis study showed that fruit and vegetable consumption, especially green leafy vegetables, is associated with a significant reduction of type 2 diabetes incidence (M. Li, Fan, Zhang, Hou, & Tang, 2014). Data from the Nurses' Health Study, Nurses' Health Study 2, and the Health Professionals Follow-up Study suggested that plant-based diets, mainly diets with high-quality plant foods, are associated with a lower risk of developing type 2 diabetes (Satija et al., 2016). A study was conducted to investigate the correlation between a vegetarian diet and the risk of diabetes in a Taiwanese population which stated that a vegetarian diet might protect against diabetes (Chiu, Pan, Lin, & Lin, 2018). The EPIC-Oxford cohort study was used to examine the association between vegetarian diets and the risk of type 2 diabetes in British adults. The study results suggested that vegetarian and low-meat diets may reduce the risk of developing diabetes development (Papier et al., 2019).

#### *1.2.1.3 Lower risk of cardiovascular diseases*

Cardiovascular diseases are the most prevalent cause of death worldwide, with more than 17.6 million deaths per year. Approximately one-third of all global deaths are caused by cardiovascular diseases ("Cardiovascular diseases (CVDs)," 2017; "Heart Disease and Stroke Statistics-2019 At-a-Glance," 2019). Plant-based diets are associated with a reduction in the risk of cardiovascular diseases and cardiovascular disease mortality (Dinu, Abbate, Gensini, Casini, & Sofi, 2017; H. Kim et al., 2019). Plant-derived foods also showed evidence of reducing heart disease risk factors such as serum total cholesterol, blood glucose, abdominal obesity, and blood pressure (Rizzo, Sabaté, Jaceldo-Siegl, & Fraser, 2011; Wang et al., 2015; Yokoyama, Barnard, Levin, & Watanabe, 2014; Yokoyama, Nishimura, et al., 2014).

The EPIC-Oxford cohort study results were used to investigate the impact of different dietary patterns on serum lipid concentration. The study showed that vegans had the lowest total cholesterol in serum compared to individuals assigned to other diet patterns (Bradbury et al., 2014). A meta-analysis of 11 randomized controlled trials found that the total low-density lipoprotein and high-density lipoprotein cholesterols were low among vegetarians. These findings, perhaps, explain why plant-based diets reduce the risk of cardiovascular disease and cardiovascular disease mortality (Wang et al., 2015). A comprehensive meta-analysis reported that vegetarian diets significantly decrease the incidence and/or mortality from ischaemic heart disease by about 25% (Dinu et al., 2017). Data from the Adventist Health Study-2 (AHS-2) were analyzed to assess the association between dietary patterns and cardiovascular disease risk factors among non-Hispanic white adults. The investigation showed that vegetarian diets were associated with significantly lower levels of cardiovascular disease risk factors than non-vegetarian diets (Matsumoto et al., 2019).

#### *1.2.1.4 Lower risk of cancers*

Cancer is a major public health issue worldwide and is the second leading cause of death globally. In 2018, the total number of deaths caused by cancer was around 9.6 million deaths, meaning that one of every six deaths was cancer-related ("Cancer," 2018). According to The American Cancer Society (ACS), the estimated number of new cancer cases and cancer deaths in the United States was 1.8 million and 606,520, respectively, in 2020 (Siegel, Miller, & Jemal, 2020). Plant-based dietary patterns have been associated with reducing the risk of cancer occurrence.

Data from the Adventist Health Study-2 was used to investigate the association between plant-based dietary patterns and the overall cancer incidence among 69,120 participants. The results showed that vegetarian diets seem to be linked with lower overall cancer risks, especially a lower risk of gastrointestinal cancer. The vegan diet appeared to confer greater protection against overall cancer incidence, especially female-specific cancer, than any other dietary patterns (Tantamango-Bartley, Jaceldo-Siegl, Fan, & Fraser, 2013). A study using the Adventist Health Study-2 data stated that vegetarian diets potentially associated with lowering the incidence of colorectal cancers (Orlich et al., 2015). Other papers reported that a vegan diet might correlate with a lower risk of prostate cancer (Tantamango-Bartley et al., 2016). A comprehensive meta-analysis study revealed that vegan and vegetarian diets significantly reduced the incidence and mortality of all cancers by around 15% and 8% respectively (Dinu et al., 2017).

Based on prior studies, it is clear that dietary patterns that are higher in plant foods and lower in animal foods are promoting general health and lowering the incidence of several diseases and metabolic disorders.

## 1.3 Gut Microbiota Modulation by Plant-Based Foods

### 1.3.1 Gut Microbiota: General Aspects

The human gastrointestinal tract harbors diverse and complex microbial community composed mainly of bacteria and termed gut microbiota. The actual numbers of microorganisms inhabiting the gastrointestinal tract have been the subject of controversies and exaggerated claims. The initial estimate of the number of bacteria inhabiting the gastrointestinal tract was $10^{14}$, which is approximately ten times more than the cells of human body (Savage, 1977);

however, a more recent calculation estimated there to be $10^{13}$ bacteria inhabiting the gastrointestinal tract making the ratio of bacteria to human cells closer to 1:1 (Sender, Fuchs, & Milo, 2016). The bacterial density and diversity in the intestinal tract are quite different depending on the location. The colon has the highest bacterial biodiversity, whereas the upper part has the lowest number of microbes (Hillman, Lu, Yao, & Nakatsu, 2017). Gut microbiota is referred to as the "hidden metabolic organ" due to its substantial impact on general human health, including metabolism, nutrition, and physiological and immune function (Thursby & Juge, 2017).

In earlier times, members of a microbial community were studied and identified using culture-dependent approaches. These methods are broadly used in the microbiology field; however, they have some limitations, especially in the study of microbial ecology. The culture-dependent methods are limited in terms of bacterial identification as a wide range of bacterial strains cannot be grown and cultured in the laboratory. Additionally, these methods are limited to distinguish bacteria at lower taxonomic levels (Morgan & Huttenhower, 2012).

Recently, culture-independent approaches, including high-throughput and low-cost sequencing methods, have been widely used to investigate numerous characteristics of microbial communities such as taxonomic diversity and functional metagenomics. 16S ribosomal RNA (rRNA) gene has been by far the most common phylogenetic marker used to study gut microbial diversity and taxonomy (Poretsky, Rodriguez-R, Luo, Tsementzi, & Konstantinidis, 2014). Many aspects make the 16S rRNA gene the ideal targeted gene for gut microbial phylogeny and taxonomy studies. These aspects include (1) the presence in all bacteria and archaea; (2) the evolution at relatively constant rates; (3) and the existence of conservative regions, used for designing amplification primers, and highly variable regions (V1-V9), used for distinguishing

and identifying bacterial species (Janda & Abbott, 2007; Mizrahi-Man, Davenport, & Gilad, 2013).

Based on 16S rRNA-based studies, human intestinal bacteria involve around 1000 bacterial species belonging to six different bacterial phyla. Bacteroidetes and Firmicutes are the most dominant phyla, whereas Actinobacteria, Proteobacteria, Verrucomicrobia, and Fusobacteria mostly present in lower proportions (Eckburg et al., 2005; Huttenhower et al., 2012; Rajilić-Stojanović & de Vos, 2014; Rowland et al., 2018). In lower taxonomic levels, bacteria like *Bifidobacterium*, *Lactobacillus*, Clostridiaceae, Lachnospiraceae, Ruminococcaceae, *Prevotella*, and *Akkermansia* are considered beneficial bacteria, while *Escherichia coli, Salmonella, Staphylococcus, Bacillus,* and *Enterococcus* are potential pathogens (Cui et al., 2019; Lavefve, Howard, & Carbonero, 2020).

Gut microbiota collaborates with the host immune system to form a complex and reciprocally beneficial relationship (Bäckhed, Ley, Sonnenburg, Peterson, & Gordon, 2005; Lazar et al., 2018; Round & Mazmanian, 2009, 2010; H.-J. Wu & Wu, 2012). Gut microbiota reinforces the function of the immune system and prevents the colonization of pathogens (Okumura & Takeda, 2017; Ubeda, Djukovic, & Isaac, 2017). On the other hand, the immune system has co-evolved to live in a cooperative relationship with the healthy microbiota and fight against pathogens (Jandhyala et al., 2015; Round & Mazmanian, 2009, 2010). In addition to the immunological protective functions, gut microbiota helps to maintain the integrity of the mucosal barrier and regulate host digestion and metabolism (Okumura & Takeda, 2018).

Changing in the gut microbial composition can lead to beneficial or harmful effects on human health (Thursby & Juge, 2017). The imbalance of the gut microbiota symbiotic

ecosystem, known as dysbiosis, links to several pathologies including IBD, celiac disease, and metabolic disorders (Biragyn & Ferrucci, 2018; Schippa & Conte, 2014). Life style changes might disrupt the gut microbiota ecosystem, which may explain the increased incidence of microbiome-linked pathologies over the past few decades (Logan, Jacka, & Prescott, 2016). Many factors can abnormally shift in the composition of gut microbiota and, in turn, increase the incidence of diseases including diet, environmental changes, physical and psychological stress, and antibiotic exposure (Karl et al., 2018). Many scientific studies have linked the domination of some gut microbiota groups with positive health outcomes, and these microbes are commonly identified as "potentially beneficial bacteria." The most common bacterial strains that fall in this category belong to the genera *Bifidobacterium, Lactobacillus, Akkermansia, Faecalibacterium, Eubacterium, Roseburia,* and *Blautia* (Hiippala et al., 2018; LeBlanc et al., 2017; Quigley, 2013; Q. Yang et al., 2020; Y.-J. Zhang et al., 2015). Other bacterial groups have been associated with the development or progression of several diseases and disorders, and these groups include some species from the genera *Clostridium, Enterobacter, Enterococcus, and Bacteroides (Alhinai, Walton, & Commane, 2019; Gagnière et al., 2016; Kowalska-Duplaga et al., 2019; Q. Yang et al., 2020).* The potential correlation between the Firmicutes and Bacteroidetes population and BMI has been intensively studied. Several studies demonstrated that the increase of the Firmicutes/Bacteroidetes ratio might contribute to the pathophysiology of obesity (Barlow, Yu, & Mathur, 2015; Fernandes, Su, Rahat-Rozenbloom, Wolever, & Comelli, 2014; Kasai et al., 2015; Koliada et al., 2017; Mathur & Barlow, 2015; Turnbaugh et al., 2006). Other studies, however, have contradictory results and showed no statistically significant difference in the Firmicutes/Bacteroidetes ratio between obese and lean individuals (Aguirre & Venema, 2015; H.-J. Hu et al., 2015; Jumpertz et al., 2011; Tims et al., 2013; Walters, Xu, & Knight, 2014). The

contradicting results from previous studies suggest that the Firmicutes/Bacteroidetes ratio may not be valid as an eventual biomarker for obesity.

### 1.3.2 Effect of Plant-Based Diets on Gut Microbiota Composition

The contribution of dietary patterns to modulate and modify the community structure and function of gut microbiota has been a hot topic in recent decades. Diet can directly influence the microbial composition in the gastrointestinal tract and play a key role in promoting or inhibiting their growth. Different dietary patterns, such as plant-based diets and the Western-style diet, have a significant impact on the gut microbiota ecology (Tomova et al., 2019). The Western-style diet, which is low in dietary fibers and high in fat content, has been linked to microbial disruptions in the digestive system and a reduction of microbial diversity (Sonnenburg et al., 2016). On the other hand, plant-based diets promote healthy and stable gut microbiota due to their high content of two critical nutrients, dietary fibers and phytonutrients (Hever & Cronise, 2017; Tomova et al., 2019).

Dietary fibers are edible carbohydrate polymers with three or more monomeric units that resist hydrolysis by digestive enzymes in the small intestine but can be fermented by microbial residents in the large intestine (Gentile & Weir, 2018; Williams, Grant, Gidley, & Mikkelsen, 2017). Gut microbiota uses dietary fibers as the primary source of energy through a metabolic process called fermentation. This fermentative activity results in the generation of bioactive metabolites such as short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate. These organic products have a beneficial impact on various physiological processes, including the regulation of host metabolism, immune system, and cell proliferation (Koh, De Vadder, Kovatcheva-Datchary, & Bäckhed, 2016). Dietary fiber intake is not only promoting a healthy

environment in the gut but also enhances the richness and diversity of the gut microbiota (Cui et al., 2019).

Phytonutrients are a wide variety of chemical compounds produced by plants. One of the major types of these plant products is dietary polyphenols. Dietary polyphenols are naturally found in many foods and beverages, namely in fruits, vegetables, cereals, tea, coffee, and wine (Kumar Singh et al., 2019). During recent decades, there has been a growing interest in the bioactivity of polyphenol-rich foods. Many clinical and preclinical studies have been published showing the antioxidant and anti-inflammatory properties of phenolic compounds. This may suggest a potential link between polyphenol-rich food consumption and the reduction of several chronic disorders including obesity, diabetes, cancers, IBD, cardiovascular disease, and neurodegenerative diseases (Costa et al., 2017; Crascì, Lauro, Puglisi, & Panico, 2018; Kinger, Kumar, & Kumar, 2018; Rahman et al., 2018).

Chemically, polyphenols are a large heterogeneous group of compounds characterized by hydroxylated phenyl moieties. Generally, polyphenols are classified into three major classes, flavonoids, stilbenoids, and phenolic acids, and flavonoids are the most prevalent class among them (Papuc, Goran, Predescu, Nicorescu, & Stefan, 2017). There are more than 9,000 different chemical structures of flavonoids that have been identified in nature. Based on their chemical structure differences, flavonoids are classified into subclasses including, anthocyanidins, flavanones, flavones, flavanonols, flavonols, flavan-3-ols or flavanols, isoflavones, neoflavonoids, and chalcones (Cardona, Andrés-Lacueva, Tulipani, Tinahones, & Queipo-Ortuño, 2013; Kawabata, Yoshioka, & Terao, 2019; Papuc et al., 2017).

The beneficial properties of dietary polyphenols are primarily reliant on their bioavailability. Gut microbial residents play a vital role in polyphenols bioavailability, especially in the colon. In general, approximately 5–10% of the total digested polyphenols are absorbed in the small intestine, while the remaining 90–95% pass to the large intestine (Peron et al., 2019). Typically, polyphenols with a low molecular weight are partially absorbed in the small intestine. However, the vast majority of polyphenols have a high molecular weight in their native state, making them unabsorbed in the small intestine (Cassidy & Minihane, 2017; Kawabata et al., 2019). The high molecular weight polyphenols and other unabsorbed low molecular weight are transported into the large intestine where the gut microbial community in the colon breaks them down into absorbable low molecular weight phenolic metabolites (Cardona et al., 2013; Cassidy & Minihane, 2017). These metabolic products might be responsible for the potential health benefits derived from polyphenol-rich foods, rather than the original native polyphenols (Cardona et al., 2013).

Consuming a diet rich in fruits and vegetables exerts an important influence on gut microbiota diversity and ecological structure. Fruits and vegetables are important foods in daily meals (Sharoba, Farrag, & Abd El-Salam, 2013). In accordance with the US Department of Health and Human Services (DHHS) and the United States Department of Agriculture (USDA), fruits and vegetables should make up half of the meal ("2015–2020 Dietary Guidelines," 2015). Due to the high content of dietary fibers and phenolic compounds, fruit and vegetable intake improves the richness and diversity of the gut microbial community. The increased interest and awareness among consumers in maintaining a healthy diet urges scientists and researchers to intensify their efforts in studying health-promoting food.

### 1.3.3 Impact of Fruits and Vegetables on Gut Microbiota Composition

Fruits and vegetables are a fundamental source of dietary fibers, polyphenols, and micronutrients such as vitamins and minerals. Many studies have investigated the potential impact of the consumption of fruits and vegetables and their extracts on the gut microbiota ecosystem. This review summarizes the latest findings on the effects of whole fruits and vegetables as well as their products, such as juices, on the gut microbiota composition and diversity.

#### *1.3.3.1 Berries*

The impact of berry consumption on the composition and diversity of the gut microbial community has been studied intensively in recent years. In general, berries are an excellent source of bioactive compounds, mainly phenolic compounds, including anthocyanins, flavonols, flavanols, tannins, or phenolic acids (Nile & Park, 2014). Recently an exhaustive was published review which covers the specific area of berries and gut microbiome and health (Lavefve et al., 2020). This review will focus only on gut microbiota composition reports **(Table 1. 1)**.

##### *1.3.3.1.1 Strawberries*

Strawberry consumption altered the gut microbial composition in diabetic *db/db* mice. The phylum Actinobacteria slightly increased after the strawberry supplementation with a significant increase of its commensal genera *Bifidobacterium*. The phylum *Verrucomicrobia and the genera Bacteroides* and *Akkermansia* were significantly reduced in the mice that ate strawberries with no impact observed to *Dehalobacterium, Dorea, Lactobacillus,* and *Turicibacter* (Petersen et al., 2019). Moreover, strawberry administration alleviated the gut microbiota dysbiosis caused by the dextran-sulfate-sodium (DSS)-induced colitis in mice.

Strawberry supplementation induced the abundance of *Bifidobacterium* and *Lactobacillus* and reduced the abundance of *Dorea*, *Bilophila, Akkermansia,* and *Bacteroides* in colitis mice compared to the control group (Y. Han et al., 2019). The increased abundance of *Bilophila*, an opportunistic pathogen, was observed in colitis mice (I. Yang et al., 2013). *Bifidobacterium* and *Lactobacillus* spp. are generally considered probiotic microorganisms (Aoki et al., 2017). Although *Akkermansia* has been claimed to associate mostly with healthier clinical profiles, recent studies showed that the relative abundance of *Akkermansia* was elevated in several diseases, such as Parkinson's disease and Alzheimer's disease (Cirstea, Radisavljevic, & Finlay, 2018; Heintz-Buschart et al., 2018; Hill-Burns et al., 2017; Vogt et al., 2017).

A randomized controlled trial showed that the mixture of strawberries, raspberries, and cloudberries potentially altered the gut microbiota composition in human subjects with symptoms of metabolic syndrome. Unfortunately, the individual variations in the gut microbiota composition were not provided (Puupponen-Pimiä et al., 2013).

*1.3.3.1.2 Cranberries*

Whole cranberries altered the gut microbiota community of a human *in vitro* by increasing the relative abundance of the families Porphyromonadaceae and Bacteroidaceae and stabilizing the commensal families Ruminococcaceae and Lachnospiraceae. Additionally, a reduction in the relative abundance of Enterobacteriaceae was observed after the cranberry treatment (O'Connor et al., 2019). A high level of the Enterobacteriaceae has often been reported in patients with IBD (Zeng, Inohara, & Nuñez, 2017).

*In vitro*, dietary cranberry (*Vaccinium macrocarpon*) ameliorated the gut microbiota dysbiosis caused by DSS-induced colitis in mice by reversing the reduction of the α-diversity. At

the genus level, cranberry consumption altered the gut microbiota composition by increasing the relative abundance of potentially beneficial bacteria, e.g., *Bifidobacterium*, *Lactobacillus,* and *Akkermansia,* and decreasing the relative abundance of potentially harmful bacteria, e.g., *Sutterella* and *Bilophila* (Cai et al., 2019).

Cranberry consumption altered gut microbiota relative abundance in a short-period (5 days) in healthy human volunteers. The Firmicutes/Bacteroidetes ratio was influenced by the cranberry intake with an increase in Bacteroidetes level and a decrease in Firmicutes level. Cranberry intake also impacts bacterial abundance in the genus level by increasing the abundance of *Lachnospira* and *Anaerostipes*, SCFAs-producing bacteria, and decreasing the abundance of *Oribacterium* (Rodríguez-Morató, Matthan, Liu, de la Torre, & Chen, 2018).

*1.3.3.1.3 Black and red raspberries*

The impact of red and black raspberries on reshaping the gut microbial composition has also been studied. Black raspberries significantly increased the genera *Akkermansia* and *Desulfovibrio* abundance in healthy rats. A number of butyrate-producing genera, such as *Anaerostipes,* were also increased after the black raspberry consumption (Pan et al., 2017). A similar impact was observed in a study using specific-pathogen-free mice where the black raspberry supplementation remarkably increased the abundance of the phylum Verrucomicrobia and its genera *Akkermansia muciniphila*. Black raspberries also altered the Firmicutes/Bacteroidetes ratio by increasing the relative abundance of Bacteroidetes and reducing Firmicutes abundance (Tu et al., 2018). *A. muciniphila* is believed to have several health benefits to humans and inversely correlated with various diseases (Cani & de Vos, 2017; Jayachandran, Chung, & Xu, 2019). Another mice study also supported the effect of black

raspberries on lowering the Firmicutes/Bacteroidetes ratio. At the genus level, *Barnesiella* and *Turicibacter* were significantly higher in mice fed a black raspberry-rich diet compared to the control group, while *Clostridium* and *Lactobacillus* were significantly lower (Gu et al., 2019). *Barnesiella*, a recently discovered genus, belongs to the Porphyromonadaceae family which was found to metabolize glucose to produce butyrate and isobutyrate (Sakamoto, Lan, & Benno, 2007; Sakamoto et al., 2009; Wylie et al., 2012). Additionally, a positive correlation between *Barnesiella* and the level of SCFAs has been reported (Ying Zhao et al., 2013). *Turicibacter* was found to have a positive correlation with butyric acid (Zhong, Nyman, & Fåk, 2015).

Supplementation with red raspberries was associated with significant changes in the fecal microbiota of obese diabetic mice. The relative abundance of Lachnospiraceae was increased in the red raspberry supplemented group. Additionally, a decrease in the relative abundance of *Lactobacillus*, *Odoribacter*, and the fiber degrader S24-7 family as well as unknown groups of Bacteroidales and Enterobacteriaceae was observed after red raspberry supplementation (Garcia-Mazcorro, Pedreschi, et al., 2018).

*1.3.3.1.4 Blueberries*

Blueberries markedly impacted the gut microbiota composition. In the IBD mouse model, a blueberry-rich diet significantly reduced *Clostridium perfringens*, *Enterococcus*, *E. coli*, and *Lactobacillus* with no impact to *Bacteroides-Prevotella-Porphyromonas* group and *Bifidobacterium* (Paturi et al., 2012). *C. perfringens* and *Enterococcus faecalis* are potentially associated with IBD (Balish & Warner, 2002; Falk et al., 2007; Paturi et al., 2012; Tannock, 2010). Furthermore, blueberry consumption led to lowering the Firmicutes/Bacteroidetes ratio in

mice. The abundance of the phylum Tenericutes increased, and the family Deferribacteres decreased in blueberry-fed mice (Wankhade et al., 2019). Another study showed that blueberry administration increased the relative abundance of *Turicibacter* sp. H121 in mice fed a high-fat and high-sucrose diet (Morissette et al., 2020).

In rats, a Lowbush wild blueberry-enriched diet influenced the gut microbiota profile by significantly increasing the relative abundance of the phylum Actinobacteria, the order Actinomycetales, and a number of genera under the family Bifidobacteriaceae and Coriobacteriaceae, and significantly decreasing the relative abundance of *Lactobacillus* and *Enterococcus*. The Lowbush wild blueberry consumption increased the *Bifidobacterium*/Coriobacteriaceae ratio compared with the control diet (Lacombe et al., 2013). The increased *Bifidobacterium*/Coriobacteriaceae ratio positively impacted plasma cholesterol levels in hamsters (Martínez et al., 2009). Blueberry juice supplementation contributed to ameliorating the alteration in the gut microbiota composition caused by carbon tetrachloride ($CCl_4$) in a $CCl_4$-induced rat liver fibrosis model (Yan et al., 2019).

In healthy humans, wild blueberry *(Vaccinium angustifolium)* consumption modulated the intestinal microbiota. The abundance of commensal *Bifidobacterium* was significantly increased following the wild blueberry treatment with no significant impact to *Bacteroides, Prevotella, Enterococcus,* and *Clostridium coccoides* (Vendrame et al., 2011). Another human study showed that the consumption of a wild blueberry drink modulated the most abundant bifidobacterial taxonomic groups with significant stimulation of *Bifidobacterium longum subsp. infantis strain* (Guglielmetti et al., 2013).

*1.3.3.1.5 Other berries*

A variety of eight Nordic berries; strawberry (*Fragaria ananassa* Senga Sengana), bilberry (*Vaccinium myrtillus*), red raspberry (*Rubus idaeus*, var. Ottawa), cloudberry (*Rubus chamaemorus*), blackcurrant (*Ribes nigrum*, var. Öjeby), and sea buckthorn berry (*Hippophae rhamnoides*) showed antimicrobial activity against selected Gram-positive and Gram-negative bacteria *in vitro*. *Staphylococcus* and *Salmonella* were the most sensitive bacteria to the berry intervention (Puupponen-Pimiä et al., 2005). *Salmonella* is a well-known foodborne pathogen and considered the leading bacterial cause of acute gastroenteritis (Majowicz et al., 2010; Rivera-Chávez et al., 2016). Sea buckthorn berry juice influenced gut microbiota *in vitro* by increasing the growth of *Bifidobacteria*, *Bacteroides/Prevotella*, and Lactic acid bacteria (Attri, Sharma, Raigond, & Goel, 2018). A similar impact on the beneficial microbial population was observed in a following *in vitro* study when the sea buckthorn berry juice supplementation stimulated the growth of *Bifidobacteria, Lactobacilli,* and *Bacteroides/Prevotella* (Attri & Goel, 2018). *Bifidobacterium* and *Lactobacillus* spp. are well-known probiotic microorganisms (Aoki et al., 2017).

Juçara *(Euterpe edulis)* significantly increased the relative abundance of *Bifidobacterium* after 24 hours of fermentation *in vitro* (Guergoletto, Costabile, Flores, Garcia, & Gibson, 2016). Similarly, juçara pulp reinstated *Bifidobacterium* content in rats fed a high-fat diet (Jamar et al., 2018). *Bifidobacterium* has been found to play a role in preventing cognitive impairment in Alzheimer's disease (Kobayashi et al., 2017).

*Lonicera caerulea* L. Berry (LCBP) modified the gut microbiota composition in a high-fat diet-induced mice model (S. Wu et al., 2018). The results revealed that supplementation with

LCBP impacted the Firmicutes/Bacteroidetes ratio by lowering the abundance of Firmicutes and elevating Bacteroidetes abundance as well as increasing the abundance of Parabacteroides and Verrucomicrobia. In the genus level, the LCBP diet induced the relative abundance of *Akkermansia,* which was suggested to play a role against obesity (Everard et al., 2013; Plovier et al., 2017). LCBP supplementation increased the relative abundance of *Bacteroides* and *Parabacteroides,* which were found to be reduced with non-alcoholic fatty liver disease and the high fat-induced diet (Del Chierico et al., 2017; Liu et al., 2016). Additionally, the relative abundance of *Staphylococcus, Lactobacillus, Oscillospira,* and *Ruminococcus* was reduced after LCBP supplementation. The increased abundance of *Lactobacillus, Ruminococcus,* and *Oscillospira* was associated with intestinal permeability, systemic inflammation, and macrophage dysfunction (Santisteban et al., 2017; Thevaranjan et al., 2017; S. Wu et al., 2018). These findings express the potential health benefits of LCBP.

Goji berry *(Lycium barbarum)* modulated the gut microbial population in IL-10-deficient mice, which will develop a chronic IBD spontaneously. Dietary Goji markedly enhanced the growth of Actinobacteria phylum and its genera *Bifidobacterium*. Butyrate-producing bacteria, including Lachnospiraceae family and its genera *Roseburia* spp. and Ruminococcaceae family and its genera *Faecalibacterium prausnitzii*, were also promoted by Goji berry (Kang, Yang, Zhang, Ross, & Zhu, 2018). These genera have been described as potentially protective against human IBD (Kumari, Ahuja, & Paul, 2013; Martín et al., 2014; Rossi et al., 2016; Tamanai-Shacoori et al., 2017). *F. prausnitzii* dysbiosis is associated with several intestinal and metabolic disorders such as IBD, irritable bowel syndrome (IBS), colorectal cancer (CRC), obesity, and celiac disease (Balamurugan, Rajendiran, George, Samuel, & Ramakrishna, 2008; De Palma et al., 2010; Furet et al., 2010; Neish, 2009; Rajilić–Stojanović et al., 2011; Sokol et al., 2008).

Furthermore, many studies have reported the anti-inflammatory effects of *F. prausnitzii* (Martín et al., 2017; Miquel et al., 2015; Quévrain et al., 2016; Rossi et al., 2015; Sarrabayrouse et al., 2014).

Lingonberries lowered the Firmicutes/Bacteroidetes ratio in *Apoe*$^{-/-}$ mice, atherosclerosis-prone mice, fed a high-fat diet. The genera *Bacteroides, Clostridium,* and *Parabacteroides,* and the species *A. muciniphila*, *Blautia producta*, *Clostridium difficile,* and *Eubacterium dolichum* were increased after Lingonberries supplementation. A decrease in the abundance of *Mucispirillum* and *Oscillospira* was observed in mice fed the high-fat diet (Matziouridou, Marungruang, Nguyen, Nyman, & Fåk, 2016). *E. dolichum* is a butyrate-producer bacterium, and butyrate has anti-inflammatory properties and can play a role against the development of IBD (M. Zhang et al., 2016).

A berry mixture is consisting of blueberries, blackberries, raspberries, Portuguese crowberries, and strawberries mitigated the gut microbiota alteration caused by a salt-induced hypertension diet in rats (Gomes et al., 2019). The Bacteroidetes population was significantly increased after the berry mixture intake while the Firmicutes and Proteobacteria were decreased. The berry mixture supplementation increased the abundance of Bacteroidaceae, S24-7, and Ruminococcaceae families. Moreover, the berry mixture intake significantly decreased the relative abundance of Erysipelotrichaceae, which has been correlated with inflammation (Dinh et al., 2015; Gomes et al., 2019).

Blackcurrants and raspberries modulated the gut microbiota composition in rats. The result showed a clear difference in the diversity index between the rats fed blackcurrants and raspberries as the diversity index was higher for the rats fed raspberries than blackcurrants.

However, the modulation in the composition of specific bacteria has not been reported (Jakobsdottir et al., 2013).

A randomized, double-blind placebo-controlled study revealed that *Schisandra shinensis* fruit modulates the gut microbiota composition in obese women. *Schisandra chinensis* fruit intake lowered the Firmicutes/Bacteroidetes ratio by increasing Bacteroidetes abundance and decreasing Firmicutes abundance. At the genus level, *Bifidobacterium, Akkermansia, Roseburia, Prevotella,* and *Bacteroides* showed a superior increase in the treatment group compared to the placebo group (Song, Wang, Eom, & Kim, 2015). These bacteria were found to be associated with reducing and controlling obesity (Dao et al., 2016; Everard et al., 2013; Hjorth et al., 2018; H. I. Kim et al., 2019; Neyrinck et al., 2012; Plovier et al., 2017; Tamanai-Shacoori et al., 2017; J. Y. Yang et al., 2017).

A variety of eight Nordic berries; bilberry (*Vaccinium myrtillus*), red raspberry (*Rubus idaeus*, var. Ottawa), cloudberry (*Rubus chamaemorus*), strawberry (*Fragaria ananassa* Senga Sengana), blackcurrant (*Ribes nigrum*, var. Öjeby) and sea buckthorn berry (*Hippophae rhamnoides*) showed antimicrobial activity against selected Gram-positive and Gram-negative bacteria, including *Staphylococcus* and *Salmonella,* which were the most sensitive to the berries (Puupponen-Pimiä et al., 2005). *Salmonella* is a well-known foodborne pathogen and considered the leading bacterial cause of acute gastroenteritis (Majowicz et al., 2010; Rivera-Chávez et al., 2016).

#### *1.3.3.2 Cherries*

Cherries contain a high level of dietary fibers and bioactive compounds, such as polyphenols (Budak, 2017; Mikulic-Petkovsek, Stampar, Veberic, & Sircelj, 2016; Redondo,

Arias, Oria, & Venturini, 2017). The impact of cherries on gut microbiota composition and diversity has been receiving attention in recent years **(Table 1. 2)**. A recent study investigated the impact of tart cherries and tart cherry juice on the human gut microbiota composition *in vitro* and *in vivo*. In the *in vitro* study, fermentation of tart cherry powder and tart cherry juice significantly increased the abundance of *Bacteroides*, *Collinsella, Veillonella, Bilophila, Escherichia, Clostridium* XIVa, and Enterobacteriaceae (Mayta-Apaza et al., 2018). In the *in vivo* study, tart cherry juice intake influenced the gut microbiota profile among healthy human volunteers. Individuals with an initial high abundance of *Bacteroides* showed a decrease in the abundance of *Bifidobacterium, Bacteroides, Parabacteroides*, and *Alistipes* after the tart cherry intervention, while *Lactobacillus,* Lachnospiraceae, *Rumainococcus, Streptococcus,* and *Collinsella* were increased. Interestingly, the treatment showed an opposite effect on the gut microbiota profile in individuals with an initial low abundance of Bacteroides. Individuals with low-*Bacteroides* abundance showed induced levels of *Bifidobacterium, Bacteroides,* and *Lactobacillus* (numerical) along with reduced levels of Lachnospiraceae, *Collinsella, Ruminococcus, Streptococcus, Dialister, Blautia,* and *Roseburia* (Mayta-Apaza et al., 2018). In another study, dark sweet cherries *(Prunus avium)* modulated the gut bacterial composition in obese diabetic *db/db* mice by increasing the abundance of *Akkermansia* spp. and fiber-degraders (the S24-7 family) and decreasing the abundance of *Lactobacillus* and Enterobacteriaceae (Garcia-Mazcorro, Lage, et al., 2018). In a more recent study, the increasing concentration of sweet and tart cherry juices resulted in significant growth of *Barnesiella* and *Akkermansia* in healthy mice (Al Othaim, Marasini, & Carbonero, 2020).

#### *1.3.3.3 Mangoes*

Mangoes exhibit a wide range of health benefits due to the high content of phytochemicals such as polyphenols, carotenoids, vitamin E, and vitamin C with excellent antioxidant properties. Additionally, mangoes haves a high content of dietary fiber; the pulp alone contains approximately 1.3–3.8g/100g total fiber (Ajila, Naidu, Bhat, & Rao, 2007; Gondi, Basha, Bhaskar, Salimath, & Prasada Rao, 2015; Masibo & He, 2009). Several studies have investigated the impact of mangoes on preventing disease and metabolic disorders through, for example, inhibiting adipogenesis, ameliorating hyperglycemia and hyperlipidemia, reducing blood glucose, and preventing the growth of colonic cancer cells (Evans et al., 2014; Gondi et al., 2015; Noratto et al., 2010; Taing et al., 2012). The high content of dietary fiber and polyphenols make mangoes promising candidate for modulating the gut microbiota profile **(Table 1. 3)**.

A study published in 2016 showed the ability of mango supplementation to restore the relative abundance of the beneficial genera *Bifidobacteria*, *Akkermansia*, and *Aldercreutzia* in the mice previously exposed to a high-fat diet. Additionally, mango supplementation increased the relative abundance of *Ruminococcus and reduced the abundance of Bacteroides* and *Parabacteroides* despite the high-fat feeding (Ojo et al., 2016). *Aldercreutzia* was characterized to produce equol, an antioxidant from dietary isoflavonoids (Maruo, Sakamoto, Ito, Toda, & Benno, 2008). A more recent study showed that mango intake caused a trend toward increasing the relative abundance of *Lactococcus lactis*, a tannase-producing bacterium. It reduced the relative abundance of *Clostridium leptum*,an endotoxin-producing bacterium, and *Bacteroides thetaiotaomicron*, an associated bacterium with obesity (Barnes et al., 2019).

#### *1.3.3.4 Apples*

Apples are a rich source of bioactive compounds such as dietary polyphenols and dietary fiber, mainly pectin, which is the primary soluble fiber in apples. Pectin resists degradation by gastric acid and intestinal enzymes and passes to the large intestine where it subjected to fermentation activity by the bacterial residents. Therefore, pectin is known to have a positive impact on gut microbiota composition and diversity and promoting health (Chung et al., 2017; Denis et al., 2016; Koutsos, Tuohy, & Lovegrove, 2015; Masumoto et al., 2016). Pectin has a prebiotic activity and has been shown to enhance beneficial bacteria such as *F. prausnitzii* (Chung et al., 2017).

To the best of our knowledge, a few studies have investigated the impact of whole apple consumption on gut microbiota **(Table 1. 4)**. Three commercial apple varieties, Renetta Canada, Golden Delicious, and Pink Lady, modulated the gut microbiota composition *in vitro*. At the phylum level, all the apple varieties significantly increased Actinobacteria proportion with no effect on Firmicutes proportion. Only Renetta Canada and Golden Delicious varieties significantly increased the abundance of Proteobacteria and significantly decreased the abundance of Bacteroidetes. At the genus level, the population of *Bifidobacterium* spp. was significantly increased after the administration of Renetta Canada and Golden Delicious. Renetta Canada administration significantly increased the abundance of *F. prausnitzii*, unlike Golden Delicious and Pink Lady, which showed no impact on *F. prausnitzii* population (Koutsos et al., 2017).

*In vivo*, apple consumption modulated the gut microbiota composition in rats fed whole apples for four and 14 weeks. In the short-term intervention, *Bacteroidetes* level was decreased

after the apple supplementation (Licht et al., 2010). In a more recent *in vivo* study, apple supplementation impacted the Firmicutes/Bacteroidetes ratio by increasing Firmicutes population and decreasing Bacteroidetes in rats fed a high-fat diet compared to the control group. Additionally, apple supplementation increased the abundance of *Blautia* in rats fed a low-fat diet compared to the control group. At lower taxonomic levels, apple supplementation significantly increased the abundance of an unknown group of Clostridiales, with a decrease in the abundance of Bacteroidaceae (Garcia-Mazcorro et al., 2019). In mice fed a high fat diet, whole apples increased the relative abundance of *Akkermansia,* whereas the apple peel supplementation increased the level of *Bacteroides* (Elkahoui, Levin, Bartley, Yokoyama, & Friedman, 2019).

In humans, apple intake significantly increased the abundance of *Bifidobacteria* with a suggestive increase in the abundance of *Lactobacillus, Streptococcus,* and *Enterococcus.* A significant reduction was observed in the abundance of Enterobacteriaceae and Lecithinase-positive clostridia, including *C. perfringens* and a suggestive decrease in the *Pseudomonas* abundance (Shinohara, Ohashi, Kawasumi, Terada, & Fujisawa, 2010). Unfortunately, this study used culture techniques to investigate the potential modulation on gut microbiota, which gives a limited description of microbial communities. Another human study showed that the whole apple consumption lowered fecal pH and resulted in differences in the denaturing gradient gel electrophoresis (DGGE) profile (Ravn-Haren et al., 2013). However, the specific modification of the gut microbiota population was not confirmed.

#### *1.3.3.5 Grapes*

Grapes are rich in bioactive polyphenolic compounds, including flavonoids, such as anthocyanins and flavan-3-ols, and non-flavonoids, such as stilbenes (Nash et al., 2018). Due to

the anti-inflammatory and antioxidant properties, grape consumption has a beneficial effect on intestinal or systemic inflammation including reducing adiposity and markers of inflammation as well as improving glucose tolerance (Collins et al., 2016; Van Hul et al., 2018). Unfortunately, a limited number of studies assess the impact of whole grapes on the gut microbiota structure and composition **(Table 1. 5)**. The vast majority of grape-based studies focused on phenolic compound extracts from grape pomace, seed, or juice (Anonye, 2017; W. Liu et al., 2017; F. Lu et al., 2019; Van Hul et al., 2018). This review will address papers that used whole grapes to investigate the potential impact on modulating the gut microbiota profile.

The grape supplementation decreased the relative abundance of sulfidogenic bacteria *Desulfobacter* spp. and *Bilophila wadsworthia* in mice fed the high-fat diet compared to control groups (Baldwin et al., 2016). Hydrogen sulfide, produced by sulfidogenic bacteria, triggers pro-inflammatory pathways and hyperproliferation and may cause colorectal cancer and other intestinal disorders, including inflammatory bowel disease and irritable bowel syndrome (Feng, Stams, De Vos, & Sánchez-Andrea, 2017; Z. Feng et al., 2017). The results also showed a suggestive increase in the abundance of *A. muciniphila*, a mucin-degrading bacterium, in mice fed the high-fat diet compared to control groups. Additionally, *Allobaculum* abundance was increased in mice fed the high-fat diet with the grape supplementation compared to the high-fat diet control group (Baldwin et al., 2016). A follow-up study also investigated the impact of grape intake on composition gut microbiota in mice fed a high-fat diet. Unlike the previous study, this study used a higher level of dietary fat and a longer intervention time-period, 16 vs. 10 weeks. In this study, the whole-grape supplementation did not have the same positive outcomes in the gut microbiota modulation in mice as in the previous study (Collins et al., 2016). More recently, an *in vivo* study showed that the supplementation of whole grapes increased the relative abundance

of various taxa belonging to the Lachnospiraceae family in mice under healthy and diseased conditions (Yiying Zhao, Nakatsu, & Jiang, 2019).

A randomized, crossover, controlled intervention study showed that red wine and de-alcoholized red wine significantly changes the gut microbiota composition in healthy volunteers. The relative abundance of *Bifidobacterium*, *Enterococcus*, *Eggerthella lenta*, the *Blautia coccoides–Eubacterium rectale* group, and the phylum Fusobacteria were significantly increased after the red wine and de-alcoholized red wine compared to the baseline (Queipo-Ortuño et al., 2012). The *Blautia coccoides-Eubacterium rectale* group, *Enterococcus* spp., and *Bifidobacterium* spp. are generally considered to be beneficial due to their anti-inflammatory and neoplastic properties (Dueñas et al., 2015). Another randomized, crossover, controlled intervention study evaluated the modulation of red wine and de-alcoholized red wine on gut microbiota profiles in obese individuals diagnosed with metabolic syndrome (MetS) (Moreno-Indias et al., 2016). The red wine and de-alcoholized red wine intake significantly increased the abundance of gut mucosal barrier protectors, such as *Bifidobacterium* spp. and *Lactobacillus* spp. Furthermore, a positive impact of the red wine and de-alcoholized red wine intake on the growth of the butyrate producers, namely the *Blautia coccoides–Eubacterium rectale* group, *Faecalibacterium prausnitzii,* and *Roseburia* was observed. The red wine and de-alcoholized red wine also showed an inhibitory effect on the *Clostridium histolyticum* group, pathogens associated with the progression of colon cancer, and the onset of IBD (Moreno-Indias et al., 2016).

#### *1.3.3.6 Kiwifruits*

Kiwifruits are rich in nutrients, such as dietary fiber and phytochemicals, which have prebiotic effects on intestinal bacteria (Leontowicz et al., 2016). Kiwifruits have shown to modify gut microbial ecology **(Table 1. 6)**. Green and gold kiwifruits increased the relative abundances of *Bifidobacterium* spp. and *Bacteroides* spp. *in vitro* (Blatchford et al., 2015). Another study showed that green and gold kiwifruits increased the total intestinal bacteria composition in rats compared to the control diet. Both green and gold kiwifruit significantly increased the abundance of Lachnospiraceae, a family that contains members among the primary producers of SCFAs and other members found to be associated with diseases (Paturi, Butts, Bentley-Hewitt, & Ansell, 2014; Vacca et al., 2020). The relative abundance of the *Bacteroides–Prevotella–Porphyromonas* group and *Enterococcus* spp. were induced with gold kiwifruit supplementation (Paturi et al., 2014). In a pig model, dietary treatment with green kiwifruits significantly increased the number of total bacteria with a significant increase in the abundance of *Bacteroides* as well as a significant decrease of *E. coli* abundance. The ratio of *Lactobacillus*/Enterobacteria was significantly increased after kiwifruit supplementation compared to the control (K. S. Han, Balan, Molist Gasa, & Boland, 2011). Therefore, kiwifruits may play a role in enhancing the relative abundance of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* as well as inhibiting the growth of opportunistic pathogens such as *E. coli* and *Enterobacteria*.

#### *1.3.3.7 Oranges*

The importance of oranges in promoting general health has long been established due to the richness in nutrients including vitamin C, dietary fiber, and other bioactive compounds such

as polyphenols. Flavonoids are among the most dominant polyphenols in oranges and orange juice (Stella, Ferrarezi, dos Santos, & Monteiro, 2011; Stinco et al., 2012). The bioactive components present in orange juice are associated with the gut microbiota metabolism (Laparra & Sanz, 2010; Pereira-Caro et al., 2015; Pereira-Caro et al., 2015). Numerous scientific papers have investigated the impact of orange juice in modulation gut microbiota ecology **(Table 1. 7)**. An *in vitro* study reported that fresh orange juice induced the growth of *Bifidobacterium* spp., *Lactobacillus* spp., *Enterococcus* spp., and *Clostridium* spp. while the juice reduced the level of the *Enterobacteria*. Pasteurized orange juice showed a similar effect in gut microbiota by increasing the growth of *Lactobacillus* spp. and reducing *Enterobacteria* (Duque, Monteiro, Adorno, Sakamoto, & Sivieri, 2016). Two different orange juices from *Citrus sinensis* variety, cv. "Cara Cara" and cv. "Bahia", influenced the composition of gut microbiota in humans in a short-term intervention. Cara Cara orange juice increased the relative abundance of Mogibacteriaceae and Tissierellaceae families while the relative abundance of Odoribacteraceae family and the *Odoribacter* genus were decreased. In the Bahia group, Mogibacteriaceae, Enterococcaceae, and Veillonellaceae families were increased, whereas the Ruminococcaceae family was decreased after the juice consumption. The Porphyromonadaceae, Odoribacteraceae, Christensenellaceae, Enterobacteriaceae, Lachnospiraceae, and Ruminococcaceae families and Parabacteroides and Butyricimonas genera were increased in Cara Cara orange juice compared to the control group. The relative abundance of the Coriobacteriaceae family and *Adlercreutzia* genus, Enterococcaceae family and *Enterococcus* genus, Clostridiaceae family and *Clostridium* genus, Ruminococcaceae family and *Anaerotruncus* genus, and Pasteurellaceae family were significantly higher in Bahia orange juice compared to the control group (Brasili et al., 2019). A long-term intervention, eight weeks, of orange juice positively modulated the gut microbiota

population in healthy volunteers by significantly increasing the relative abundance of *Bifidobacterium* spp. and *Lactobacillus* spp. (Lima et al., 2019).

#### *1.3.3.8 Pomegranates*

Pomegranate fruits and products are widely consumed and recognized for their health benefits (Z. Li, Henning, et al., 2015). The health benefits are attributed to the presence of phenolic compounds, mainly ellagitannins (Henning et al., 2017; Z. Li, Henning, et al., 2015). Several studies have shown the impact of pomegranate fruits and products on the gut microbiota composition **(Table 1. 8)**. The residual materials, which are left after the pomegranate juice production, are pressed and extracted with water to make the pomegranate by-product (POMx) which is commercially available as a dietary supplement (Bialonska et al., 2010). POMx was reported to increase the total bacteria and the level of *Bifidobacterium* spp. and *Lactobacillus* spp. *in vitro* (Bialonska et al., 2010). In agreement with the previous study, another *in vitro* study showed that the pomegranate (POM) juice and POMx increased the level of *Bifidobacterium* and *Lactobacillus*. *B. fragilis group, clostridia,* and Enterobacteriaceae was significantly inhibited by the POM juice and POMx in a dose–response manner (Z. Li, Summanen, et al., 2015).

In an *in vivo* study, an increase in the relative abundance of Ruminococcaceae, a family that has been reported to have anti-inflammatory activities (X. Zhang et al., 2012), was observed in rats supplemented with the pomegranate beverage compared to the control group (Kim et al., 2017). Moreover, pomegranate juice abolished the dysbiosis in gut microbiota caused by acrolein in apoE−/− mice (Rom et al., 2017).

POMx supplementation significantly increased the relative abundance of the commensal bacterium *A. muciniphila* in healthy volunteers (Henning et al., 2017). Furthermore, POMx

supplementation significantly increased the Actinobacteria population and decreased the Firmicutes population in healthy individuals. At the genus level, *Lactobacillus, Akkermansia, Prevotella, Butyrivibrio, Enterobacter, Escherichia, Serratia,* and *Veillonella* were increased after POMx intake, whereas *Collinsella* significantly decreased (Z. Li, Henning, et al., 2015).

#### *1.3.3.9 Avocados*

Avocados are rich in dietary fiber, monounsaturated fat, and a selection of essential phytochemicals, including lutein (Dreher & Davenport, 2013; Q.-Y. Lu et al., 2009). A few studies have investigated the potential impact of avocado administration on shaping the intestinal microbiota **(Table 1. 9)**. A recent study showed that the Firmicutes/Bacteroidetes ratio was maintained by avocado consumption in healthy overweight and obese individuals. A significant increase was observed in the abundance of Veillonellaceae and Sutterellaceae with a suggestive increase in the abundance of Ruminoccocaceae and its genera *Ruminococcus* and Prevotellaceae and its genera *Prevotella* after the avocado intervention. The genera *Bacteroides* and *Methanosphaera* were decreased in the avocado group with a significant increase in the abundance of *Dialister, Sutterella, Bilophila, Holdemanella, Herbaspirillum,* and *Acetivibrio,* of which several have the capability to ferment fiber (Bang et al., 2018; Fouhse, Gänzle, Beattie, Vasanthan, & Zijlstra, 2017; Henning et al., 2019). A randomized controlled trial study showed that the avocado consumption significantly increased the relative abundances of *Lachnospira* and suggestively elevated the abundance of *Faecalibacterium* in overweight or obese adults (Thompson, Edwards, Reeser, Khan, & Holscher, 2019). *Lachnospira* and *Faecalibacterium* are thought to play a role in maintaining gut homeostasis and epithelial integrity due to their ability to produce butyrate (Ferrario et al., 2014; Vital, Howe, & Tiedje, 2014). *Lachnospira* belongs to Lachnospiraceae, which has been reported to restrain the growth of the opportunistic pathogen,

*Clostridium difficile* (Bhaduri, Dubey, & Basu, 2019; Reeves, Koenigsknecht, Bergin, & Young, 2012).

#### *1.3.3.10 Bitter melons*

Bitter melon fruit is an excellent source of physiologically active compounds, including dietary fiber and polyphenols, mainly catechin (Bai, Zhu, & Dong, 2016; Hwang, 2018; Ri, Magbagbeola, Akinwande, & Okunowo, 2010). A good number of studies reported the impact of the bitter melon fruit in hypoglycemic, hypolipidemic, and improving insulin resistance in the obese (Bao et al., 2013; Juan, Ying, & Ying, 2017; Xu et al., 2014; S. J. Yang et al., 2015; Zhu, Bai, Zhang, Xiao, & Dong, 2016). However, a few studies have investigated the potential impact on gut microbiota ecology, most of which were conducted with obesity-induced diets (Bai et al., 2016; Nerurkar, Orias, Soares, Kumar, & Nerurkar, 2019; Zhu et al., 2016) **(Table 1. 10)**. In obese rats, the level of the families Desulfovibrionaceae and Enterobacteriaceae were reduced by the bitter melon supplemented diet compared to the high-fat diet, whereas the Odoribacteraceae, a butyrate producer, level was significantly increased after the dietary intervention (Bai et al., 2016). The association of Enterobacteriaceae and Desulfovibrionaceae families with a systemic inflammatory state have been reported (Hakansson & Molin, 2011; Zeng et al., 2017). At the genus level, the abundance of *Escherichia*, which contains opportunistic pathogens, was significantly decreased in the group supplemented with bitter melons compared to the high-fat diet group. This was coordinated with an increase in the abundance of the butyrate-producing genera *Faecalibacterium, Allobaculum, Butyricimonas,* and *Odoribacter* (Bai et al., 2016). A follow-up study showed that the bitter melon powder supplementation increased the Verrucomicrobia population in obese rats fed a high-fat diet. At the genus level, the bitter melon powder treatment significantly increased the relative abundance of *Blautia, Anaerotruncus,*

*Lactococcus, Allobaculum,* and *Oceanobacillus* and decreased *Prevotella* and *Anaeroplasma* (Bai, Zhu, & Dong, 2018). *Blautia* and *Allobaculum* are SCFAs producing-bacteria (Greetham et al., 2004; Park, Kim, Roh, & Bae, 2012). Bitter melon juice improved the ratio of Firmicutes/Bacteroidetes with a reduction in Actinobacteria abundance in mice fed a high-fat diet compared to the high-fat control group. Additionally, an increase in the abundance of Clostridiaceae, Lactobacillales, and *Lactobacillus* and a decrease in *Bacteroides* abundance was reported after bitter melon juice administration compared to the control group (Nerurkar et al., 2019). Lactobacillales is negatively correlated with colonic injury as well (Ritchie et al., 2015).

#### *1.3.3.11 Broccoli*

Broccoli is a good source of dietary fiber and phytochemicals, including glucosinolates and polyphenols (X. Liu et al., 2017). Glucosinolates are relatively non bioactive in their native state. However, the gut microbial residence can hydrolyze glucosinolates to form the bioactive glucosinolates derivatives, mainly isothiocyanates (Angelino et al., 2015; Tian, Liu, Lei, Zhang, & Shan, 2018). Several studies showed that broccoli consumption could alter the composition of gut microbiota **(Table 1. 11)**. Cooked broccoli supplementation significantly increased the relative abundance of the genera *Akkermansia* and *Oscillospira* as well as the family Ruminococcaceae. The broccoli consumption significantly decreased the relative abundance of *Blautia, Clostridium,* and *Dorea* in rats (X. Liu et al., 2017). A controlled feeding and randomized crossover study showed that daily consumption of cooked broccoli in human volunteers increased the relative abundance of Bacteroidetes and its genus *Bacteroides* and decreased the relative abundance in Firmicutes (Kaczmarek et al., 2019). An *in vivo* study reported that the broccoli supplementation decreased the abundance of *C. perfringens* and *E. coli,* bacteria that are known to be associated with IBD, in *mdr1a−/−* mice, a model for IBD.

The broccoli-supplemented diet also lowered the abundance of *Enterococcus* spp., *Lactobacillus* spp., and *F. prausnitzii* compared to the control diet (Paturi et al., 2012).

#### *1.3.3.12 Mushrooms*

Mushrooms contain a high content of bioactive components, including dietary fiber and polyphenols (Amirullah, Abidin, & Abdullah, 2018; Sánchez, 2017). Beside the anti-inflammation and antioxidant effects, whole mushroom supplementation has been reported to influence the gut microbial ecosystem in animal models (Iuchi et al., 2015; S. P. Therkelsen, G. Hetland, T. Lyberg, I. Lygren, & E. Johnson, 2016; Stig Palm Therkelsen, Geir Hetland, Torstein Lyberg, Idar Lygren, & Egil Johnson, 2016; Vetvicka & Vetvickova, 2014) **(Table 1. 11)**. White button mushrooms increased the Bacteroidetes population and decreased Firmicutes in mice. At the class level, the mushroom supplementation increased the abundance of Mollicutes, Proteobacteria sub-classes Delta, Gamma, and Epsilon, and Verrucomicrobia and decreased the abundance of Erysipelotrichi, Deferribacteres, as well as the potentially pathogenic bacteria Clostridia (Varshney et al., 2013). The dietary supplementation of white button mushrooms altered the composition of gut microbiota in pigs (Solano-Aguilar et al., 2018). A mushroom treatment enriched the abundance of the family Ruminococcaceae and its genera *Oscillibacter* and *Butyricicoccus* and the family Lachnospiraceae and its genera *Fusicatenibacter, Robinsoniella,* and *Eisenbergiella*, which have a role in plant fibers degradation and SCFAs production (Chassard, Delmas, Robert, Lawson, & Bernalier-Donadille, 2012; Meehan & Beiko, 2014). In addition, the white button supplementation increased the abundance of *Fusicatenibacter, Eisenbergiella, Lachnobacterium,* and *Robinsoniella* witch were found to be associated with many health outcomes, including lowering levels of cholesterol, correcting the dysbiosis caused by obesity, and improving outcomes for patients with primary biliary cirrhosis

(Lv et al., 2016; Prieto et al., 2018; Togo et al., 2016). Feeding pigs with mushrooms reduced the relative abundance of *Bifidobacterium* and increased the relative abundance of *Prevotella*, which was found to be associated with improving glucose metabolism (Kovatcheva-Datchary et al., 2015). In contrast, the *Armillariella tabescens* administration promoted *Lactobacillus* spp. and *Bifidobacterium* spp. and inhibited *E.coli* growth (Chen et al., 2017). A variety of mushrooms increased the Firmicutes and Actinobacteria population and decreased the population of Bacteroidetes and Proteobacteria in mice fed a high-fat diet compared to the high-fat diet group. Furthermore, there was a tendency toward an increased relative abundance of SCFAs-producing bacteria, *Allobaculum, Bifidobacterium,* and *Ruminococcus*, as well as lactic acid-producing bacteria, *Lactobacillus, Lactococcus,* and *Streptococcus,* in mice fed mushrooms compared to the high-fat diet group. Additionally, *Adlercreutzia* and *Sutterella* showed a suggestive increase after the mushrooms intake, while a suggestive decrease was observed in *Bacteroides, Prevotella, Mucispirillum, Dorea, Roseburia, Anaerotruncus, Oscillospira, Escherichiam,* and *Akkermansia* (Shimizu, Mori, Ouchi, Kushida, & Tsuduki, 2018). More recently, *Pleurotus eryngii* mushrooms significantly increased the richness and diversity of gut microbiota and partially reversed the dysbiosis induced by dextran-sodium-sulfate (DSS)-treatment in mice (Q. Hu et al., 2019). *P. eryngii* administration significantly increased the relative abundance of S24-7 and Odoribacteraceae at the family level, and *Adlercreutzia, Akkermanisa, Lactobacillus, Anaerostipes,* and *Allobaculum* at the genus level compared to the DSS group. *Adlercreutzia* reduction was observed in patients with IBD (Shaw et al., 2016). *P. eryngii* supplementation also reduced the abundance of Actinobacteria, Mollicutes, Desulfovibrionaceae, Enterococcaceae, *Turici*bacter, Dorea, rc4-4, *Bacteroides,* and *Prvotella* compared to the DSS group. It has been reported that the family Desulfovibrionacea, a sulfate-reducing bacteria, is a potential endotoxin

producer and might be associated with the low grade and chronic inflammation (Xiao et al., 2014).

#### *1.3.3.13 Jerusalem artichokes*

Jerusalem artichokes, also called sunchoke, sunroot, or earth apple, are rich in dietary fiber, mainly Inulin-type fructans. The inulin content in Jerusalem artichokes ranges from 7 to 30% for fresh weight or from 60 to 85.5% for dry weight (Aduldecha et al., 2016; Samal, Chaturvedi, & Pattanaik, 2017). A few studies have investigated the potential effect of Jerusalem artichoke on gut microbiota composition **(Table 1. 11)**. Results from *an in vivo* study showed that Jerusalem artichoke supplementation increased the level of *Lactobacillus* spp. and *Bifidobacterium* spp. in the cecal, colonic, and rectal digesta in healthy rats. The Jerusalem artichoke supplementation decreased the Coliforms population in rats (Samal et al., 2017). More studies, especially *in vivo* studies, need to be done to assess the potential impact of Jerusalem artichoke on the gut microbiota composition and diversity.

## 1.4 Conclusion

Fruit and vegetable consumption profoundly modifies and shapes the gut microbiota ecosystem due to the intense content of dietary fibers and bioactive compounds, including polyphenols. Several health outcomes have been linked to reshaping the gut microbiota and their associated functions by fruit and vegetable supplementation, including promoting the host's general health, reducing the risk of several diseases and metabolic disorders. Phytochemicals and fibers are the compounds found in fruits and vegetables that impact the intestinal microbiota composition and diversity. A majority of nutritional and microbiological researches have focused on investigating the effect of extracted compounds on gut microbiota profiles instead of the

whole foods.  Berries have been more commonly used to investigate the impact of whole foods on gut microbiota. More studies of whole fruits and vegetables, and especially in combination, are needed due to potential synergies between compounds and interactions with the whole fruits or vegetables matrices.

**Table 1. 1:** Modulation of the gut microbiota by berries.
**HTS**: High throughput sequencing; **qPCR**: Quantitative polymerase chain reaction; **DGGE**: Denaturing gradient gel electrophoresis; **FISH**: Fluorescent in situ hybridization; **SHIME**: Simulator of the human intestinal microbial ecosystem; **PCR**: polymerase chain reaction.

| **Berries** | **Study type** | **Dosage** | **Treatment duration** | **Analytical method** | **Changes in gut microbiota** | **Ref.** |
|---|---|---|---|---|---|---|
| Strawberry | Animal study (diabetic mice) | 2.35% of dried strawberry powder | 10 weeks | HTS (Illumina Miseq) | Phylum level:<br>↓ Verrucomicrobia<br>Genus level:<br>↑ *Bifidobacterium*<br>↓ *Bacteroides* and *Akkermansia*<br>↔ *Dehalobacterium, Dorea, Lactobacillus,* and *Turicibacter* | (Petersen et al., 2019) |
| Strawberry | Animal study (colitic mice) | 0, 2.5, or 5% (w/w) freeze-dried strawberry powder | 37 days | HTS (Illumina Miseq) | Genus level:<br>↑ *Bifidobacterium* and *Lactobacillus*<br>↓ *Akkermansia, Dorea, Bilophila,* and *Bacteroides* | (Y. Han et al., 2019) |
| Cranberry | *In vitro* (Dynamic Fermentation Models, SHIME) | 1 mg/mL of whole cranberry powder | every 24 hours for 5 days | Ion Torrent PGM | Family level:<br>↑ Porphyromonadaceae and Bacteroidaceae<br>↓ Enterobacteriaceae<br>↔ Ruminococcaceae and Lachnospiraceae | (O'Connor et al., 2019) |
| Cranberry | Animal study (colitic mice) | 1.5% (w/w) freeze-dried whole cranberry powder | 37 days | HTS (Illumina Miseq) | Phylum level:<br>↑ Verrucoicrobia and Proteobacteria<br>↓ Actinobacteria<br>Genus level:<br>↑ *Bifidobacterium* and *Lactobacillus*<br>↓ *Bilophila Sutterella,* and *Akkermansia* | (Cai et al., 2019) |
| Cranberry | Human study (healthy individuals) | 30 g of dried cranberry powder | 5 days | HTS (Illumina Miseq) | Phylum level:<br>↑ Bacteroidetes<br>↓ Firmicutes<br>Genus level:<br>↑ *Lachnospira* and *Anaerostipes*<br>↓ Oribacterium | (Rodríguez-Morató et al., 2018) |
| Blueberry | Animal study (mice, inflammation bowel disease model) | 10% of dried blueberry powder) | 21 weeks | qPCR | ↔ *Bacteroides-Prevotella-Porphyromonas, Bifidobacterium, Bacteroides vulgatus,* and *Faecalibacterium prausnitzii*<br>↓ *Clostridium perfringens, Enterococcus, Lactobacillus,* and *Escherichia coli* | (Paturi et al., 2012) |

**Table 1. 1 (Cont.)**

| Berries | Study type | Dosage | Treatment duration | Analytical method | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Blueberry | Animal study (healthy mice) | 5% of dried blueberry powder) | 4 weeks | HTS (Illumina Miseq | Phylum level:<br>↑ Bacteroidetes and Tenericutes<br>↓ Firmicutes<br>Family level:<br>↓ Deferribacteres | (Wankhade et al., 2019) |
| Blueberry | Animal study (obese mice) | 4% of freeze-dried whole blueberry powder | 8 weeks | HTS (Illumina Hiseq) | ↑ *Turicibacter* sp. H121 | (Morissette et al., 2020) |
| Blueberry | Animal study (healthy rats) | 8% of dried blueberry powder) | 6 weeks | HTS (Illumina Hiseq) | Phylum level:<br>↑ Actinobacteria<br>ordar level:<br>↑ Actinomycetale<br>Family level:<br>↑ Bifidobacteriaceae and Coriobacteriaceae<br>Genus level:<br>↓ *Lactobacillus* and *Enterococcus* | (Lacombe et al., 2013) |
| wild blueberry *(Vaccinium angustifolium)* | Human study (healthy male) | 25 g of wild blueberry powder in 250 mL of water) | 6 weeks | qPCR | ↑ *Bifidobacterium, Lactobacillus acidophilus*<br>↔ *Bacteroides, Prevotella, Enterococcus,* and *Clostridium coccoides* | (Vendrame et al., 2011) |
| wild blueberry *(Vaccinium angustifolium)* | Human study (healthy male) | 25 g of wild blueberry powder in 250 mL of water) | 6 weeks | qPCR | ↑ *Bifidobacterium longum* subsp. *Infantis*<br>↔ *Bifidobacterium adolescentis, B. longum subsp. longum, B. catenulatum, B. breve,* and *B. bifidum* | (Guglielmetti et al., 2013) |
| Black raspberries | Animal study (healthy rats) | 5% of dried black raspberries powder | 6 weeks | Pyrosequencing | ↑ *Akkermansia, Desulfovibrio,* and *Anaerostipes* | (Pan et al., 2017) |
| Black raspberries | Animal study (specific-pathogen free mice) | 10% of dried black raspberries powder | 7 weeks | HTS (Illumina Miseq) | Phylum level:<br>↑ Bacteroidetes and Verrucomicrobia<br>↓ Firmicutes<br>Species level:<br>↑ *Akkermansia muciniphila* | (Tu et al., 2018) |
| Black raspberries | Animal study (healthy mice) | 10% of dried black raspberries powder | 6 weeks | HTS (Illumina Miseq) | ↑ Bacteroidetes, *Barnesiella*, and *Turicibacter*<br>↓ Firmicutes, *Clostridium,* and *Lactobacillus* | (Gu et al., 2019) |
| Red raspberries | Animal study (obese diabetic *db/db* mice) | 5.3% of dried red raspberry | 8 weeks | qPCR | Order level:<br>↓ Bacteroidales<br>Family level:<br>↑ Lachnospiraceae<br>↓ Enterobacteriaceae and S24-7<br>Genus level:<br>↓ *Lactobacillus* and *Odoribacter* | (Garcia-Mazcorro, Pedreschi, et al., 2018) |

**Table 1. 1 (Cont.)**

| Berries | Study type | Dosage | Treatment duration | Analytical method | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Strawberry, bilberry, red raspberry, cloudberry, blackcurrant, and sea buckthorn berry | *In vitro* (batch-culture fermentation) | 2 and 10 mg $ml^{-1}$ of dried berry powder | 24 hours | Culture methods | ↓ *Salmonella enterica,* and *Staphylococcus aureus* | (Puupponen-Pimiä et al., 2005) |
| Sea buckthorn berry | *In vitro* (batch-culture fermentation) | 250 mg lyophilized fraction of small intestine digested berries | 72 hours | PCR-DGGE | ↑ *Bifidobacteria, Bacteroides/Prevotella* group, and Lactic acid bacteria | (Attri et al., 2018) |
| Sea buckthorn berry | *In vitro* (batch-culture fermentation) | 10% of berry juice | 1 week (continuous gut model) | PCR-DGGE | ↑ *Bifidobacteria, Lactobacilli,* and *Bacteroides/Prevotella* | (Attri & Goel, 2018) |
| Juçara *(Euterpe edulis)* | *In vitro* (batch-culture fermentation) | 1% of dried juçara pulp powder | 24 hours | FISH | ↑ *Bifidobacterium, Clostridium clusters XIVa, Bacteroides, Eubacterium rectale,* and *Prevotella* | (Guergoletto et al., 2016) |
| Juçara *(Euterpe edulis)* | Animal study (rats fed a high-fat diet) | 0.5 and 0.25% of dried berry powder | 7 days | qPCR | ↑ *Bifidobacterium* | (Jamar et al., 2018) |
| *Lonicera cerula* L. berry | Animal study (mice fed a high-fat diet) | 1% of dried *Lonicera cerula* L. berry powder | 45 Days | HTS (Illumina Miseq) | <u>Phylum level:</u><br>↑ Bacteroidetes, Parabacteroides, and Verrucomicrobia<br>↓ Firmicutes<br><u>Genus level:</u><br>↑ *Akkermansia, Bacteroides,* and *Parabacteroides*<br>↓ *Staphylococcus, Lactobacillus, Oscillospira,* and *Ruminococcus* | (S. Wu et al., 2018) |
| Goji berry *(Lycium barbarum)* | Animal study (colitis mice model) | 1% of dried Goji berry powder | 10 weeks | HTS (Illumina Miseq) | <u>Phylum level:</u><br>↑ Actinobacteria<br><u>Family level:</u><br>↑ Lachnospiraceae and Ruminococcaceae<br><u>Genus level:</u><br>↑ *Bifidobacterium, Roseburia,* and *Faecalibacterium prausnitzii* | (Kang et al., 2018) |

**Table 1. 1 (Cont.)**

| Berries | Study type | Dosage | Treatment duration | Analytical method | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Lingonberry | Animal study (mice fed a high-fat diet) | 44% of dried Lingonberry powder | 8 weeks | HTS (Illumina Miseq) | Phylum level:<br>↑ Bacteroidetes<br>↓ Firmicutes<br>Genus level:<br>↑ *Bacteroides*, *Clostridium*, and *Parabacteroides*<br>↓ *Mucispirillum* and *Oscillospira*<br>Spices level:<br>↑ *Akkermansia muciniphila, Blautia producta, Clostridum difficile*, and *Eubacterium dolichum* | (Matziouridou et al., 2016) |
| A berry mixture (blueberries, blackberries, raspberries, Portuguese crowberry, and strawberry tree fruit) | Animal study (rats fed a salt-induced hypertension diet) | 2 g of lyophilized berry mixture | 9 weeks | HTS (Illumina Miseq) | Phylum level:<br>↑ Bacteroidetes<br>↓ Firmicutes and Proteobacteria<br>Familly level:<br>↑ Bacteroidaceae , S24-7, and Ruminococcaceae<br>↓ Erysipelotrichaceae | (Gomes et al., 2019) |
| *Schisandra chinensis* fruit | Human study (obese women) | 100 ml, twice a day | 12 weeks | qPCR | Phylum level:<br>↑ Bacteroidetes<br>↓ Firmicutes<br>Genus level:<br>↑ *Bifidobacterium, Akkermansia, Roseburia, Prevotella*, and *Bacteroides* | (Song et al., 2015) |

**Table 1. 2:** Modulation of the gut microbiota by cherries.
**HTS**: High throughput sequencing; **qPCR**: Quantitative polymerase chain reaction.

| Cherries | Study type | Dosage | Treatment duration | Analytical method | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Tart Cherry | *In vitro* (batch-culture fermentation) | 5 ml of concentrated tart cheery or 5g of tart cherry powder | 48 Hours | Culture methods | ↑ *Bacteroides, Veillonella, Bilophila, Escherichia, Clostridium* XIVa, and Enterobacteriaceae | (Mayta-Apaza et al., 2018) |

**Table 1. 2 (Cont.)**

| Cherries | Study type | Dosage | Treatment duration | Analytical method | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Tart Cherry | Human study (healthy individuals) | 8 oz. of tart cherry juice daily | 5 days | HTS (Illumina Miseq) | Individuals with initial high-*Bacteroides* level:<br>↑ *Lactobacillus,* Lachnospiraceae, *Ruminococcus, Streptococcus,* and *Collinsella*<br>↓ *Bifidobacterium Bacteroides, Parabacteroides,* and *Alistipes*<br>Individuals with initial low-*Bacteroides* level:<br>↑ *Bifidobacterium, Bacteroides,* and Lactobacillus (numerical)<br>↓ Lachnospiraceae, *Collinsella*, *Ruminococcus, Streptococcus, Dialister, Blautia*, and *Roseburia* | (Mayta-Apaza et al., 2018) |
| Dark sweet cherry *(Prunus avium)* | Animal study (obese diabetic *db/db* mice) | 10% of dark sweet cherry powder | 12 weeks | qPCR | ↑ *Akkermansia* spp. and fiber-degraders (the S24-7 family)<br>↓ *Lactobacillus* and Enterobacteriaceae | (Garcia-Mazcorro, Lage, et al., 2018) |
| Sweet and tart cherries juices | Animal study (Healthy mice) | Increasing concentration of the juices (1/20 v/v, 1/15 v/v, 1/10 v/v, 1/7 v/v, and 1/4 v/v) every 5 days | 25 days | HTS (Illumina Miseq) | ↑ *Barnesiella* and *Akkermansia*<br>↓ *Bacteroides* | (Al Othaim et al., 2020) |

**Table 1. 3:** Modulation of the gut microbiota by mangos.
**HTS**: High throughput sequencing; **qPCR**: Quantitative polymerase chain reaction.

| Mangos | Study type | Dosage | Treatment duration | Analytical methods | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Mango | Animal study (mice fed a high-fat diet) | 1% or 10% of dried mango | 12 weeks | HTS (Illumina Miseq) | ↑ *Bifidobacteria, Akkermansia, Aldercreutzia,* and *Ruminococcus*<br>↓ *Bacteroides* and *Parabacteroides* | (Ojo et al., 2016) |
| Mango | Human study (healthy lean and obese individuals) | 400g of dried mango daily | 6 weeks | qPCR | ↑ *Lactococcus lactis*<br>↓ *Clostridium leptum* and *Bacteroides thetaiotaomicron* | (Barnes et al., 2019) |

**Table 1. 4:** Modulation of the gut microbiota by apples.
**HTS**: High throughput sequencing; **DGGE**: Denaturing gradient gel electrophoresis.

| Apples | Study type | Dosage | Treatment duration | Analytical method | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Apple | Animal study (healthy rat) | 10 g/day | 4 weeks | PCR-DGGE | ↓ *Bacteroides* | (Licht et al., 2010) |

**Table 1. 4 (Cont.)**

| Apples | Study type | Dosage | Treatment duration | Analytical method | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Apple varieties (Renetta Canada, Golden Delicious, and Pink Lady) | *In vitro* (batch-culture fermentation) | 2g of apple, a final concentration of 1% (w/v) | 24 hours | HTS (Illumina Miseq) and FISH | Phylum level:<br>↑ Actinobacteria (with all apple varieties)<br>↑ Proteobacteria (with Renetta Canada and Golden Delicious)<br>↓ Bacteroidetes (with Renetta Canada and Golden Delicious)<br>↔ Firmicutes (with all apple varieties)<br>Genus level:<br>↑ *Bifidobacterium spp.* (with Renetta Canada and Golden Delicious)<br>↑ *Faecalibacterium prausnitzii* (with Renetta Canada) | (Koutsos et al., 2017) |
| Apple | Animal study (rat fed a high-fat or low-fat diet) | 5% of freeze dried apple | 6 weeks | HTS (Illumina Miseq) | Phylum level (high-fat diet group):<br>↑ Bacteroidetes<br>↓ Firmicutes<br>Other levels (hight-fat diet group):<br>↑ Clostridiales<br>↓ Bacteroidaceae<br>Genus level (low-fat diet group):<br>↑ *Blautia* | (Garcia-Mazcorro et al., 2019) |
| Apple | Animal study (mice fed a high-fat or low-fat diet) | 40% of freeze dried whole RedDel apple | 4 weeks | HTS (Illumina Miseq) | ↑ *Akkermansia* | (Elkhaoui et al., 2019) |
| Apple (apple peel) | Animal study (mice fed a high-fat or low-fat diet) | 20% of freeze dried RedDel apple peel | 4 weeks | HTS (Illumina Miseq) | ↑ *Bacteroides* | (Elkhaoui et al., 2019) |
| Apple | Human study (healthy individuals) | Two apples daily | 2 weeks | Culture methods | ↑ *Bifidobacteria*, *Lactobacillus*, *Streptococcus*, and *Enterococcus.*<br>↓ Enterobacteriaceae, Lecithinase-positive clostridia, including *C. perfringens*, and *Pseudomona* | (Shinohara et al., 2010) |

**Table 1. 5:** Modulation of the gut microbiota by grapes.
**HTS**: High throughput sequencing; **DGGE**: Denaturing gradient gel electrophoresis; **PCR**: polymerase chain reaction.

| Grapes | Study type | Dosage | Treatment duration | Analytical method | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Grape | Animal study (mice following a high fat diet) | 3% or 5% powdered grapes | 11 weeks | HTS (Illumina Miseq) | ↑ *Akkermansia muciniphila* and *Allobaculum*<br>↓ *Desulfobacter* spp. and *Bilophila wadsworthia* | (Baldwin et al., 2016) |

**Table 1. 5 (Cont.)**

| Grapes | Study type | Dosage | Treatment duration | Analytical method | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Grape | Animal study (mice fed a high-fat diet) | 5% powdered grapes | 16 weeks | HTS (Illumina Miseq) | No significant impact was reported | (Collins et al., 2016) |
| Red wine and de-alcoholized red wine | Human study (healthy individuals) | 272 mL of red wine or de-alcoholized red wine daily | 20 days | PCR-DGGE | Phylum level:<br>↑ Fusobacteria<br>Genus level:<br>↑ *Bifidobacterium* and *Enterococcus*<br>Species level:<br>↑ *Eggerthella lenta, Blautia coccoides–Eubacterium rectale* group | (Queipo-Ortuño et al., 2012) |
| Red wine and de-alcoholized red wine | Human study (obese individuals) | 272 mL of red wine or de-alcoholized red wine daily | 30 days | PCR-DGGE | Phylum level:<br>↑ Fusobacteria and Bacteroidetes<br>↓ Firmicutes<br>Other levels:<br>↑ *Lactobacillus, Bifidobacterium,Blautia coccoides–Eubacterium rectale* group, *Faecalibacterium prausnitzii*, *Roseburia, Eggerthella lenta,and Prevotella*<br>↓ *Clostridium, Clostridium histolyticum* group, *Bacteroides, Escherichia coli* and *Enterobacter cloacae* | (Moreno-Indias et al., 2016) |

**Table 1. 6:** Modulation of the gut microbiota by kiwifruits.
**qPCR**: Quantitative polymerase chain reaction; **DGGE**: Denaturing gradient gel electrophoresis; **PCR**: polymerase chain reaction.

| Kiwifruits | Study type | Dosage | Treatment duration | Analytical method | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Kiwifruit (green and gold kiwifruit) | *In vitro* (batch-culture fermentation) | 1% of green or gold kiwifruit | 48 hours | qPCR | ↑ *Bifidobacterium* spp. and *Bacteroides* spp. | (Blatchford et al., 2015) |
| Kiwifruit (green kiwifruit) | Animal study (healthy rats) | 10% of green kiwifruit | 4 weeks | PCR-DGGE | ↑ Lachnospiraceae | (Paturi et al., 2014) |
| Kiwifruit (green kiwifruit) | Animal study (healthy pigs) | 25% of dried green kiwi | 14 days | PCR-DGGE | ↑ *Bacteroides*<br>↓Enterobacteria and *Escherichia coli* | (K. S. Han et al., 2011) |

**Table 1. 7:** Modulation of the gut microbiota by oranges.
**DGGE**: Denaturing gradient gel electrophoresis; **SHIME**: Simulator of the human intestinal microbial ecosystem.

| **Oranges** | **Study type** | **Dosage** | **Treatment duration** | **Analytical method** | **Changes in gut microbiota** | **Ref.** |
|---|---|---|---|---|---|---|
| Orange juice | *In vitro* (Dynamic Fermentation Models, SHIME) | 105 ml of fresh orange juice | 14 days | PCR-DGGE | ↑ *Bifidobacterium* spp., *Lactobacillus* spp., *Enterococcus* spp., and *Clostridium* spp.<br>↓ *Enterobacteria* | (Duque et al., 2016) |
| | *In vitro* (Dynamic Fermentation Models, SHIME) | 105 ml of pasteurized orange juice | 14 days | PCR-DGGE | ↑ *Lactobacillus* spp.<br>↓ *Enterobacteria* | |
| Orange juice (Cara Cara) | Human study (healthy individuals) | 500 mL of orange juice | 7 days | HTS | .<u>Family level:</u><br>↑ Mogibacteriaceae, Tissierellaceae Porphyromonadaceae, Christensenellaceae, Enterobacteriaceae, Lachnospiraceae, and Ruminococcaceae<br>↓ Odoribacteraceae<br><u>Genus level:</u><br>↑ *Parabacteroides* and *Butyricimonas*<br>↓ Odoribacter | (Brasili et al., 2019) |
| Orange juice (Bahia) | Human study (healthy individuals) | 500 mL of orange juice | 7 days | HTS | <u>Family level:</u><br>↑ Mogibacteriaceae, Enterococcaceae, Veillonellaceae, Coriobacteriaceae, Enterococcaceae, Clostridiaceae, and Pasteurellaceae<br>↓ Ruminococcaceae<br><u>Genus level:</u><br>↑ *Adlercreutzia, Enterococcus, Clostridium*, and *Anaerotruncus* | |
| Orange juice | Human study (healthy individuals) | 300 mL of orange juice | 8 weeks | PCR-DGGE | ↑ *Bifidobacterium* spp. and *Lactobacillus* spp. | (Lima et al., 2019) |

**Table 1. 8:** Modulation of the gut microbiota by pomegranates.
**HTS**: High throughput sequencing; **qPCR**: Quantitative polymerase chain reaction; **FISH**: Fluorescent in situ hybridization.

| **Pomegranates** | **Study type** | **Dosage** | **Treatment duration** | **Analytical method** | **Changes in gut microbiota** | **Ref.** |
|---|---|---|---|---|---|---|
| Pomegranate (POMx) | *In vitro* (batch-culture fermentation) | 1.5 mL of POMx | 0, 5, 10, 24 and 48 hours | FISH | ↑ *Bifidobacterium* spp. and *Lactobacillus* spp. | (Bialonska et al., 2010) |
| Pomegranate beverage | Animal study (healthy rats) | *ad libitum* | 2 weeks | qPCR | ↑ Ruminococcaceae | (Kim et al., 2017) |

**Table 1. 8**

| Pomegranates | Study type | Dosage | Treatment duration | Analytical method | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Pomegranate (POMx) | Human study (healthy individuals) | 1000 mg of POMx | 4 weeks | qPCR | ↑ *Akkermansia muciniphila* | (Henning et al., 2017) |
| Pomegranate (POMx) | Human study (healthy individuals) | 1000 mg of POMx | 4 weeks | HTS (Illumina Miseq) | Phylum level:<br>↑ Actinobacteria<br>↓ Firmicutes<br>Genus level:<br>↑ *Lactobacillus, Akkermansia, Prevotella, Butyrivibrio, Enterobacter, Escherichia, Serratia,* and *Veillonella*<br>↓ *Collinsella* | (Z. Li, Henning, et al., 2015) |

**Table 1. 9:** Modulation of the gut microbiota by avocados.
**HTS**: High throughput sequencing.

| Avocados | Study type | Dosage | Treatment duration | Analytical method | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Avocado (Hass Avocado) | Human study (healthy overweight/obese individuals) | 1 Hass avocado daily | 12 weeks | HTS (Illumina Miseq) | Phylum level:<br>↑ Bacteroidetes<br>↓ Firmicutes<br>Family level:<br>↑ Veillonellaceae, Sutterellaceae, Ruminoccocaceae, and Prevotellaceae<br>Genus level:<br>↑ *Ruminococcus, Prevotella, Dialister, Sutterella, Bilophila, Holdemanella, Herbaspirillum,* and *Acetivibrio*<br>↓ *Bacteroides* and *Methanosphaera* | (Henning et al., 2019) |
| Avocado (Hass Avocado) | Human study (healthy overweight/obese individuals) | 1 Hass avocado daily | 12 weeks | HTS (Illumina Miseq) | ↑ *Lachnospira* and *Faecalibacterium* | (Thompson et al., 2019) |

**Table 1. 10:** Modulation of the gut microbiota by bitter melons.
**HTS**: High throughput sequencing.

| Bitter melons | Study type | Dosage | Treatment duration | Analytical method | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Bitter melon (Momordica charantia L.) | Animal study (obese rats) | 300 mg of bitter melon powder /kg | 8 weeks | HTS (Illumina Miseq) | Phylum level:<br>↑ Odoribacteraceae<br>↓ Desulfovibrionaceae and Enterobacteriaceae<br>Genus level:<br>↑ *Faecalibacterium, Allobaculum, Butyricimonas,* and *Odoribacter*<br>↓ *Escherichia* | (Bai et al., 2016) |

**Table 1. 10**

| Bitter melons | Study type | Dosage | Treatment duration | Analytical method | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Bitter melon | Animal study (obese rats) | 400 mg of bitter melon powder /kg | 8 weeks | HTS (Illumina Miseq) | Phylum level:<br>↑ Verrucomicrobia<br>Genus level:<br>↑ *Blautia, Anaerotruncus, Lactococcus, Allobaculum,* and *Oceanobacillus*<br>↓ *Prevotella, Anaeroplasma* | (Bai et al., 2018) |
| Bitter melon (dried juice) | Animal study (mice fed a high-fat diet) | 1.5% of freeze-dried bitter melon juice | 16 weeks | HTS | Phylum level:<br>↑ Bacteroidetes<br>↓ Firmicutes<br>↓ Actinobacteria<br>Other levels:<br>↑ *Lactobacillus*, Lactobacillales, and Clostridiaceae<br>↓ *Bacteroides* | (Nerurkar et al., 2019) |

**Table 1. 11:** Modulation of the gut microbiota by vegetables.
**HTS**: High throughput sequencing; **qPCR**: Quantitative polymerase chain reaction.

| Vegetables | Study type | Dosage | Treatment duration | Analytical method | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Broccoli | Animal study (mice, inflammation bowel disease model) | 10% of dried broccoli powder) | 21 weeks | qPCR | ↔ *Bacteroides-Prevotella-Porphyromonas, Bifidobacterium,* and *Bacteroides vulgatus*<br>↓ *Clostridium perfringens, Enterococcus, Lactobacillus, Escherichia coli,* and *Faecalibacterium prausnitzii* | (Paturi et al., 2012) |
| Broccoli | Animal study (healthy rats) | 10% of dried cocked broccoli powder) | 4, 7, or 14 days | HTS (Illumina Miseq) | ↑ *Akkermansia, Oscillospira,* and Ruminococcaceae<br>↓ *Blautia*, *Clostridium*, and *Dorea* | (X. Liu et al., 2017) |
| Broccoli | Human study (healthy individuals) | 200 g of cocked broccoli daily | 15 days | qPCR | Phylum level:<br>↑ Bacteroidetes<br>↓ Firmicutes<br>Genus level:<br>↑ *Bacteroides* | (Kaczmarek et al., 2019) |
| Mushrooms (*Agaricus bisporous*, commonly called white button mushrooms) | Animal study (healthy rats) | 1% of dried mushroom | 6 weeks | 454 Titanium sequencer | Phylum level:<br>↑ Bacteroidetes, Mollicutes, Proteobacteria sub-classes Delta, Gamma, and Epsilon, and Verrucomicrobia<br>↓ Firmicutes, Erysipelotrichi, Deferribacteres, and Clostridia | (Varshney et al., 2013) |

**Table 1. 11 (Cont.)**

| Vegetables | Study type | Dosage | Treatment duration | Analytical method | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Mushrooms (white button mushrooms) | Animal study (healthy pigs) | 75 g or 150 g of dried mushrooms | 6 weeks | Multi-Tag Sequencing | Family level:<br>↑ Ruminococcaceae, Lachnospiraceae, Veillonellaceae, Succinivibrionaceae, and Clostridiaceae<br>Genus level:<br>↑ *Prevotella, Oscillibacter, Butyricicoccus, Fusicatenibacter, Robinsoniella, Eisenbergiella, Fusicatenibacter, Eisenbergiella, Lachnobacterium, Robinsoniella, Schwartzia, Selenomonas, Anerovibrio, Succinivibrio, Sarcina,* and *Clostridium sensu stricto*<br>↓ *Bifidobacterium* | (Solano-Aguilar et al., 2018) |
| Mushrooms (*Armillariella tabescens*) | Animal study (early-weaned pigs) | 0.1, 0.3, or 0.9% of dried *Armillariella tabescens* | 30 days | qPCR | ↑ *Lactobacillus* spp. and *Bifidobacterium* spp.<br>↓ *E.coli* | (Chen et al., 2017) |
| Mushrooms (*Bunashimeji, Shiitake, Maitake,* and *Eringi*) | Animal study (mice fed a high-fat diet) | 0.5% or 3% of dried mushrooms | 4 weeks | HTS (Illumina Miseq) | Phylum level:<br>↑ Firmicutes and Actinobacteria<br>↓ Bacteroidetes and Proteobacteria<br>Genus level:<br>↑ *Allobaculum, Bifidobacterium, Ruminococcus, Lactobacillus, Lactococcus, Streptococcus, Adlercreutzia,* and *Sutterella*<br>↓ *Bacteroides, Prevotella, Mucispirillum, Dorea, Roseburia, Anaerotruncus, Oscillospira, Escherichiam,* and *Akkermansia* | (Shimizu et al., 2018) |

**Table 1. 11 (Cont.)**

| Vegetables | Study type | Dosage | Treatment duration | Analytical method | Changes in gut microbiota | Ref. |
|---|---|---|---|---|---|---|
| Mushrooms (*Pleurotus eryngii*) | Animal study (IBD mice model) | 1.5% or 3% of *Pleurotus eryngii* mushroom powder | 6 weeks | HTS (Illumina Miseq) | Phylum level:<br>↓ Actinobacteria<br>Class level:<br>↓ Mollicutes<br>Family level:<br>↑ S24-7 and Odoribacteraceae<br>↓ Desulfovibrionaceae and Enterococcaceae<br>Genus level:<br>↑ *Adlercreutzia*, *Akkermanisa*, *Lactobacillus*, *Anaerostipes*, and *Allobaculum*<br>↓ *Turicibacter*, *Dorea*, *rc4-4*, *Bacteroides*, and *Prvotella* | (Q. Hu et al., 2019) |
| Jerusalem artichoke | Animal study (healthy rats) | 2, 4, or 6% of Jerusalem artichoke powder | 12 weeks | Culture method | ↑ *Lactobacillus* spp. and *Bifidobacterium* spp.<br>↓ Coliforms | (Samal et al., 2017) |

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**CHAPTER 2:**

# Impact of Increasing Concentration of Tart and Sweet Cherries Juices Concentrates on Healthy Mice Gut Microbiota

Ayoub Al Othaim[1,2], Daya Marasini[3,†], and Franck Carbonero[1,3,4*]

[1]Cell and Molecular Biology Program; University of Arkansas, Fayetteville, AR, 72701 USA; franck.carbonero@wsu.edu
[2]Department of Medical Laboratories, College of Applied Medical Sciences, Majmaah University, Al-Majmaah, 11952, Saudi Arabia
[3]Department of Food Science; University of Arkansas, Fayetteville, AR, 72704 USA
[4]Department of Nutrition and Exercise Physiology, Elson Floyd School of Medicine, Washington State University-Spokane, 412 East Spokane Falls Boulevard, Spokane, WA, 99202 USA
***** Correspondence: franck.carbonero@wsu.edu
† Current affiliation: IHRC INC, Atlanta, GA, United States of America.

## Abstract

Cherries are an excellent source of dietary polyphenols and are marketed as sports and health dietary supplements. However, there is still limited direct evidence for their purported nutritional and health benefits. Gut microbiota modulation ought to be tested since most polyphenols reach the colon where they undergo microbial metabolization to become bioactive and bioavailable metabolites. In the present study, varying dilutions of three concentrate cherry juices, Montmorency tart cherry juice, Balaton tart cherry juice, and sweet cherry juice, were examined to determine their potential effect on the murine gut microbiota composition. Forty-five mice were randomly assigned to three different groups. Each group received an increased concentration of an assigned juice (1/20 v/v, 1/15 v/v, 1/10 v/v, 1/7 v/v, and 1/4 v/v) every five days for 25 days. Fecal samples were collected after each administration for microbiota characterization by high throughput 16S rRNA gene sequencing using Illumina Miseq. The

results revealed consistent gut microbiota modulation based on the concentration regardless of the juice type, but not in a true dose-dependent manner. The two median and the two higher concentrations were significantly different from each other and from the baseline and lowest concentration. Increasing cherry consumption consistently resulted in a significant increase of the relative abundance of *Barnesiella* and *Akkermansia*, whereas *Bacteroides* abundance was negatively correlated with the concentration of the juice. Overall, we demonstrate that cherries induce an overall beneficial modulation of the murine gut microbiota, and that amounts of fruits consumed need to be considered to devise appropriate health and nutrition studies.

**Keywords:** Tart cherries, Sweet cherries, Gut microbiota, Polyphenols, Dietary supplement.

## 2.1 Introduction

Cherry fruits belong to the genus *Prunus* from the family Rosaceae. The most common types of cherries consumed by humans are *Prunus cerasus*, designated as sour or tart cherries, and *Prunus avium*, designated as sweet cherries (Kelley, Adkins, & Laugero, 2018; Mayta-Apaza, Marasini, & Carbonero, 2017). Cherry production has been steadily increasing worldwide in the last few decades (Cásedas, Les, Gómez-Serranillos, Smith, & López, 2016), with the average world cherry production 27% higher from 2010 to 2014 than it was from 1995 to1999 according to data from Food and Agriculture Organization (FAO). Furthermore, data from The United States Department of Agriculture (USDA) showed that the average cherry production in the United States, the second leading global producers and suppliers of cherries, during 2014 to 2016 was nearly double the average production during the 1990s (Perez, Ferreira, & Minor, 2017), and in 2019, the US total cherries production was more than one billion pounds ("Crop Production," 2019). This dramatic growth of cherries production is probably due to the

increasing awareness among food industries and consumers about the purported health-promoting properties of cherries (Blando & Oomah, 2019; Kelley et al., 2018; Mayta-Apaza et al., 2017).

Cherries are nutrient-dense foods and an excellent source of bioactive components including phytochemicals, mainly polyphenols, with relatively low caloric and sugar content (Kelley et al., 2018; Khoo, Clausen, Pedersen, & Larsen, 2011; McCune, Kubota, Stendell-Hollis, & Thomson, 2010; Wojdyło, Nowicka, Laskowski, & Oszmiański, 2014). These properties give cherries unique organoleptic characteristics and desirable nutritional value leading to consumer attractiveness and cash crop status (Bak et al., 2010; P. G. Bell, McHugh, Stevenson, & Howatson, 2014; Mayta-Apaza et al., 2017). A growing body of research indicates that polyphenols have antioxidant and anti-inflammatory properties and may exhibit a wide range of potential health benefits through the regulation of metabolism, chronic disease, weight, and cell proliferation (Cory, Passarelli, Szeto, Tamez, & Mattei, 2018; Levers et al., 2016; Tomás-Barberán, Selma, & Espín, 2016). Generally, both sweet and tart cherries have higher concentrations of total phenolic compounds (Commisso et al., 2017; Ferretti, Bacchetti, Belleggia, & Neri, 2010; McCune et al., 2010). In fact, the total phenolic content in tart cherries is roughly twice higher than sweet cherry, while sweet cherries contain more anthocyanins (Kim, Heo, Kim, Yang, & Lee, 2005; McCune et al., 2010). Cherries are rich in phenolic compounds, mainly, anthocyanins, flavonoids (anthocyanins, flavan-3-ols and flavonols) which are the major class of polyphenolics in tart cherry, and phenolic acids (hydroxycinnamic acids or Hydroxycinnamates) which are the major class of polyphenolics in sweet cherry (Abrankó & Szilvássy, 2015; Chaovanalikit & Wrolstad, 2004; Damar & Ekşi, 2012; Gonçalves, Bento, Jesus, Alves, & Silva, 2018; Mayta-Apaza et al., 2017; Wojdyło et al., 2014). Several studies

have reported potential health benefits of cherry consumption which includes promoting cardiovascular health and reducing the risk of diabetes, obesity, and metabolic syndrome (Blando & Oomah, 2019; Chai, Davis, Wright, Kuczmarski, & Zhang, 2018; Csiki et al., 2015; Juhasz et al., 2013; Lachin, 2014; Nemes et al., 2019). Furthermore, the use of tart cherry juice has been rapidly increasing in sports medicine due to its beneficial impact on improving muscle function and reducing muscle soreness, muscle damage, oxidative stress, and inflammation in athletes (Gao & Chilibeck, 2020; Vitale, Hueglin, & Broad, 2017)

Polyphenols, in general, are relatively large molecules with high molecular weights (Costa et al., 2017; Peron et al., 2019; Santhakumar, Battino, & Alvarez-Suarez, 2018). As a consequence, the absorption of these compounds is relatively low in the upper intestinal tract (Espín, González-Sarrías, & Tomás-Barberán, 2017). The vast majority of polyphenols persist into the large intestine where they undergo biotransformation by bacterial members of gut microbiota, mainly in the colon (Edwards et al., 2017; Ozdal et al., 2016; Peron et al., 2019). The complex interplay between gut microbiota and food indigestible nutrients and phytochemicals, particularly polyphenols, largely contributes to the host's overall health (Kalt et al., 2020). There is a reciprocal relationship between gut microbiota and polyphenols (Gowd, Karim, Shishir, Xie, & Chen, 2019). The gut microbial residence actively drives the metabolism of polyphenols and modulates the biological activity of polyphenols by converting them into phenolic metabolites (Cardona, Andrés-Lacueva, Tulipani, Tinahones, & Queipo-Ortuño, 2013; Kumar Singh et al., 2019). On the other hand, polyphenols and their metabolites can impact the intestinal ecosystem and modulate gut microbiota composition (Braune, Engst, & Blaut, 2016; Gowd et al., 2019; Marín, Miguélez, Villar, & Lombó, 2015; Mayta-Apaza et al., 2018; Nash et al., 2018). However, the knowledge on how dietary polyphenols influence the gut microbiota and its

metabolic potential is still scarce with an emphasis on specific berries and grapes (Espín et al., 2017; Lavefve, Howard, & Carbonero, 2020). In addition to the prevailing focus on specific food products or extracts, there is still limited knowledge about the impact of the concentration or amounts of polyphenols on the gut microbiota. Often, amounts of food used are selected arbitrarily and would be unrealistically high or low concerning a typical human dietary intake. Tart cherry juice, for instance, has been used in most research studies, mainly in sports medicine, in a wide range of dosage and concentrations, mostly from 1oz of concentrated juice to 12oz of non-concentrated juice, with dilution ratio ranges from 1/3 v/v to 1/8 v/v of concentrated juice in water, twice a day. (Phillip G. Bell, Stevenson, Davison, & Howatson, 2016; Phillip G. Bell, Walshe, Davison, Stevenson, & Howatson, 2014; Phillip G. Bell, Walshe, Davison, Stevenson, & Howatson, 2015; Bowtell, Sumners, Dyer, Fox, & Mileva, 2011; Howatson et al., 2012; Kent et al., 2017; Lynn et al., 2014; Mayta-Apaza et al., 2018). That range of tart cherry juice dose seems efficient in accelerating strength recovery after exercise and decreasing blood markers of inflammation and oxidative stress in athletes, but some concentrations may only be realistic for consumption as a dietary supplement. In particular, the optimal bioactive dose to modulate the gut microbiome with a desired response is most often unclear because of the lack of studies including a dose-dependent experimental design. To the best of our knowledge, only two human studies have investigated the potential impact of tart cherry juice on modifying the composition of gut microbiota (Lear et al., 2019; Mayta-Apaza et al., 2018). Choosing the ideal dosage is quite challenging as the phenolic content depends on many factors including product preparation, processing, and shelf-life (Vitale et al., 2017). Therefore, the goal of this study was to investigate the impact of varying amounts of concentrated tart and sweet cherries juices on the murine gut microbiota composition.

## 2.2 Materials and Methods

### 2.2.1 Animals

The animal study was conducted at the Animal House Facility of the University of Arkansas after receiving approval from the Institutional Animal Care and Use Committee (IACUC). Forty-five (45) male mice (Mus musculus strain C57BL/6J) aged ten weeks were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Mice were housed in stainless steel cages under standard pathogen-free conditions at a temperature of 22 °C with 50% humidity and exposed to a 12 h light/12 h dark cycle. Before the dietary intervention, mice were provided with standard Teklad chow pellets (Envigo, Madison, WI) and water *ad libitum* for one week.

### 2.2.2 Treatments

Commercially available pure cherry juices concentrates were used in this study; (i) Montmorency tart cherry juice (MTC) (King Orchards, Central Lake, MI), (ii) Balaton tart cherry juice (BTC) (Royal Farms, Inc., Ellsworth, MI), and (iii) sweet cherry juice (SC) (Plentiful Planet Brands, Boulder, CO).

### 2.2.3 Experiment intervention

Mice were randomly assigned into three different groups ($n$ = 15 per treatment group), and each major group was split into five sub-groups of three. The groups had been assigned based on the type of juice they received. In this study, group (1) received (MTC), group (2) received (BTC), and group (3) received (SC). The experiment was performed for a duration of 23 days. Animals had *ad libitum* access to chow pellets throughout the experiment. Furthermore, groups received an increased concentration of their assigned juices added to their drinking water

(1/20 v/v, 1/15 v/v, 1/10 v/v, and 1/7 v/v) every five days; except the final intervention where the animals received the highest juice concentration (1/4 v/v) for only three days **(Figure 2. 1)**. At the end of each period, mice were transferred from stainless steel cages into metabolic cages (Tecniplast Cdd, 170013) for 6 hours in order to collect fecal samples at day 0, 5, 10, 15, 20, and 23, where day 0 samples represented the baseline. Samples were stored at −20 °C until used for microbiome analysis.

### 2.2.4 DNA extraction

A cell lysis step was performed initially to ensure full disruption of microbial cells. Briefly, 220 mg of fecal content were weighed and transformed to 2 ml tube containing 0.1 g of 0.1 mm diameter and 0.1 g of 0.5 mm diameter zirconia-silica beads (BioSpec Products, Bartlesville, OK, United States), and 1 mL of InhibitEX Buffer (from the QIAamp® Fast DNA Stool Mini Kit) was added to the tubes. The tubes were then subjected to a bead-beater for 60 seconds at maximum speed using a FastPrep®-24 bead-beater (MP Biomedicals, Santa Ana, CA, United States) and then heated to 70°C for 5 min in a Thermomixer (Eppendorf, Montesson, France). Microbial DNA was then extracted using a commercial QIAamp DNA stool mini kit (QIAGEN, Valencia, CA) following the manufacturer's protocol.

The NanoDropTM 1000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, United States) was used to determine the DNA concentration. Extracted DNA was then visualized following electrophoresis in a 2% agarose gel and 1X TAE (Tris-acetate ethylenediaminetetraacetic acid) buffer (AMRESCO®, Cleveland, OH, United States).

### 2.2.5 Universal polymerase chain reaction

16S ribosomal RNA gene was amplified by PCR (95 °C for 3 min, followed by 35 cycles at 98 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min and a final extension at 72 °C for 5 min) using primers (8F 5' - AGAGTTTGATCCTGGCTCAG - 3' and 1541R 5' - AAGGAGGTGATCCAGCCGCA - 3'). PCR reactions were performed in 25 µL mixture containing 3 µL of DNA template; 1 µL of each universal primer (8F and 1541R); 12.5 µL of GoTaq® Green Master Mix (Promega™ Corporation, Wisconsin, USA); and 7.5 µL of Nuclease-Free Water. Agarose gel electrophoresis was then performed to check the success of the amplification by using 1 µL of SYBR Safe DNA Gel Stain (Thermo Fisher Scientific, USA) fluorescent dye.

### 2.2.6 Libraries Preparation and Sequencing

Index PCR was performed by targeting the V4 region of the bacterial 16S rRNA gene using the dual-index strategy for primer design as previously described (Kozich, Westcott, Baxter, Highlander, & Schloss, 2013). Briefly, dual-index PCR was performed in 25 µL reaction components with 3 µL of DNA template, 2.5 µL of AccuPrime™ PCR buffer II, 0.5 µL of each index primers, 0.1 µL of AccuPrime™ Taq DNA Polymerase (Invitrogen, USA), and 18.4 µL of Nuclease-Free Water following the manufacture's protocol. The PCR consisted of initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 30 sec, primer annealing at 55 °C for 30 sec, and an extension at 72 °C for 1 min, with a final extension step at 72 °C for 5 min. After successful amplification, the correct size amplicons were subjected to purification and normalization by using SequalPrep kits (Invitrogen, USA) following the

manufacturer's protocol. Amplicon concentrations were measured by Agilent bioanalyzer after pooling the library.

For a very accurate concentration, the amplicons library was quantified by qPCR using the same primers and PerfeCta NGS library quantification kits (Quanta Biosciences, USA) following manufacturer instructions. The library was then denatured and diluted to appropriate concentrations and sequenced on an Illumina MiSeq.

The sequencing reads were downloaded from the Illumina Basespace server in Fastq files format. The sequences were demultiplexed in read 1 and read 2 with approximately 250bp in length. Operational taxonomic units (OTUs) with 97% of identity were determined and classified against the SILVA database as a reference. The Mothur v.1.41.1 pipeline was used for further analysis (Schloss et al., 2009). Non-metric multidimensional scaling (NMDS) plots based on the Bray-Curtis index were obtained in PAST 3.18 software (Hammer, Harper, & Ryan, 2001).

### 2.2.7 Statistical analysis

Statistical analysis including *t*-test, ANOVA followed by Tukey post hoc, Mann-Whitney U test, and Kruskal-Wallis were performed to detect significant differences between groups (by convention, differences were considered significant when $P < 0.05$). Non-Metric Multidimensional scaling (NMDS) based on count distance metrics (Bray-Curtis similarity index; ANOSIM: $P < 0.05$) were considered to be significant similarities between groups using Past4 software.

## 2.3 Results and Discussion

### 2.3.1 Nutritional analyses

It was observed that the addition of juice to drinking water resulted in increased consumption, but mainly for the median concentration (1/15 and 1/10 v/v; **Figure 2. 2 A**). This would be ascribed to the sweetness of the juices making the water more palatable, as well as a source of calories. Notably, at higher concentration, palatability decreased markedly; and it was noticed that mice started spitting out the water. Such concentrations are tolerable by human consumers when drinking cherry juice as a supplement. We elected to provide juices in drinking water to limit the stress on the animals, but these observations showed that there are limits of such an approach depending on the food tested, and tart cherries, in particular, being astringent with a strong flavor (Clausen, Pedersen, Bertram, & Kidmose, 2011).

The type of cherry juice consumed significantly affected mice weight trajectories, regardless of the concentration in drinking water **(Figure 2. 2 B).** Specifically, the Balaton and sweet cherries juices resulted in significant weight gain, whereas the Montmorency juice resulted in stable weight. Montmorency cherries are known to have lower sugar content, which would directly explain this observation, also supporting their potential as a low-calorie dietary supplement (Blando & Oomah, 2019; Wang et al., 1997).

### 2.3.2 Gut microbiota modulation

Without considering the concentration, Bray-Curtis dissimilarity-based non-metric multidimensional scaling (NMDS) revealed no significant clustering of the gut microbiota profile based on juice type **(Figure 2. 3 A)**. This is somewhat unexpected given the difference in sugars, carbohydrates, and possibly phytochemicals in the different juices. However, it is clear

from our observation that the gut microbiota modulation was almost entirely the same with each cherry type. While caution should be used with animal data, this observation suggests that other cherries than the Montmorency variety may provide similar benefits, at least from the gut microbiota perspective.

Regardless of juice type, there was significant clustering based on concentration. Specifically, the two median concentrations (1/15 and 1/10 v/v) and the two higher (1/7 and 1/4 v/v) were significantly different from each other and from the baseline and lowest concentration **(Figure 2. 3 B, C, D)**. It is interesting to note that this response does not fulfill the definition of a true dose-dependent response which had been suggested by previous reports (Li et al., 2015) based on comparing low and high doses only. It can thus be postulated that dietary modulation of the gut microbiota probably follows a multimodal response to any nutrient/food dose, and that the amounts used for nutrition and microbiome research need to be tested and validated.

Diversity indices were influenced differently by the concentration of the different juices. MTC induced a consistent decline in diversity regardless of concentration **(Figure 2. 4 A and B)**. BTC resulted in fluctuating diversity, but a significant decline for the two highest concentrations **(Figure 2. 4 C and D)**. In stark contrast, SC brought diversity down at the median concentrations, especially the 1/10 v/v **(Figure 2. 4 E and F)**. Interestingly, these results were inconsistence with another cherry-based study result which showed that obese diabetic mice that were fed the dark sweet cherry supplementation had the highest diversity compared to obese and lean controls (Garcia-Mazcorro et al., 2018).

In this experiment, the mice gut microbiota were initially dominated by relatively similar relative abundance of Bacteroidetes and Firmicutes, followed by Verrucomicrobia,

Proteobacteria, Actinobacteria, and Tenericutes, in line with commonly described murine gut microbiota profiles (Hugenholtz & de Vos, 2018; Nagpal et al., 2018; Nguyen, Vieira-Silva, Liston, & Raes, 2015). While the different juices exerted various dynamics of the phyla, Firmicutes relative abundances were consistently and significantly decreased and replaced by Verrucomicrobia with increasing concentration of the juices, while Bacteroidetes stayed relatively stable **(Figure 2. 5 A and B)**. The negative correlation between the abundance of Firmicutes and Verrucomicrobia was also reported in healthy mice supplemented black raspberry as well as in high-fat fed mice consuming *Lonicera caerulea* L. berry for seven weeks and 45 days, respectively (Tu et al., 2018; Wu et al., 2018). Blueberry consumption for four weeks also significantly decreased Firmicutes population in mice (Wankhade et al., 2019).

While Bacteroidetes were stable, there were still significant changes among the genera, with a decrease in *Bacteroides* and an increase in *Barnesiella* **(Figure 2. 6 A and B)**. The decrease in *Bacteroides* is consistent with what we observed in human subjects who had initially high *Bacteroides* levels after tart cherry consumption (Mayta-Apaza et al., 2018). The increase in *Barnesiella* was particularly marked at the highest concentrations (from non-detectable to 5-10%). This increase by cherry consumption may be one explanation for tart cherry benefits for exercise recovery, as *Barnesiella* has been reported to increase with exercise and being possibly beneficial (Fielding et al., 2019; Liu et al., 2017).

*Lactobacillus* has been reported, with wide variation, to respond significantly to berries and other polyphenol-rich fruit consumption (Han & Xiao, 2020; Lavefve et al., 2020). In this study, the increase of *Lactobacillus* was observed only for specific and different concentrations for each different juice **(Figure 2. 6 C)**. While these erratic dynamics are difficult to interpret,

they may also be a good illustration of the reason for the large variation in *Lactobacillus* bloom in response to polyphenol-rich fruits.

The clear and consistent impact of the cherry consumption was a steep increase of *Akkermansia* **(Figure 2. 6 D)**. The increase was specifically marked at higher concentrations, with the BTC even inducing the changes at the median concentrations. In agreement with our findings, the *Akkermansia* spp. relative abundance was increased in obese diabetic mice fed dark sweet cherry powder compared to obese and lean control groups (Garcia-Mazcorro et al., 2018). *Akkermansia muciniphila* is atype species of the genus typically detected in intestinal samples (Derrien, Belzer, & de Vos, 2017; Everard et al., 2013; van Passel et al., 2011). This genus/species has been increasingly reported as highly responsive to polyphenol-rich food consumption in murine models (Anhê et al., 2015; Tu et al., 2018; Wu et al., 2018; Zhang et al., 2017). However, such a response has not been commonly described, at least not as remarkable, in human subjects, which needs to be accounted when discussing such a bloom. *Akkermansia* has also recently been associated with exercise and exercise recovery (Munukka et al., 2018), and this effect of cherry juices is again noteworthy in that light.

## 2.4 Conclusion

We report that cherry juices concentrations in normal drinking water impacted weight gain and palatability in mice, which is important to consider for future animal studies. The impact of cherry juices on gut microbiota was notably influenced by the variation in the concentrations of the juices, but not significantly according to the type of cherry. Increasing concentration of juices was positively correlated with some bacterial genera, including *Akkermansia* and *Barnesiella*, whereas the opposite response was observed for *Bacteroides*

population. These consistent trends can be considered positive, and specifically *Akkermansia* and *Barnesiella* have recently been suggested to play roles in improved exercise recovery, which would make sense knowing that cherries as dietary supplements have been shown to provide such benefits.

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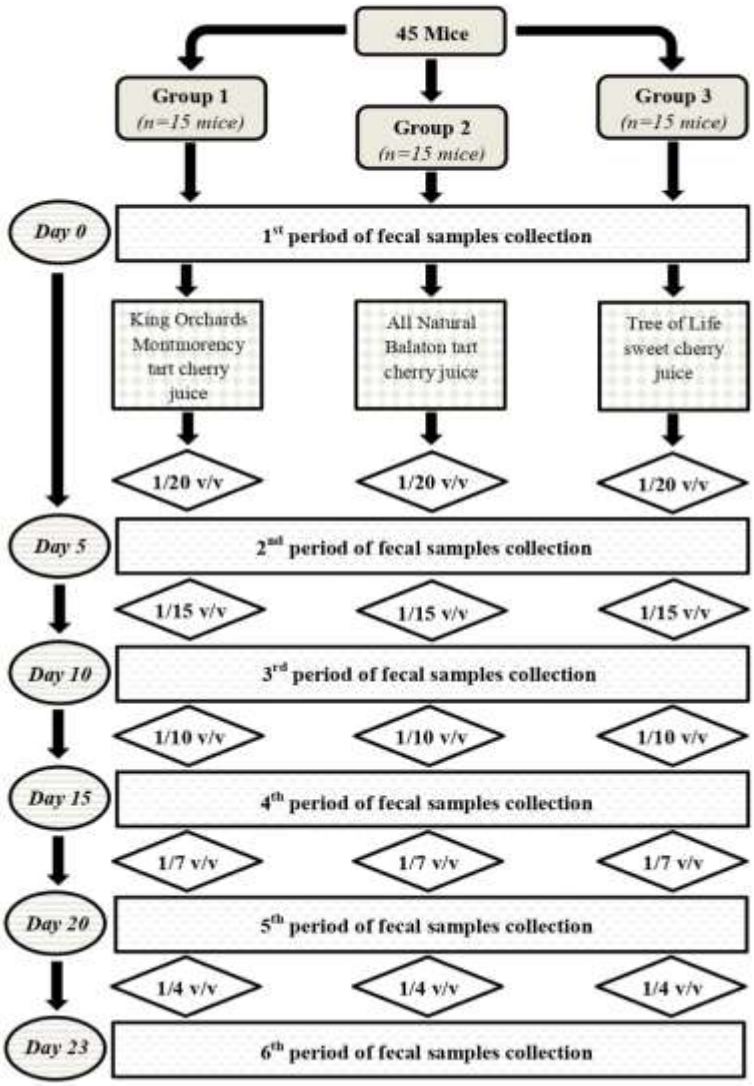


**Figure 2. 1:** Study design and intervention.

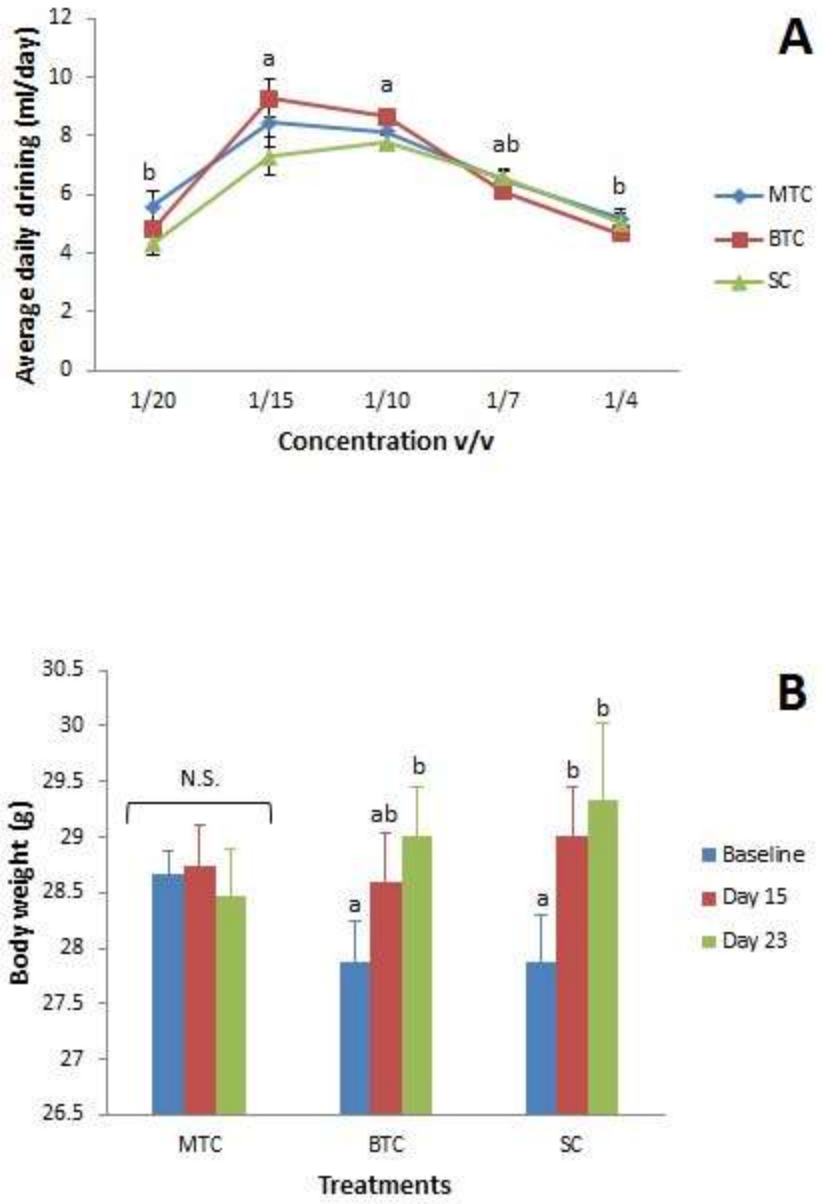


**Figure 2. 2: (A)** Palatability of cherries juices concentrates as represented by daily water consumption. Values are expressed as mean ± standard error (SE) ($n$ = 15). Different letters indicate significant difference ($P$ < 0.05) between concentrations for (MTC) group. **(B)** The impact of cherries juices consumption on weight trajectories for 23 days. Values are expressed as mean ± standard error (SE) ($n$ = 15). Different letters indicate significant difference ($P$ < 0.05) while (N.S.) indicates no significant difference.

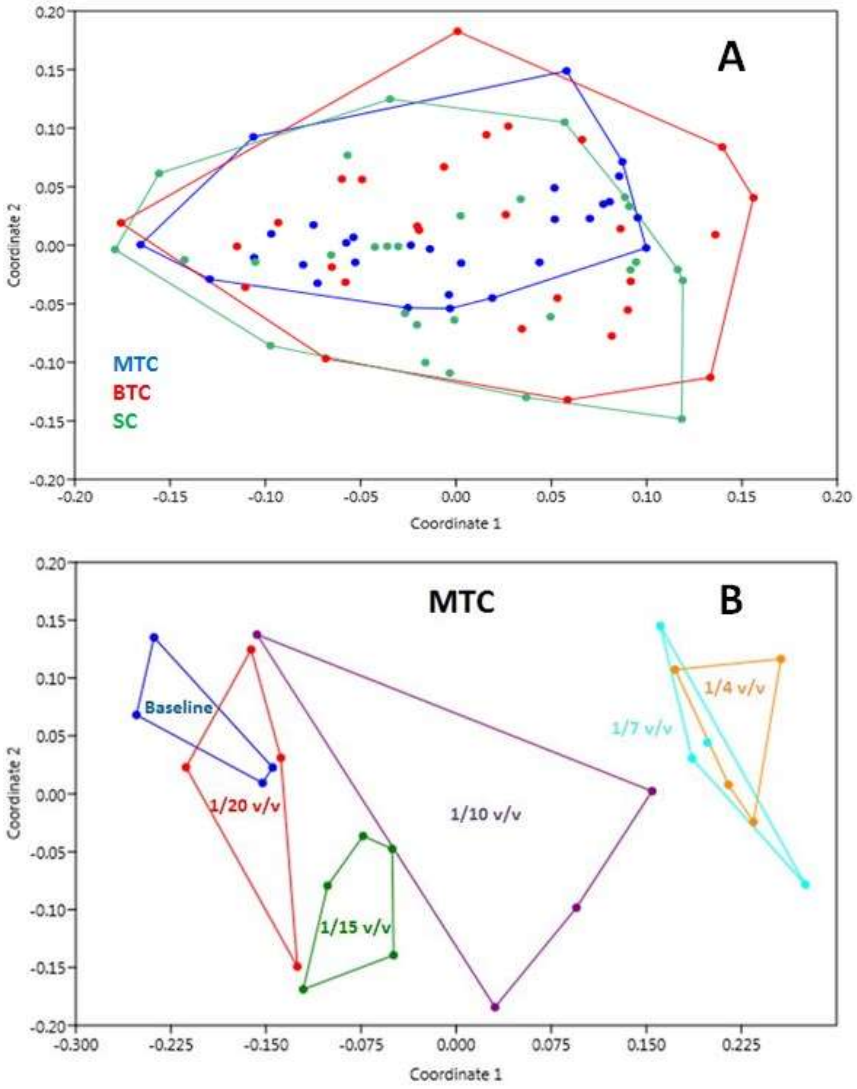


**Figure 2. 3:** Cherries type **(A)** have less impact on gut microbiota than concentration and amounts consumed **(B, C, and D)**. Gut microbiota data are shown on non-metric multidirectional scaling (NMDS) (Bray-Curtis similarity index; ANOSIM: ($P < 0.05$)).

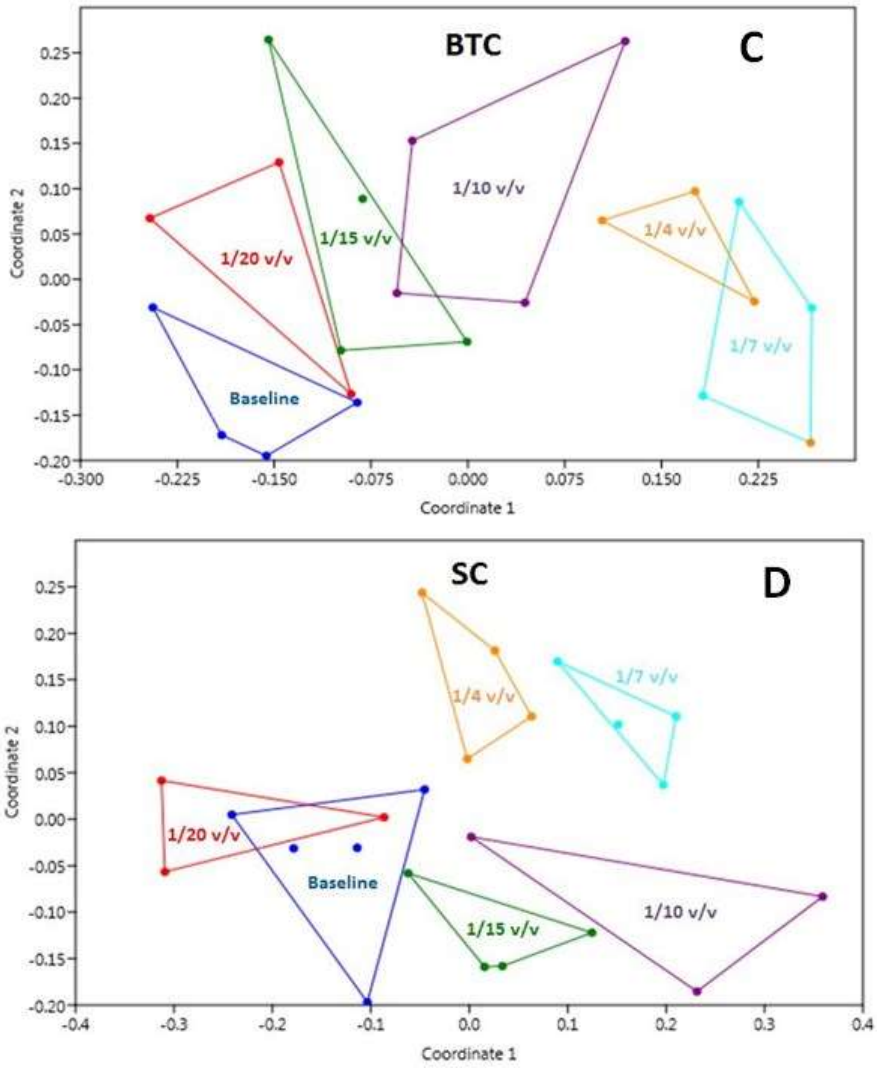


**Figure 2. 3 (Cont.)**

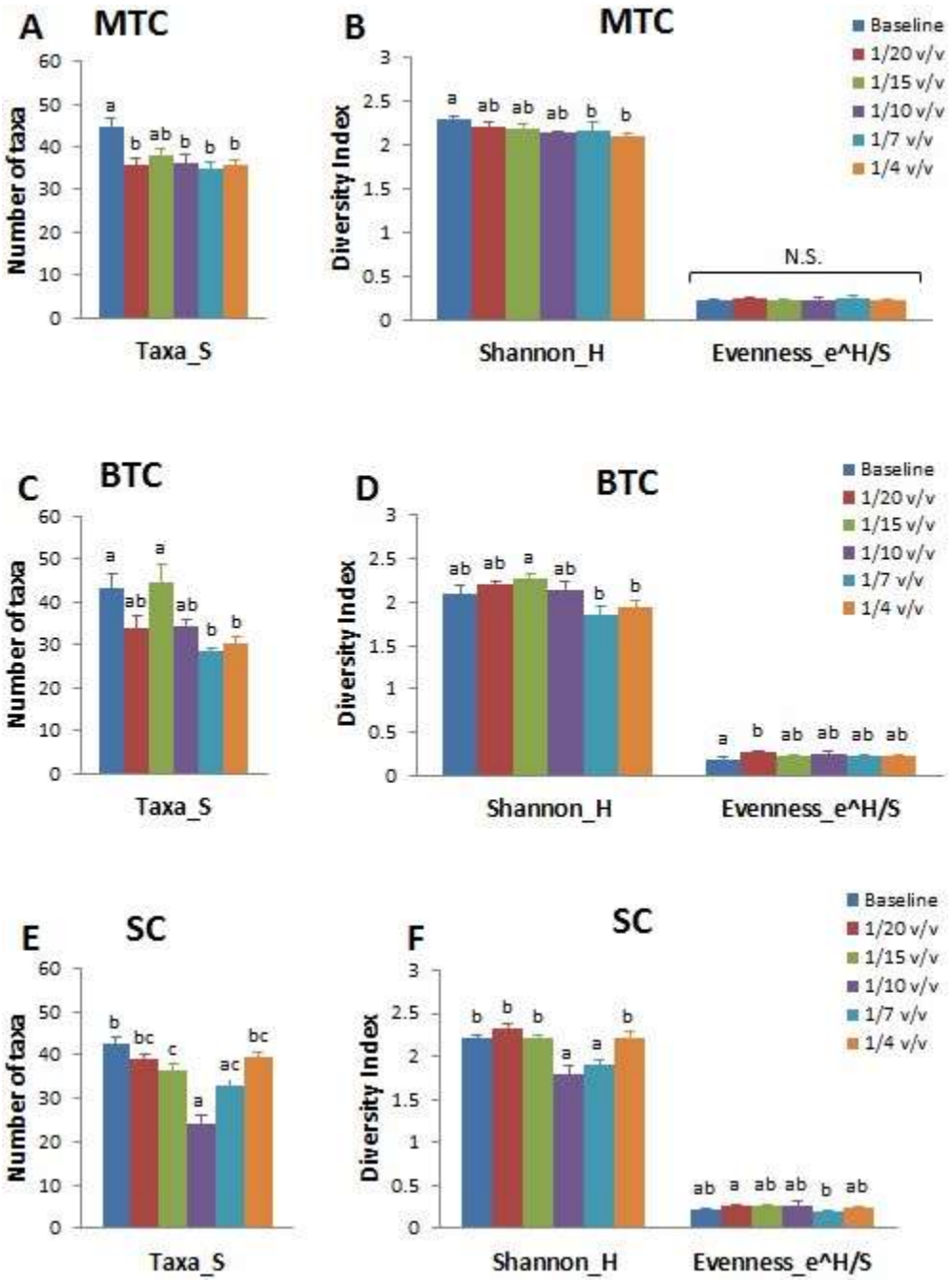


**Figure 2. 4:** The impact of concentration of cherries juices on gut microbiota diversity. **(A)** represents the impact of varying concentration of (MTC) on the number of taxa. **(B)** represents the impact of varying concentration of (MTC) on Shannon_H and Evenness_e^H/S indexes. **(C)** represents the impact of varying concentration of (BTC) on the number of taxa. **(D)** represents the impact of varying concentration of (BTC) on Shannon_H and Evenness_e^H/S indexes. **(E)** represents the impact of varying concentrations of (SC) on the number of taxa. **(F)** represents the impact of varying concentration of (SC) on Shannon_H and Evenness_e^H/S indexes. Values are expressed as mean ± standard error (SE) ($n$ = 15). Different letters indicate significant difference ($P < 0.05$) while (N.S.) indicates no significant difference.

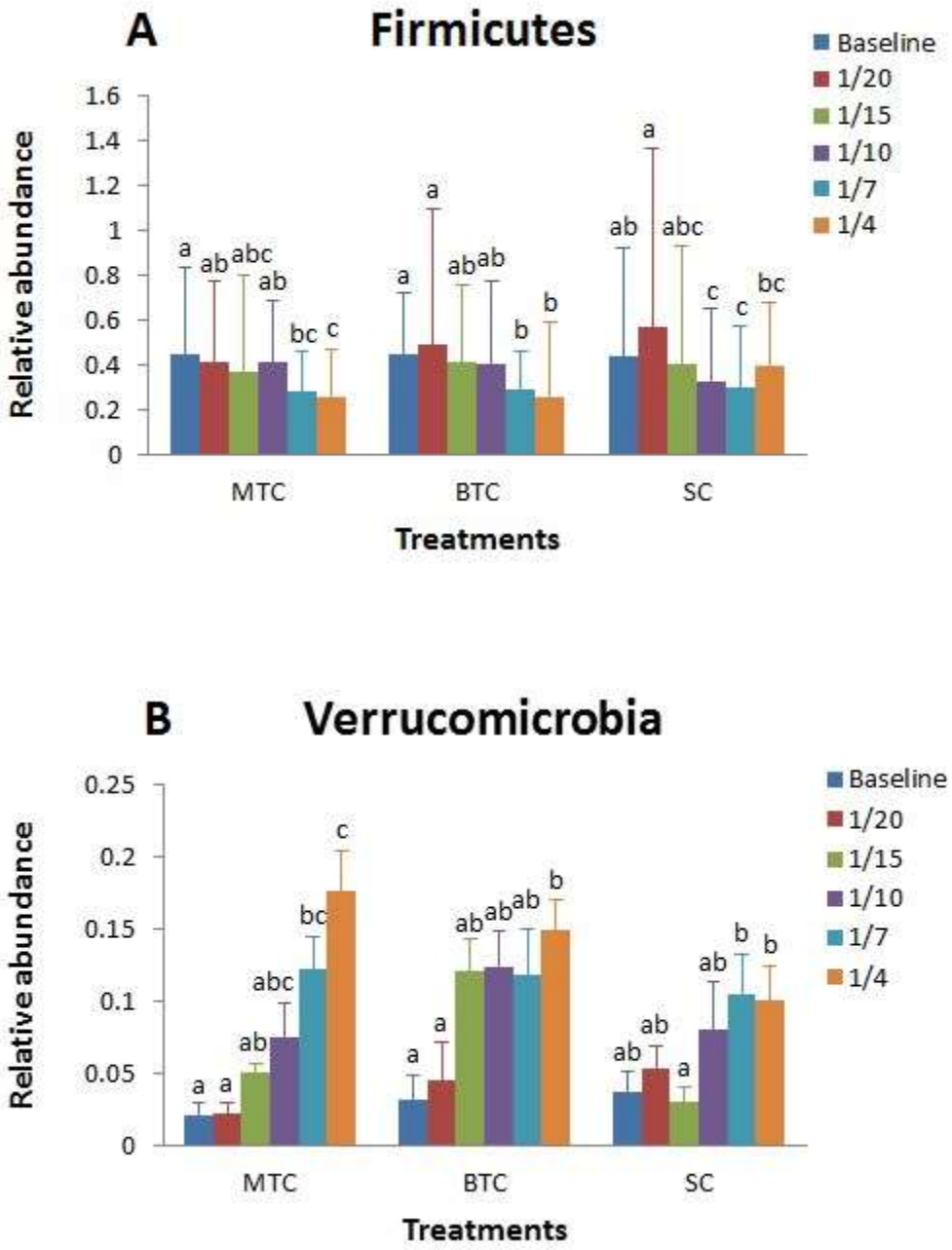


**Figure 2. 5:** Impact of varying concentrations of cherries juices on the phylum level. Increasing concentration of cherries juices result in a decrease of Firmicutes **(A)** and an increase of Verrucomicrobia **(B)**, with a slight variation by cherries type. Values are expressed as mean ± standard error (SE) ($n$ = 15). Different letters indicate significant difference ($P < 0.05$).

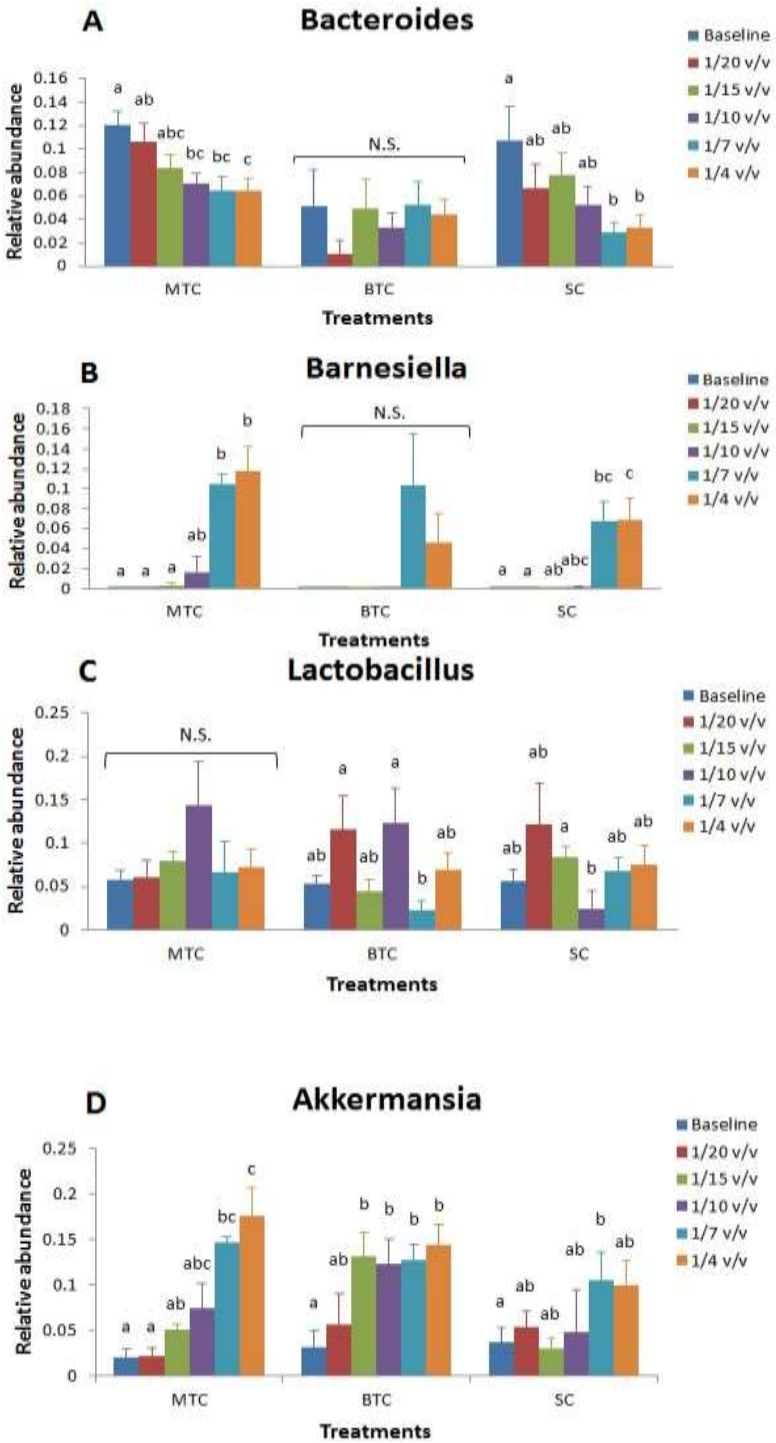


**Figure 2. 6:** Impact of varying concentrations of cherries juices on the genus level. Cherries consumption consistently resulted in decreased **(A)** *Bacteroides* and increased **(B)** *Barnesiella* and **(D)** *Akkermansia* in healthy mice. Values are expressed as mean ± standard error (SE) ($n$ = 15). Different letters indicate significant difference ($P < 0.05$) while (N.S.) indicates no significant difference.

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**CHAPTER 3:**

# Impact of Cranberry Juice Consumption on Gut and Vaginal Microbiota in Postmenopausal Women

Ayoub Al Othaim[1,2], Daya Marasini[3,†], and Franck Carbonero[1,3,4,5*]

[1]Cell and Molecular Biology Program; University of Arkansas, Fayetteville, AR, 72701 USA; franck.carbonero@wsu.edu
[2]Department of Medical Laboratories, College of Applied Medical Sciences, Majmaah University, Al-Majmaah, 11952, Saudi Arabia
[3]Department of Food Science; University of Arkansas, Fayetteville, AR, 72704 USA
[4] Department of Food Science, Washington State University
[5] Department of Nutrition and Exercise Physiology, Elson Floyd School of Medicine, Washington State University-Spokane, 412 East Spokane Falls Boulevard, Spokane, WA, 99202 USA

***** Correspondence: franck.carbonero@wsu.edu
† Current affiliation: IHRC INC, Atlanta, GA, United States of America.

## Abstract

Cranberries have long been purported to provide protection against urinary tract infections (UTIs). There is a line of evidence suggesting that causal pathogens might be seeded from the bacteria reservoirs in the intestinal and vaginal tracts. We tested the hypothesis whether cranberry intake would reshape bacteria taxa in the gut, as well as the vaginal ecosystem. A total of 25 postmenopausal women were enrolled into a randomized, double-blind, placebo-controlled study. Stool samples and vaginal swabs were collected at baseline and after 15 days of consumption of placebo or cranberry beverages, microbiota analyses were performed by Illumina Miseq sequencing following a double index 16S rRNA gene amplicon. All baseline stool samples generally fell in the *Bacteroides* enterotype. Significant increases of *Prevotella* ($P$ = 0.04), *Clostridium* XIVa members ($P$ = 0.04), *Eggerthella* ($P$ = 0.03), and *Bifidobacterium* ($P$ =

0.02) were shown following the cranberry juice intervention; this indicates modulation of the gut microbiota by cranberry components. Baseline vaginal microbiotas fell in three distinct patterns, *Lactobacillus* dominant, diversified microbiome, and *Streptococcus* dysbiosis. Compared with the placebo, the cranberry intervention significantly reduced the abundance of pathogenic *Streptococcus* ($P = 0.04$) in the dysbiosis group and increased commensal bacteria *Anaerococcus, Finegoldia*, *Actinomyces,* and *Corynebacterium* in the diversified microbiome and dysbiosis groups. Overall, these data suggest that cranberry consumption may improve vaginal microbiota composition in individuals with dysbiosis. Gut-borne taxa stimulation by the combination of cranberry oligosaccharides and polyphenols present in the cranberry product potentially mediates these beneficial properties.

**Keywords:** Cranberries; Urinary Tract Infection, Gut microbiota, Vaginal microbiota; Dysbiosis

### 3.1 Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections among women in general, and postmenopausal women are at in increased risk of recurrent infections (Fu, Liska, Talan, & Chung, 2017; Jung & Brubaker, 2019). It is estimated that more than 50% of women will have at least one occurrence of a UTI during their life spans and approximately 20–30% are affected with recurrent infections (Beerepoot & Geerlings, 2016; Fu et al., 2017). While UTIs are generally benign, their prevalence poses medical and economic issues resulting in millions of visits yearly and an estimated cost of $2.8 billion in medical care expenses in the United States (Simmering, Tang, Cavanaugh, Polgreen, & Polgreen, 2017). In addition, the need for antibiotic treatment is problematic both for the patient's natural microbiota stability and for the community and environment, since antibiotic-resistance can result from such treatments

(Ianiro, Tilg, & Gasbarrini, 2016; Koutzoumis et al., 2020; Morgun et al., 2015; Stapleton, 2016).

While pathogenic bacteria, most often *Escherichia coli* and *Streptococcus*, are the main UTI causative bacteria, it has been proposed that a reduction in the abundance of commensal vaginal microbes may also contribute to an increased UTI risk in postmenopausal women (Reid, 2018; Stapleton, 2016). It is now well understood that the normal vaginal microbiome is dominated by *Lactobacilli*, and the vaginal tract is naturally populated with a relatively dense and diverse collection of bacteria (Mendling, 2016; Ravel et al., 2011; White, Creedon, Nelson, & Wilson, 2011; Witkin & Linhares, 2017). An imbalance of the vaginal microbiome is not only a common cause for bacterial vaginosis (BV) (Stapleton, 2016; White et al., 2011) but may directly affect the potential pathogens to colonize the urethra and/or bladder (Czaja et al., 2009; Stapleton, 2016). Thus, it would appear that asymptomatic changes in the vaginal microbiome may trigger UTI development, either by seeding pathogens or by creating a niche for pathogens to thrive (Czaja et al., 2009; Stapleton, 2016).

The factors shaping the vaginal microbiota composition and stability are still not well known (Braundmeier et al., 2015). In this context, we hypothesize that the vaginal microbiome is strongly influenced by the gut microbiome. There are different lines of evidence to support this hypothesis. First, because of the anatomical proximity, gut microbes in the rectum and distal colon can easily come in contact with the vaginal tract, and indeed several vaginal *Lactobacillus* species are also found in the digestive tract (Marchesi, Silva, Wiese, & Nader-Macías, 2020; Pan, Hidalgo-Cantabrana, Goh, Sanozky-Dawes, & Barrangou, 2020). Second, it has become increasingly evident that metabolites produced by the gut microbiome are highly bioavailable and distributed to any organ/region of the human body and these metabolites may influence the

vaginal ecosystem as well (Possemiers, Bolca, Verstraete, & Heyerick, 2011). Since the gut microbiome is strongly individualized, shaped by external factors, and naturally evolving to some extent, this would in part explain why the underlying causes of UTI and BV are often challenging to identify.

It is plausible that the different responses to cranberry intervention might be the consequence of inter-individual differences in gut microbiome composition. During the last decade, reports of potential positive modulation of the human gut microbiota by berries have emerged (Cai et al., 2019; Lavefve, Howard, & Carbonero, 2020; O'Connor et al., 2019; Rodríguez-Morató, Matthan, Liu, de la Torre, & Chen, 2018). Polymeric cranberry phenolic compounds generally have poor bioavailability. However, when they reach the distal intestine, those molecules are broken down and metabolized by microbiota under anaerobic conditions, a process known as fermentation. Compared to their parent compounds, these bacteria-derived metabolites are highly bioavailable and bioactive. Previous studies showed that γ-valerolactone and its sulphate conjugates (Mena et al., 2017) phenylacetic acid, 3,4-dihydroxyphenylacetic acid (González de Llano et al., 2015), hippuric and α-hydroxyhippuric acids (González de Llano, Liu, Khoo, Moreno-Arribas, & Bartolomé, 2019) have anti-bacteria effects under experimental conditions. These findings highlight the critical need to understand how cranberries could affect the possible crosstalk between intestinal and other microbiome ecosystems, thereby modulating the impact of dietary cranberries on UTI prevention. This project aimed to assessing the relationships between the gut microbiome and vaginal microbiome, and how cranberry consumption affecting the gut microbiome may also impact the vaginal microbiome. It is expected that in the long term, nutritional studies using other dietary elements (such as prebiotics) may be designed to optimize and maximize the beneficial effects of cranberries.

## 3.2 Materials and Methods

### 3.2.1 Subjects

Twenty-three (23) women volunteered and participated in this study. The eligibility (no menstrual period for at least 12 months) of the participants was determined using a questionnaire. The participants were postmenopausal women between the ages of 50-75 years old who met the inclusion criteria including no recent antibiotics or immunosuppressive therapies; surgery of the stomach, small or large intestines; appendectomy, gastric bypass or gastric banding in the previous 6 months, and diagnosis with any autoimmune diseases.

### 3.2.2 Study Design and sample collection

This study was a randomized, placebo-controlled, crossover, dietary intervention. Ocean Spray Cranberries, Inc. provided the experimental and placebo beverages, which have been validated and used in previous studies (Hsia, Zhang, Beyl, Greenway, & Khoo, 2020; Haiyan Liu, Howell, Zhang, & Khoo, 2019; Maki et al., 2016; Zhao, Liu, Su, Khoo, & Gu, 2020). Both beverages were similar in appearance, taste, and aroma and assigned to volunteers according to computer-generated random orders. Both investigators and participants were blind to the assignment and the products. The products were identified by a random 3-digit code pre-printed on the cap. The participants consumed either cranberry juice or the placebo beverage daily (8 fl oz per day) for 15 days **(Table 3. 1)**. Analytical methods for testing the products were previously described (Maki et al., 2016). Biological samples were collected at baseline and after each intervention **(Figure 3. 1)**. Subjects were provided with polyester tipped swabs with a neutralizing buffer solution using the Environmental Sampling Kit (Puritan Diagnostics, USA) for collecting vaginal samples and the Precision™ Stool Collector (Cardinal Health, USA) for

collecting stool samples. They were also provided with instructions for self-sampling at each sampling event. Vaginal swabs and stool samples were stored at −20 °C until used for microbiome analysis.

### 3.2.3 DNA extraction

Microbial DNA was extracted from all samples with the QIAamp DNA stool kit (QIAGEN, Valencia, CA) following the manufacturer's protocol with the addition of cell lysis step to rapidly and completely disrupt cells in the samples to release DNA. The cell lysis was performed by adding 0.1 g of 0.1 mm diameter and 0.1 g of 0.5 mm diameter zirconia-silica beads (BioSpec Products, Bartlesville, OK, United States) to 2 ml tubes. Stool and vaginal samples were transferred to the 2 ml tubes containing zirconia-silica beads. One ml of InhibitEX Buffer (from the QIAamp® Fast DNA Stool Mini Kit) was then added to the tubes. The tubes were subjected to a bead-beater for 60 seconds at maximum speed using a FastPrep®-24 bead-beater (MP Biomedicals, Santa Ana, CA, United States).

The NanoDropTM 1000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, United States) was used to determine the DNA concentration. Extracted DNA was then visualized following electrophoresis on a 2% agarose gel in 1X TAE (Tris-acetate ethylenediaminetetraacetic acid) buffer (AMRESCO®, Cleveland, OH, United States).

### 3.2.4 Universal polymerase chain reaction

16S ribosomal RNA gene was amplified by PCR (95 °C for 3 min, followed by 35 cycles at 98 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min and a final extension at 72 °C for 5 min) using primers (8F 5' – AGAGTTTGATCCTGGCTCAG – 3' and 1541R 5' – AAGGAGGTGATCCAGCCGCA - 3') (Carbonero, Oakley, & Purdy, 2014). PCR reactions

were performed in 25 µL mixture containing 3 µL of DNA template; 1 µL of each universal primer (8F and 1541R); 12.5 µL of GoTaq® Green Master Mix (Promega™ Corporation, Wisconsin, USA); and 7.5 µL of Nuclease-Free Water. Amplicons were run on agarose gel electrophoresis to confirm the success of the PCR by using 1 µL of SYBR safe DNA Gel Stain (Thermo Fisher Scientific, USA) fluorescent dye.

### 3.2.5 Libraries Preparation and Sequencing

For 16S rRNA gene sequencing analyses, a dual-indexed amplicon strategy was performed using universal primers tagged with Illumina adapters and barcodes as previously described (Aljahdali et al., 2017; Kozich, Westcott, Baxter, Highlander, & Schloss, 2013; Mayta-Apaza et al., 2018). Briefly, PCR was set up in 25 µL reaction components with: 3 µL of the DNA template; 2.5 µL of the AccuPrime™ PCR buffer II; 0.5 µL of each index primers; 0.1 µL of AccuPrime™ Taq DNA Polymerase (Invitrogen, USA); and 18.4 µL of Nuclease-Free Water following the recommended protocol. The PCR consisted of initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 30 sec, primer annealing at 55 °C for 30 sec, and an extension at 72 °C for 1 min, with a final extension step at 72 °C for 5 min. Correct size amplicons were subjected to purification and normalization with the Invitrogen SequalPrep kits following the manufacturer's protocol. Amplicons were pooled into two different libraries and concentrations were measured by Agilent bioanalyzer and qPCR using the same primers and PerfeCta NGS library quantification kits (Quanta Biosciences, USA). Libraries were then denatured and diluted to appropriate concentrations and sequenced on an Illumina MiSeq.

Resulting sequencing files were analyzed using the Mothur software. Operational taxonomic units (OUT) were determined and classified against reference databases (Ribosomal

Database Projet (RDP), SILVA, GenBank). Basic and multivariate statistical analyses were performed in PAST.

### 3.2.6 Statistical analyses

Statistical analyses including Wilcoxon signed-rank test and Mann-Whitney U test (depending on the availability of fully paired sample sets) were performed to detect significant differences between groups (by convention, differences were considered significant when $P < 0.05$). Non-metric multidimensional scaling (NMDS) and analysis of similarities (ANOSIM) based on count distance metrics (Bray-Curtis similarity index; ANOSIM: $P < 0.05$) were obtained with Past4 software (Al Othaim, Marasini, & Carbonero, 2020; Hammer, Harper, & Ryan, 2001).

## 3.3 Results and Discussion

The human study was completed with 23 volunteers, and 21of witch provided the full set of samples. The volunteers' demographics are described in **Table 3. 2**.

For the baseline samples, 23 stool and 23 vaginal swabs were collected, 22 of each for the first period samples, and 21 of each for the second period. Therefore, a total of 66 stool samples and 66 vaginal swabs have been collected. However, for several subjects, self-sampling of stool resulted in problematic samples for at least one of the three samples. As these samples did not yield satisfactory levels of microbial DNA, only eight subjects with the full set of samples were included in the analyses.

### 3.3.1 Impact of the cranberry juice on the gut microbiota

Cranberry juice significantly increased the numbers of *Bifidobacterium* ($P = 0.02$) and *Eggerthella* ($P = 0.03$), two Actinobacteria members that have been shown previously to be able to grow and metabolize dietary polyphenols (Fogliano et al., 2011; Koutsos & Lovegrove, 2015; Morais, de Rosso, Estadella, & Pisani, 2016; Queipo-Ortuño et al., 2012). *Bifidobacterium* are also known as probiotics and the increased abundance is considered a sign of improved intestinal health (Cuervo et al., 2014; Rajkumar et al., 2014; Singh et al., 2017). Cranberry juice also resulted in a significant increase of *Prevotella* ($P = 0.04$), which is considered a beneficial genus associated with polysaccharides and fiber consumption (David et al., 2014; De Filippis et al., 2016; Wu et al., 2011) **(Figure 3. 2)**.

The most remarkable trend among the Firmicutes was a significant increase of *Clostridium* XIVa ($P = 0.04$) and other known butyrate producers (*Eubacterium*, *Flavonifractor*, and *Subdoligranulum*) after the consumption of cranberry juice (El Aidy, Van den Abbeele, Van de Wiele, Louis, & Kleerebezem, 2013; Rivière, Selak, Lantin, Leroy, & De Vuyst, 2016; Walker, Duncan, Louis, & Flint, 2014) **(Figure 3. 2)**. Butyrate is a short-chain fatty acid shown to offer benefits to not only the local intestinal environment, but also contributes to systemic metabolism (Hu Liu et al., 2018; O'Keefe, 2016). *Akkermansia*, a genus that has been associated with a number of health properties, was increased with cranberry consumption in animal models (Anhê et al., 2017; Rodríguez-Daza et al., 2020) and to some extent in humans (Bekiares, Krueger, Meudt, Shanmuganayagam, & Reed, 2018), it showed a slight but non-significant increase between cranberry and placebo beverages (Dao et al., 2016; Png et al., 2010) **(Figure 3. 2)**. It should be noted that all those trends were strongly individualized, and further validation is

warranted in more clinical studies with a larger sample size. Overall, cranberry consumption appears to stimulate beneficial genera.

### 3.3.2 Impact of the cranberry juice on the vaginal microbiota

The vaginal microbiotas were found to be extremely heterogeneous at baseline, and have therefore been stratified into three groups based on the most abundant genera and their known relation to vaginal health **(Figure 3. 3)**:

Group 1 comprises individuals whose baseline vaginal microbiotas were strongly dominated by *Lactobacillus*, which is considered the ideal healthy state (Ravel et al., 2011). Group 2 comprises individuals whose baseline vaginal microbiotas were characterized by diverse and relatively well distributed high number of different taxa, without obvious dominant detrimental genus. Group 3 comprises individuals whose baseline vaginal microbiotas were characterized by high abundance of *Streptococcus*, which is strongly indicative of microbial dysbiosis that could potentially lead to vaginosis or UTI (Leclercq et al., 2016; Tan, Ulett, Steele, Benjamin, & Ulett, 2012; Ulett et al., 2009). Individuals were roughly equally distributed among the 3 groups which allowed statistical analyses for each group.

#### *3.3.2.1 Lactobacillus dominant vaginal microbiota*

Consumption of both products appeared to have little impact on the vaginal microbiota of volunteers that have *Lactobacillus* as the major bacterial taxa **(Figure 3. 4)**.

#### *3.3.2.2 Diverse baseline vaginal microbiota*

As expected with the criterion used to segregate the groups, the vaginal microbiota of these subjects was significantly more diverse **(Figure 3. 5)**. The number of taxa was significantly

decreased after the consumption of cranberry juice, and even more with the placebo. However, the decrease of the Shannon index was only significant in the placebo group. It can be hypothesized that loss of diversity not associated with *Lactobacillus* dominance may be detrimental to some extent; but the loss of the diversity does not appear to be different between the interventions and thus, it is likely not associated with cranberry components.

Several significant changes were observed in the bacteria abundance at genus level **(Figure 3. 6)**. Compared with baseline, consumption of cranberry juice allowed for the maintenance of several moderately abundant Firmicutes (*Lactobacillus*, *Anaerococcus*, *Finegoldia*, and *Peptoniphilus*) and Actinobacteria (*Actinomyces*, *Varibaculum*, *Actinomycetales*, and *Corynebacterium*). In contrast, aforementioned bacteria were significantly reduced following placebo beverage consumption. Different responses between the cranberry and placebo beverage from baseline were statistically significant. The status of these genera is somewhat controversial, but they would be considered as mostly harmless to vaginal health (Boskey, 2001; Ma, Forney, & Ravel, 2012). The placebo beverage led to an unexplainable increase of *Prevotella*, which was different from the response following cranberry beverage intake. *Prevotella* in the gut microbiota are associated with plant-based diets with a possible role in digestion of complex carbohydrates and production of short-chain fatty acids (Geva-Zatorsky et al., 2017; Precup & Vodnar, 2019; Zhu, Sunagawa, Mende, & Bork, 2015). However, the function of *Prevotella* in the vaginal tract is less clear. Some lines of evidence suggested that high levels of SCFAs might promote dysbiosis and inflammation in the vaginal tract (Aldunate et al., 2015; Amabebe & Anumba, 2020). To conclude, cranberry juice appears to maintain the diversity of vaginal microbiota which is a presumably healthier profile for these volunteers.

#### *3.3.2.3 Streptococcus dysbiosis*

Several women (*n* =7) were found to have a strong dysbiosis of their vaginal microbiota, characterized by a high initial abundance 22% to 81% of *Streptococcus*, a well-known causative agent of vaginosis and UTI (Jass & Reid, 2009; Leclercq et al., 2016; Tan et al., 2012; Ulett et al., 2009).

As suggested by the diversity trends, cranberry juice had a very strong and specific impact on the dysbiosed vaginal microbiota **(Figure 3. 7)**. Most notably, although both beverages reduced the abundance of *Streptococcus* compared to baseline, the consumption of cranberry juice significantly reduced the relative abundance of *Streptococcus* to a greater extent, compared to the placebo beverage. Intriguingly, it appeared that *Streptococcus* has been replaced with a range of different Firmicutes (*Anaerococcus* and *Finegoldia*) and Actinobacteria (*Actinobaculum*, *Actinomyces*, *Varibaculum*, and *Corynebacterium*; **Figure 3. 8**). *Streptococcus*, both group A and B, colonization has been associated with an increased risk of vaginitis, bacterial vaginosis, through the female life span (Clark & Atendido, 2005; Verstraelen, Verhelst, Vaneechoutte, & Temmerman, 2011). The presence of this genus also significantly increased chances of vertical transmission to neonates during pregnancy (Puopolo, Lynfield, Cummings, & Committee On Infectious, 2019). The exact mechanism of the reduction of the abundance of *Streptococcus* by cranberries is unknown. However, Feliciano and colleagues showed that cranberry proanthocyanidins (PAC) reduced extra-intestinal pathogenic *E. coli* agglutination and inhibited the invasion of enterocytes (Feliciano, Meudt, Shanmuganayagam, Krueger, & Reed, 2014). Cranberry-derived PACs might impair virulence and quorum sensing of *Pseudomonas aeruginosa*, according to another study (Maisuria, Lopez-de Los Santos, Tufenkji, & Déziel, 2016). In the research performed by O'May and Tufenkji, cranberry PAC and other tannin-

containing materials blocked swarming motility of *P. aeruginosa*, possibly via inhibition of the flagellin gene expression (O'May & Tufenkji, 2011). It can be hypothesized that cranberries and metabolites generated and absorbed in the gut affected *Streptococcus* through multiple mechanisms, inhibition of bacteria growth or viability, disruption of cytoskeleton structure, and etc. These hypotheses will warrant future experiments to explore.

### 3.4 Conclusion

These findings suggest a beneficial impact of cranberry juice on vaginal microbiota with dysbiosis, and possibly its contribution to vaginal health. It can be speculated that *Streptococcus* dysbiosis could be improved directly within the local niche environment by increasing competition of other commensal bacteria or indirectly by decreasing the bacteria migration from the gut reservoir, and thereby restoring a healthier vaginal microbiota. Due to that possible relationship, the beneficial impact of cranberry juice on the gut microbiota is also encouraging for vaginal health. As reported previously, the ageing gut microbiota tends to be less diverse and responsive to dietary changes. Herein, a relatively short-term consumption and daily consumption of cranberry juice shows promising prebiotic effect and may help maintain levels of a wide range of beneficial genera (*Prevotella*, *Bifidobacterium*, and butyrate-producing Firmicutes).

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**Author Contributions:** Conceptualization, Franck Carbonero; Data curation, Ayoub Al Othaim, Daya Marasini and Franck Carbonero; Formal analysis, Ayoub Al Othaim, Daya Marasini and Franck Carbonero; Funding acquisition, Franck Carbonero; Methodology, Ayoub Al Othaim,

Daya Marasini and Franck Carbonero; Project administration, Daya Marasini and Franck Carbonero; Supervision, Daya Marasini and Franck Carbonero; Visualization, Franck Carbonero; Writing – original draft, Ayoub Al Othaim and Franck Carbonero; Writing – review & editing, Ayoub Al Othaim, Daya Marasini and Franck Carbonero.

**Table 3. 1:** The analytical data for the cranberry and placebo beverages. ND indicates non-detectable

| Analytical | Cranberry juice | Placebo | Unit |
|---|---|---|---|
| Anthocyanins | 3.09 | ND | mg |
| Flavonols | 5.68 | ND | mg |
| Phenolic acids | 11.60 | ND | mg |
| Total Phenolics | 161 | ND | mg |
| Proanthocyanidins | 141 | ND | mg |
| Organic acid | 1.74 | 1.67 | g |
| Sugar | 5.94 | 6.3 | g |

**Table 3. 2:** Volunteers demographics.

| | |
|---|---|
| **Age (years)** | 61±7 (min=51; max=74) |
| **BMI** | 28.5±7.8 (min=18; max=46) |
| **Height (inches)** | 64.2±2.3 (min=60.2; max=72.2) |
| **Weight (lbs)** | 170.5±49.7 (min=112; max=278) |

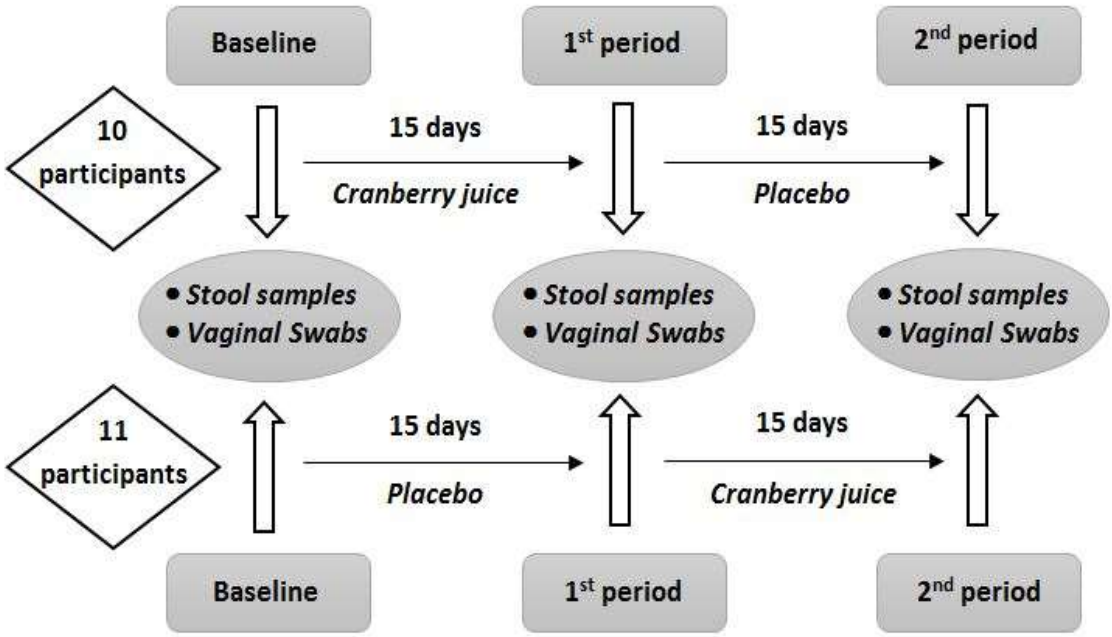


**Figure 3. 1:** Timeline of intervention.

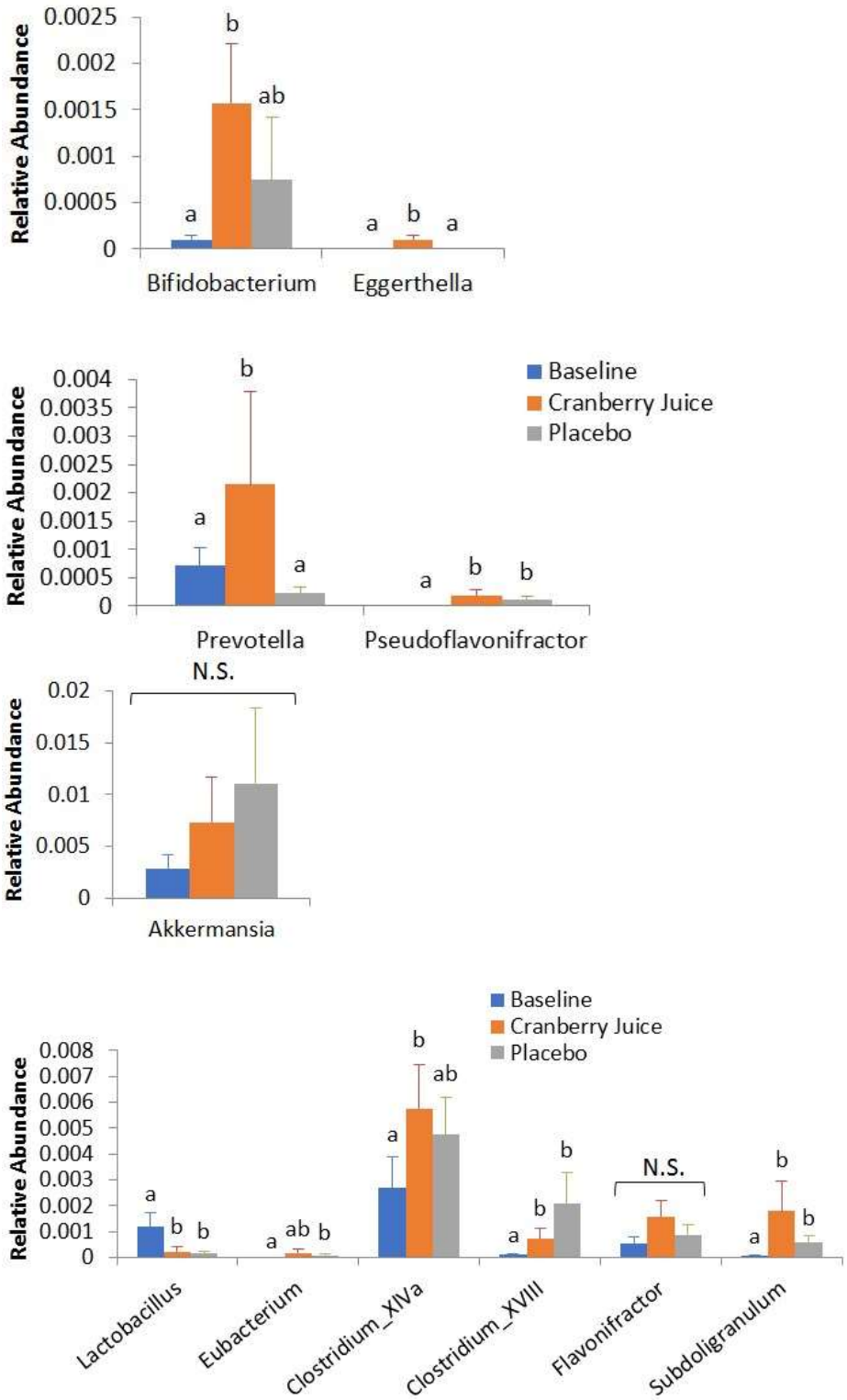


**Figure 3. 2:** Impact of cranberry juice and placebo on the relative abundance of genera from Actinobacteria, Bacteroidetes, Verrucomicrobia, and Firmicutes. Values are expressed as mean ± standard error (SE) ($n$ = 8). Different letters indicate significant difference ($P < 0.05$), whereas N.S. indicates no significant difference.

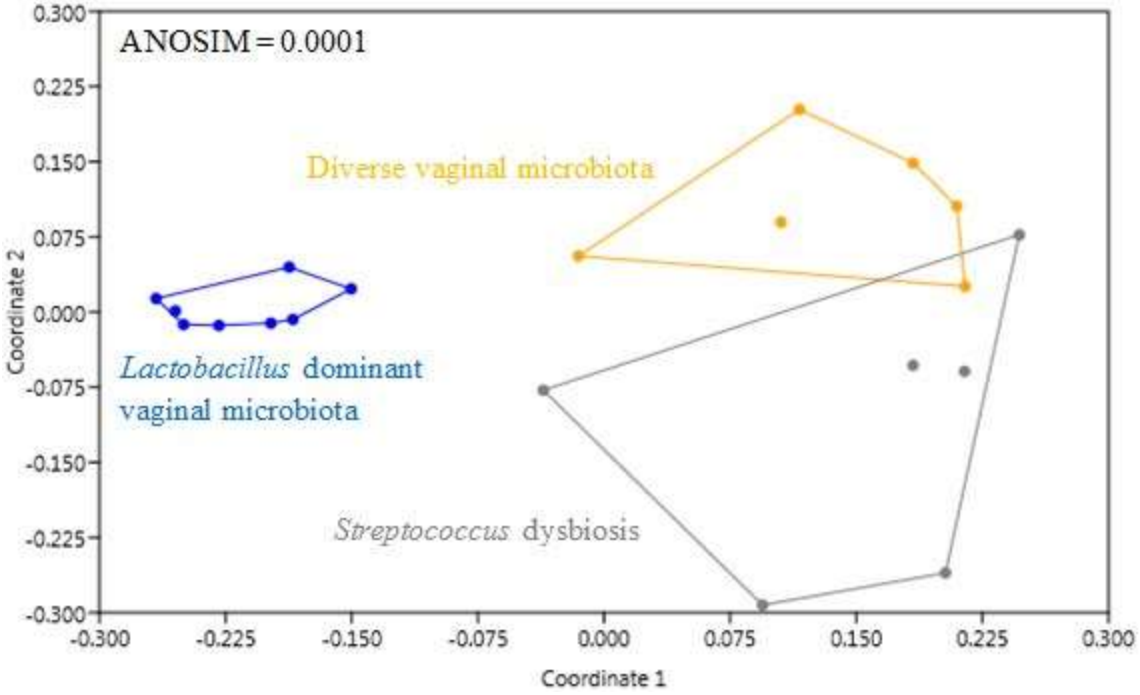


**Figure 3. 3:** Non-metric Multidimensional Scaling (Bray-Curtis index) sowing the three different clusters of baseline vaginal microbiota.

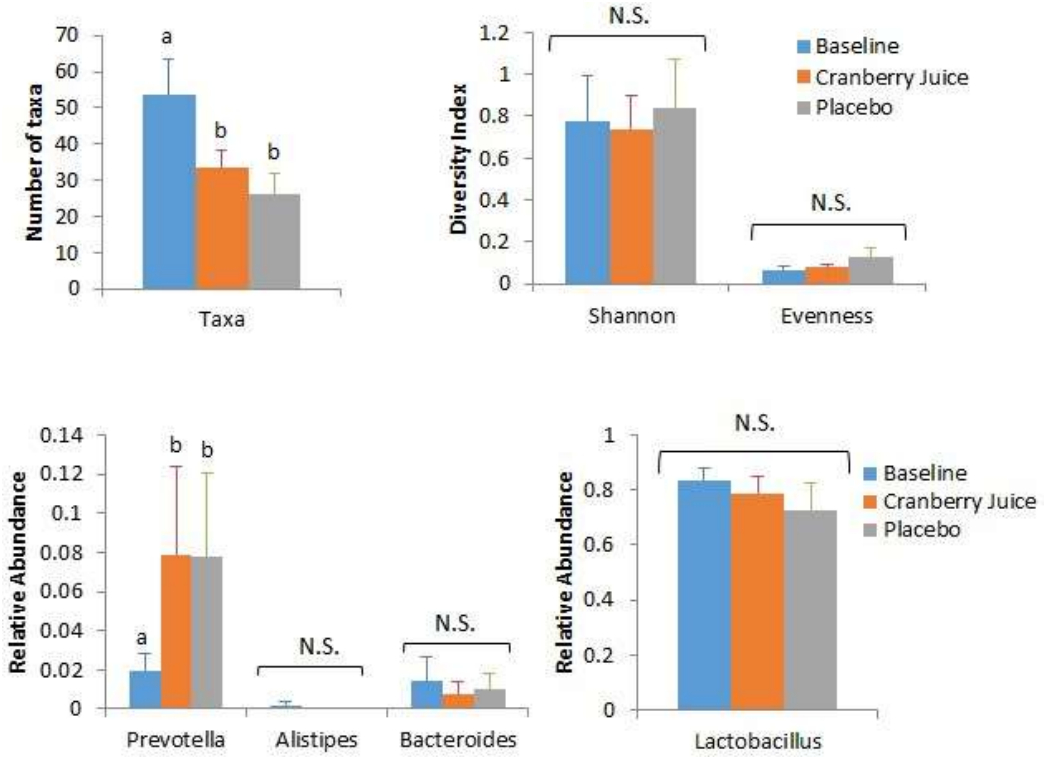


**Figure 3. 4:** Diversity indices and changes in relative abundances in volunteers with *Lactobacillus*-dominated vaginal microbiota. Values are expressed as mean ± standard error (SE) ($n$ = 8). Different letters indicate significant difference ($P < 0.05$), whereas N.S. indicates no significant difference.

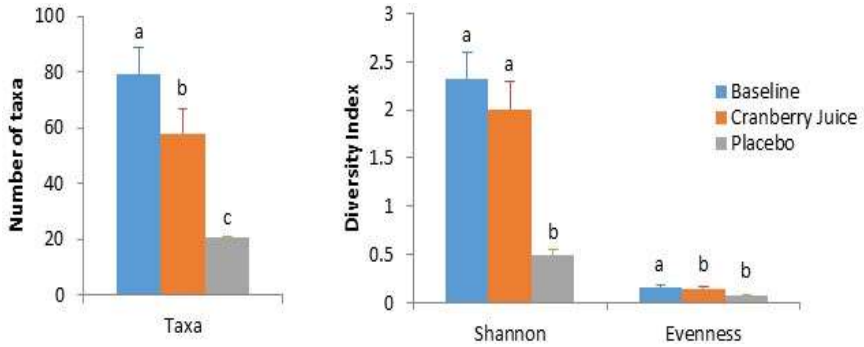


**Figure 3. 5:** Diversity indices changes in subjects with diverse vaginal microbiota at baseline. Values are expressed as mean ± standard error (SE) ($n$ = 6). Different letters indicate significant difference ($P < 0.05$).

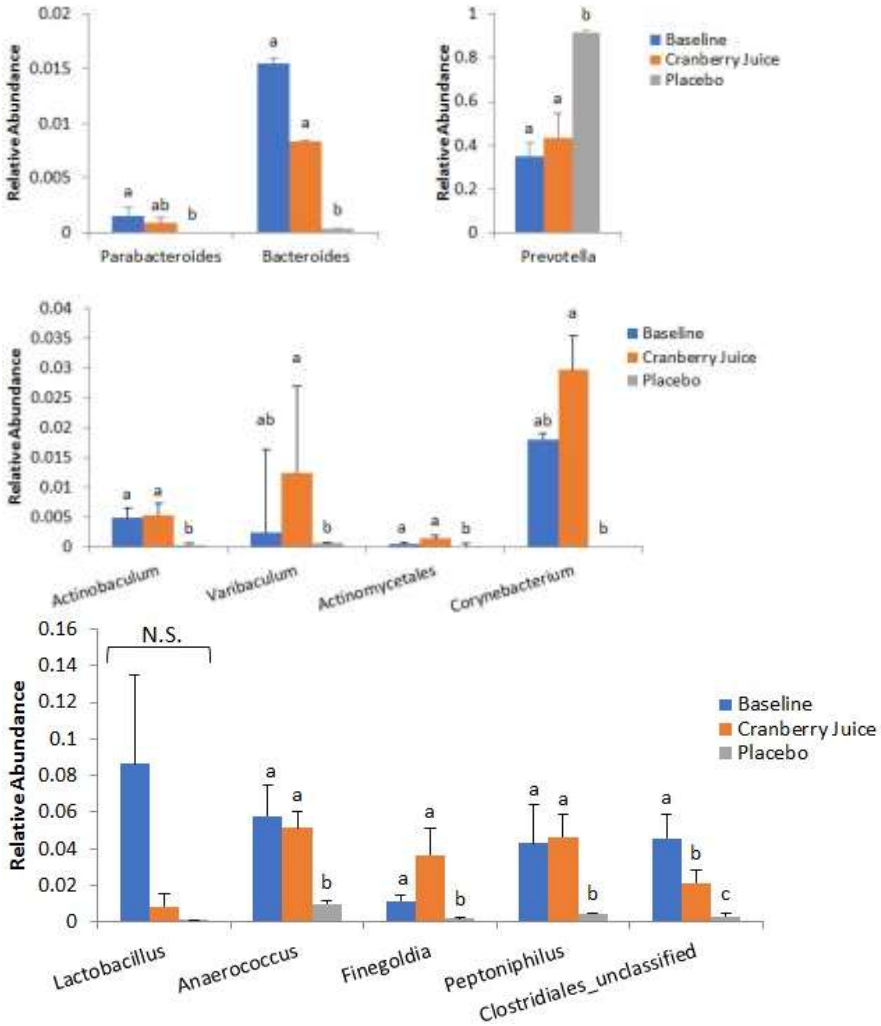


**Figure 3. 6:** Impact of cranberry juice and the placebo on the relative abundance of the major genera in subjects with diverse vaginal microbiota at baseline. Values are expressed as mean ± standard error (SE) ($n$ = 6). Different letters indicate significant difference ($P < 0.05$), whereas N.S. indicates no significant difference.

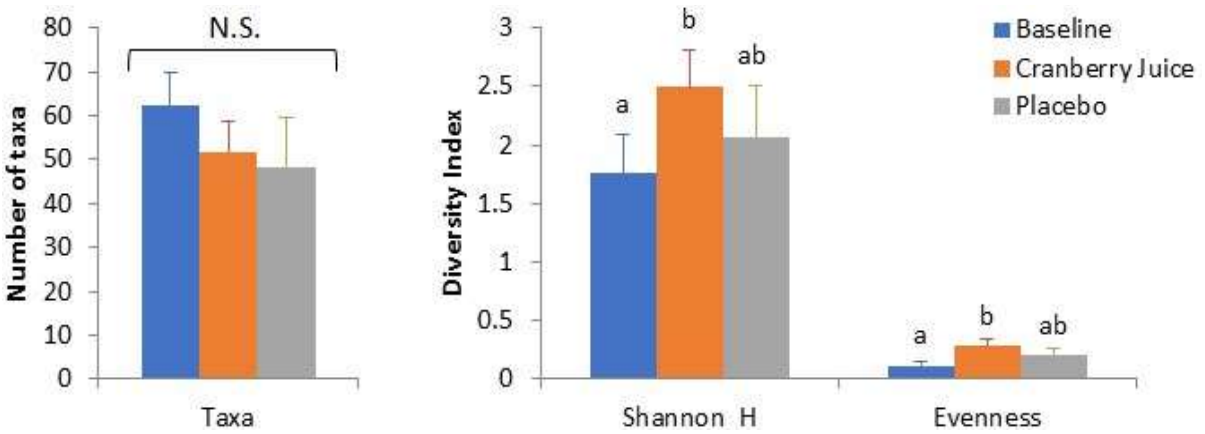


**Figure 3. 7:** Diversity indices changes in subjects with *Streptococcus* dysbiosis. Values are expressed as mean ± standard error (SE) ($n$ = 7). Different letters indicate significant difference ($P$ < 0.05), whereas N.S. indicates no significant difference.

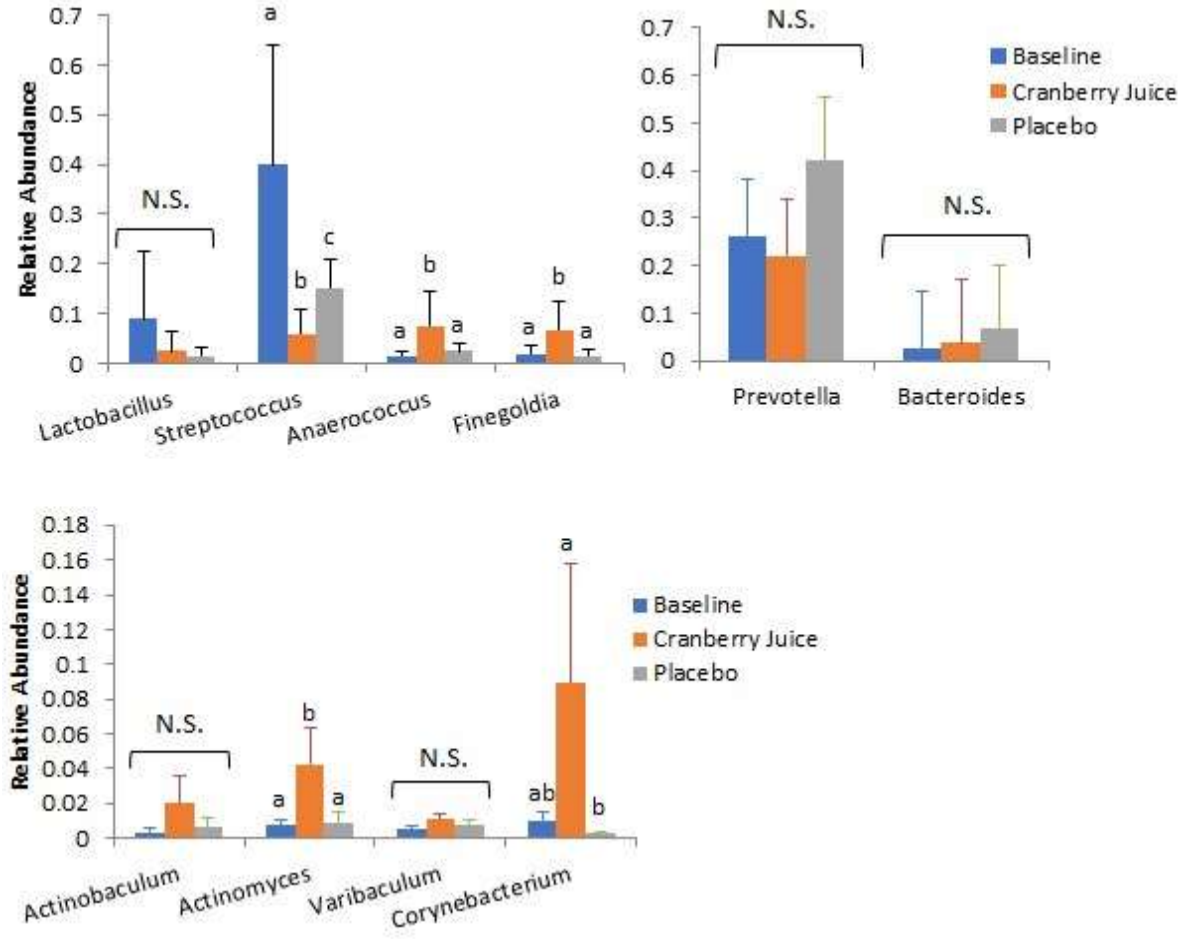


**Figure 3. 8:** Impact of cranberry juice and the placebo on the relative abundance of the major genera in subjects with *Streptococcus* dysbiosis at baseline. Values are expressed as mean ± standard error (SE) ($n$ = 7). Different letters indicate significant difference ($P$ < 0.05), whereas N.S. indicates no significant difference.

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**CHAPTER 4:**

# Botanical Diversity and Amount of Dietary Fruits and Vegetables Distinctly Affect the Human Gut Microbiome


## Abstract

While increasing fruit and vegetable (FV) intake is a near-universal recommendation for improved health outcomes, information on dietary FV the impact amount and diversity on health biomarkers is scarce. FV are a major dietary source of accessible gut microbiota carbohydrates and phytochemicals, however, most studies have focused on single food items or their extracted components, with few holistic studies available. Here, two separate randomized dietary interventions were used to assess the impact of low vs. adequate FV intake and low vs. high botanical diversity on gut microbiota profiles in healthy adults. We hypothesized that increasing FV would result in beneficial modulations to gut microbiota with further increases to benefit those consuming FV from diverse botanical families. The SMART-Diet study was a pilot, randomized, cross-over dietary intervention where male volunteers were randomized to start diets of low FV or adequate FV over 9 days. Stool samples were obtained at day 0, 3, 6 and 9 for each treatment period. In the SMART-Valid study, 21 individuals were provided a low FV lead-in diet for 4 days and then randomly assigned to a high FV diet with either low (11 families) or high botanical diversity (24 families) for an additional 4 days. Stool was collected at baseline and after each diet intervention. Gut microbiota was analyzed using 16S rRNA sequencing performed on an Illumina MiSeq. The Mothur pipeline was used for preliminary data analysis, followed by statistical analyses in PAST. In the SMART-Diet study, the low FV treatment resulted in minimal microbiota alterations, while a significant increase in Bacteroidetes and

decrease in Firmicutes (which peaked at day 6) was observed in the adequate FV treatment group. Intriguingly, the low group experienced a short-term increase in *Bifidobacterium* and *Lactobacillus*, and both treatments incurred significant increases in *Bacteroides* and *Akkermansia* and a decline of *Faecalibacterium*. In the SMART-Valid study, the transition from low to high FV resulted in similar trends as the SMART-Diet study. However, the high botanical treatment resulted in a more diverse gut microbiota, characterized by increased relative abundances of beneficial Firmicutes (Lachnospiraceae, *Faecalibacterium*, and *Clostridium* XIVa). Our results suggest that both the amounts of FV consumed and botanical diversity modulate the gut microbiota. Determining better FV combinations from a gut microbiota perspective, thus, appears as a possible task for future research.

**Keywords:** Fruits, Vegetables, Gut microbiota, Fibers, Polyphenols.

### 4.1 Introduction

A balanced diet is a substantial factor in promoting human health and limiting chronic disease risks (Cena & Calder, 2020; Fischer, Pallazola, Xun, Cainzos-Achirica, & Michos, 2020; Slavin & Lloyd, 2012; World Health, 2019). Fruits and vegetables (FV) are fundamental components of a healthy diet and have been the keystone foods of dietary recommendations for decades (Dietary Guidelines Advisory, 2015; Lampe, 1999; Phe, 2016; Trichopoulou et al., 2003; Vanderlee, McCrory, & Hammond, 2015; Wallace et al., 2019; World Health, 2019). The U.S. Departments of Health and Human Services (DHHS) and Agriculture (USDA) recommended that half of a meal consists of FV (Dietary Guidelines Advisory, 2015). Generally, FV contain a diverse collection of nutrients and biologically active components that provide potential health benefits, including dietary fibers, phytochemicals, and vitamins (Sharoba,

Farrag, & Abd El-Salam, 2013; Slavin & Lloyd, 2012; Yahia, García-Solís, & Celis, 2019). Epidemiologic studies have overwhelmingly confirmed the role of FV in the reduction of systemic inflammation and the prevention of various chronic diseases and metabolic disorders such as obesity, diabetes, high blood pressure, cardiovascular diseases, and cancers (Bertoia et al., 2015; Farvid et al., 2016; Farvid et al., 2019; Gehlich et al., 2020; Gluba-Brzózka, Franczyk, & Rysz, 2017; Jurikova et al., 2017; Lee & Park, 2017; Muraki et al., 2013; Mursu, Virtanen, Tuomainen, Nurmi, & Voutilainen, 2014; Tome-Carneiro & Visioli, 2016; Wang et al., 2014; Yokoyama et al., 2014).

FV are an excellent source of dietary fibers, which are primarily credited with imparting the health benefits associated with adequate consumption (Korcz, Kerényi, & Varga, 2018). Many types of dietary fibers support gastrointestinal and digestive health by modulation of the gut microbiota. The gut microbiota has recently emerged as an important regulator of human health, and fermentable fibers serve as a critical energy source for colonic microorganisms, which subsequently result in the production of bioactive metabolites, such as short chain fatty acids (SCFAs) (Flint, Duncan, & Louis, 2017; Holscher, 2017; Makki, Deehan, Walter, & Bäckhed, 2018). A series of meta-analyses and systematic reviews recently summarized in the Lancet concluded that dietary fiber intake is inversely associated with the risk of developing non-communicable diseases (Reynolds et al., 2019); and the strong dose-responsiveness of risk reduction suggests a causal link between fiber and disease protection. Furthermore, fiber rich FV are also high in other bioactive compounds, such as phytochemicals, which may be partially responsible for the widely acknowledged reduction in disease risks and other benefits to human health.

Phytochemicals are a chemically diverse array of secondary metabolites produced by plants. In nature, they play a role in the plant's growth and defense against competitors, pathogens, predators, or environmental factors (Bartwal, Mall, Lohani, Guru, & Arora, 2013; Rasmann & Agrawal, 2009). Phytochemicals are organized according to their chemical structure; therefore, phytochemicals in the same group typically have similar mechanisms of action in the human body (Produce for Better Health, 2015). They exert various physiologic effects in humans, including acting as anti-inflammatory and anti-oxidant agents (Hussain et al., 2016; Li et al., 2014; Williamson, 2017; H. Zhang & Tsao, 2016). Major categories of health-promoting phytochemicals include phenolic compounds, terpenoids, alkaloids, and other nitrogen-containing compounds and sulphur-containing compounds. While some of these compounds, such as polyphenols, are widely distributed among FV (Abbas et al., 2017; Abuajah, Ogbonna, & Osuji, 2015; Edwards et al., 2017; Williamson, 2017), others classes, such as alkaloids and glucosinolates, are much more restricted in occurrence. Like dietary fiber, phytochemicals can positively alter the gut microbiota composition both through provision of conjugated sugars that can be used as an energy source as well as through anti-microbial activity that can selectively inhibit some microorganisms and reduce competition within mixed microbial populations. These effects on the microbiota, along with direct anti-inflammatory and anti-oxidant activity within the intestines, can promote homeostasis within the gastrointestinal tract (Cardona, Andrés-Lacueva, Tulipani, Tinahones, & Queipo-Ortuño, 2013; Kumar Singh et al., 2019; Lavefve, Howard, & Carbonero, 2020; Ozdal et al., 2016). Furthermore, the metabolism of these compounds within the gut may also increase their bioavailability and bioactivity, suggesting that inter-individual variability in microbial composition and capacity to metabolize phytochemicals can influence their response to exposure to these compounds.

Despite near-universal recommendations regarding the intake of FV to improve health outcomes, the average vegetable intake was found to be below the recommendations of 240 g/day in approximately 88% of the countries (Kalmpourtzidou, Eilander, & Talsma, 2020). In the U.S., only one in 10 adults report adequate intake of FV (Lee-Kwan, Moore, Blanck, Harris, & Galuska, 2017). Not only are Americans falling short in their FV consumption, but the variety and botanical diversity of commonly consumed FV is also low. The most consumed whole fruits by Americans include bananas, apples, and berries, while the most consumed vegetables include white potatoes, lettuce, onions, and tomatoes (Produce for Better Health, 2015). Among, these most popular vegetables are provided similar phytochemicals. Therefore, not only is FV intake generally inadequate, but the lack of phytochemical diversity in consumed foods may be limiting potential health benefits, both in gut health and in overall disease prevention. Here, we aim to identify the impact of adequate versus inadequate FV intake on the gut microbiota as well as identify whether botanical diversity can promote microbial diversity in the gut. In this study, two separate randomized short-term dietary interventions were used to assess the impact of low vs. adequate FV intake, according to the USDA recommendations (Dietary Guidelines Advisory, 2015), and low vs. high botanical diversity on gut microbiome profiles in healthy adults. We hypothesized that increasing FV in meals would result in beneficial modulations to the gut microbiota such as increased diversity and increased relative abundance of beneficial genera, with even more marked benefits in those consuming FV from diverse botanical families.

## 4.2 Materials and Methods

### 4.2.1 Subjects

#### *4.2.1.1 SMART-Diet study*

This study was a pilot, randomized, cross-over dietary intervention and was conducted to investigate the impact of low vs. adequate FV intake on the gut microbiota ecosystem. Eleven (11) males between 18-35 years of age at Colorado State University participated in this study; however, only a full set of samples from six (6) participants were used for gut microbiota analysis, and that is what is used for the sequence data. Recruitment was conducted by word of mouth, email, and flyers. Inclusion and exclusion criteria are detailed in **Table 4. 1**. Study protocol was approved by the Colorado State University Institutional Review Board (CSU IRB), Protocol #13-4278H and all participants consented in writing before participating in the study.

#### *4.2.1.2 SMART-Valid study*

This study was a pilot, parallel-arm dietary intervention which was conducted to assess the impact of low vs. high botanical diversity on gut microbiome profiles. This study was completed by twenty-one (21) healthy volunteers, nine (9) males and twelve (12) females between 18-65 years old with BMI's between 20-29.9. Eligibility was determined using a questionnaire. Inclusion and exclusion criteria are outlined in **Table 4. 1**. All participants provided written consent, and the study protocol was approved by the CSU IRB, Protocol #17-7214H.

### 4.2.2 Study Design and sample collection

#### *4.2.2.1 SMART-Diet study*

Participants were randomized into adequate (five cups of FV daily) and low (two cups of FV daily) starting treatments at the beginning of the study. Starting diet treatments spanned nine days followed by a twelve-day wash-out period and a second nine-day treatment intervention **(Figure 4. 1)**. Biological samples (blood and stool) and anthropometric measures were recorded during clinical visits. All samples were stored at -80°C prior to processing. Dietary intake information was collected using three-day food logs, which included two weekdays and one weekend day. Diet data was collected prior to the start of both diet treatments (baseline) and during each treatment period (compliance). FV were provided, but remaining foods were self-selected by participants. Participants were given menu suggestions, developed by registered dieticians at Colorado State University's Kendall Reagan Nutrition Center (KRNC), for the remainder of their intake. A sample menu for each treatment is provided in **Appendix 1**. Participants were asked to consume all of the provided foods and the remainder of their diet was *ad libitum*. All provided FV were prepared in kitchen facilities located in the KRNC. Participants were provided with new portions of FV every three days during their clinical visits. To measure compliance, diet interviews were conducted at each clinical visit and food containers and any uneaten food portions (including peels, pits, and skins) were returned.

#### *4.2.2.2 SMART-Valid study*

Participants were assigned to the two intervention groups based upon schedule availability. Those who were available during the earlier intervention schedule followed the low botanical diversity protocol and those who requested the later intervention schedule followed the

high botanical diversity protocol. Baseline diet was assessed using two 24-hour recalls using the ASA24 tool (https://epi.grants.cancer.gov/asa24/) the week before the start of the intervention period. Prior to starting the diet intervention, participants collected baseline stool samples, and returned them to the clinic the following day. Participants were instructed to store all stool samples in the refrigerator until they were delivered to the lab. Baseline anthropometric measures were collected as well as a fasting blood sample.

After completing the clinic visit, all participants consumed a pre-specified lead-in low FV diet for a period of four days. Following this, participants provided a second stool sample on the morning of day five. They then began one of two treatment groups: high FV intake with low biodiversity, or high FV intake with high biodiversity. They followed their intervention diets for four days. On day nine of the intervention, participants provided a third stool sample **(Figure 4. 2)**. All samples were stored at -80°C prior to processing.

The treatment meals were specified by a pre-designed menu and provided varying amounts of FV depending on the respective treatment groups **(Appendix 2, 3)**. The low FV diets were comprised of two to three cups of FV per day coming from six different botanical families. Both of the high FV diets contained five to nine cups of FV daily, with the high biodiversity diet containing foods from 24 different botanical families and the low diversity diet containing foods from 11 different botanical families. All foods were provided through CSU's Housing and Dining Services. Compliance with prescribed menus was determined through pre- and post-meal photos of all meals and snacks.

### 4.2.3 DNA extraction

Bacterial DNA extraction from stool samples was conducted using commercial QIAamp DNA stool mini kits (QIAGEN, Valencia, CA) following the manufacturer's protocol with the addition of a cell lysis step to rapidly and completely disrupt cells in the samples to release DNA. The cell lysis was performed by adding 0.1 g of 0.1 mm diameter and 0.1 g of 0.5 mm diameter zirconia-silica beads (BioSpec Products, Bartlesville, OK, United States) to 2 ml tubes. Subsequently, Stool content was transferred to the 2 ml tubes containing zirconia-silica beads, and 1 ml of InhibitEX Buffer (from the QIAamp® Fast DNA Stool Mini Kit) was added to the tubes. The tubes were then subjected to a bead-beater for 60 seconds at maximum speed using a FastPrep®-24 bead-beater (MP Biomedicals, Santa Ana, CA, United States).

The NanoDropTM 1000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, United States) was used to determine the DNA concentration. Extracted DNA was then visualized following electrophoresis in a 2% agarose gel and 1X TAE (Tris-acetate ethylenediaminetetraacetic acid) buffer (AMRESCO®, Cleveland, OH, United States).

### 4.2.4 Universal polymerase chain reaction

16S ribosomal RNA gene was amplified by the polymerase chain reaction (PCR) (95 °C for 3 min, followed by 35 cycles at 98 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min and a final extension at 72 °C for 5 min) using primers (8F 5' - AGAGTTTGATCCTGGCTCAG - 3' and 1541R 5' - AAGGAGGTGATCCAGCCGCA - 3'). PCR reactions were performed in 25 µL mixture containing 3 µL of DNA template; 1 µL of each universal primer (8F and 1541R); 12.5 µL of GoTaq® Green Master Mix (Promega™ Corporation, Wisconsin, USA); and 7.5 µL of Nuclease-Free Water. Agarose gel electrophoresis was then accomplished to confirm the success

of the PCR by using 1 μL of SYBR Safe DNA Gel Stain (Thermo Fisher Scientific, USA) fluorescent dye.

### 4.2.5 Amplicon Library Preparation and Sequencing

For 16S rRNA gene sequencing (ITag) analyses, universal primers tagged with Illumina adapters and barcodes were used for PCR from the DNA extracts (Kozich, Westcott, Baxter, Highlander, & Schloss, 2013). Briefly, the PCR dual-indexed strategy was performed in 25 μL reaction components with 3 μL of the DNA template; 2.5 μL of the AccuPrime™ PCR buffer II; 0.5 μL of each index primers; 0.1 μL of AccuPrime™ Taq DNA Polymerase (Invitrogen, USA); and 18.4 μL of Nuclease-Free Water following the manufacture's protocol. The PCR consisted of initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 30 sec, primer annealing at 55 °C for 30 sec, and an extension at 72 °C for 1 min, with a final extension step at 72 °C for 5 min. Correct size amplicons were subjected to purification and normalization using Invitrogen SequalPrep kits following the manufacturer's protocol. Amplicons concentrations were measured by Agilent bioanalyzer after pooling as two different libraries.

For accurate concentration, amplicon libraries were quantified by qPCR using the same primers and PerfeCta NGS library quantification kits (Quanta Biosciences, USA) following manufacturer instructions. Libraries were then denatured and diluted to appropriate concentrations and sequenced on an Illumina MiSeq.

Resulting sequencing files were analyzed using the Mothur v.1.41.1 pipeline (Schloss et al., 2009). Operational taxonomic units (OTU) were determined and classified against reference databases (Ribosomal Database Project (RDP), SILVA,)). Basic and multivariate statistical

analyses were performed in PAST (Al Othaim, Marasini, & Carbonero, 2020; Hammer, Harper, & Ryan, 2001).

### 4.2.6 Short-chain fatty acids analyses

Frozen stool samples from the SMART-Valid study were extracted for SCFAs by mixing 0.75 g of frozen feces with acidified water (pH 2.5) and sonicating for 10 min. as described previously (Sheflin et al., 2015; Weir et al., 2013). Briefly, samples were analyzed on a 6890 series Gas Chromatograph with a flame ionization detector (GC_FID; Agilent). Samples were injected at a 10:1 split ratio; the inlet was held at 22°C and the transfer line was held at 230°C. Separation of SCFAs was achieved on a 30m TG-WAX-A column (0.25-mm ID, 0.25-µm film thickness; Thermo Scientific) by using a temperature program of 100°C for 1 min, increased by 8°C/min to 180°C, held at 180°C for 1 min, increased by 200°C at 20°C/min, and held at 200°C for 5 min. Helium carrier flow was held at 1.2 mL/min. Commercial standards of acetic acid, propionic acid and butyric acid, (Sigma, St. Louis, MO, USA) were used to confirm compound identities.

### 4.2.7 Statistical analysis

Statistical analyses including Wilcoxon signed-rank test and Mann-Whitney U test (depending on the availability of fully paired sample sets) were performed to detect significant differences between groups (by convention, differences were considered significant when $P < 0.05$). Non-Metric Multidimensional scaling (NMDS) based on count distance metrics (Bray-Curtis similarity index; ANOSIM: $P < 0.05$) were considered to be significant similarities between groups using Past4 software.

## 4.3 Results and Discussion

### 4.3.1 SMART-Diet study: Longitudinal assessment of the impact of low and adequate FV intake

Using NMDS visualization of Bray-Curtis distances, short-term changes to FV intake did not significantly impact the general gut microbiota profiles **(Figure 4. 3)**. These results are in line with other studies which revealed no significant impact from a vegetarian diet on the microbial community composition in healthy and obese individuals (Kim, Hwang, Park, & Bae, 2013; Losasso et al., 2018). Likewise, a study showed that short-term dietary changes could alter the gut in as little as 24 hours, but that the diet high in animal products had a much greater immediate impact on the microbiota than the plant-based diet (David et al., 2014). It is clear from the NMDS that the individuality of each subject had a stronger impact than the diet assignment; specifically, half of the subjects were responders to the diet change, while the other half were not responders **(Figure 4. 4)**. This observation is also logical considering the numerous reports of metabotypes with differing response to dietary phytochemicals (Cortés-Martín et al., 2020; Mayta-Apaza et al., 2018) as well as differential responses to fiber and other plant-derived oligosaccharides (Davis, Martínez, Walter, Goin, & Hutkins, 2011; G. D. Wu et al., 2011).

Both diet interventions significantly increased the gut microbiota diversity (Shannon index), but the increase was gradual with the low FV diet **(Figure 4. 5)**. Improving the gut microbiota diversity is generally considered a healthy outcome, whereas dysbiosis, loss of gut microbiota, was associated with many diseases and metabolic disorders (Kriss, Hazleton, Nusbacher, Martin, & Lozupone, 2018). A study reported that the fiber intake increased the gut

microbiota diversity and richness in overweight pregnant women (Röytiö, Mokkala, Vahlberg, & Laitinen, 2017).

When comparing phyla-level changes across the different FV intake periods, both treatments showed similar trends. The low FV diet had more heterogeneity, probably because of responder/non-responder effects. Generally, both diets showed reductions in the Firmicutes/Bacteroidetes ratio, although the maximum change from baseline occurred on different days (peak at day 6 for adequate; peaks at day 3 and 9 for low). During the low FV diet, the Firmicutes relative abundance was significantly decreased from baseline to day 9, although the Bacteroidetes were not significantly changed. In comparison, from baseline to day 9 on the adequate FV diet, there was a decrease in Firmicutes which became apparent by day 6, and an increase in Bacteroidetes, which appeared by day 3 of the diet intervention **(Figure 4. 6).** Alteration of the Firmicutes/Bacteroidetes ratio, associated with adequate FV intake, may indicate a beneficial change to the gut microbiota. Many animal and human studies have demonstrated that the Firmicutes/Bacteroidetes ratio is increased in the microbiota of obese individuals compared to lean individuals, making it a potential indicator/biomarker for obesity (Barlow, Yu, & Mathur, 2015; Kim et al., 2013; Koliada et al., 2017; Xu, Li, Zhang, & Zhang, 2012; Zou et al., 2020). However, other studies have not observed this correlation with obesity (Hu et al., 2015; Magne et al., 2020; Tims et al., 2013; Walters, Xu, & Knight, 2014). A recent review suggests that differences in sample processing, participant demographics, and lack of control in other variables that influence the microbiota may account for some of these discrepancies in the literature, but that it is currently difficult to interpret the Firmicutes/Bacteroidetes ratio in light of human health and obesity status (Magne et al., 2020).

Genus-level microbial dynamics were mostly mirrored in both diet interventions. Among Bacteroidetes, the *Bacteroides, Prevotella, Alistipes,* and *Parabacteroides* genera were increased by both diet interventions compared to baseline, whereas *Faecalibacterium, Blautia, Dorea,* and Lachnospiraceae_unclarified, which belong to Firmicutes phyla, were decreased **(Figure 4. 7, 4. 8)**. Both adequate and low FV diets induced the relative abundance of butyrate producing bacteria *Oscillibacter, Clostridium*_IV, and *Clostridium*_XIVa (Gophna, Konikoff, & Nielsen, 2017; Moens & De Vuyst, 2017). A significant increase in *Akkermansia* relative abundance with a slight decrease in *Bifidobacterium* relative abundance was observed after the adequate FV diet **(Figure 4. 9, 4. 10)**. The gut commensal *Akkermansia muciniphila* is a potential probiotic bacterium that is associated with systemic effects on host metabolism and immune response (Ansaldo et al., 2019; T. Zhang, Li, Cheng, Buch, & Zhang, 2019). It promises to be a therapeutic target in microbiota-related diseases, such as colitis, metabolic syndrome, immune diseases and cancer (T. Zhang et al., 2019).

### 4.3.2 SMART-Valid: Impact of botanical diversity of a high FV diet

In line with SMART-Diet study, we observed that the strong gut microbiota individuality prevented the detection of significantly different clusters by NMDS and ANOSIM **(Figure 4. 11)**.

Both diets increased taxonomic diversity, but only significantly in the case of the high botanical diversity **(Figure 4. 12)**. It can be hypothesized that this is a reflection of the diversity of glycans and phytochemicals leading to the stimulation of rare taxa with very specific metabolic abilities. The gut microbiota diversity (Shannon index) increased slightly after both

botanical diversity diets but was more marked with high botanical diversity **(Figure 4. 12)**. This observation was not significant possibly because of the short dietary intervention period.

Regardless of botanical diversity, similar trends were observed at the phylum level. The Firmicutes/Bacteroidetes ratio was induced by increasing the relative abundance of Firmicutes and decreasing the relative abundance of Bacteroidetes. The Proteobacteria level was reduced in both intervention but was only significant in the low botanical diversity diet. It is commonly considered that higher abundance of Proteobacteria is a microbial signature of disease (Rizzatti, Lopetuso, Gibiino, Binda, & Gasbarrini, 2017). Similar response of Actinobacteria was observed in both groups. The low FV diet increased the abundance of Actinobacteria; however, the trends dropped with the high FV diet **(Figure 4. 13)**.

Surprisingly, the low botanical diversity diet led to a decrease of the relative abundance of *Prevotella* and *Butyricimonas*, whereas the relative abundance of *Alistipes* was increased **(Figure 4. 14)**. It has been reported that *Butyricimonas*, a butyrate producing bacteria, plays a potential role in maintaining the gut function and enhancing the intestinal barrier integrity (Tomsett et al., 2020; E. Wu, Jingzhu, Lingpeng, & Yaqin, 2020). The reduction of the abundance of *Butyricimonas* was found to be linked with the incidence of irritable bowel syndrome (IBS) (E. Wu et al., 2020; Yang et al., 2017). It has been fund that the higher-fiber diet increased the relative abundance of *Butyricimonas* and *Prevotella* (de Moraes et al., 2017; Tomsett et al., 2020).

The low botanical diversity diet, however, led to a significant increase in the relative abundance of the butyrate producers *Faecalibacterium* and Lachnospiraceae_unclarified as well as a suggestive increase in the *Clostridium*_XIVa level. The results also showed a significant

decrease in the relative abundance of some other genera belonging to Firmicutes phyla including *Blautia, Ruminococcus, Dorea,* and *Clostridium*_XI with a suggestive decrease in the *Clostridium*_IV abundance. Similar trends were observed for the high botanical diversity diet but they were not significant **(Figure 4. 15)**. Regardless of the botanical diversity, high amounts of FV interestingly limited the increase of commensal bacteria *Bifidobacterium* and *Akkermansia* **(Figure 4. 16)**. The possible explanation is that these genera were outcompeted with higher abundance of other bacteria in the presence of higher amounts of FV in the diets. The low botanical diversity diet significantly decreased the relative abundance of the family Enterobacteriaceae **(Figure 4. 16)**. The decrease of Enterobacteriaceae abundance was not significant with the high botanical diversity diet, but the abundance was very low in the baseline. Enterobacteriaceae family includes opportunistic pathogens that are known to cause morbidity and mortality among humans (Sassone-Corsi et al., 2016). It was reported that the relative abundance of Enterobacteriaceae was significantly low in vegan individuals (Zimmer et al., 2012).

A general limitation of DNA-based microbiota analysis is that any trends observed do not necessarily reflect the fermentation metabolic rates (Fricker, Podlesny, & Fricke, 2019; Wurm et al., 2018). To better assess them, we measured the SCFAs in stool samples from subjects of this study **(Figure 4. 17)**. Remarkably, while only slight microbiota changes were observed, SCFAs levels were strikingly different between the low and high botanical diversity group. SCFAs intriguingly trended slightly down in response to low botanical diversity. The high botanical diversity resulted in significantly higher SCFA levels. It can be hypothesized that effective fermentative metabolism improvements require a higher botanical diversity in concert with a

higher amount of dietary FV. The composition of diet has rarely been the focus in such studies, and our data demonstrate it should be.

On the other hand, acetate, propionate and particularly butyrate were each significantly increased by the high botanical diversity diet. This outcome clearly indicates that botanical diversity is likely to be beneficial in stimulating the gut microbiota and the fermentative metabolism. All SCFAs have been associated with beneficial health properties (Chambers, Preston, Frost, & Morrison, 2018; De Filippis et al., 2016; Gill, Van Zelm, Muir, & Gibson, 2018). Models derived from different animal models can explain this observation, with cross-feeding and intra-genus diversity potentially at play (Ríos-Covián et al., 2016; Solden et al., 2018).

### 4.4 Conclusion

General trends were similar in terms of diversity indices which indicate the potential impact of FV consumption in enhancing the gut microbiota ecosystem for the participants regardless of the amount. Intriguingly, at phylum level, changes were almost opposite between the two studies as the SMART-Diet study showed a reduction of the Firmicutes/Bacteroidetes ratio whereas the SMART-Valid study resulted in an increase in the Firmicutes/Bacteroidetes ratio. This might be due to differences in the type and the amount of meals provided to the participants in each study.

**Table 4. 1:** Inclusion/Exclusion criteria for the SMART-Diet and SMART-Valid Studies.

| | SMART-Diet | SMART-Valid |
|---|---|---|
| **Inclusion Criteria** | • Male<br>• 18-40 years old<br>• BMI between 20-29.9<br>• Non-smoker | • 18-65 years old<br>• BMI between 20-29.9<br>• Non-smoker |
| **Exclusion Criteria** | • Female<br>• Outside of the age and BMI requirements<br>• Smokers<br>• Dietary restrictions and/or food allergies<br>• Metabolic or intestinal disease diagnosis<br>• Use of antibiotics, probiotics, dietary supplements and/or prescription drugs in previous 2 months<br>• Inability to abstain from recreational drugs and limit alcohol to 1 beverage/day. | • Outside of the age and BMI requirements<br>• Smokers<br>• Strong food aversions<br>• Dietary restrictions and/or food allergies<br>• Metabolic or intestinal disease diagnosis<br>• Use of antibiotics, probiotics, dietary supplements and/or prescription drugs in previous 2 months<br>• Inability to abstain from recreational drugs and limit alcohol, coffee, and tea to 1 beverage total/day. |

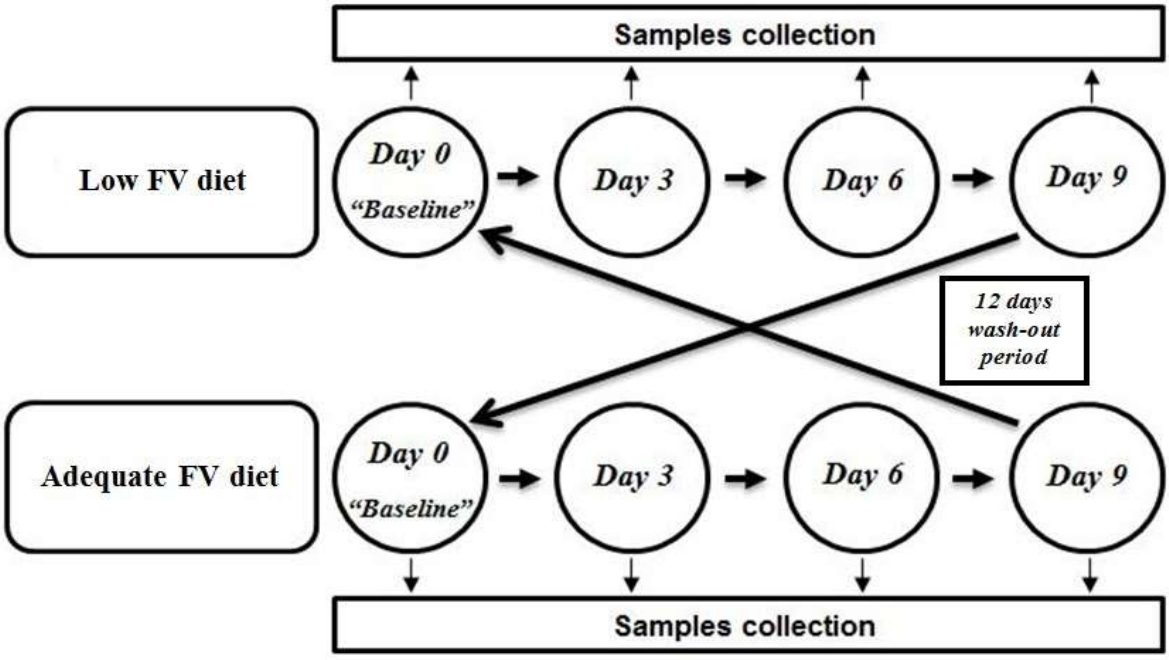



**Figure 4. 1:** SMART-Diet study design and intervention.

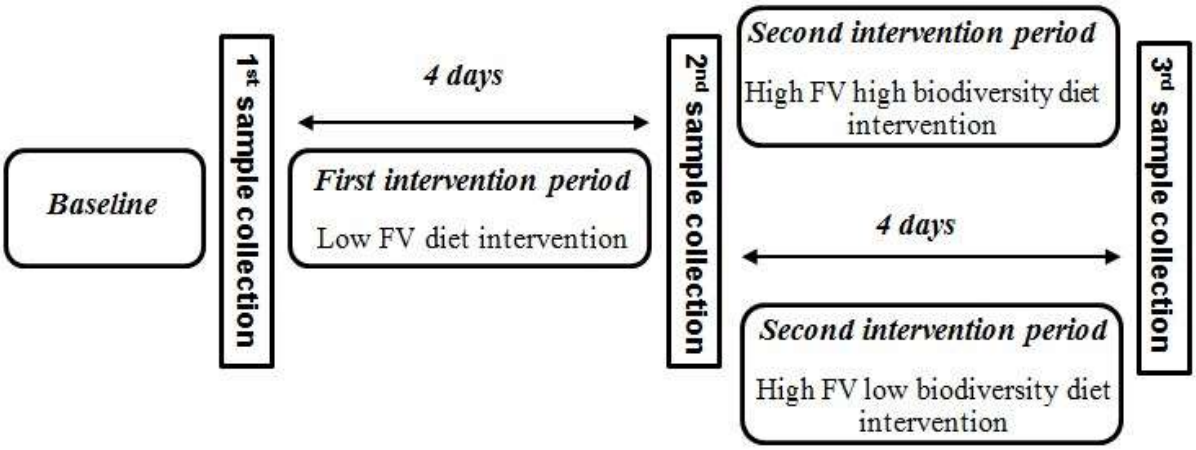



**Figure 4. 2:** SMART-Valid study design and intervention.

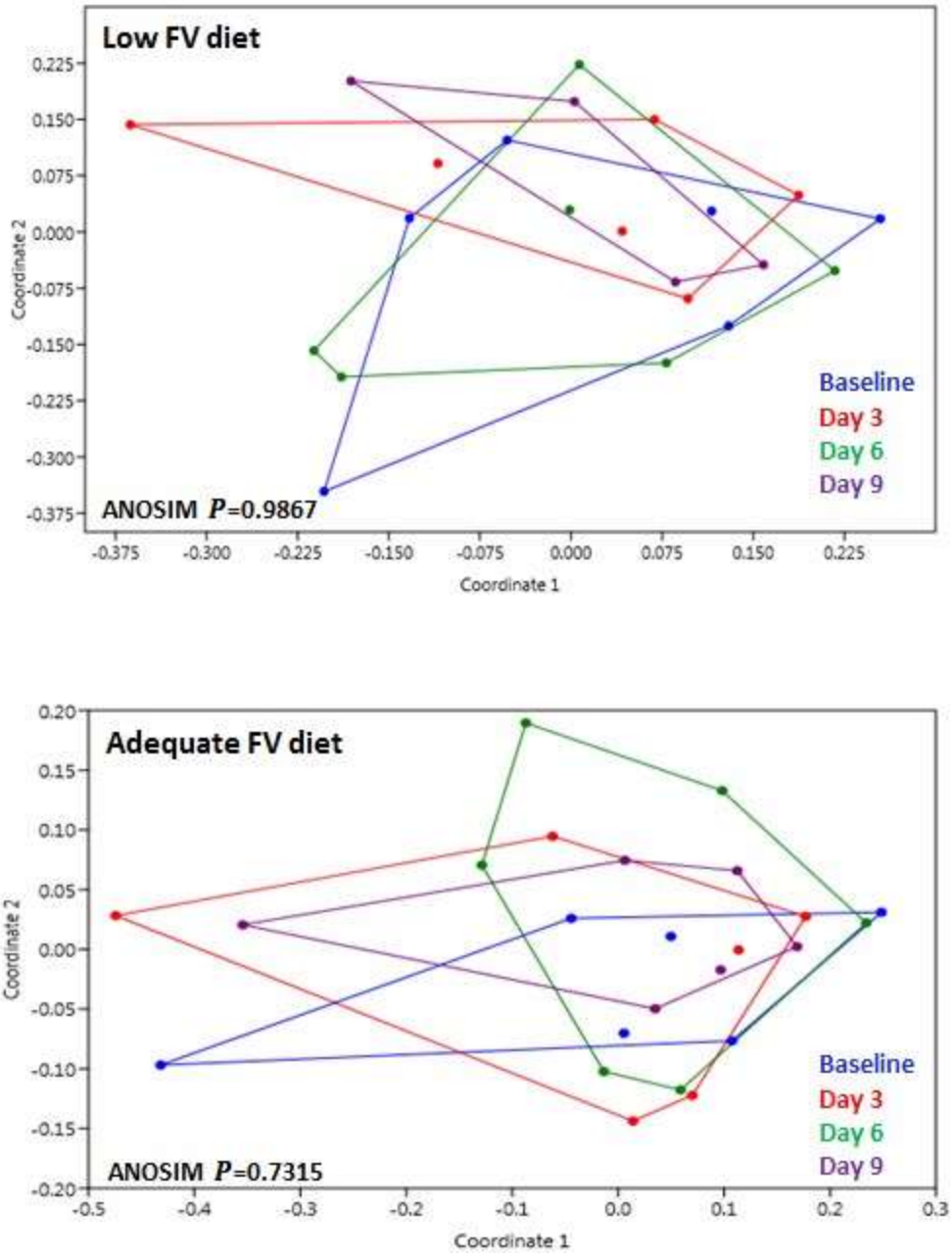



**Figure 4. 3**: Impact on general microbiota profiles of the low and adequate FV treatment interventions at day 3, 6, and 9 compared to subjects' baseline. Gut microbiota data are shown on non-metric multidirectional scaling (NMDS) (Bray-Curtis similarity index; ANOSIM: ($P <$ 0.05)).

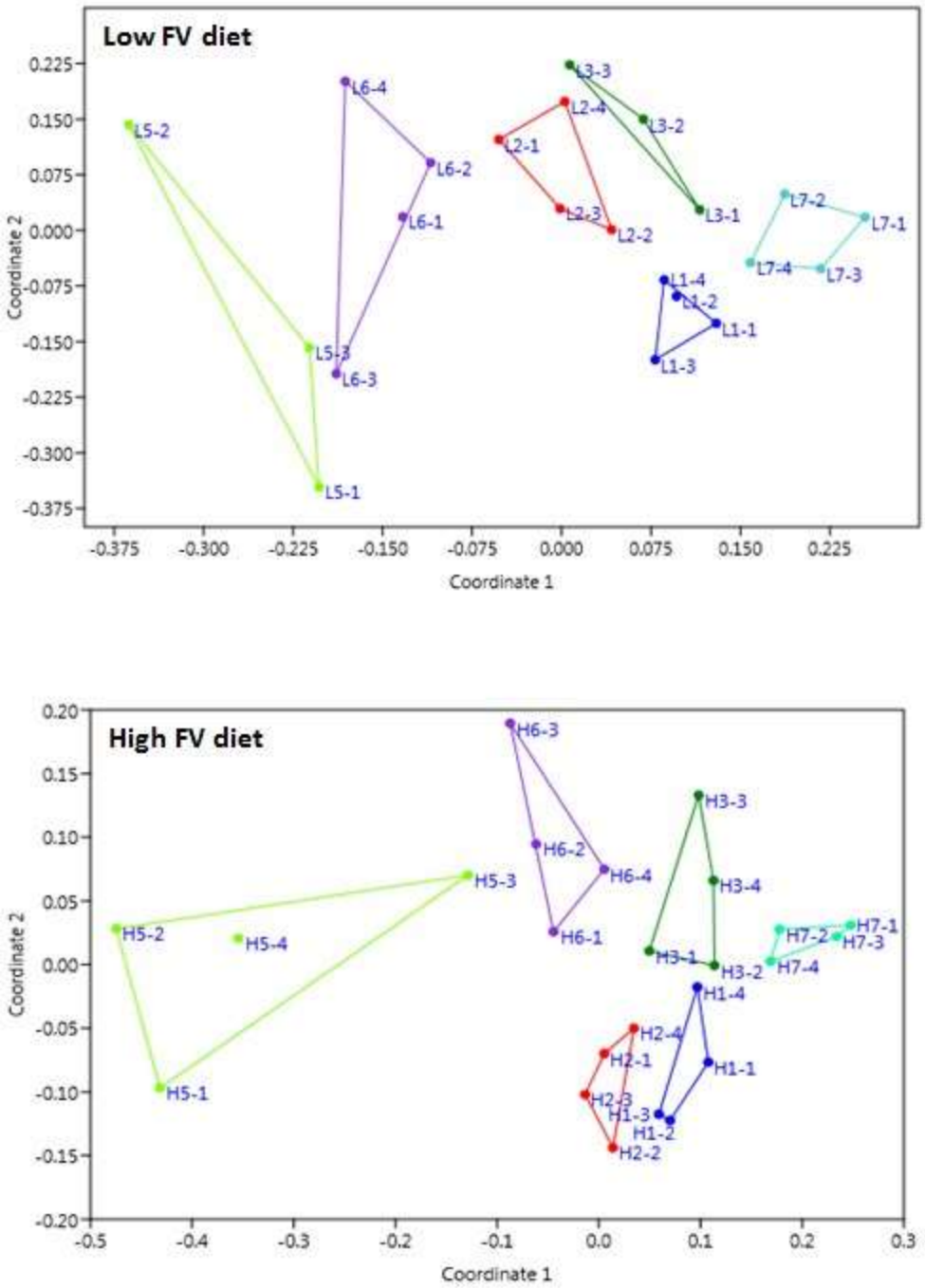



**Figure 4. 4:** Impact on individuals' gut microbiota profiles (NMDS) of the low and high FV botanical biodiversity diet interventions. Gut microbiota data are shown on non-metric multidirectional scaling (NMDS) (Bray-Curtis similarity index; ANOSIM: ($P < 0.05$)).

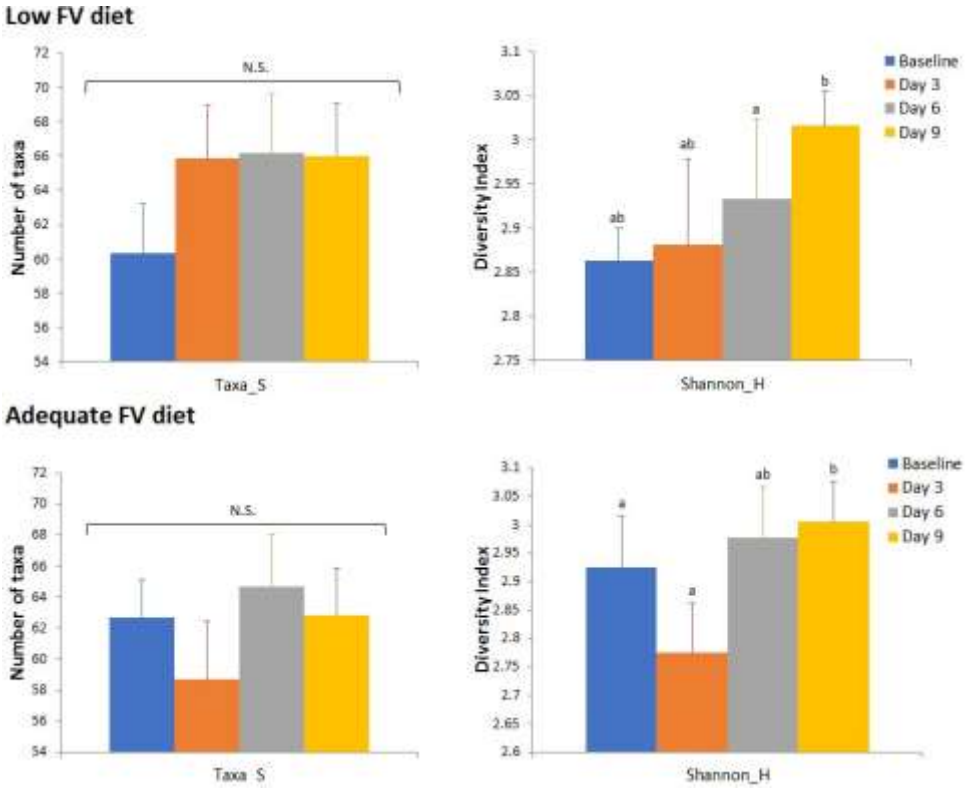



**Figure 4. 5:** Impact on diversity indices (Number of taxa, Shannon index) of the low and adequate FV treatment interventions at day 3, 6, and 9 compared to subjects' baseline. Values are expressed as mean ± standard error (*SE*). Different letters indicate significant difference ($p < 0.05$), whereas N.S. indicates no significant difference.

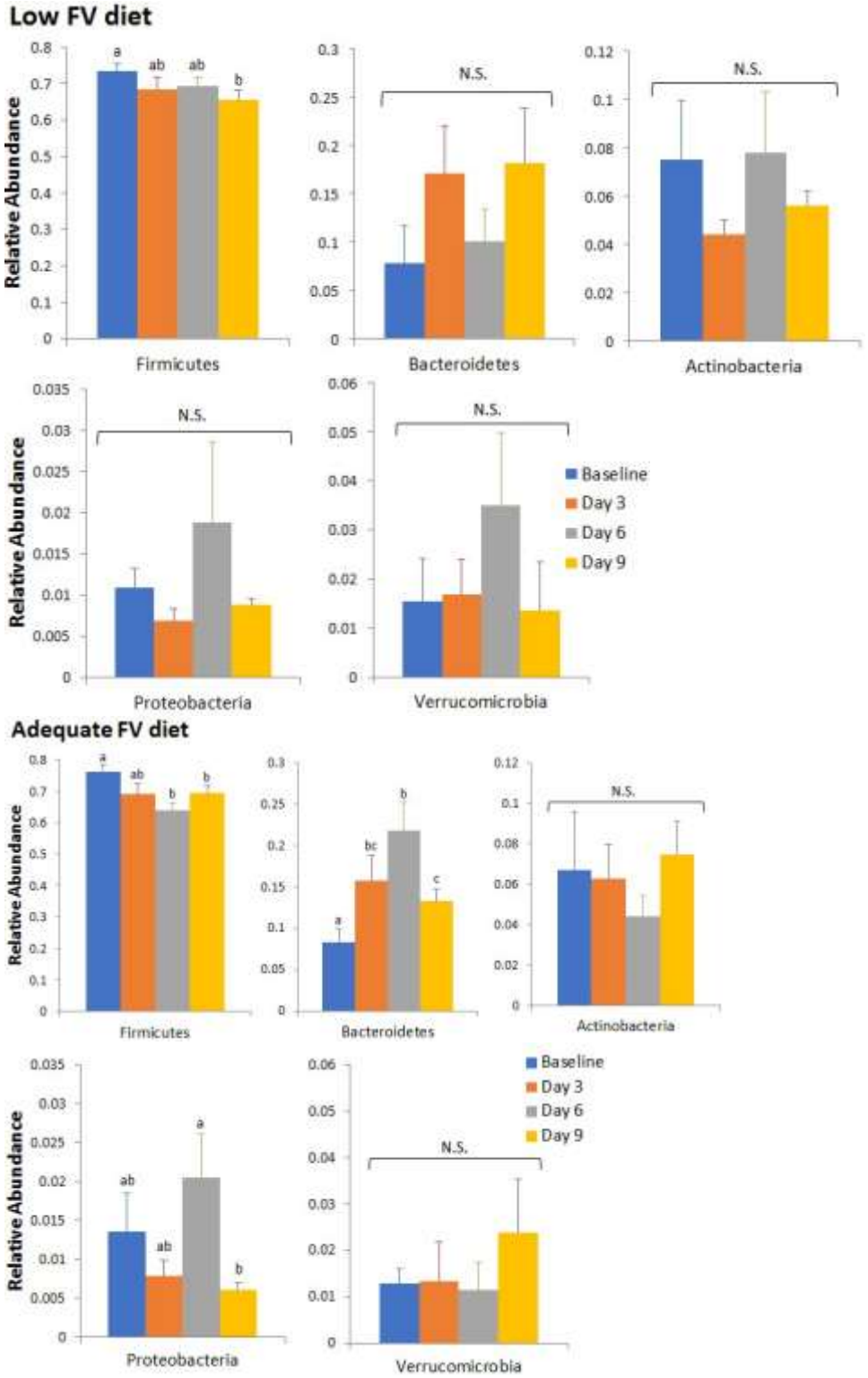



**Figure 4. 6:** Impact of low FV and adequate FV diets at the phylum level for each time point. Values are expressed as mean ± standard error (SE). Different letters indicate significant difference ($p < 0.05$), whereas N.S. indicates no significant difference.

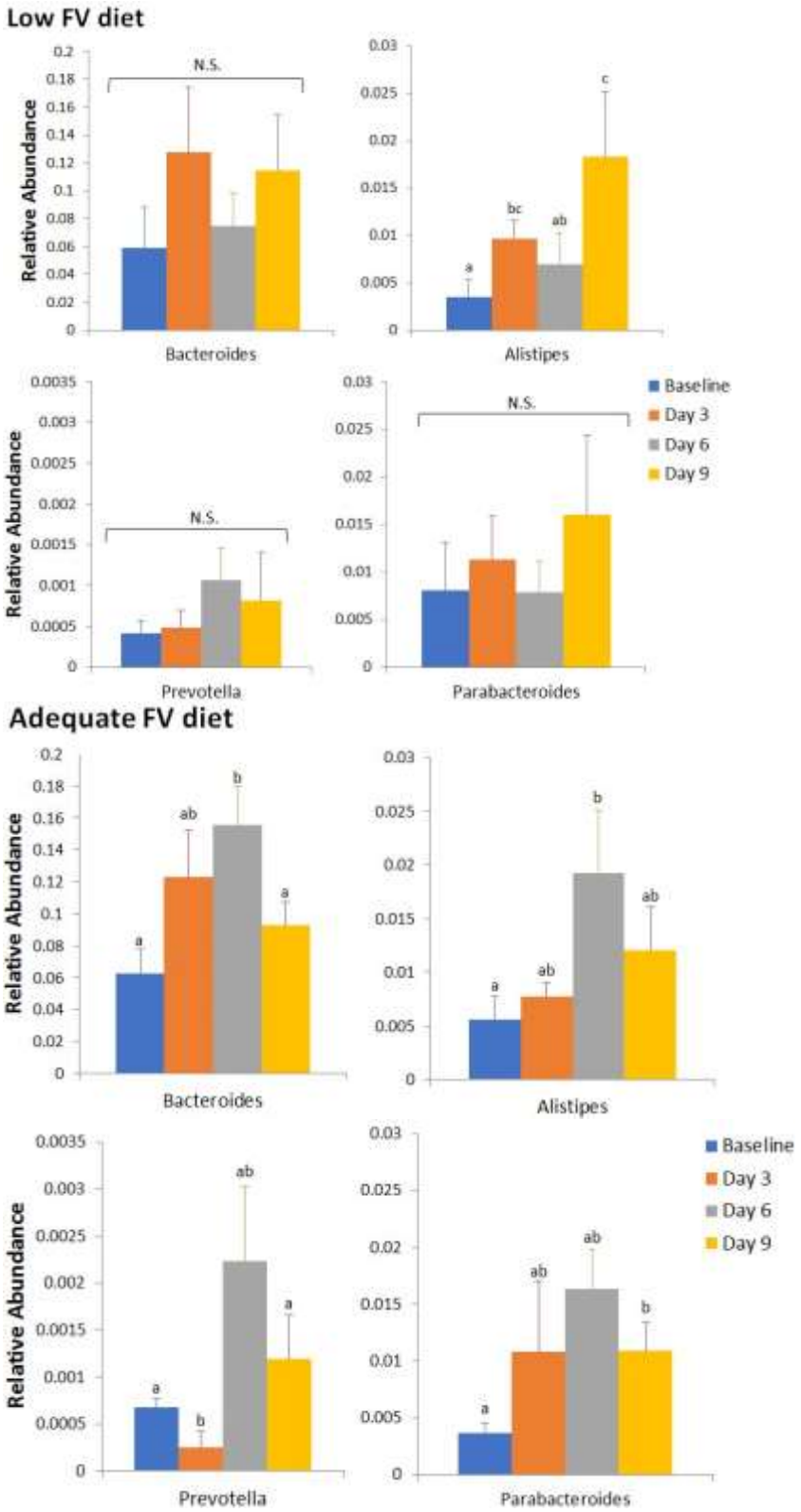



**Figure 4. 7:** Impact of low FV and adequate FV diets on the composition of genera that belong to the Bacteroidetes phyla. Values are expressed as mean ± standard error (SE). Different letters indicate significant difference ($p < 0.05$), whereas N.S. indicates no significant difference.

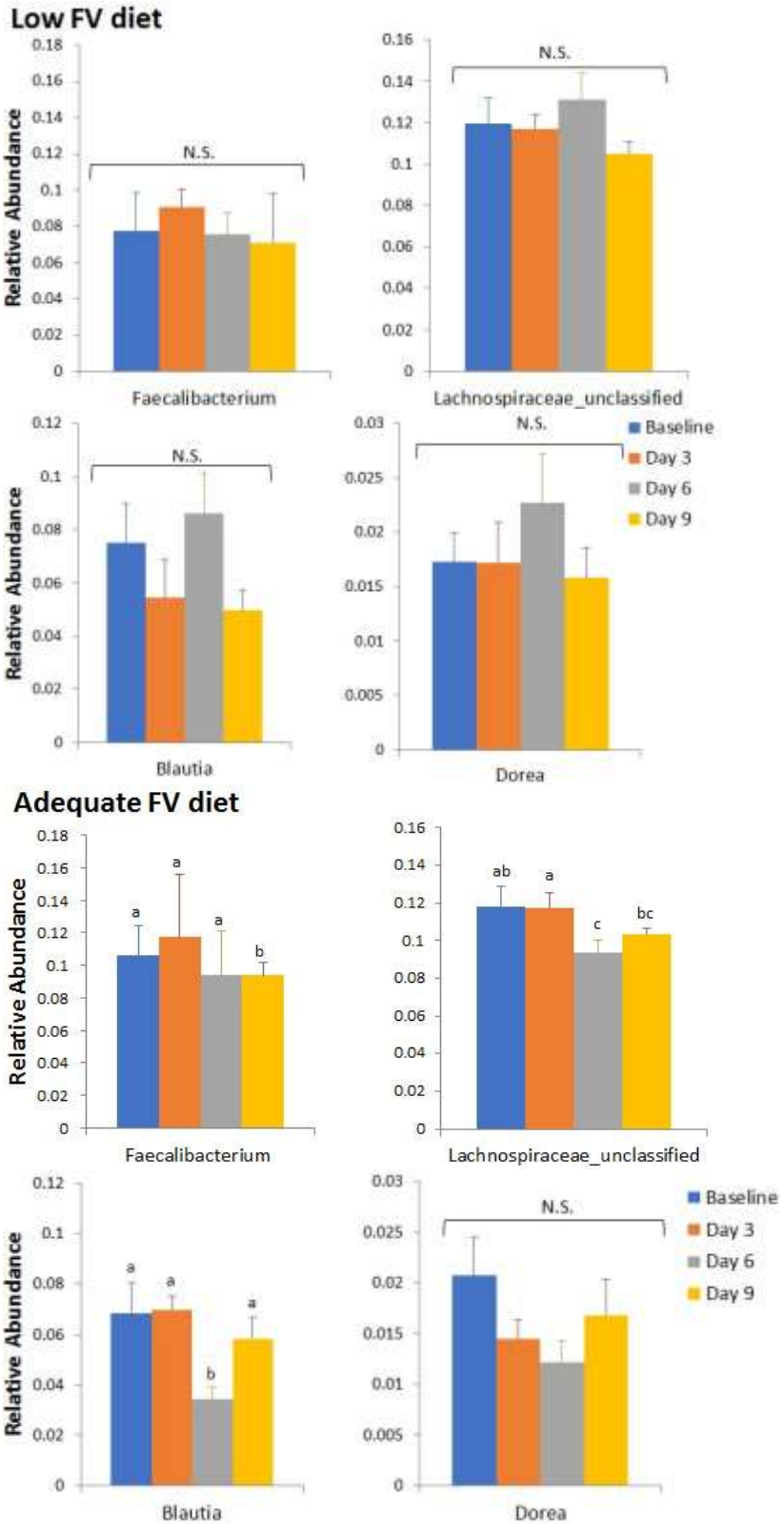



**Figure 4. 8:** Impact of low FV and adequate FV diets on the composition of genera that belong to the Firmicutes phyla. Values are expressed as mean ± standard error (SE). Different letters indicate significant difference ($p < 0.05$), whereas N.S. indicates no significant difference.

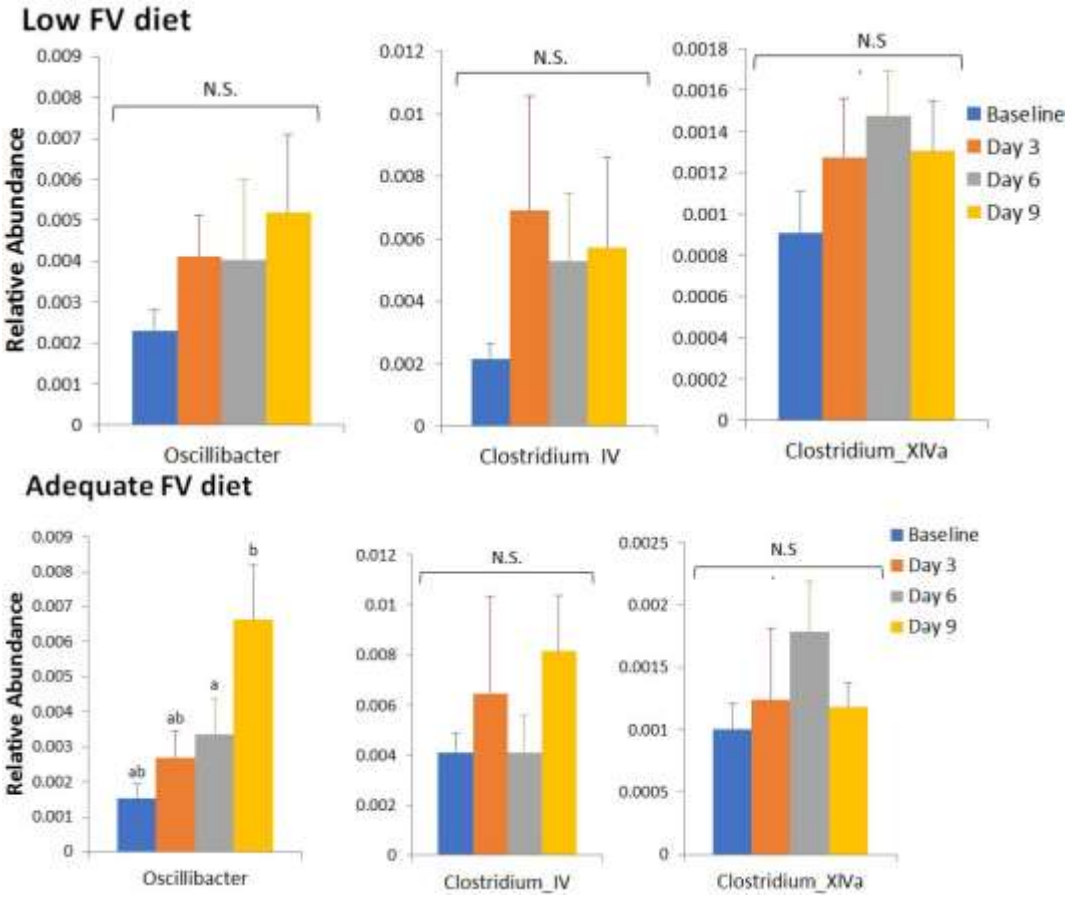



**Figure 4. 9:** Impact of low FV and adequate FV diets on the composition of genera that belong to the Firmicutes phyla. Values are expressed as mean ± standard error (SE). Different letters indicate significant difference ($p < 0.05$), whereas N.S. indicates no significant difference.

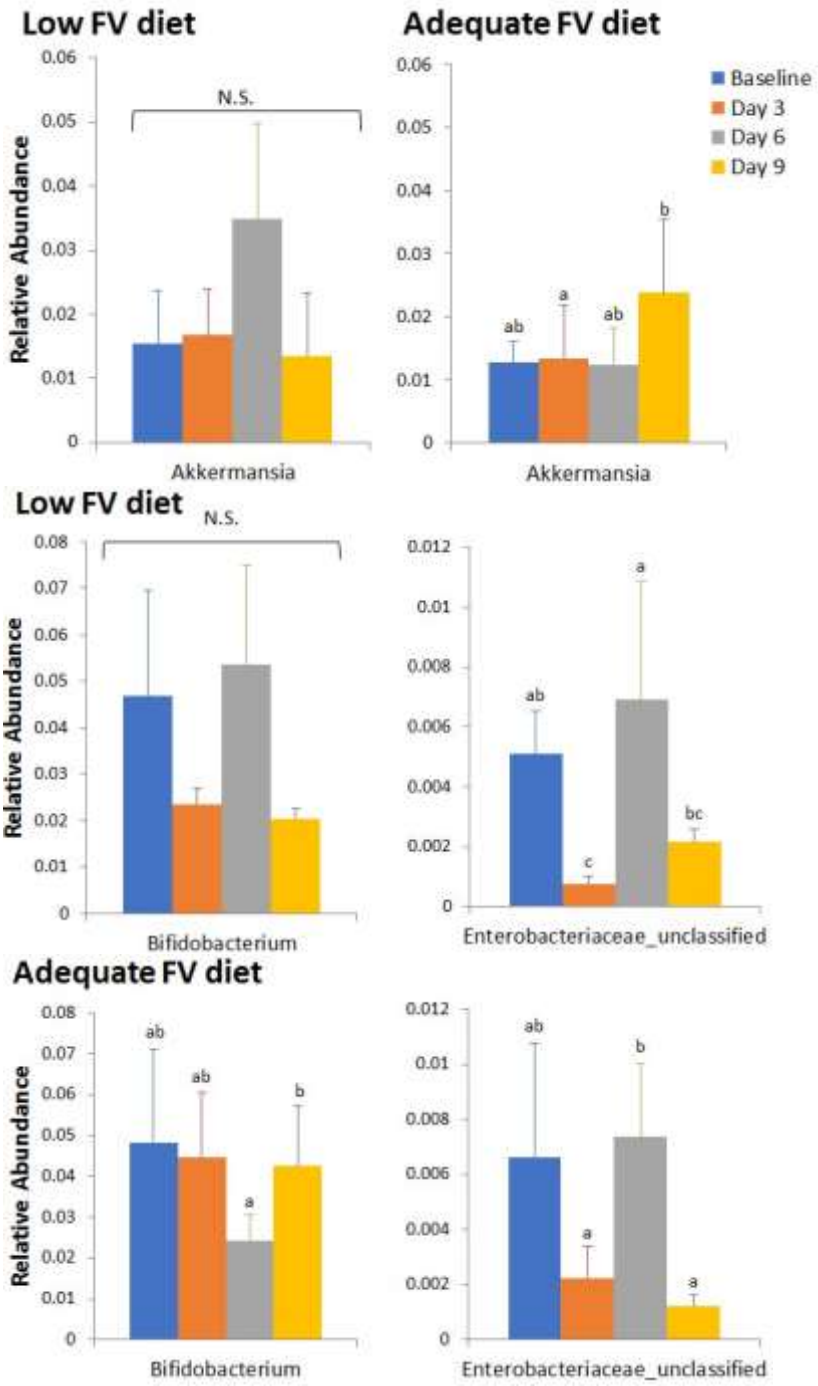
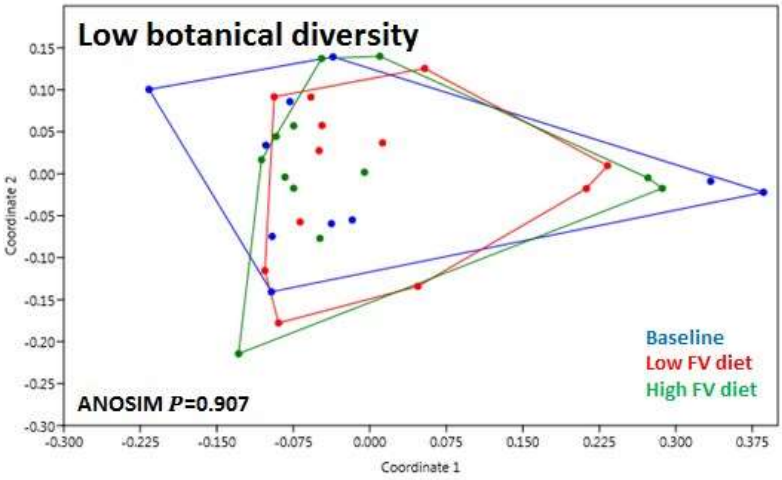



**Figure 4. 10:** Impact of low FV and adequate FV diets on the composition of *Bifidobacterium*, *Akkermansia*, and Enterobacteriaceae. Values are expressed as mean ± standard error (SE). Different letters indicate significant difference ($p < 0.05$), whereas N.S. indicates no significant difference.



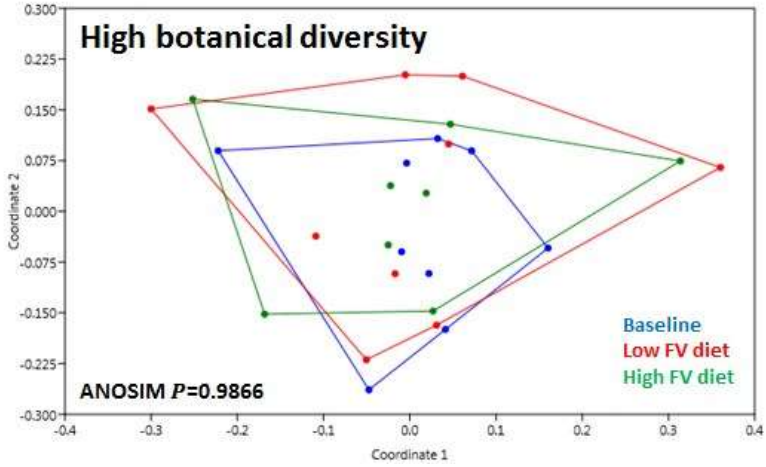




**Figure 4. 11:** Impact on general microbiota profiles (NMDS) of the low and high FV botanical biodiversity diet interventions. Gut microbiota data are shown on non-metric multidirectional scaling (NMDS) (Bray-Curtis similarity index; ANOSIM: ($P < 0.05$)).

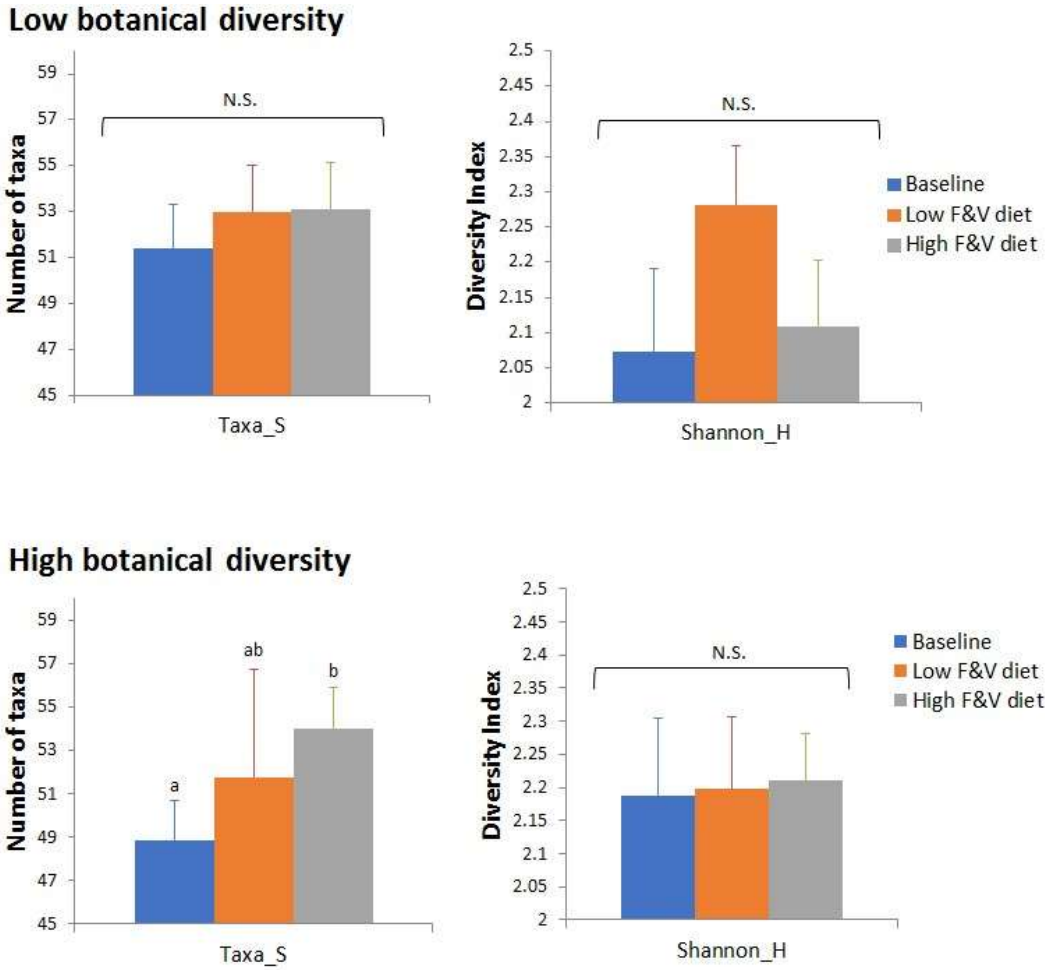



**Figure 4. 12:** Impact on diversity indices (Number of taxa, Shannon index) of the low and high FV botanical diversity diet interventions. Values are expressed as mean ± standard error (*SE*). Different letters indicate significant difference ($p < 0.05$), whereas N.S. indicates no significant difference.

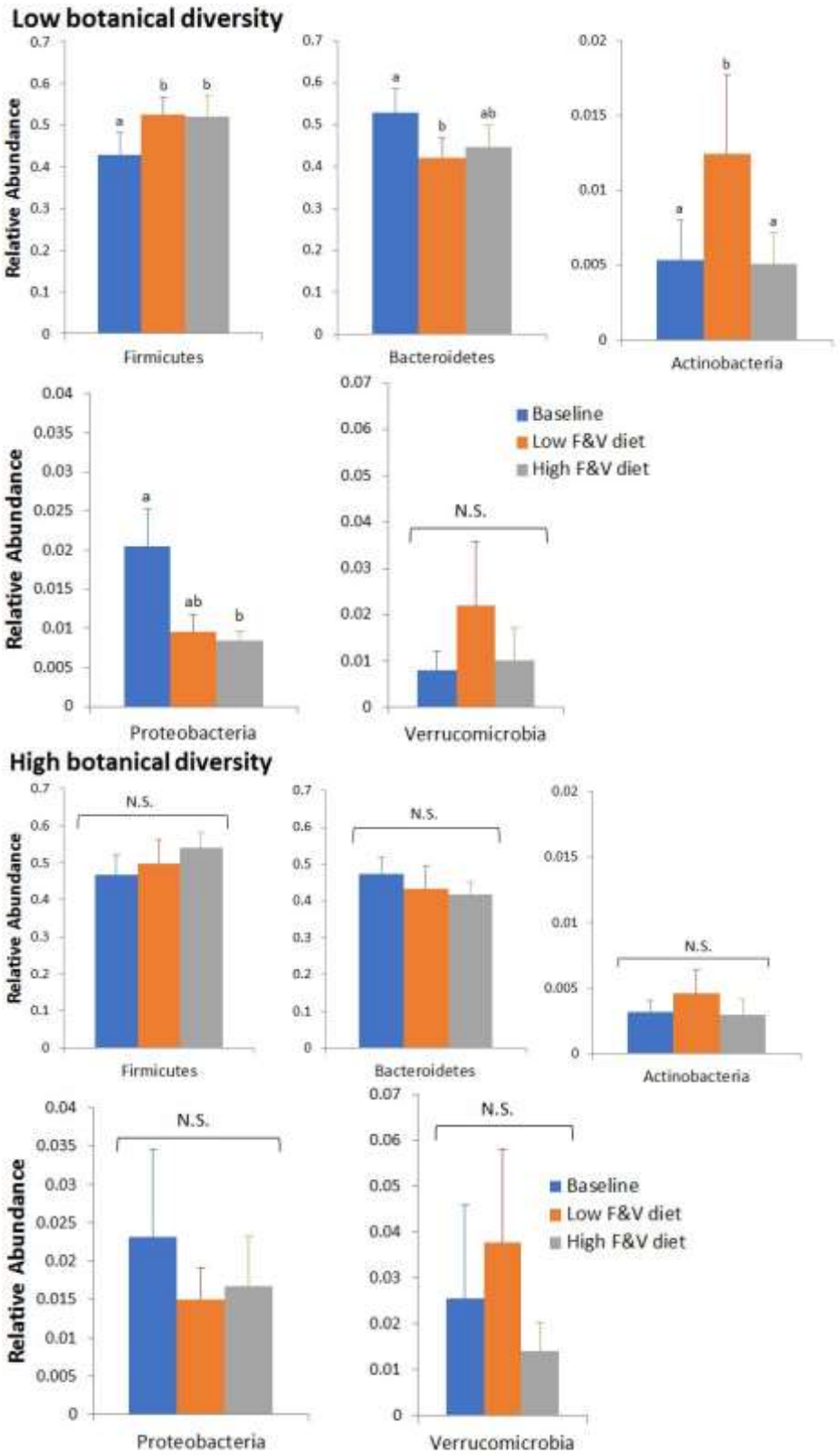



**Figure 4. 13:** Impact of the low and high FV botanical diversity consumption at the phylum level. Values are expressed as mean ± standard error (SE). Different letters indicate significant difference ($p < 0.05$), whereas N.S. indicates no significant difference.

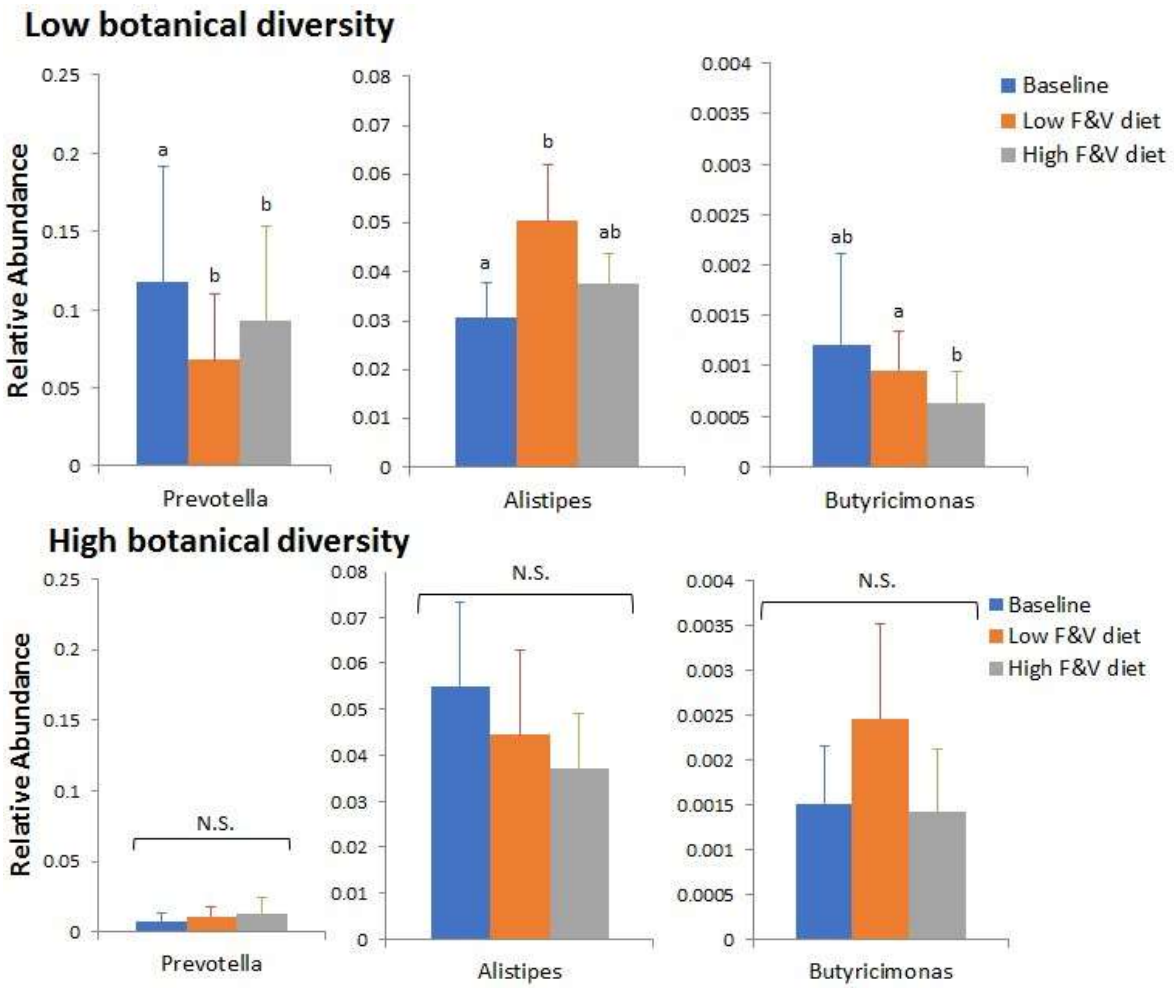



**Figure 4. 14:** Impact of the low and high FV botanical diversity consumption of the genera belong to the Bacteroidetes phyla. Values are expressed as mean ± standard error (SE). Different letters indicate significant difference ($p < 0.05$), whereas N.S. indicates no significant difference.

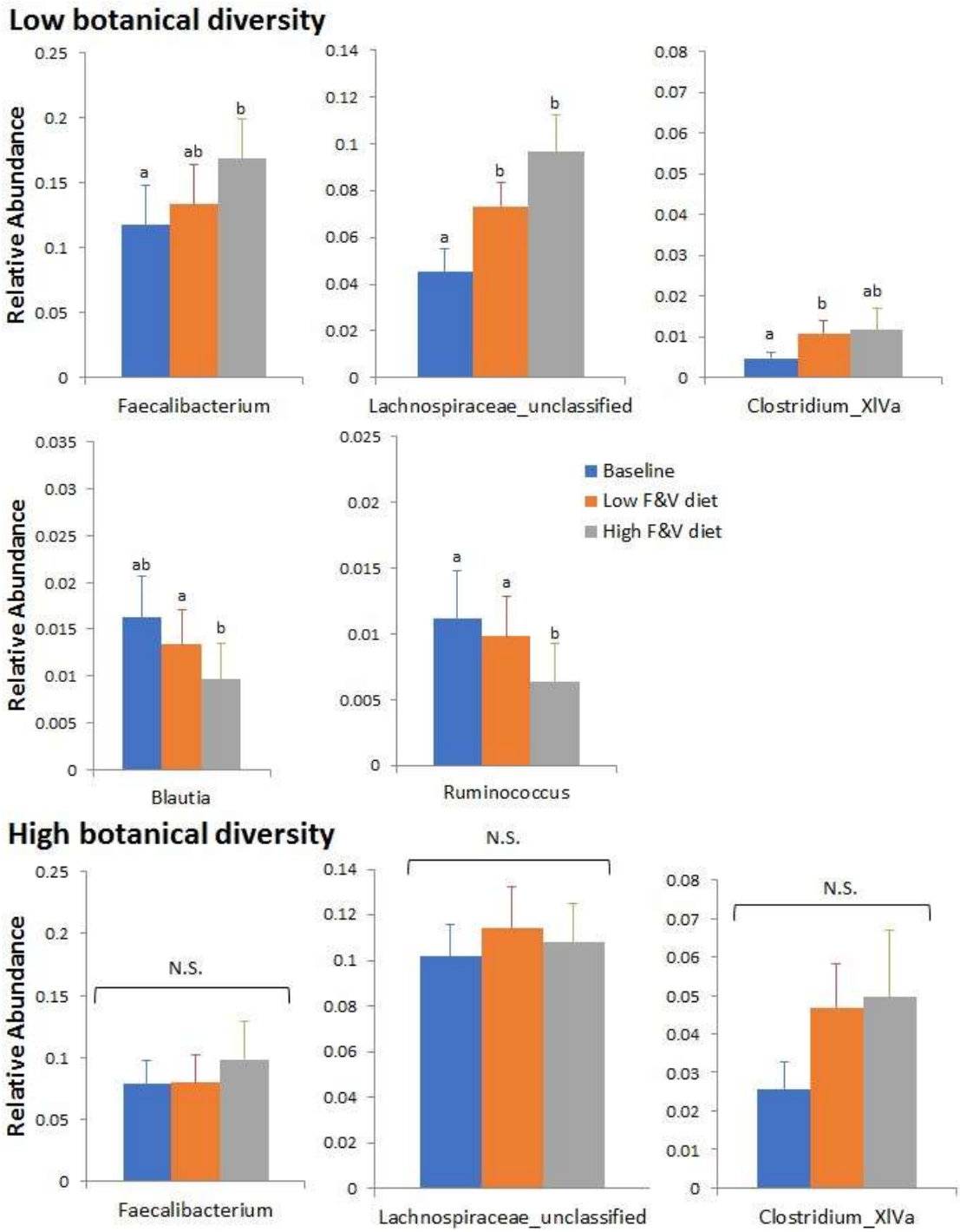



**Figure 4. 15:** Impact of the low and high FV botanical diversity consumption of the genera belong to the Firmicutes phyla. Values are expressed as mean ± standard error (SE). Different letters indicate significant difference ($p < 0.05$), whereas N.S. indicates no significant difference.

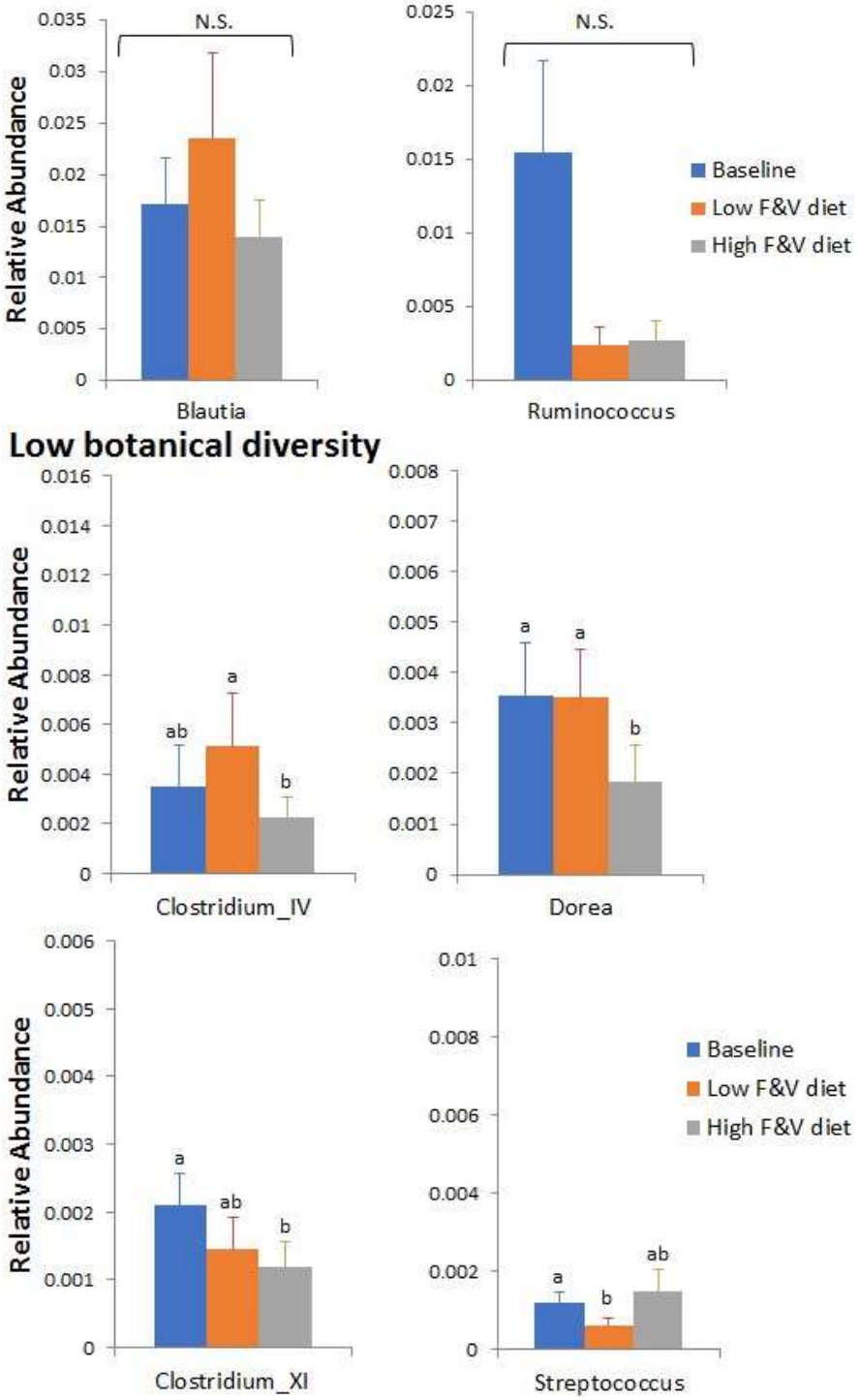



**Figure 4. 15 (Cont.)**

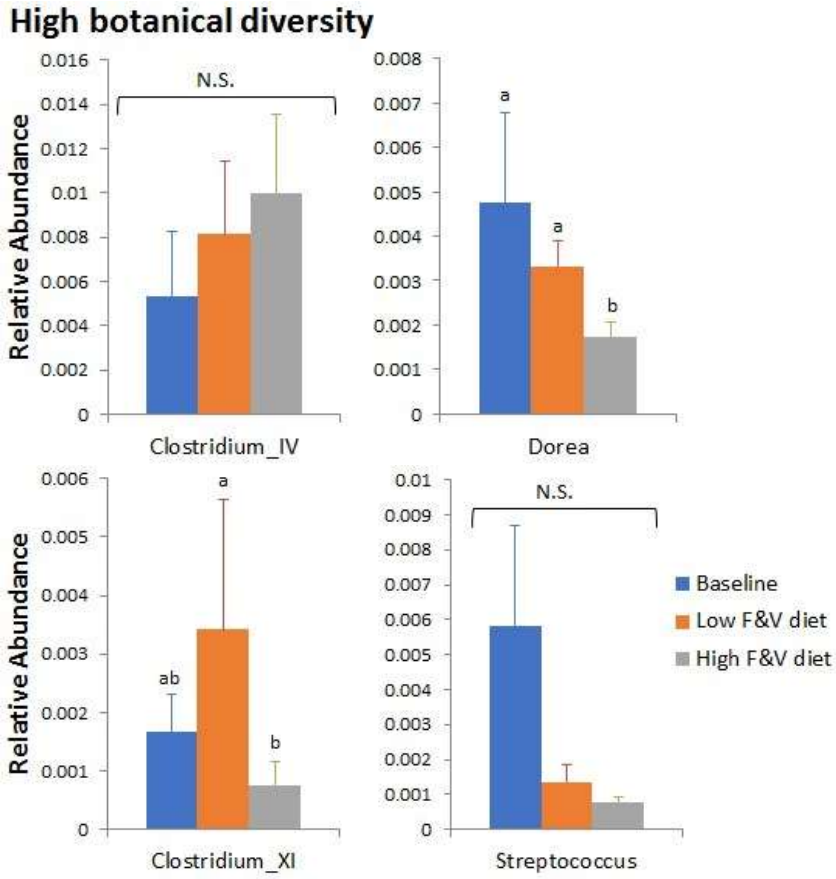



**Figure 4. 15 (Cont.)**

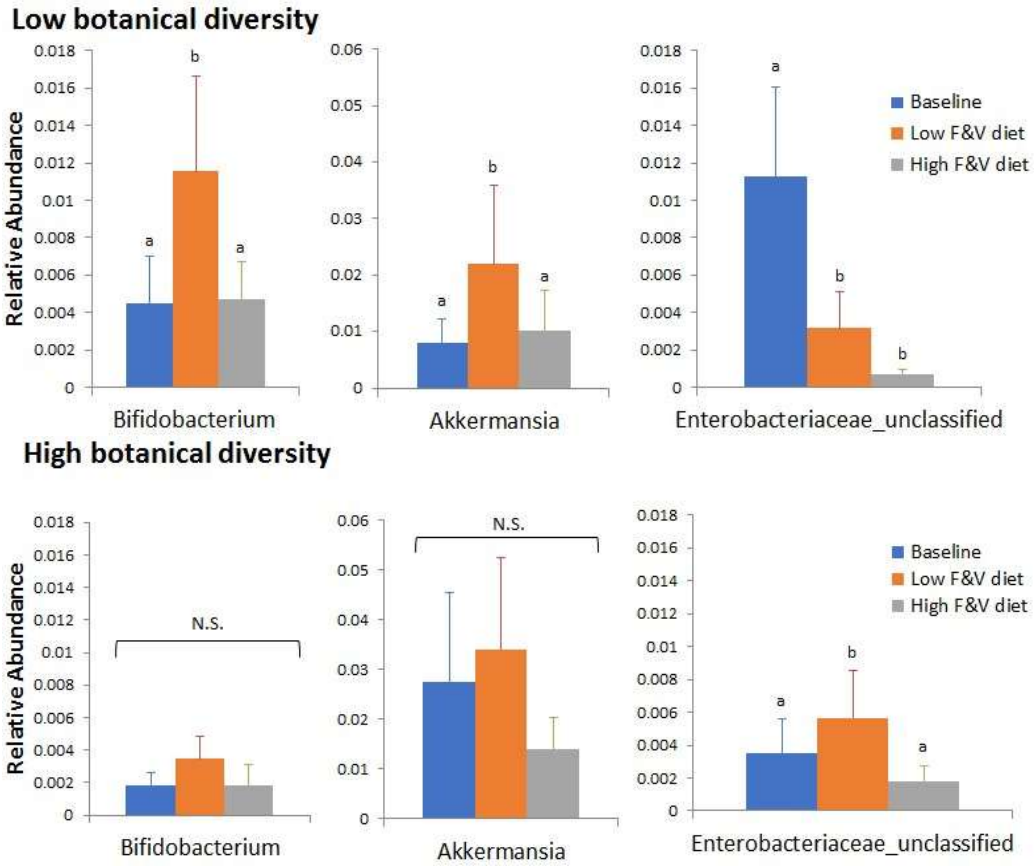



**Figure 4. 16:** Impact of the low and high FV botanical diversity consumption of *Bifidobacterium*, *Akkermansia*, and Enterobacteriaceae Unclassified. Values are expressed as mean ± standard error (SE). Different letters indicate significant difference ($p < 0.05$), whereas N.S. indicates no significant difference.

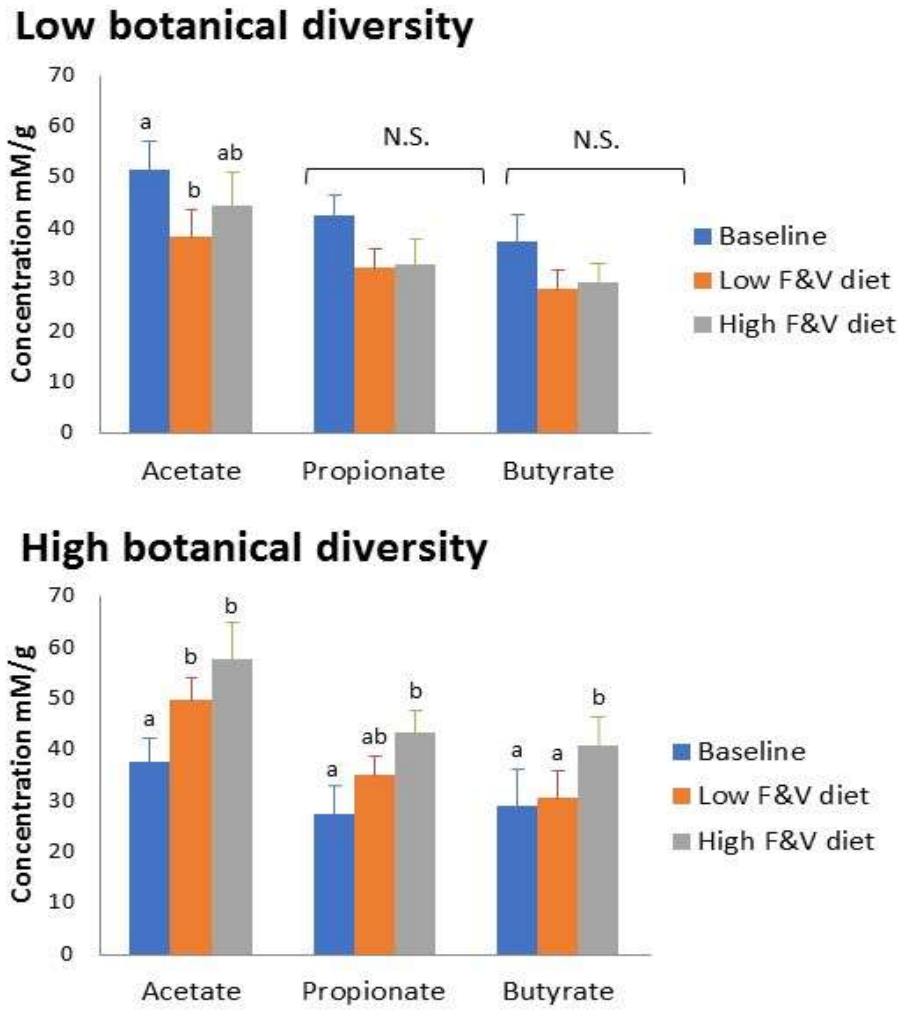



**Figure 4. 17:** Impact of low and high FV botanical diversity consumption on SCFAs levels. Values are expressed as mean ± standard error (SE). Different letters indicate significant difference ($p < 0.05$), whereas N.S. indicates no significant difference.

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## GENERAL CONCLUSION

The relation between gut microbiota and human health has been increasingly recognized in the last few decades. Gut microbiota plays critical roles in the overall health of the host by, for instance, contributing to metabolism, maintaining homoeostasis, providing energy, and educating the immune system. Fruits and vegetables are rich in dietary fibers and polyphenols which aid in the modifying and reshaping of the gut microbiota ecosystem. Most of the microbiological and nutritional studies have traditionally focused on investigating the impact of extracted compounds on gut microbiota profiles instead of whole foods. Therefore, this work brings attention to the possible impact of whole foods — including cherry juices, cranberries juice, and the amounts and botanical diversity of fruit and vegetable diets — on the gut microbiota composition.

The impact of cherry juices in gut microbiota in mice was notably influenced by the variation in the juices concentrations. Increasing cherry juices concentration was positively correlated with some considerably beneficial bacteria, including *Akkermansia* and *Barnesiella*, whereas the opposite response was observed for the *Bacteroides* population.

Cranberry juice consumption impacted the gut and vaginal microbiota in postmenopausal women. Cranberry juice intake resulted in an increase of the relative abundance of *Bifidobacterium*, a well-known probiotic bacterium. The clear beneficial impact of cranberry juice was the improvement of dysbiosed vaginal microbiota by reducing the relative abundance of *Streptococcus*.

Fruit and vegetable consumption enhanced the gut microbiota ecosystem for the participants regardless of the amount. Intriguingly, the effects of fruit and vegetable consumption at the phylum level were almost opposite between the two studies, the SMART-Diet and SMART-Valid. The SMART-Diet study showed a reduction of the Firmicutes/Bacteroidetes

ratio whereas the SMART-Valid study resulted in an increase in the Firmicutes/Bacteroidetes ratio. Remarkably, a higher botanical diversity of fruits and vegetables was clearly associated with higher short-chain fatty acids levels, suggesting that this botanical diversity may be needed to fully realize health benefits mediated by the microbiome fermentative activities.

**APPENDIXES**

**Appendix 1:** SMART-Diet Sample Menu.

| **HIGH** | | | | | |
|---|---|---|---|---|---|
| **Day** | **Food Item** | **Serving** | **Day** | **Food Item** | **Serving** |
| 1 | Pre-Washed Salad Mix | 1 Cup | 1 | Grapes | 1/2 Cup |
| | Frozen Corn-Off Cob | 1 Cup | | Banana | 1 Cup |
| | Baby Carrots | 1 Cup | | Blueberries | 1/2 Cup |
| 2 | Chopped Celery Stalks | 1 Cup | 2 | Peach or Green Apple (Group 2) | 1 Cup |
| | Tomato Cucumber Salad | 1/2 Cup | | Strawberries | 1/2 Cup |
| | Chicken Vegetable Stir Fry | 1 1/2 Cups | | Pineapple Chunks | 1/2 Cup |
| 3 | Broccoli Floret & Cucumber Mix | 1 Cup | 3 | Orange | 1 Cup |
| | Asparagus Spears | 1 Cup | | Red Apple | 1 Cup |
| | Marinara Sauce (No Meat) | 1 Cup | | | |
| **LOW** | | | | | |
| **Day** | **Food Item** | **Serving** | **Day** | **Food Item** | **Serving** |
| 1 | Pre-Washed Salad Mix | 1 Cup | 1 | Green Apple | 1 Cup |
| 2 | Pre-Washed Salad Mix | 1 Cup | 2 | Banana | 1 Cup |
| 3 | Pre-Washed Salad Mix | 1 Cup | 3 | Orange | 1 Cup |

**Appendix 2:** Low Diversity Cohort Menus.

Low Diversity Cohort Menu:

**Lead-in: 6 Total Botanical families:** *Rutaceae (*orange*), Roasaceae (*apple), *Solanaceae* (peppers, potato, tomato), *Asteraceae* (lettuce), *Poaceae* (corn), *Alliaceae* (onion).

**High FV: 11 Total Botanical families:** *Rutaceae (*orange*), Roasaceae (*apple, peaches, pears, berries), *Solanaceae* (peppers, potato, tomato), *Asteraceae* (lettuce), *Poaceae* (corn), *Alliaceae* (onion), *Muscaceae* (banana), *Brassicaceae* (broccoli, cauliflower), *Fabaceae/Leguminosae* (beans, soybeans, chickpeas), *Cucurbitaceae* (cucumber, watermelon, squash), Apiaceae/Umbelliferae (carrots, celery).

| **Condition** | **Day** | **Meal** | **Menu** |
|---|---|---|---|
| Lead in | 1 | Breakfast | 1/4 plate (1/2 cup) scrambled eggs, wheat bagel with tablespoon plain cream cheese or butter, 1/4 plate (1/2 cup) breakfast potatoes, 8 oz glass of skim milk |
| | | Lunch | Deli turkey/ham sandwich (3oz meat) on wheat bread (1 slice cheese, mustard or light mayo, lettuce, tomato), apple with 2 tablespoons peanut butter |
| | | Dinner | ½ plate (2 cups) penne pasta with 1/2 cup red meat sauce with 2 tablespoons of parmesan cheese, 1 breadstick |
| | | Snack/Dessert | Cup of ice cream |

**Appendix 2 (Cont.)**

| Condition | Day | Meal | Menu |
|---|---|---|---|
| | 2 | Breakfast | 1/2 plate Scrambled eggs with cheese, 2 slices of wheat toast lightly buttered, 8oz glass of orange juice |
| | | Lunch | 2 slices of pepperoni pizza, small side salad (romaine lettuce or iceberg lettuce) with tablespoon of dressing, cup of corn chowder soup (1 ½ ladle fulls) |
| | | Dinner | 1 piece garlic grilled salmon, ¼ plate (1/2 cup) potato au gratin dinner roll |
| | | Snack/Dessert | Cookie (any kind but oatmeal raisin) with 8oz glass of skim milk |
| | 3 | Breakfast | cup of oatmeal (approximately 1 ½ ladle fulls) with ~1 oz milk, 2 tablespoons brown sugar or honey, 2 turkey sausage |
| | | Lunch | 3 beef tacos w/ salsa, ½ cup tortilla chips, 2 tablespoons nacho cheese |
| | | Dinner | 2 plum glazed chicken thighs, baked potato w/ tablespoon chz and tablespoon sour cream, side salad (romaine or iceberg lettuce) with dressing, 8oz glass of skim milk |
| | | Snack/Dessert | apple with 2 tablespoons peanut butter |
| | 4 | Breakfast | 2 pancakes/french toast tablespoon butter and syrup, 8oz glass of milk, 2 turkey sausage |
| | | Lunch | Hamburger (no cheese), ¼ (1/2 cup) plate fries with 2 tablespoons of ketchup, orange, ½ cup flavored yogurt |
| | | Dinner | 3 slices of cheese pizza, 1/2C bell peppers or side salad (romaine or iceberg lettuce) with tablespoon ranch, apple |
| | | Snack/Dessert | None |
| High FV | 1 | Breakfast | ¼ plate (1/2 cup) scrambled eggs with cheese, 2 pieces of wheat toast lightly butter, banana |
| | | Lunch | Chicken burrito- (1 whole wheat tortilla, ask for extra grilled veggies, shredded cheese, lettuce, salsa), 1/4 plate (1/2cup) of beans, ¼ plate (1/2 cup) rice |
| | | Dinner | (2 pieces or "boats") Zucchini stuffed with tomato, and faro, dinner roll, 1 cup of berries (no blueberries) or melon, 8oz glass of skim milk |
| | | Snack/Dessert | 2 cookies (any kind but oatmeal raisin) |

**Appendix 2 (Cont.)**

| Condition | Day | Meal | Menu |
|---|---|---|---|
| | 2 | Breakfast | ¼ plate scrambled eggs with cheese, ¼ plate breakfast potatoes, 2 veggie sausage, 2 slices of toast lightly buttered or with peanut butter, 1 cup of melon, |
| | | Lunch | 2 black bean tamales with 2 tablespoons salsa, 1 cup (1 ½ ladle fulls) butternut squash bisque, side salad, (no spinach) with dressing, cookie (any kind but oatmeal raisin) |
| | | Dinner | carved roast sirloin (3oz) with tablespoon creamy horseradish, ½ plate (1 cup) steamed fresh vegetables, dinner roll, apple, 8oz glass skim milk |
| | | Snack/Dessert | ½ cup or 1 Cup n celery/ carrots/ broccoli/ bell peppers |
| | 3 | Breakfast | ¼ plate scrambled eggs with salsa, 2 pieces of toast lightly buttered, orange |
| | | Lunch | ~ ¼ plate (1 cup) whole wheat pasta with (1/4 cup) red meat sauce, and tablespoon of parmesan cheese, ½ plate (1 cup) steamed broccoli, breadstick, ½ cup yogurt with ¼ cup berries, (no blueberries) |
| | | Dinner | tandoori chicken, ¼ plate (½ cup) basmati rice, ¼ plate (1/2 cup) roasted cauliflower, 8oz glass of milk, side salad, (no spinach) with dressing |
| | | Snack/Dessert | banana, with tablespoon peanut butter |
| | 4 | Breakfast | 1 cup plain Greek yogurt, ½ cup peaches or berries (no blueberries), 1/2 cup granola, 2 veggie sausages |
| | | Lunch | Falafel, gyro sandwich with tzatiki, ¼ plate (½ cup) quinoa tabbouleh, 1 cup cucumbers, with tablespoon hummus |
| | | Dinner | Cobb Salad, 1 piece of cornbread with tablespoon butter, 8oz glass of chocolate milk, 1 cup watermelon |
| | | Snack/Dessert | ½ cup carrots /bell peppers /broccoli /celery |

**Appendix 3:** High Diversity Cohort Menus.

<u>High Diversity Cohort Menu:</u>

**Lead-in: 6 Total Botanical families:** *Rutaceae (*orange*), Roasaceae (*apple), *Solanaceae* (peppers, potato, tomato), *Asteraceae* (lettuce), *Poaceae* (corn), *Alliaceae* (onion).

**High FV: 24 Total Botanical Families:** *Rutaceae (*orange*), Roasaceae (*apple/berries/peach/pear), *Solanaceae* (peppers, potato, tomato), *Asteraceae* (lettuce), *Poaceae* (corn), *Alliaceae* (onion), *Muscaceae* (banana), *Brassicaceae* (broccoli, cauliflower), *Fabaceae/Leguminosae* (beans, peas, soybeans), *Cucurbitaceae* (cucumber, melon, squash), Apiaceae/Umbelliferae (carrots, celery), *Chenopodiaceae* (spinach), *Actinidaceae* (kiwi), *Ericaceae* (blueberry, cranberry), *Convolvulaceae* (sweet potatoes), *Vitaceae* (grape), *Anacardiaceae* (mango), *Arecaceae* (date, coconut), *Bromeliaceae* (pineapple), *Caricaceae* (papaya), *Asparaaaceae* (asparagus), *Dioscoreaceae* (yam), *Agaricaceae* (mushrooms), *Grossulariaceae* (black currant).

| Condition | Day | Meal | Menu |
|---|---|---|---|
| Lead in | 1 | Breakfast | 1/4 plate (1/2 cup) scrambled eggs, wheat bagel with tablespoon low fat plain cream cheese or butter, 1/4 plate (1/2 cup) jalapeno cheddar potato rounds, 8 oz glass of skim milk |
| | | Lunch | Deli turkey/ham sandwich (3oz meat) on wheat bread (1 slice cheese, mustard or light mayo, lettuce, tomato), apple, with 2 tablespoons peanut butter, ¼ plate (1/2 cup) steamed corn |
| | | Dinner | ½ plate (2 cups) penne pasta with 1/2 cup meat sauce, and 2 tablespoons of parmesan cheese, 1 breadstick |
| | | Snack/Dessert | Cup of ice cream |
| | 2 | Breakfast | 1/2 plate (1 cup) Scrambled eggs with cheese, 2 slices of wheat toast lightly buttered, 8oz glass of orange juice |
| | | Lunch | 2 slices of pepperoni pizza, small side salad, (romaine or iceberg lettuce) with tablespoon of dressing (no raspberry vinaigrette), 2 focaccia sticks, ½ cup (roughly size of tennis ball) greek yogurt drizzled with honey |
| | | Dinner | Tempura chicken with 2 tablespoons honey mustard, ¼ plate (1/2 cup) mashed potatoes , dinner roll |
| | | Snack/Dessert | Cookie (any kind but oatmeal raisin) with 8oz glass of skim milk |
| | 3 | Breakfast | cup of oatmeal (approximately 1.5 ladle fulls) with ~1 oz milk, 2 tablespoons brown sugar, 1 turkey sausage patty |
| | | Lunch | 3 tacos w/ red salsa or pico de galo, ½ cup (roughly size of tennis ball) tortilla chips, 2 tablespoons nacho cheese |
| | | Dinner | Spicy marinated cod, baked potato, with tablespoon cheese and tablespoon sour cream, side salad, (romaine or iceberg lettuce) with dressing (not raspberry vinaigrette), 8oz glass of skim milk |
| | | Snack/Dessert | Apple with 2tbsp peanut butter |
| | 4 | Breakfast | 2 cinnamon vanilla pancakes with butter and syrup, 8oz glass of skim milk, 1/4 cup scrambled eggs or hard boiled egg |
| | | Lunch | Hamburger (no cheese), ¼ plate fries (1/2 cup) with 2 tablespoons of ketchup, orange |
| | | Dinner | 2 slices of cheese pizza, ½ cup bell peppers or side salad (romaine or iceberg lettuce), with tablespoon ranch, apple |
| | | Snack/Dessert | lowfat vanilla yogurt |

**Appendix 3 (Cont.)**

| Condition | Day | Meal | Menu |
|---|---|---|---|
| High FV | 1 | Breakfast | ¼ plate (1/2 cup) plate scrambled eggs with cheese, ~15 grapes, 2 veggie sausage, oatmeal yam muffin |
| | | Lunch | Vegetarian burrito bowl: cilantro lime rice, black beans, 2 tablespoons shredded cheese, extra peppers and onions, lettuce, salsa, w/ tropical smoothie (made with 1C frozen papaya & mango, 4oz OJ, 1/4C plain greek yogurt) |
| | | Dinner | Chicken Florentine, ½ plate (1 cup) roasted potatoes and vegetables (sweet potatoes), spinach side salad (include sliced mushrooms), with dressing |
| | | Snack/Dessert | 1/2C carrots/celery and ½ cup dried figs, 8oz glass of skim milk |
| | 2 | Breakfast | 1 cup (1 ½ ladle full) oatmeal with 1oz milk, ¼ cup dried black currants, and 2 tablespoons brown sugar, ½ cup pineapple |
| | | Lunch | Chipotle lime tilapia with ¼ cup chipotle pineapple salsa, ¼ plate (½ cup) Jamaican peas and rice, ¼ plate (½ cup) asparagus |
| | | Dinner | Meatloaf, ¼ plate (1/2 cup) mashed potatoes, ½ plate, (1 cup) sautéed spinach w/ garbanzo beans, 8oz lowfat chocolate milk |
| | | Snack/Dessert | ½ cup of mushrooms and banana with 1- 2 tablespoons peanut butter |
| | 3 | Breakfast | ¼ plate scrambled eggs, bagel with tablespoon cream cheese, 1 cup melon |
| | | Lunch | Salmon with ¼ cup tropical fruit salsa, ¼ plate (½ cup) aztec blend or rice pilaf, ½ plate (1 cup) steamed fresh vegetables |
| | | Dinner | Plate of salad, (include raisins, cheese, and nuts of some sort) cup of vegetable soup of the day, (1 ½ ladles full), 1 piece of cornbread, 8 oz glass of skim milk |
| | | Snack/Dessert | 2 kiwi, yam muffin, 2 date coconut bites |

**Appendix 3 (Cont.)**

| Condition | Day | Meal | Menu |
|---|---|---|---|
| | 4 | Breakfast | Banana walnut pancakes topped w/ whipped cream, ½ cup peaches/pears/apple/strawberries, 8oz glass of milk |
| | | Lunch | 2 fish or chicken tacos with ~ 3 tablespoons mango salsa, ½ cup black beans |
| | | Dinner | ¼ plate (1 cup) pasta with marinara sauce, and 2 tablespoons parmesan, ½ plate (1 cup) roasted cauliflower, side salad (romaine lettuce, sliced mushrooms, craisins, any other veg) with tablespoon dressing |
| | | Snack/Dessert | 1 cup greek yogurt with 1 cup blueberries and sweet potato chips |

**Appendix 4:**

UNIVERSITY OF ARKANSAS

Office of Research Compliance

| | |
|---|---|
| To: | Franck Carbonero |
| FR: | Craig Coon |
| Date: | March 10th, 2017 |
| Subject: | IACUC Approval |
| Expiration Date: | March 9th, 2018 |

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **17057:** *Impact of varying amounts of concentrate tart cherry juice on healthy mice gut microbiota.*

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond March 9th, 2018 you can submit a modification to extend project up to 3 years, or submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: . Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem

**Appendix 5:**

UNIVERSITY OF ARKANSAS

Office of Research Compliance
Institutional Review Board

May 9, 2017

MEMORANDUM

| | |
|---|---|
| TO: | Franck Carbonero<br>Ayoub AlOthaim<br>Daya Marasini |
| FROM: | Ro Windwalker<br>IRB Coordinator |
| RE: | New Protocol Approval |
| IRB Protocol #: | 17-04-631 |
| Protocol Title: | *Cranberry Juice and its Effects on Vaginal and Gut Microbiota in Postmenopausal Women: A Pilot Cross-Over Trial of a Dietary Preventive for Urinary Tract Infection* |
| Review Type: | ☐ EXEMPT ☒ EXPEDITED ☐ FULL IRB |
| Approved Project Period: | Start Date: 05/08/2017 Expiration Date: 04/27/2018 |

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form *Continuing Review for IRB Approved Projects*, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (https://vpred.uark.edu/units/rscp/index.php). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

**This protocol has been approved for 40 participants.** If you wish to make *any* modifications in the approved protocol, including enrolling more than this number, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 109 MLKG Building, 5-2208, or irb@uark.edu.

109 MLKG • 1 University of Arkansas • Fayetteville, AR 72701-1201 • (479) 575-2208 • Fax (479) 575-6527 • Email irb@uark.edu