Summer 8-16-2024

The Antioxidants and Anti-inflammatories Benefit of Broccoli Sprout Diets and Their Relation to Health

Tolu Esther Alaba
University of Maine, tolu.adekeye@maine.edu

THE ANTIOXIDANTS AND ANTI-INFLAMMATORIES BENEFIT OF BROCCOLI SPROUT DIETS AND THEIR RELATION TO HEALTH.

By

Tolu Esther Alaba

B. Tech. Ladoke Akintola University of Technology, Ogbomosho, 2011

MSc. University of Ibadan, Ibadan, 2015

A DISSERTATION

Submitted in partial fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

(in Biomedical Sciences)

The Graduate School

The University of Maine

August 2024

Advisory Committee:

Suzanne Ishaq, Associate Professor of Microbiomes, Advisor

Yanyan Li, Assistant Professor of Pharmaceutical Sciences

Melody Neely, Associate Professor of Molecular and Biomedical Science

Tao Zhang, Assistant Professor of Pharmaceutical Sciences

Abigail Johnson, Assistant Professor, Division of Epidemiology and Community Health

THE ANTIOXIDANTS AND ANTI-INFLAMMATORIES BENEFIT OF BROCCOLI SPROUT DIETS AND THEIR RELATION TO HEALTH.

By Tolu Esther Alaba

Dissertation Advisor: Dr. Suzanne Ishaq

An Abstract of the Dissertation Presented
in Partial Fulfillment of Requirements for the
Degree of Doctor of Philosophy
(in Biomedical Sciences)
August 2024

Inflammatory bowel disease is a chronic intestinal condition characterized by severe inflammation, oxidative stress, microbial dysbiosis, and cellular structure and barrier damage. The complex mechanism of pathologies makes it challenging to manage the disease effectively. Most current treatments target only inflammation using steroids and antibiotics with side effects. Therefore, recent research has focused on safe and effective nutritional options. Broccoli sprouts, *Brassica oleracea* variety italica, is a member of the *Brassicaceae* family and has documented antioxidant, anti-inflammatory, and gut protective benefits against inflammatory bowel diseases. These benefits have been associated mainly with sulforaphane from dietary glucosinolates present in broccoli sprouts. However, many other dietary and microbially derived metabolites are present in broccoli sprouts that are yet to be identified and explored against inflammatory bowel diseases. Commensal bacteria such as *Bifidobacterium and Bacteroides* have been reported to participate in hydrolyzing dietary metabolites for gut benefits. However, there is a need for more studies on the differential effects of broccoli sprouts on metabolite concentration and microbial communities in diverse patients with these gut conditions. Additionally, the interactions of these metabolites with

microbial communities in specific gut regions still need to be clarified. Therefore, this project utilized dietary assessments, computational analyses, and a global metabolomics approach to identify beneficial metabolites and microbial interactions across different age groups and genders associated with broccoli sprouts diet as a potential nutritional intervention against inflammatory bowel diseases.

First, I assessed the role of high consumption of dark green vegetables diets such as *Brassicas*, fruits, and fiber on the healthy eating index using dietary history surveys and the effect of steamed broccoli sprouts intervention on the microbial response of *Bacteroides thetaiotaomicron* genes for benefit in healthy individuals with self-reported gut problems. *Bacteroides thetaiotaomicron* genes are associated with increased conversion of glucosinolates from broccoli sprouts into isothiocyanates, such as sulforaphane, for anti-inflammatory and antioxidant effects against inflammatory bowel disease. The data showed a healthy eating index of >70 in individuals who consumed at least twice daily dark green vegetables may have a high expression of *Bacteroides thetaiotaomicron* genes. This result was particularly evident before, at the end, and seven days after the broccoli sprout diet in young and middle-aged persons with gut conditions who consumed high amounts of dark green vegetables, fiber, and fruits above the daily values recommended by the dietary guidelines for Americans. These vital results provide information for future research to explore personalized approaches for dietary recommendations for individuals with gut conditions in compliance with dietary guidelines for Americans and its beneficial effect on diet-microbial responsiveness, particularly in young adults.

Second, I utilized untargeted metabolomics and computational tools to examine the effects of steamed broccoli sprouts intervention on the concentrations of beneficial dietary and microbial-derived metabolites. Also, I investigated the association between the metabolites, commensal

bacteria, and *Bacteroides thetaiotaomicron* genes in ulcerative colitis mice. Metabolites annotated in fecal samples after broccoli sprouts diet includes sulforaphane, short-chain fatty acids, tryptophan, indoles, glutamic, and polyphenols with antioxidant, anti-inflammatory, and gut-protective effects against mouse models of inflammatory bowel diseases. These metabolites positively correlate with commensal bacteria, B*acteroides, Intestinimonas*, *Oscillibacter*, and *Lachnospiraceae species* in the colon, cecal and jejunum regions. These findings open up exciting avenues for future research, particularly in targeting region-specific benefits of broccoli sprouts intervention against inflammatory bowel diseases.

Lastly, I examined the differential effect of two different preparations, steamed and mildly heated broccoli sprouts diets, as interventions for ulcerative colitis in female and male, young and adult mice. Metabolic profiles of plasma samples revealed increased concentrations of glucoraphanin and glucoerucin, iso-ferulic acid, flavanone-4-glucuronide, and denticulaflavonol are which may be associated with decreased disease activity and recovery from colitis observed in adult female mice. Adult male and younger mice may benefit more from mildly heated broccoli sprouts with increased concentrations of catechin, proanthocyanidins, short-chain fatty acids, and glutathione metabolites. Novel information generated from this study could guide the targeted application of broccoli intervention as nutritional management for specific age and gender groups with inflammatory bowel condition

DEDICATION

I dedicate this dissertation to God, my parents, family, and friends, who supported, prayed, and motivated me throughout my doctoral studies.

ACKNOWLEDGEMENTS

This dissertation would not have been achieved without the input and support of many amazing people. Completing this project was made possible by the incredible support and mentorship of my advisor, Dr. Sue Ishaq. Thank you so much for the opportunity to work in your lab, the timely feedback, and the thorough insights you provided regarding my career development and goals during the dissertation journey. My sincere gratitude goes to my co-advisor, Dr. Yanyan Li. Your thoroughness, support, and encouragement have helped me to learn a lot. I appreciate Dr. Melody Neely, who has been a member of my committee since 2019; your unwavering commitment to mentorship is indispensable. I could not have done this without you. Despite all odds, you worked continuously to support and encourage me to greater heights. My thesis committee member, Dr. Tao Zhang, has been a supportive collaborator, especially with the early life metabolomics data that facilitated my interest and gave me speed on this journey. Thank you so much, Dr. Abigail Johnson; you have coached and inspired me in dietary data analysis to improve my knowledge for success. I sincerely appreciate my colleague and co-author, Johanna Holman, for her incredible support in ensuring samples are ready for metabolomics analysis. Every member of Ishaq's laboratory is appreciated for your teamwork and support. The Mass spectrometry manager at the University of California, Irvine, Felix Grun, and the facility supervisor, Ben Katz, were of tremendous help with the metabolomics data analysis. The metabolomics manager at the University of California, Amancio Jose de Souza, and the SUNY staff, Yan Sun, were of tremendous guidance during the early life metabolomics data analysis. Also, my sincere appreciation goes to Lynn Atkins, Trish Costello, Peg Killian, and Drs. Dieffenbacher-Krall, Kass, and Dastoor, thank you for your support during my teaching assistantship position. I appreciate UMaine's Office of International Programs, particularly Sarah Joughin and Mireille Le Gal, for

their constant support and encouragement. You made my stay at UMaine homely and filled me with beautiful memories. I appreciate everyone who has guided me through this journey, Drs. Townsend, Blaszk, Weber, VandenAkkar, Klimis-Zacas, Mason, Talbot, and Tsakiroglou. Thank you for your support; my departmental administrators, Zane Long, Jennifer Chiarell, and Drs. Henry, Kaye, and Cox. A heartfelt thank you goes to my siblings (Tayo, Tobi, and Toba) and parents (Pastors Adekeye and Alaba) for their constant prayers, sacrificial love, and enduring support. As a family, you all have always believed in me and my goals. There are no words to express the depth of my gratitude to all relatives and friends (Adekeyes, Alabas, Olatundes, Adios, Olaitans, Amodus, Okoties, Adeyemos, Wiafe-Kwakyes, Emes, Chivhenges, Alayos, Oluwoles, Funmi Akinrelere, Abolaji Akinyele and Shola Awoyemi), and spiritual support groups (Old Baptist church, The Rock church Bangor, Chi-Alpha D-Group, Peace House New England Brethren, Christ Life fellowship and Westminster Church Assembly). Most importantly, I greatly thank my hardworking, highly supportive, incredibly loving, and handsome husband, Oluwafemi Alaba, my son, Emmanuel Alaba, and my daughter, Bethel Alaba, for their patience, sacrifice, and amazing support that brought me joy, laughter, and strength throughout my studies. I could not have achieved this success without your support. To God Almighty, the creator of all things, Jesus Christ, my Lord, and the Holy Spirit, the great comforter, be all the Glory for the success of this PhD journey.

TABLE OF CONTENTS

DEDICATION..... iii

ACKNOWLEDGEMENTS..... iv

LIST OF FIGURES..... ix

LIST OF TABLES..... xi

GLOSSARY OF TERMS..... xii

Chapter

1. CURRENT KNOWLEDGE ON THE PREPARATION AND BENEFITS OF CRUCIFEROUS VEGETABLES AS RELATES TO IN VITRO, IN VIVO AND CLINICAL MODELS OF INFLAMMATORY BOWEL DISEASE..... 1

1.1 Abstract..... 1

1.2 Introduction..... 2

1.3 Discussion..... 9

1.4 Conclusion..... 28

2. EFFECT OF CRUCIFEROUS CONSUMPTION ON HEALTHY EATING INDEX AND OVERALL NUTRITIONAL QUALITY IN DIET HISTORY ASSESSMENT OF HEALTHY AND SELF-REPORTED IBD SUBJECTS..... 41

2.1 Abstract..... 41

2.2 Introduction..... 42

2.3 Methods..... 46

2.4 Results..... 50

2.5 Discussion..... 58

2.6 Conclusion, limitations and future direction..... 62

3. STEAMED BROCCOLI SPROUTS DIET ALLEVIATES INFLAMMATORY BOWEL DISEASE BY INCREASING ANTI-INFLAMMATORY, ANTIOXIDANT AND GUT PROTECTIVE METABOLITES IN DSS-MICE..... 65

3.1 Abstract..... 65

3.2 Introduction..... 65

3.3 Methods..... 69

3.4 Results..... 72

3.5 Discussion..... 83

3.6 Conclusion, limitations and future direction..... 89

4. BROCCOLI SPROUTS ALLEVIATE ULCERATIVE COLITIS IN MICE BY INCREASING DIETARY AND MICROBIAL METABOLITES: DIFFERENTIAL EFFECTS IN YOUNG AND ADULT MALE AND FEMALE MICE..... 90

4.1 Abstract..... 90

4.2 Introduction..... 91

4.3 Methods..... 94

4.4 Results..... 98

4.5 Discussion..... 103

4.6. Conclusion, limitations and future direction..... 108

REFERENCES..... 110

APPENDICES..... 153

Appendix A. Supplementary Figures..... 153

Appendix B. Supplementary Files..... 157

BIOGRAPHY OF THE AUTHOR.. 164

LIST OF FIGURES

Figure 1.1: Inflammatory bowel disease is a chronic condition characterized by inflammation, oxidative stress, and dysbiosis..... 11

Figure 1.2: Cruciferous vegetables or their purified compounds can ameliorate inflammatory symptoms through multiple pathways..... 15

Figure 2.1: Bar graphs graphically display the demographics and healthy eating index distribution of participants..... 51

Figure 2.2: Food nutrients and healthy eating index differed among participants by age and sex..... 53

Figure 2.3: Bar charts of dietary fiber, dark green vegetables, and total fruit intake comparing female and male participants that filled the DHQ3 survey..... 54

Figure 2.4: Heat map of Spearman correlation of HEI with fiber, vegetables, saturated fats, sugar, and salt..... 56

Figure 2.5: Scattered bars illustrate the expression of BT2160-2156 genes in response to broccoli diet among the twenty participants..... 57

Figure 3.1: Fecal metabolites were differentially present by steamed broccoli sprout diet and DSS treatment..... 73

Figure 3.2: Hierarchical clusters analysis comparing the metabolites among the treatment groups and the control..... 74

Figure 3.3: Bar plots showing the concentrations of fecal metabolites with significant effects in mice fed with a 10%SS diet, 2.5%DSS treatment, 2.5%DSS10%SS, compared with control groups (n=3 mice/group)..... 77

Figure 3.4: Correlogram of fecal metabolites, bacterial diversity metrics, IBD-associated bacterial taxa, and B. thetaiotaomicron genes..... 83

Figure 4.1: Mildly heated or steamed broccoli sprout diets improve weight gain and colon growth and reduce disease activity index by age and sex of mice with chemically induced colitis..... 99

Figure 4.2: Serum metabolites were differentially present in mice of different ages and sexes, and the preparation of broccoli sprouts..... 101

Figure 4.3: Serum metabolites were differentially abundant in mice according to age and sex, and preparation of the broccoli sprouts..... 102

LIST OF TABLES

Table 1.1: Potential benefits of Cruciferous vegetables in IBD in vitro models..... 52

Table 1.2: Potential benefits of Cruciferous vegetables in IBD in vivo models..... 29

Table 1.3: Potential benefits of Cruciferous vegetables in IBD clinical studies..... 36

Table 2.1: The demographics of the participants and the healthy eating index score of those who filled out the diet history questionaries..... 50

Table 2.2: The correlation values of participants' healthy eating index scores with their daily nutrition values in grams and compliance differences compared with dietary guidelines for Americans in percentages..... 55

Table 3.1: Liquid Chromatography conditions for metabolomics..... 71

Table 3.2: Open source information about annotated fecal metabolites of interest, Human Metabolomics Database or PubChem identification number, chemical formula, average molecular weight and CAS number..... 77

Table 4.1: Classification of experimental groups based on broccoli diet, age and sex..... 95

Table 4.2: Liquid Chromatography conditions for metabolomics..... 97

GLOSSARY OF TERMS

Abbreviation	Definition	Abbreviation	Definition
AHR	Aryl Hydrocarbon Receptor	IL6	Interleukin-6
CCL	Chemokine (C-C) Ligand	IL23R	Interleukin-23 receptor
CCR	Chemokine (C-C) Receptor	iNOS	Inducible Nitric Oxide Synthase
CD	Crohn's Disease	ITC	Isothiocyanates
CG	Cysteine-Glycine	LPS	Lipopolysaccharides
COX-2	Cyclooxygenase	NAC	N-acetyl-L-Cysteine
CXCL	Chemokine(CXC) Ligand	NFkB	Nuclear Factor kappa B
DPPH	2,2-Diphenyl-1-picrylhydrazyl	Nrf2	Nuclear Factor Erythroid 2-related Factor
DSS	Dextran Sulfate Sodium	NOD	Nucleotide oligomerization domain (NOD)
ERN	Erucin	OCTN	Organic Cation Transporter
GLR	Glucoraphanin	SCFA	Short Chain Fatty Acid
GPX	Glutathione Peroxidase	SFN	Sulforaphane
GSH	Glutathione	SS	Steamed broccoli Sprouts
GSL	Glucosinolates	TLR	Toll-Like Receptor
IBD	Inflammatory bowel Disease	TNFα	Tumor Necrosis Factor-alpha
ICAM	Intercellular-Adhesion Molecule	TNFSF	Tumor-Necrosis-Factor Superfamily
IL10	Interleukin-10	UC	Ulcerative Colitis
IL1β	Interleukin-1 beta	ZO-1	Zonula Occluden-1

CHAPTER 1

CURRENT KNOWLEDGE ON THE PREPARATION AND BENEFITS OF CRUCIFEROUS VEGETABLES AS RELATES TO IN VITRO, IN VIVO AND CLINICAL MODELS OF INFLAMMATORY BOWEL DISEASE.

This chapter was published in *Current Developments in Nutrition* and has undergone minor edits according to the dissertation format for consistency (Alaba et al. 2024)

1.1 Abstract

Inflammatory bowel disease is a chronic condition with a significant economic and social burden. The disease is complex and challenging to treat because it involves several pathologies, such as inflammation, oxidative stress, dysbiosis, and intestinal damage. The search for an effective treatment has identified cruciferous vegetables and their phytochemicals as potential management options for inflammatory bowel disease, as they contain prebiotics, probiotics, and anti-inflammatory and antioxidant metabolites essential for a healthy gut. This critical narrative style review provides a robust insight into the pharmacological effects and benefits of crucifers and their documented bioactive compounds in in vitro and in vivo models, as well as clinical inflammatory bowel disease. The review highlights the significant impact of crucifer preparation and the presence of glucosinolates, isothiocyanates, flavonoids, and polyphenolic compounds, which are essential for the anti-inflammatory and antioxidative benefits of cruciferous vegetables, as well as their ability to promote the healthy microbial community and maintain the intestinal barrier. This review may serve as a viable nutritional guide for future research on methods and features essential to developing experiments, preventions, and treatments for inflammatory bowel

disease. There is limited clinical information and future research may utilize current innovative tools, such as metabolomics, for adequate knowledge and effective translation into clinical therapy.

Key words: Inflammatory bowel disease, cruciferous vegetables, oxidative stress, inflammation, microbiomes, glucosinolates, isothiocyanates, polyphenols and flavonoids.

1.2 Introduction

1.2.1 Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a chronic condition that can last a lifetime and reoccur, causing inflammation, diarrhea, rectal bleeding, and abdominal pain due to severe anatomical damage, immune over-activation, oxidative stress, and microbiome disruption (Seyedian et al. 2019; Tian et al. 2017). Ulcerative colitis (UC) primarily affects the rectum and colon, leading to inflammation, pain, and diarrhea, including mucousy and bloody stool, resulting in anemia and iron deficiency (Seyedian et al. 2019; El Amrousy et al. 2022) as well as a greater risk factor for colon cancer, the second leading cause of cancer death globally (Gates et al. 2023; Siegel et al. 2021). Crohn's disease (CD) involves chronic inflammation of the distal gut, leading to lesions along the entire intestine, immune intolerance of commensals, fever, tiredness, and complications such as stenosis, fistulas, and strictures (Caparrós et al. 2021). Patients often experience remission and recurring flares resulting in surgical removal of damaged intestinal regions (Petagna et al. 2020). IBDs create a continuous pathological circle of comorbidities: malabsorption and malnutrition can lead to adipogenesis, obesity, diabetes, and non-alcoholic fatty liver disease (Lomer et al. 2019; Saroli Palumbo et al. 2019); IBD stress is associated with 8-9% of pregnancy loss or preterm birth (Nielsen et al. 2022); and it can impact psychological well-

being, including anxiety, depression, obsessive disorder, and teasing from peers (Barberio et al. 2021; Quick et al. 2015).

IBD is a significant economic and social burden, particularly in Europe and North America where rates are high (Kaplan 2015; The Lancet Gastroenterology Hepatolog...); in the US, > 3 million people suffer from IBD, with an annual cost > $30 billion. Cases of IBD have dramatically increased in many countries (Caviglia et al. 2023), with various factors contributing, including diet and the switch to ultra-processed foods (Dolan and Chang 2017), lifestyle, urbanization and exposure to environmental pollutants (Benchimol et al. 2017), and stress (Kaplan and Ng 2017), as well as a few genetic markers (Liu et al. 2015; Ahern et al. 2010). Many studies suggest that diet is a major factor, including one which reported a 70% relationship between IBD and a switch to self-reported unhealthy diets with high sugar and saturated fats and less fiber, fruits, and vegetables (Damas et al. 2018).

IBD management focuses on alleviating inflammation, oxidative stress, or microbial dysbiosis, and is challenged by severe side effects, or individualized presentation of symptoms. Clinical medicine, such as immunosuppressants, antibiotics and steroids, and surgery (Lewis et al. 2018; Sandborn WJ, Ghosh S, Panes J, et al....), can induce remission, but many patients become less responsive over time. Patients can spontaneously enter remission, which masks medication's loss of efficacy. Individuals can experience repetitive relapse (Miyatani Y 2023), and patients with recurrent surgery often experience a high mortality rate (Lewis et al. 2018; Hudesman DP, Chakravarty SD, Emond B,...). Thus, a low-cost and efficient approach is essential for IBD management to alleviate oxidative stress, inflammation, and host-microbial dysbiosis is essential for IBD management (Hudesman DP, Chakravarty SD, Emond B,...; Dahlhamer et al. 2016). This critical-narrative review style highlights primary research articles documenting the preparation,

use, and mechanisms of bioactive compounds derived from cruciferous vegetables as a potential management for IBD. There is evidence that a person's response to plant fibers or secondary compounds can be highly individualized and reliant on their gut microbiota, thus any ranking of the effectiveness of different brassicas would not be broadly applicable. This critical narrative style of review highlights that crucifer's preparation affects the effectiveness and potential for targeting benefits in the gut.

1.2.2 Comorbidities of oxidative stress and inflammation during IBD

The complex relationship between oxidative stress and inflammation could create a vicious cycle of chronic inflammation when immune cells produce reactive oxygen species (ROS) in response to intestinal injury, free radicals, and reactive nitrogen species (Tian et al. 2017) in the gut to recruit more inflammatory cells to exacerbate IBD (Pereira et al. 2015; Nuñez et al. 2021). Some critical pathways in the relationship between inflammation and oxidative stress are nuclear Factor kappa B (NFkB) and nuclear factor erythroid 2-related factors 2/Kelch-like ECH-associated protein (NRF2/KEAP) . The coupling of NRF2 and KEAP proteins within the intestinal cells could prevent the NRF's transcription of antioxidants and anti-inflammatory proteins (Moura et al. 2020; Geertsema et al. 2023). Meanwhile, NFkB could increase mitochondrial production of ROS by proton loss during intracellular enzymatic reactions, leading to uncontrolled oxidative stress, inflammation, and intestinal damage (Piechota-Polanczyk A 2014).

The gut epithelial barrier is critical to enforcing selective permeability to permit essential nutrients but prevent harmful substances (Schulzke et al. 2009). Damaged barriers cause excess ROS, inadequate production of antioxidants, and leaky gut conditions (van Duijn W, et al. 2003) in which an influx of pathogenic bacteria activates NFkB epithelial and recruits immune cells which disrupts the gut-microbiota homeostasis (Wullaert 2010; Zuo and Ng 2018).

Similarly, the gut's vasculature contains several tight junctions crucial for selective permeability and vascular dilation (Deban L, Correale C, Vetrano S, Males...; Song Y, He Y, Rong L, et al. 2023). Mucosal endothelial cells can express vascular endothelial cell adhesion molecules (VCAM), intercellular adhesion molecules (ICAM), chemokines, and fractalkines to recruit and retain inflammatory cells (Gu et al. 2017). Inflammatory signals, toll-like receptors (TLR), and nucleotide oligomerization domains NOD1/2 via endothelial NFkB and mitogen-activated protein kinases could trigger sphingosine 1-phosphate receptor-2 for oxidative stress and further damage (Schirbel et al. 2013). The endothelial-immune cell interaction within the gut could ultimately lead to platelet aggregation, intestinal coagulation, ischemia, and dysbiosis (Schirbel et al. 2013; Roberts et al. 2010).

1.2.3 Microbial dysbiosis during IBD

Microorganisms inhabit the human gastrointestinal tract, from the oral cavity to the rectum (Turnbaugh et al. 2007), colonizing the gut early in life through the parent's microbiota and the living environment (Dominguez-Bello et al. 2010; Dill-McFarland et al. 2019), and play an essential role in developing the child's gut, cellular barrier, and mucosal layer (Selma-Royo et al. 2021; Selma-Royo et al. 2020), and tolerance to gut microbiota (Knoop et al. 2017). The composition of microbial communities varies by anatomical location and diet, which can either protect or predispose the gut to IBD (Nishino K, Nishida A, Inoue R, et al....; Holman et al. 2023). Microbial populations in healthy and IBD patients are highly individualized, and changes associated with UC and CD are distinct. Moreover, there are differences between mucosal and luminal microbiota in inflamed and non-inflamed tissues from IBD patients (Walker et al. 2011).

Significant causal relationships exist between the gut immune system, oxidative stress, and loss of commensal bacteria (Borrelli et al. 2018; Rana et al. 2014; Schaubeck et al. 2016). Dysregulated inflammatory processes can cause inappropriate responses to regular gut commensals and lead to altered microbial architecture - dysbiosis. It is situationally specific whether dysbiosis precedes inflammation or dysbiosis leads to inflammation (Aldars-García et al. 2021). In UC, pathogenic microbiota can initiate IBD-like inflammation in germ-free mice (Abeles et al. 2016; Schaubeck et al. 2016), and some bacterial species of *Clostridium*, *Escherichia coli*, and *Shigella flexneri* can induce pathogenicity of commensals in the early development of IBD (Ma et al. 2023). Similarly, in CD, pathogen infection and gut microenvironmental cues can cause the pathogenesis of commensal colonies to trigger dysbiosis (Schaubeck et al. 2016). Conversely, studies in mice showed that knockout of genes associated with host-microbe tolerance and increased copies of immune defense genes protected against gastrointestinal inflammation (Li et al. 2023), indicating that inflammation can lead to dysbiosis.

Studies show that IBD patients are likely to have lower microbial diversity (Hansen et al. 2012), featuring more pathogenic bacteria and fewer beneficial species (Kang et al. 2010; Shaw et al. 2016) which reduces short-chain fatty acid (SCFA) production but increases sulfur metabolism (Machiels K, Joossens M, Sabino J, et ...; de Vos WM. 2013). Therefore, dysbiosis is more pronounced in CD with increased pathogens: *Enterococcus* spp., *Lactobacillus fermentum*, *Clostridioides difficile*, *Shigella flexneri*, and *Listeria* spp. and decreased commensals such as *Faecalibacterium prauznitzii*, *Eubacterium rectale*, *Ruminococcus* spp., *Bacteroides fragil*is and *B. vulgatus* (Nishino K, Nishida A, Inoue R, et al....; Holman et al. 2023; Hoarau G, Mukherjee PK, Gower-Roussea...; Heinken et al. 2021). Meanwhile, reports from UC patients showed increased bacterial phyla *Pseudomonadota (*formerly *Proteobacteria), Actinobacteria, and*

Prevotella spp. and lowered *Bacteroidota (*formerly *Bacteroidetes)* (Aldars-García et al. 2021). Dysbiosis persists during disease inactivity, so decreased *F. prausnitzii* and *Clostridium coccoides* can predict relapse (Rajca et al. 2014).

1.2.4 The role of diet in IBD development and treatment

The development of dysbiosis, inflammation, and oxidative stress during IBD can be modulated by diet, about five times more closely associated than genetic factors (Tian et al. 2017; David et al. 2014; Khalili et al. 2018; Ho et al. 2019). High consumption of dietary fat and sugar can increase mucosal dysbiosis, inflammation and horizontal gene transfer in microbiome to increase pathogenic colonization, such as *Pseudomonadota* (formerly *Proteobacteria*) and *Bacillota* (formerly *Firmicutes*) phyla (Agus et al. 2016; Groussin et al. 2021), and decrease SCFA-producing bacteria *Roseburia spp*., *Eubacterium rectale*, and *Ruminococcus bromii* (da Rocha et al. 2023; De Filippo et al. 2010). Dysbiosis and damaged intestinal cells promote the influx of ROS and inflammasomes from impaired mitochondria biogenesis (Das and Ganesh 2023; Saint-Georges-Chaumet and Edeas 2015): the hypoxic epithelial cells and inflammatory cytokines induce mitochondrial leaky electron transfer chain and peroxidase production for oxidative stress (Lee et al. 2020; Clark and Mach 2017), which results in inadequate energy production and tiredness (Clark and Mach 2017). Mice fed with a high-fat and sugar diet exhibit severe edema, high leukocyte scores, and the presence of chemokines and cytokines, such as tumor necrosis factor-alpha (TNFα), Interleukin-6 (IL6), and chemokine (C-C) ligand2 (CCL2), in their colon (Shon et al. 2023), and fecal samples revealed more pathogenic bacteria, TLR expression, and neutrophil-to-lymphocyte ratio genes (Shon et al. 2023). The same study also reported elevated C-reactive proteins and monocytes in plasma of people consuming an ultra-processed high-fat and sugar diet, indicating IBD-like high-fat-diet-induced systemic inflammation (Shon et al. 2023).

High fat diets can reprogram innate immune cells via NOD-like receptor protein-3 signaling to promote systemic inflammation (Christ et al. 2018), and fat- and protein-rich diets triggered IL4, TNFα, and monocytes chemoattractant protein-1 and generated harmful peroxides (Tayyem et al. 2021; Keewan et al. 2020). Obesity is a significant link between type 2 diabetes and IBD severity with increased inflammation with decreased tight junction protein such as epithelial cadherin. These markers reflect epithelial permeability and inflammatory influx and retention within the colon (Francis et al. 2023). Therefore, unhealthy diets promote dysbiosis, inflammation, and mitochondria-induced oxidative stress.

Meanwhile, healthy diets rich in fiber, antioxidants, and anti-inflammatory metabolites (Fritsch et al. 2021; Rahmani et al. 2019) can increase SCFA-producing bacteria to metabolize dietary micronutrients and phytochemicals for IBD treatment (Caban and Lewandowska 2023; Guo et al. 2022; Facchin et al. 2020). Vegetables with high content of glucosinolates and polyphenols can promote microbiota diversity (Li et al. 2020; Wu et al. 2021), increase antioxidant activity, and reduce inflammation via increased dietary cysteine and microbiota production of SCFA (Li et al. 2015; Scarano et al. 2017; Holman et al. 2023; Barton et al. 2017; Kumar et al. 2022). SCFA can bind G-protein coupled receptors to alter inflammatory genes and increase the antioxidant activities of glutathione (Kaisar et al. 2017; Flint et al. 2012; Rom et al. 2020; Mardinoglu et al. 2015). Despite these benefits, there is a need for a comprehensive review of the preparation methods, models, and specific metabolic effects of cruciferous vegetables as a guide for translational research. Therefore, the discussion of this review will focus on the role of crucifers and their phytochemicals, which have bioactive properties against IBD development.

1.2.5 Search criteria

The search criteria for this review include articles published between 2003 and 2023 in PubMed and Web of Science. We found 625 articles based on the keyword's combination with inflammatory bowel disease, cruciferous vegetables, Brassicaceae, broccoli, inflammation, oxidative stress, microbiomes, microbiota, bioactive compounds, glucosinolates, isothiocyanates, polyphenols, flavonoids, gut barrier, and colon damage. Excluding criteria such as cancer, microbial neuroinflammation, pharmacological or drug interventions in IBD, a disease associated with cruciferous plants, and non-cruciferous sources of polyphenols and flavonoids which reduced the searched articles to 220 publications. After checking for content relevance, we finally selected only 35 primary articles for the discussion section by excluding reviews, inadequate studies based on the reported information on extraction methods, and applications not associated with this review's objectives.

1.3 Discussion

1.3.1 Cruciferous vegetables and bioactive compounds that mitigate IBD

Cruciferous vegetables belong to the mustard family, and are rich in bioactive compounds beneficial to human health (Zhang et al. 2018; Wang et al. 2022) via prebiotic, probiotic (i.e., when fermented), anti-inflammatory and antioxidant effects for regulating gut microbiota communities and alleviating pathogenic signals of IBD (Wu et al. 2019; Wu et al. 2023; Dolan and Chang 2017). They include broccoli and broccoli sprouts (*Brassica oleracea* variety italica), Brussels sprouts (*B. oleracea* var. gemmifera), cabbage (*B. oleracea* var. capitata), cauliflower (*B. oleracea* var. botrytis), collard greens (*B. oleracea* var. viridis), kale (*B. oleracea* var. *Acephala* or *B. napus*), bok choy (*Brassica rapa* cultivar Chinensis), mustard greens (*B. rapa and B. juncea*), turnips (*B. rapa*, var. *rapa*), the mustard Virginia pepperweed/peppergrass (*Lepidium virginicum*), arugula

(*Eruca vesicaria sativa*), and wasabi (Wasabia japonica, W. koreana, and *Eutrema japonicum*) (Zhang et al. 2018; Wang et al. 2022). The presence of glucosinolates (GSLs), flavonoids, and polyphenols in crucifers provide antioxidants to scavenge ROS and anti-inflammatory agents to alleviate IBD symptoms and heal intestinal damage (Cicio et al. 2022; Mueller et al. 2013).

Cruciferous diet can supply GSLs such as glucoraphanin (GLR), sinigrin, and glucoerucin, which are metabolized by plant-sourced myrosinase and gut microbiota-sourced enzymes into isothiocyanates (ITCs), such as sulforaphane (SFN) and erucin (see Figure 1.1), for intestinal and systemic health benefits (Tian et al. 2018; Saha et al. 2012; Zhang et al. 2023). SFN is effective against IBD by maintaining Nrf2 redox homeostasis, protecting tight junctions, recruiting commensal bacteria, and increasing antioxidants and anti-inflammatory markers (Wei et al. 2022; Geertsema et al. 2023; He et al. 2022). Broccoli sprouts possess more antioxidants, total phenolic content (TPC), and GSLs that provide ITCs than other crucifers (Amron NA 2018; Cartea ME, Francisco M, Soengas P, Ve...). Meanwhile, broccoli, kale, radish, and cabbage are flavonoids-rich with quercetin and cyanidin, and polyphenols like ferulic, sinapic, and caffeic acids for prebiotic, endothelial and epithelial barrier protection, antioxidant, anti-inflammatory and anti-adhesive potential to complement the effect of ITCs against IBD (Choe et al. 2018; Kim et al. 2021; Choi et al. 2016; Ayaz et al. 2008; Bian et al. 2018; Jeon et al. 2016). Importantly, cooking or other preparations of crucifers affect bioavailability of these phytochemicals (Figure 1.2), and where in the GI tract they will be absorbed. Future research should investigate the combined effect of phytochemicals from crucifers as a holistic approach to developing treatment or supplements for the clinical management of IBD.

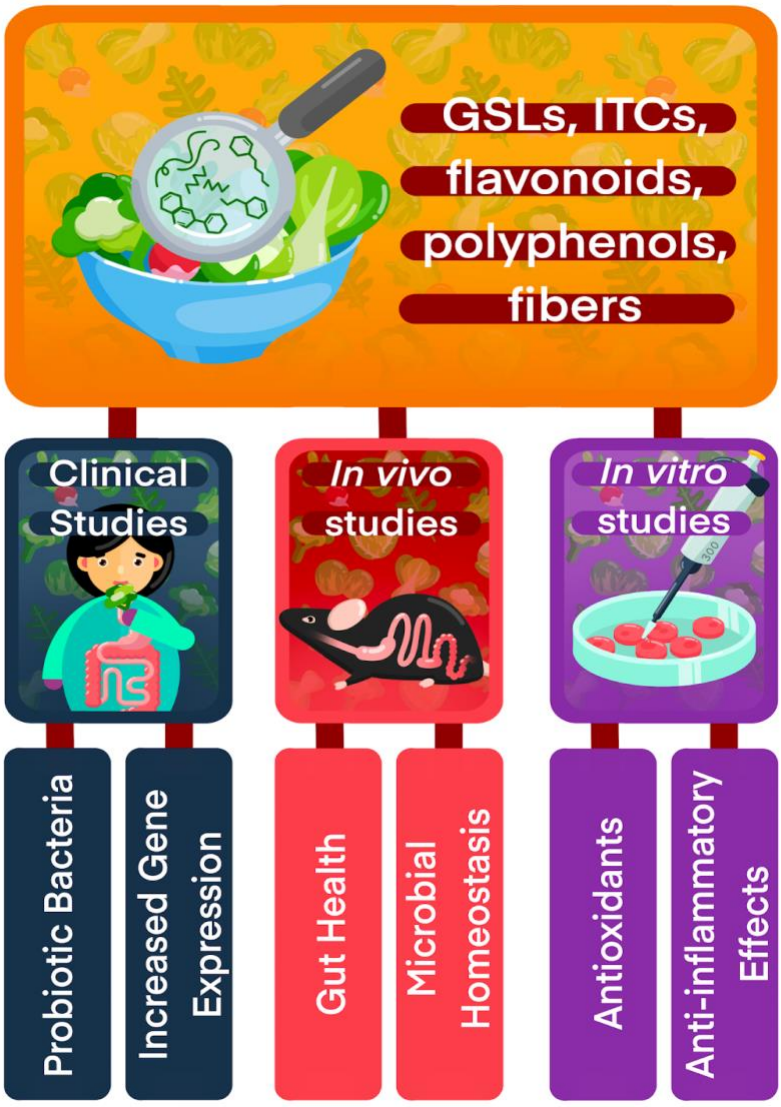


Figure 1.1: Inflammatory bowel disease is a chronic condition characterized by inflammation, oxidative stress, and dysbiosis. Clinical, in vivo, and in vitro studies have shown that dietary cruciferous vegetables and their phytochemicals, such as glucosinolates (GSLs), isothiocyanates (ITCs), flavonoids, and polyphenols, may modulate inflammation, oxidative stress, dysbiosis, and gut barrier to alleviate inflammatory bowel disease. These dietary phytochemicals may promote probiotic bacteria, microbial homeostasis, gut health, as well as anti-inflammatory and antioxidant genes, as potential dietary management for inflammatory bowel disease patients.

1.3.2 *In vitro* studies

1.3.2.1 Broccoli and broccoli sprouts

Broccoli has been widely studied for its anti-inflammatory and antioxidant properties against *in vitro* IBD models (Table 1.1 and Figure 1.1). One study showed that broccoli-derived nanoparticles (BDN), made of broccoli juice and lipids, prevented dendritic cell (DC) activation by upregulating AMPK anti-inflammatory pathways, thereby reducing chemoattractant molecules like chemokine (C-C) receptors (CCR), chemokine (CXC) ligand (CXCL), and CCLs in the DCs and monocytes (Deng et al. 2017). Similarly, broccoli-derived vesicles rich in GLR, glucobrassicin and neoglucobrassicin decreased oxidative stress (Hossain et al. 2022) in Caco-2 cells (colon cells derived from a 72-year old man with colorectal adenocarcinoma). The SFN treatment transformed the white blood cell monocytes (M1) to anti-inflammatory macrophages (M2) (Sun et al. 2022; Pal and Konkimalla 2016), through IL10 and signal-transducer and activator of transcription-3 pathways to ameliorate inflammation in bone marrow-derived macrophages (Sun et al. 2022), and decreased COX-2 and iNOS activities and increased IL10 and CD36 against chronic inflammation in human cells (Pal and Konkimalla 2016; Fernandez-Prades et al. 2023).

Cold-pressed broccoli seed extracts (BSE) exhibited prebiotic, anti-inflammatory, and antioxidant properties against LPS-induced inflammation in J774A.1 mouse cancer cells (Choe et al. 2018). The treatment increased *Bacteroidetes/Firmicutes* ratio and production of SCFA and decreased commensal bacteria *Akkermansia spp.*, *Lactobacillus spp.*, and *Bifidobacterium spp.* (Choe et al. 2018). BSE increased the antioxidant activities of ORAC, HOSC, and ABTS scavengers and inhibited COX-2 and IL1β cytokines above the control (Choe et al. 2018). The benefits of BSE may be attributed to the presence of GLR, Glucoerucin, and quercetin-3-glucoside.

1.3.2.2 Other cruciferous vegetables

Culture media containing cabbage juice exhibited *in vitro* anti-inflammatory and prebiotic properties associated with indole-3-carbinol, a metabolite of glucobrassicin (Jeong et al. 2023; Jeon et al. 2016). LPS RAW264.7 cells (macrophage-like, Abelson leukemia virus-transformed cell line derived from BALB/c mice) were co-cultured with vegetable waste from green and kimchi cabbage. The kimchi inhibited 20% of IL6 activities more than green cabbage. However, both types of cabbage had similar inhibitory effects against LPS-induced nitric oxide inflammation. Furthermore, kimchi decreased *E. coli* significantly, while green cabbage had more effect against *S.aureus* (Jeong et al. 2023).

The cold-water extract of cauliflower exhibited higher antioxidant activities of DPPH scavenger, while the ethanol extract possessed more flavonoids and polyphenols than both hot and cold water extracts (Bhatt et al. 2020). Furthermore, all three extracts had prebiotic effects and promoted the growth of six *Lactobacilli* strains. Only hot and cold-water extract significantly increased *L. acidophilus* microbial flora (Bhatt et al. 2020), which can promote a healthy gut.

One study identified nine phenolic acids in kale, with ferulic and caffeic acids being the most abundant (Ayaz et al. 2008). In the study, phenolic fractions of kale increased DPPH scavengers and decreased pathogenic bacteria, *S. aureus* and *E. coli* (Ayaz et al. 2008). An ethanolic extract from kale inhibited LPS-induced inflammation in RAW264.7 macrophages and relevant cytokines: IL6, iNOS, TNFα, and TLR-4 (Raychaudhuri et al. 2023). In a similar study, kale digesta rich in phenolic acids inhibited LPS and TNFα-induced intestinal epithelial cell inflammation and upregulated catalase, GSH, and superoxide dismutase antioxidant levels against cellular oxidative stress (Kaulmann et al. 2016).

One study investigated different radish extracts with chloroform, hexane, butanol, ethyl acetate, and water-soluble fraction on LPS-induced inflammation in RAW264.7 macrophages. The 100 μg/mL chloroform extract exhibited the most significant inhibition against NFkB, COX-2, iNOS, IL6, and TNFα levels. Interestingly, the treatment also prevented platelet aggregation by decreasing cellular prostaglandin E-2 concentration (Park and Song 2017). Similarly, radish sprouts ethanolic extract (RSE) decreased IL6 and chemoattractant protein-1 levels in monocytes: the 100μg/mL decreased IL1β and TNFα proteins the most, but both 50 and 100μg/mL doses inhibited iNOS. The RSE 100μg/mL significantly inhibited NFkB protein by decreasing its association with inflammatory subunits, p-IkBα and p65, while increasing the expression of the inhibitory subunit, IkBα (Kim et al. 2021).

Wasabi, a Japanese and Korean crucifer, exhibited anti-inflammatory properties and may repair intestinal barriers. Wasabi extracts co-cultured with LPS-treated macrophages and Caco-2 cells inhibited NFkB and decreased IL1β and IFN-γ levels, and increased the expression of tight junction proteins zonula occludens-1 (ZO-1) and claudin, but not occludin, in Caco-2 cells (Kang et al. 2017). In conclusion, these in vitro studies suggest the beneficial effects of cruciferous vegetables through prebiotic, anti-inflammatory, antioxidants, and gut barrier protective mechanisms, determined primarily by the crucifer's preparation, bioactive compounds, dosage, and treatment period (See Table 1.1, Figure 1.1 & 1.2).

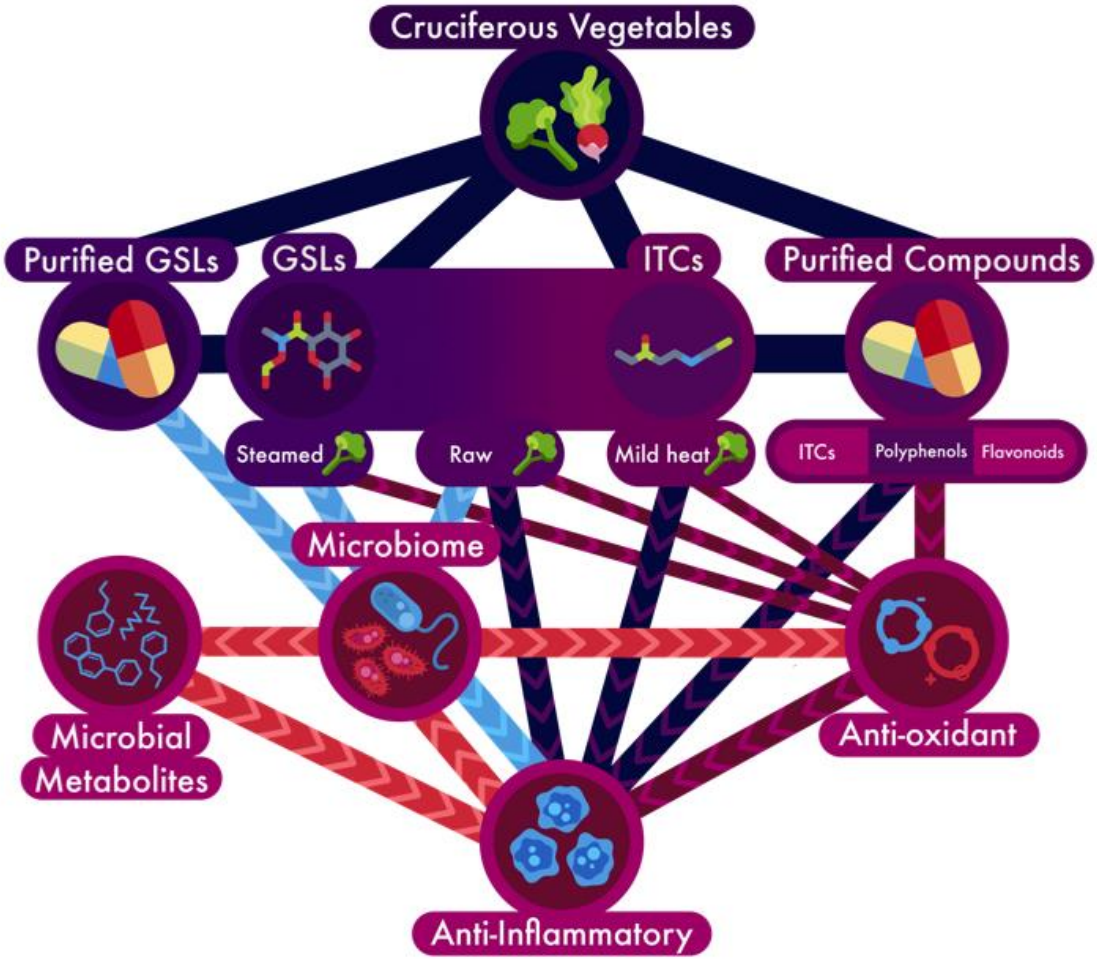


Figure 1.2: Cruciferous vegetables or their purified compounds can ameliorate inflammatory symptoms through multiple pathways. Fiber**,** glucosinolates (GSLs), isothiocyanates (ITCs), flavonoids, and polyphenols, can reduce inflammation and immune activation, oxidative stress and barrier damage, and can promote functional microbial communities. Each pathway has direct and indirect benefits.

1.3.3 *In vivo* studies

Similar to the findings from in vitro studies, broccoli has been reported to have anti-inflammatory, antioxidant, and prebiotic effects against IBD in animal models (see Table 1.2 and Figure 1.2). Both the UC and CD models use chemical triggers or genetic knockouts. The zymosan-mouse is a model of generalized gut inflammation using colon injection of zymosan to enhance the regeneration of immune-specific receptors for colitis and mucosal damage symptoms.

Similarly, a generalized inflammation model is made using AHR, which is a ligand-activated transcription factor of the aryl hydrocarbon receptor with high ligand affinity or sensitivity in the Ahrb/b mice model (alleviates inflammation) and low ligand affinity or sensitivity in the Ahrd/d model (promotes inflammation).

The DSS mouse or rat is the most commonly used UC model, with dextran sulfate sodium in drinking water used to chemically induce colitis with pathologies similar to human UC. The DNBS rat model uses intra-rectal administration of dinitrobenzene sulfonic acid to induce diet- and stress-associated inflammation. MDR1a-/- mice are a UC model with genetically deleted multiple drug resistance genes to develop colitis spontaneously. Rag1-/- mice are a recombination activating gene1 knock-out mouse model for chronic gut inflammation and fatal microbial infection. The IL10-knockout is the most popular immunological model for CD development. The TNBS rat model uses intra-rectal administration of trinitrobenzene sulfonic acid to long-lasting pathologies and clinical symptoms of CD through the NOD2 pathway. In addition to these models to generate colitis, these mice and rats can be associated with human microbiota to assess gut microbial disorder.

1.3.3.1 Broccoli and broccoli sprouts

A study reported the effect of a broccoli diet rich in SFN and GLR against oxidative stress and inflammation in DSS-mice. The diet decreased pathological score and TNFα, IL1β, and IL18. Meanwhile, it increased AMPK and PGC-1α pathways and promoted Nrf2, HO-1, and 8-hydroxydeoxyguanosine (8-OHDG) activities against oxidative stress and DNA damage within the colon (Tian et al. 2022). A similar anti-inflammatory effect was observed in DSS-mice fed raw and lightly cooked broccoli diets (Wang et al. 2018). Both diets decreased proinflammatory levels

of IL6 and CCR2 but not TNFα in the colon, even though cooked broccoli had low SFN levels because myrosinase was inactivated and thus GLR conversion is reliant on gut bacteria. The diets improved mucosal regeneration and decreased neutrophil infiltration, disease activity index (DAI), and colonic damage scores. Interestingly, increased levels of tight junction proteins such as claudin-2, occludin, and ZO-1 and decreased VCAM-1 level may restore epithelial tight junction and risk of IBD flare (Wang et al. 2018). Though this study confirmed that broccoli preparations can affect the immediacy of SFN bioavailability (Li et al. 2013), cooking, which inactivates myrosinase and preserves GLR, can utilize gut microbiota to convert it to SFN directly in the colon (Zhang et al. 2023) and other phytochemicals in any preparation could enhance the beneficial effects observed in cooked broccoli (Wang et al. 2018).

SFN from broccoli increased IL10 levels and inhibited IL6 and TNFα activities via signal-transducer and activator of transcription-3 and Nrf2 pathways in DSS-mice (Sun et al. 2022; He et al. 2022). The prebiotic effect of SFN treatment upregulated commensal bacteria such as *Bifidobacterium* and *Lactobacillus* bacteria in DSS mice (He et al. 2022), species which can metabolize GLR for SFN and SFN-N acetylcysteine bioavailability (Wu et al. 2023), and increased protective epithelial barrier proteins such as ZO-1, occludin, and claudin. In another DSS mouse model, *Bifidobacterium* increased anti-inflammatory IL10 levels, inhibited TLR4, and decreased TNFα, IL1β, and IL8 cytokines in colon tissues. *Bifidobacterium bifidum* treatment decreased the pathogenic colonization of *Streptococcus* and *Enterococcus* and increased beneficial *Actinobacteria* to alleviate dysbiosis (Shang et al. 2022).

Broccoli is a rich source of aryl hydrocarbon receptor (AHR) in the gut of mice that are sensitive to specific environmental toxicants and develop mucosal disorders and GI tumors, *Ahrb/b,* or the less sensitive, *Ahrd/d* mice (Hubbard et al. 2017). Broccoli diet increased duodenal

AHR-associated cytochrome P450, family 1, subfamily A, polypeptide 1 (Cyp1a1) and decreased IL1β, IL6, prostaglandin-endoperoxide synthase (Ptgs), CXCL5 levels and increased IL1O mainly in *Ahrb/b* mice to abrogate inflammation (Hubbard et al. 2017). The broccoli diet was associated with beneficial bacteria, such as *Actinobacteria* and *Alistipeds*, and metabolic pathways, such as those involved in drug metabolism, valine/leucine/isoleucine biosynthesis, vitamins, and cofactors. At the same time, RNA seq analysis revealed Reg1, Reg2, Tiff2, and CCL28 genes for gut barrier homeostasis, cell cycle regulation, cyclins, and checkpoints (Hubbard et al. 2017). Thus, a broccoli diet may prevent barrier damage and DNA deregulation associated with intestinal stress.

Broccoli seed extract (BSE) exhibited prebiotic, anti-inflammatory, antioxidant, and tight junction benefits in DSS-mice. BSE promoted beneficial bacteria, such as species of *Lactobacillus*, *Bifidobacterium*, and *Alistipeds*, to increase SCFA production (Wu et al. 2023). The BSE treatment decreased the oxidative level of malondialdehyde and increased the antioxidant levels of superoxide dismutase and GSH, inhibited inflammatory cytokines such as IL1β, IL6, and TNFα, and increased anti-inflammatory IL10. The diet protected gut barriers by increasing claudin-1, occludin, and ZO-1 levels in the colon's tight junctions (Wu et al. 2023). Similar prebiotic and anti-inflammatory effects of freeze-dried broccoli decreased inflammation, histologic injury score, and pathogenic bacteria such as *E. coli* and *Enterococcus* in the *mdr1a-/-* mice model which lack a multiple drug resistance gene and spontaneously develop colitis. A 10% broccoli diet induced beneficial cecal microbial communities which increased SCFA richness, such as butyric and propionic acids (Paturi et al. 2012).

Raw broccoli extract did not alleviate inflammation and oxidative stress in DSS-mice (Mueller et al. 2013), but interestingly, broccoli juice decreased serum IL8 levels to alleviate

intestinal and systemic inflammation in DSS-mice (Samuel et al. 2015), again highlighting the importance of preparation's effect on symptoms. Meanwhile, BDN increased AMPK to inhibit DCs and decreased mucosal inflammatory score. The colon level of IL10 increased, but TNFα, IL17A, and interferon-gamma (IFN-γ) were decreased in DSS-mice. The study highlighted the role of SFN in activating AMPK and transforming DCs from immunogenic types to regulatory cells in the DSS and Rag1-/- mice (Deng et al. 2017).

Meanwhile, broccoli sprouts showed better effects against IBD due to higher GLR and SFN content. A steamed broccoli sprouts diet inhibited TNFα, IL1β, and IL6 and promoted bacteria richness such as *Bacillota (formerly Firmicutes)*, *Pseudomonadota (formerly Proteobacteria)*, *Bacteroides*, and *Verrucomicrobiota* against dysbiosis and IBD in DSS-mice (Holman et al. 2023). Microbial hydrolases and commensal microbiota promoted the beneficial effects of SFN (Li Y, Zhang T, Holman J, et al. 2022) and SCFA production and decreased colonic ulceration (Zhang et al. 2023). Interestingly, eating broccoli sprouts early in life can improve microbiota richness. The study used IL10-knockout mice as a CD model and found higher serum SFN and microbial diversity in young mice than in adult mice. The diet decreased IBD symptoms, such as diarrhea, fecal blood, and pathobiont bacteria like *E. coli* and *Helicobacter*, to alleviate dysbiosis and inflammation (Holcomb et al. 2023).

1.3.3.2 Other cruciferous vegetables

Brussels sprouts exhibited prebiotic effects similar to broccoli sprouts, as reported in human microbiota-associated rats with improved gut microbiota diversity with increased commensal bacteria such as *Bifidobacterium* and *Lactobacillus* (Humblot et al. 2005) and β-glucuronidase for SCFA production against intestinal toxicity and oxidative stress (Humblot et al.

2004). Meanwhile, broccoli sprouts and bok choy increased the expression of antioxidants such as Nrf2, NADH-Quinone oxidoreductase 1, Gstm1, Srxn1, and GPx2 to improve colitis symptoms (Lippmann et al. 2014).

Red cabbage alleviated colitis and intestinal inflammation by decreasing IL6, IL1β, TNFα, iNOS and COX-2 levels in DSS-mice (Kim et al. 2018). The juice extracts increased microbiota diversity and *Bacteroidetes/Firmicutes* ratio due to the richness of cyanidin, a major flavanol from crucifiers (Wu et al. 2023). Interestingly, *Lactobacillus* isolated from Chinese cabbage increased IL10 anti-inflammatory activity against colitis in DSS-mice (Jo et al. 2016). Kale exhibited similar anti-inflammatory and prebiotic effects in DSS mice. Freeze-dried powder of kale improved DAI scores and restored colon length. The diet also decreased inflammatory score, NFkB, IL6, IL1β, TNFα levels, and macrophage infiltration marker F4/80. Interestingly, kale promoted gut barrier integrity by increasing claudin-1 and occludin, decreased pathogenic *Pseudomonadota (formerly Proteobacteria)* such as *E. coli* and *Enterobacter* by 13%, and increased beneficial *Bacillota (formerly Firmicutes)* (Raychaudhuri et al. 2023).

Radish has antioxidant, anti-inflammatory, and prebiotic benefits to alleviate IBD. Water extract of radish (RWE) reversed colitis and decreased the rats' body weight, colon length, DAI, and inflammatory damage scores. The diet suppressed inflammation and epithelial adhesion molecules by decreasing IL1β, TNFα, iNOS, NFkB, monocytes chemoattractant protein-1, and ICAM-1 levels in the colon. The RWE increased GSH and decreased malondialdehyde levels to attenuate oxidative stress (Choi et al. 2016). Comparably, ethanol extract of radish decreased colon atrophy, inflammation, and prostaglandin E-2 platelet aggregation and improved microbiota diversity by increasing *Bacteroidota (formerly Bacteroidetes)* and *Akkermansia* spp. and decreasing *Pseudomonadota (formerly Proteobacteria)* such as *E. coli* and *Enterobacter* spp. The

high content of sinapic acid, a polyphenol, was associated with the benefits of radish (Kim et al. 2021). In contrast, the anti-inflammatory effect of fermented black radish was attributed to the presence of α-linolenic acid and omega-6 acid (Kim et al. 2020).

Virginia pepperweed alleviated colitis and inflammation in the 2,4-dinitrobenzene sulfonic acid (DNBS) colitis rat. Its ethanolic extract was effective against IBD when administered intraperitoneally (i.p) or orally. However, only the i.p treatment completely restored weight loss and diarrhea and reversed intestinal damage by decreasing ulcers, edema, and inflammatory cells. Interestingly, both i.p and oral treatments alleviated bloody stool, while only the i.p decreased inflammatory levels of MPO, CXCL2, IL1β, and TNFα (Cruz-Muñoz et al. 2022). This study established the differential benefits of the two administration routes and the need to investigate further the various routes of cruciferous intervention in IBD studies.

A wasabi diet decreased colon inflammatory cells and serum TNFα levels to alleviate intestinal and systemic inflammation. Additionally, the diet reduced IBD-associated pain and anxiety behaviors in mice due to high sinapic acid content (Park et al. 2017). Similar effect was observed with sinigrin, a polyphenol from wasabi, inhibited NFkB and decreased inflammatory IL1β and TNFα levels. Interestingly, the compound also repaired the colonic barrier and increased the tight junction protein, ZO-1 (Kang et al. 2017). In conclusion, these in vivo studies suggest that cruciferous vegetables possess prebiotic, anti-inflammatory, antioxidant, and gut barrier protective benefits in animal models of IBD based on the preparation method, bioactive compounds, dosage, and treatment period (See Table 1.2, Figure 1.1 & 1.2).

1.3.4 Clinical studies

There are few clinical studies in the literature; here we highlight the effects of various crucifer preparations on metabolites' bioavailability and potential antioxidant, anti-inflammatory, and prebiotic benefits, as shown in Table 1.3 and Figure 1.1.

1.3.4.1 Broccoli and broccoli sprouts

In a randomized crossover clinical study, fresh and frozen broccoli soup had differential SFN bioavailability and metabolism. Healthy subjects who ate the fresh broccoli soup had ten times more SFN in their plasma and urine than those who ate frozen diets (Saha et al. 2012). Interestingly, the fresh broccoli soup had higher erucin and SFN conjugates in urine and fecal samples, which may be due to the gut microbial metabolism of GLR (Saha et al. 2012). Similarly, participants who ate cooked broccoli with mustard seed powder had increased urine content of SFN and SFN-NAC four times more than broccoli alone (Okunade et al. 2018) due to exogenous myrosinase from mustard powder.

A blinded crossover study on broccoli sprouts and supplements revealed differential SFN and ERN bioavailability (phase 1: 150 μmoles glucoraphanin and 71 μmoles glucoerucin; phase 2: 121 μmoles glucoraphanin and 40 μmoles glucoerucin) (Clarke et al. 2011). Participants who ate cooked sprouts showed maximum SFN and ERN levels in their plasma and urine after 3 and 6 hours, respectively, while those who ate supplements had SFN and ERN peaks after 6 and 12 hours (Clarke et al. 2011). Participants who ate sprouts had higher urine and plasma levels of SFN-Cys, SFN-NAC, ERN-Cys, and ERN-NAC. Meanwhile, supplements increased urine SFN/ERN ratio more than cooked sprouts, which may indicate more benefits (Clarke et al. 2011). Similarly, participants who ate myrosinase-treated broccoli sprouts had maximum urine and plasma SFN

levels 3-6 hours ahead of those who had cooked broccoli, again due to the immediate availability of SFN in raw or treated sprouts but the reliance on gut microbes to produce SFN in the colon using cooked sprouts. SFN-Cys and SFN-CG were majorly expressed at 3-6 hours, while SFN-NAC was abundant after 12 hours. However, two doses of sprouts diet at 12-hour intervals had three times more SFN than a single dose per day. Meanwhile, broccoli sprouts, and myrosinase-treated diets increased HDAC and HQ1 antioxidant levels against DNA oxidation at two-dose consumption. Only the sprouts diet increased p21 gene expression, which activates the Nrf2 antioxidant response. These studies revealed the importance of preparation, dose, and time on broccoli's benefit against oxidative stress (Atwell et al. 2015).

1.3.4.2 Combined effect of crucifers

In a randomized crossover study, ten healthy adults who consumed 200g of cooked broccoli and 20g of fresh winter radish for 17 days showed microbial diversity in their feces and metabolite diversity in their urine samples. The broccoli diet increased participants' urinal *Bacteroidetes/Firmicutes* ratio by 37% to promote gut diversity with increased GSL metabolites, especially in subjects with low BMI <26 kg/m2, but the control diet decreased it by 5%, (Kaczmarek et al. 2019). Broccoli consumption also increased *Bacteroides* spp. by 8%, which is necessary for GSL microbial interconversion to ITCs (Kaczmarek et al. 2019). A similar study reported a significant increase in plasma and urine metabolites of SFN and ERN after 4-18 hours of consuming 200g of cooked broccoli diet (providing 147.6 μmol of glucoraphanin and 3.6 μmol of glucoerucin) in individuals with high BMIs >26 kg/m2 compared with individuals with low BMIs (Charron et al. 2020). Another randomized crossover study examined the prebiotic effect of broccoli sprouts, radish sprouts, cauliflower, and cabbage as basal diets of low fruits and

vegetables; a single diet of 7g cruciferous vegetables; a double diet of 14g cruciferous vegetables; a mixed diet of 7g cruciferous and 4g apiaceous vegetables (Li et al. 2009). The double cruciferous diets had higher SFN levels associated with beneficial gut microbiota such as *Bacteroidota (formerly Bacteroidetes)* to *Bacillota (formerly Firmicutes)* ratio and *Actinobacteria* in healthy subjects (Li et al. 2009). A similar study revealed a differential prebiotic effect of low (0.16 mmol GSLs) and high (2.7 mmol GSLs) broccoli and cauliflower diets. Participants who ate a high broccoli diet had microbial diversity with increased *Lactobacillus species,* which may help protect against intestinal damage. In contrast, subjects who consumed low broccoli or only a cauliflower diet had no differential beta diversity (Kellingray et al. 2017), perhaps due to the low serving levels.

Consuming crucifers such as broccoli, cabbage, cauliflower, Chinese cabbage, arugula, watercress, horseradish, mustard sauce, and wasabi may have both beneficial and adverse effects in Crohn's patients, via the expression of genes associated with oxidative stress, inflammation, and gut barrier damage (Laing et al. 2013). This study, conducted in New Zealand, reported interesting correlations between gene expression and self-reported adverse effects of crucifers, including broccoli diet and GPX3, IL23R, TNFα, and OCTN1/2; cabbage and GPX2, GPX3, cadherin-29, ICAM1, signal-transducer and activator of transcription-3, IL23R, IL12B, TLR9 and claudin-12; cauliflower and GPX3, TNFSF15, cadherin-29, Janus kinase-2, nucleotide-binding oligomerization domain-2 and OCTN1/2; Chinese cabbage and IL23R and claudin-12; rocket arugula with IL6, ICAM1, GPX3 and claudin-2; watercress and ICAM1 and TNFSF15; mustard greens and IL6, ICAM1, nucleotide-binding oligomerization domain-2, GPX3, GPX2, IL12B, TNFSF15 and OCTN2; horseradish and OCTN1/2; and wasabi correlated with IL12B and OCTN2 (Laing et al. 2013). For example, GPX3, a gene with protective effect against oxidative stress, had

beneficial effects with broccoli and cauliflower diets but had an adverse effect with mustard sauce. Meanwhile, NOD2, a gene important for bacterial inflammatory response, had beneficial effects with mustard powder but had an adverse effect with cauliflower. Similarly, CDH29, a gene important for barrier junction organization, had a beneficial effect with cabbage but an adverse effect with cauliflower. This study revealed both positive and negative nutrigenomic effects of crucifers and the need for adequate experimental measures in cruciferous and IBD intervention. Further information, such as dietary data, lifestyle, specific dose, and preparation of the vegetables, could be more helpful in understanding the gene-diet relationship. In conclusion, the clinical studies suggest the importance of crucifers' preparation method, presence of bioactive compounds, dosage, and treatment period on their respective prebiotic, anti-inflammatory, and antioxidant benefits in healthy and CD patients (See Table 1.3, Figure 1.1 & 1.2).

1.3.5 The role of metabolomics tools in cruciferous dietary IBD treatment

Metabolomics analysis is an effective tool for exploring dietary interactions with genetics, environment, gut microbiota, and signaling pathways (Ho et al. 2019; Barnett et al. 2014; Tiffany et al. 2021). Analyzing metabolite concentrations can identify pathophysiological pathways relevant to clinical IBD treatment (Gallagher et al. 2021). A metabolomics profiling showed a positive correlation between dietary metabolites and the pathogenic expression of microbes: there were increased amino acids and Pseudomonadota (formerly proteobacteria) species and decreased riboflavin and Faecalibacterium species in children with CD dysbiosis compared with non-dysbiosis children (Heinken et al. 2021).

Existing metabolomics have demonstrated the importance of dietary metabolites derived from cruciferous vegetables as modulators against IBD. For example, untargeted metabolomics

revealed the differential expression of antioxidants and polyphenolic metabolites in raw and lactic acid bacteria (LAB)-fermented broccoli puree, using two strains of LABs, *Lactiplantibacillus plantarum* (LAB1) and *Lactiplantibacillus pentosus* (LAB10), relevant in phytochemical metabolism, which were isolated from fermented broccoli (Hou et al. 2023). The LAB-fermented broccoli had more antioxidant activities than the unfermented broccoli, after 60 hours, with 20% increase in DPPH activities and 70% increase in FRAP and ABTS capacities. Also, the LAB-fermented broccoli had abundant phenolic metabolites after 24 and 48 hours, especially tryptophan and kaempferol (Hou et al. 2023). Tryptophan alleviated clinical IBD symptoms of fatigue and inflammation (Truyens et al. 2022), and kaempferol upregulated anti-inflammatory, antioxidants, and barrier protection markers (Bian et al. 2020). This metabolomic analysis suggests that LAB fermentation may improve phenolics bioavailability and antioxidant benefits of broccoli intervention against IBD (Hou et al. 2023).

A comprehensive metabolomic and microbiota profiling revealed an important correlation between indole-3-carbinol, glucobrassicin metabolite, *Roseburia species*, a butyrate-producing bacteria and IL22, an anti-inflammatory cytokine, for gut protection against colitis (Busbee et al. 2017). Interestingly, decreased butyrate-producing bacteria, such as *Faecalibacterium* and *Roseburia species*, have been identified in humans suffering from constipation (Zhuang et al. 2019). Human fecal samples co-cultured with digesta from broccoli and brussels sprouts revealed upregulated dietary metabolites. Metabolites, phenolic acids, and flavonoids, such as azelaic acid, sinapic acids, suberic acids, coumaric acids, kaempferol, flavonoid-O-3-glucosides, and indole acetic acids, showed positive relationships with both beneficial and detrimental microbiota (Bouranis et al. 2022). *Bifidobacterium* spp. were increased, which helps GLR microbial

transformation to SFN, while the *Intestinibacter and Clostridiaceae* genera were mostly unannotated (Bouranis et al. 2022).

It is essential to employ appropriate metabolomics analysis to efficiently identify metabolites with low sensitivity. For example, untargeted metabolomics identified the presence of glutathione metabolites in healthy human plasma samples, which was associated with the antioxidant benefits of the broccoli diet (Atwell et al. 2015). However, glutathione and its precursors, glutamine and cysteine, were transiently downregulated at 6 and 12 hours after the broccoli diet (Housley et al. 2018), while fatty acid metabolites associated with lipid metabolism were consistently downregulated (Housley et al. 2018). There is a need for further investigation into this physiological effect of the broccoli diet against intestinal lipid peroxidation. Untargeted metabolomics did not reveal SFN and its metabolites in the urine of individuals who consumed 200g of raw broccoli (Sun et al. 2020). However, target metabolomics identified SFN, SFN-CYS, SFN-NAC, SFN-GSH, and other metabolites associated with SFN precursors mercapturic pathway in the same samples (Sun et al. 2020). Similarly, a sensitive targeted LC/MS metabolomics method quantified plasma and urine SFN in humans after eating a 200g broccoli diet. The authors synthesized SFN and mercapturic metabolites in the lab. They identified increased SFN, SFN-CYS, SFN-NAC, and SFN-GSH in specific quantities during intraday and interday plasma and urine measurements compared with control after the four-week broccoli diet (Hauder et al. 2011). Therefore, metabolomics profiling may be a significant tool to better understand the importance of dietary crucifers for gut health.

1.3.6 Literature gaps, limitations and perspectives for future work

The central gap in this literature review highlights the need for substantial publications on cruciferous vegetables' translational and clinical effects on IBD management, as searching for clinical IBD studies generated articles primarily about immunosuppressant interventions and steroid therapies. Nutritional researchers may find it challenging to recruit IBD participants for studies if the medical institutions do not appreciate nutritional management of IBD with fibers such as cruciferous vegetables. Enlightening clinicians about the potential results of crucifers in IBD management by highlighting specific pathways of action would further encourage more participation in clinical studies. Proper documentation of cruciferous methods of preparation, extraction of biochemicals, dosage, application, and mechanisms of action in vitro and in vivo models will further encourage clinical studies and provide adequate information to the public and clinicians about the potential benefits of cruciferous consumption in IBD management.

1.4 Conclusion

This review highlights cruciferous vegetables and phytochemicals with potential antioxidants, anti-inflammatory, and prebiotic effects that may prevent or alleviate IBD. Although many studies suggest that GSLs, ITCs, polyphenols, and flavonoids may benefit IBD patients, there is a limited report on the mechanisms of polyphenols and flavonoids. Nonetheless, this information shows that flavonoids and phenolic compounds have beneficial properties that may work with GSL-derived SFN and conjugates to restore gut health and prevent IBD. The data in this review suggests broccoli and sprouts are the most widely studied crucifers with high bioactive compounds associated with the most reported IBD benefits. Future research should carefully consider diet preparation, dose and mode of administration, and intervention timeline as crucial factors that could impact crucifers' benefit in IBD research. Metabolomics is one of the significant

tools currently being explored to identify and correlate the influence of dietary intervention on diseases. We recommend annexing metabolomics tools to investigate critical metabolites from cruciferous vegetables and their association with IBD pathways. This holistic approach may yield groundbreaking results for the clinical development of cruciferous vegetables as supplements and treatment recommendations for IBD patients.

Table 1.1: Potential benefits of Cruciferous vegetables in IBD in vitro models.

Cruciferous vegetables	**Image**	**Bioactive compounds**	**Preparation**	**Dose**	**Treatment period**	**Pathways**	**Benefits**	**Model**	**References**
purified SFN	NA	SFN	purified SFN	10,1 and 0.1 µmol/L	N.R.	IL10 activation of STAT3	Anti-inflammatory	Bone marrow derived macrophages	(Sun et al. 2022)
Broccoli		SFN, lipids	Edible nanoparticles	N.R., Average diameter of 32.4 nm	2 and 3 hrs	CCRs, CXCLs and CCLs	Anti-inflammatory	DCs and monocytes	(Deng et al. 2017)
		Glucoraphanin, Glucobrassicin, Neoglucobrassicin	Nano-vesicles	5 and 22 µg/mL	24 hrs	Hydrogen peroxide	Anti-oxidative	Caco-2 and NCI-H441 cells	(Hossain et al. 2022)
		SFN	N.R.	100, 50, 20,10 and 1 µmol	24 hrs	COX-2 and iNOS, IL10 and CD36	Anti-inflammatory	Immortalized human blood monocytes	(Pal and Konkimalla 2016)
		Glucoraphanin, Glucoerucin, Quercetin-3-glucoside	Cold pressed seed	0.1% (v/v)	0, 2, 4, 5, and 6 hrs time point	*Bacteroidota/Bacillota* ratio; ORAC, HOSC and ABTS scavenging activities. COX-2 and IL1β cytokines	Antioxidant, Prebiotic, Anti-inflammatory	J774A.1 macrophages	(Choe et al. 2018)

Table 1.1 continued

Green cabbage and kimchi		Polyphenols	Freeze dried	0.2, 0.5, 1 and 2 mg/ml	24 hrs	MCP-1, IL1β and TNFα, E. coli, S. aureus	Probiotic (kimchi), Anti-inflammatory	LPS-macrophages	(Jeong et al. 2023)
Cauliflower		Polyphenols, Flavonoids	Ethanolic, hot and cold-water extract	1% and 50%(w/w)	24 hrs	DPPH, Lactobacilli	Antioxidant, Prebiotic	*Lactobacilli strains*	(Bhatt et al. 2020)
Kale		SFN, Beta Carotene	Ethanolic extract	100 µg/mL	28 hrs	IL6 and TNFα	Anti-inflammatory	LPS-macrophages	(Ayaz et al. 2008)
		Polyphenols: Ferulic and Caffeic acids	Frozen powder	100 µL	18 hrs	DPPH, *E. coli* and *S. aureus* suppression	Antioxidant, Antimicrobial	Microorganisms	(Raychaudhuri et al. 2023)
		Total Polyphenol and Vitamins C	Freeze dried powder and *in vitro* digestion	27mg /100g of polyphenols	18 hrs	Catalase, GSH and SOD	Antioxidant	Caco-2, HT-29-MTX and THP-1 cells	(Kaulmann et al. 2016)

Table 1.1 continued

Radish		Polyphenols	Chloroform, hexane, butanol, ethyl acetate and water-soluble fractions	0, 1, 10, 100 and 200 µg/mL	48 hrs	PGE2, NFkB, COX-2 and iNOS, TNFα,	Anti-inflammatory	LPS-macrophages	(Park and Song 2017)
Radish Sprouts		Polyphenol: sinapic acid	Ethanol extract	10, 25, 50 and 100µg/mL	4 hrs	NFkB, p-IkBα, IkBα, p65, iNOS, IL1β and TNFα	Anti-inflammatory	LPS-macrophages	(Kim et al. 2021)
Wasabi		Polyphenol: sinigrin acids	Ethanolic extract	100 and 500 µg/mL	18 hrs	NFkB, IL1β, TNFα, ZO-1, and occludin	Anti-inflammatory and barrier repair	Caco-2 cells and LPS-macrophages	(Kang et al. 2017)

This review suggests that the effects of cruciferous vegetables depend on the preparation and presence of bioactive compounds, which can alleviate inflammation, oxidative stress, dysbiosis, and gut barrier damage pathways during in vitro inflammatory bowel disease models. NA means not available. N.R means data not reported in the article

Table 1.2: Potential benefits of Cruciferous vegetables in IBD in vivo models.

Cruciferous vegetables	Image	Bioactive compounds	Preparation	Dose	Treatment period	Pathways	Benefits	Model	References
purified SFN	NA	SFN	purified compound	10, 20 and 40 mg/kg	14 days	IL10 activation of STATs	Anti-inflammatory	DSS mice	(Sun et al. 2022)
purified SFN	NA	SFN	purified compound	2.5, 5, 10 and 20 mg/kg	19 days	IL6 and TNFα, STAT3 and Nrf2, Bifidobacterium and Lactobacillus spp.	Prebiotic, Anti-inflammatory	DSS mice	(He et al. 2022)
Broccoli		SFN, GLR	purified	600 ppm	4 wks	IL1β and IL18, AMPK and PGC-1α, Nrf2, HO-1, 8-OHdG	Anti-inflammatory, Antioxidant	DSS mice	(Tian et al. 2022)
		SFN	Raw and slightly cooked	10%(w/w)	2 wks	IL6, CCR2 and VCAM-1	Anti-inflammatory	DSS mice	(Wang et al. 2018)
		GLR, ITCs	Freeze dried	4.1 mg/g	7 days	NQO1	Antioxidant	C57BL/6 mice	(Wu et al. 2019)
		SFN	Seed extract	370 mg/kg/day	2 wks	Bifidobacterium spp., Nrf2 signaling	Prebiotic, Anti-inflammatory	DSS mice	(Wu et al. 2023)

Table 1.2 continued

		Glucobrassicin	Freeze dried	15%(w/w)	24 days	AHR, Cyp1a1, IL1β, IL10, IL6, Ptgs and CXCL5, increased Actinobacteria and Alistipes	Prebiotic, Anti-inflammatory	Ahrb/b and Ahrd/d mice	(Hubbard et al. 2017)
		GLR, SFN and SFN-NAC	Raw seed extract	370mg/kg/day	2 wks	IL1β, IL6 and TNFα, IL10, SOD, GSH, MDA, claudin-1, occludin and ZO-1, Bifidobacterium and Alistipes, SCFAs	Prebiotic, Anti-inflammatory, Antioxidant	DSS mice	(Wu et al. 2023)
		N.R.	Freeze dried	10%(w/w)	21 wks	histologic injury score, E. coli and Enterococcus spp.	Prebiotic, Anti-inflammatory	mdr1a-/- mice	(Paturi et al. 2012)
		SFN	Crude extract	4%	12 days	NFkB, TNFα, IL10, MCP-1 and COX 2	No significance	DSS-rat	(Mueller et al. 2013)
		N.R.	Juice	1.5mL	7 days	IL8	Anti-inflammatory	DSS mice	(Samuel et al. 2015)

Table 1.2 continued

		SFN, Lipids	Edible nanoparticles	Average diameter 32.4nm	10 and 13 days	AMPK, IL10, TNFα, IL17A and IFN-γ	Anti-inflammatory	DSS mice	(Deng et al. 2017)
		SFN, Lipids	Edible nanoparticles	Average diameter 32.4nm	10 days	TNFα, IL6 and IL23	Anti-inflammatory	Rag1-/- mice	(Deng et al. 2017)
Broccoli sprouts		GLR, SFN	Steamed and freeze dried	10%(w/w)	34 days	Bacillota, Pseudomonadota, Bacteroidota and Verrucomicrobiota; TNFα, IL1β and IL6	Prebiotic, Anti-inflammatory	DSS mice	(Holman et al. 2023)
		SFN, GLR	Streamed	5%(w/w)	13 days	microbial hydrolase, Bacillota	Prebiotic	DSS mice	(Zhang et al. 2023)

Table 1.2 continued

		SFN	raw	10%(w/w)	2 wks	E. coli and Helicobacter	Prebiotic, Anti-inflammatory	IL10-KO mice	(Holcomb et al. 2023)
Broccoli sprouts and seed		GLR, Glucoiberin, Glucoerucin, SFN-lysine	powder	100mg	4 wks	Nrf2, NQO1, Gstm1, Srxn1 and GPx2	Anti-inflammatory and antioxidant	DSS mice	(Lippmann et al. 2014)
Brussels Sprouts		GLR	Cooked and freeze dried	10%(w/w)	4 wks	Bifidobacterium and Lactobacillus	Prebiotic	human microbiota associated rats	(Humblot et al. 2005)
		GLR	cooked	10%(w/w)	4 wks	B-glucuronidase, SCFA	Antioxidant, Prebiotic	human microbiota associated rats	(Humblot et al. 2004)
Bok Choy		Glucobrassicin, gluconapin, Progoitrin	powder	100mg	4 wks	AHR, Cyp1a1, Nrf2, NQO1, Gstm1, Srxn1 and GPx2	Anti-inflammatory and antioxidant	DSS mice	(Lippmann et al. 2014)

Table 1.2 continued

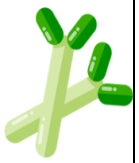
Red Cabbage		Cyanidin 3,5-diglucoside	Freeze dried powder	5% (w/w)	2 wks	IL6, IL1β, TNFα, iNOS, and COX-2	Anti-inflammatory	DSS mice	(Kim et al. 2018)
Kale		SFN, Beta Carotene	Freeze dried powder	4.5% (w/w)	2 wks	NFkB, IL6, IL1β and TNFα, claudin-1 and occludin, Bacillota	Prebiotic, Anti-inflammatory	DSS mice	(Raychaudhuri et al. 2023)
Radish		Polyphenol: sinapic acid	Water extract	10, 40, 70 and 100 mg/kg	7 days	GSH, MDA, IL1β, TNFα, iNOS, NFkB, MCP-1 and ICAM-1	Antioxidant, Anti-inflammatory	DSS and TNBS- Rat	(Choi et al. 2016)
Radish Sprouts		Polyphenol: sinapic acid	Ethanol extract	10, 50 and 100 mg/kg	14 days	NFkB, COX-2, iNOS, IL6, TNFα and PGE2, Bacteroidota, Pseudomonadota	Anti-inflammatory, prebiotic	DSS mice	(Kim et al. 2021)

Table 1.2 continued

Black Radish		α-linolenic acid	Fermented	30 and 60 mg/kg	7 days	Histologic injury and colitis score	Anti-inflammatory	DSS mice	(Kim et al. 2020)
Virginia pepperweed		Polyphenols Flavonoids	Ethanolic extract	3, 30, and 100 mg/kg	4 days	MPO and CXCL2, IL1β and TNFα	Anti-inflammatory	DNBS- rat	(Cruz-Muñoz et al. 2022)
Wasabi		Sinapic acid and ITCs	Ethanolic extract	100, 200 and 400 mg/kg	14 days	TNFα, Anxiety and pain test.	Anti-inflammatory	Zymosan-mice	(Park et al. 2017)
		Polyphenol: sinigrin acids	Ethanolic extract	100 and 500 µg/mL	7 days	NFkB, IL1β, TNFα and ZO-1	Anti-inflammatory	DSS mice	(Kang et al. 2017)

This review suggests that the effects of cruciferous vegetables depend on the preparation and presence of bioactive compounds, which can alleviate inflammation, oxidative stress, dysbiosis, and gut barrier damage pathways during in vivo inflammatory bowel disease models. NA means not available. N.R means data not reported in the article.

Table 1.3: Potential benefits of Cruciferous vegetables in IBD clinical studies.

Cruciferous vegetables	Image	Bioactive compounds	Preparation	Dose	Treatment period	Pathways	Benefits	Subjects	References
BroccoMax supplement pill	NA	SFN, ERN, GLR, Glucoerucin	Supplement	Six supplement pills	48 hours	SFN, SFN-CG and ERN-CG	SFN and ERN metabolites bioavailability	Healthy participants	(Clarke et al. 2011)
Broccoli		SFN, GLR	Fresh and Frozen	100g	5 days	SFN, glutathione, SFN-CG, SFN-Cys and SFN-NAC	SFN, ERN and SFN conjugates bioavailability	Healthy participants	(Saha et al. 2012)
		SFN, SFN-NAC	cooked	200g	24 hours	SFN-NAC	SFN metabolites bioavailability	Healthy participants	(Okunade et al. 2018)
		GSL metabolites	cooked	200g	18 days	Bacteroidota/ Bacillota ratio	Prebiotic	Healthy participants with high and low BMI	(Kaczmarek et al. 2019)
		GSL metabolites	cooked	200g	17 days	SFN-Cys, SFN-NAC, SFN-GSH, ERN-Cys, ERN-GSH, ERN-NAC	SFN and ERN metabolites bioavailability	Healthy participants with high and low BMI	(Charron et al. 2020)

Table 1.3 continued

		GSL metabolites	Frozen and steamed	300g and 84g	2 wks	Lactobacillus species, microbial diversity	Prebiotic	Healthy participants	(Kellingray et al. 2017)
		N.R.	N.R., self-reported consumption	N.R., self-reported consumption	N.R., self-reported consumption	GPX3, IL23R, TNFα, and OCTN1/2	oxidative stress, inflammation, gut barrier	CD patients	(Laing et al. 2013)
Broccoli sprouts		SFN, ERN, GLR, Glucoerucin	Freeze dried,	40 g	48 hours	SFN-Cys, SFN-NAC, ERN-Cys and ERN-NAC	SFN and ERN metabolites bioavailability	Healthy participants	(Clarke et al. 2011)
		SFN, SFN-Cys, SFN-CG, and SFN-NAC	Fresh or myrosinase treated extract	200 μmol/day	24 hrs	P21, HDAC and HQ1	Antioxidant	Healthy participants	(Atwell et al. 2015)
		SFN	cooked	45.7% (w/w)	14 days	Bacteroidota, Bacillota, Actinobacteria	Prebiotic	Healthy participants	(Li et al. 2009)

Table 1.3 continued

Cabbage		SFN	raw	15.7% (w/w)	14 days	Bacteroidota, Bacillota, Actinobacteria	Prebiotic	Healthy participants	(Li et al. 2009)
		N.R.	N.R., self-reported consumption	N.R., self-reported consumption	N.R., self-reported consumption	GPX3, GPX2, cadherin-29, ICAM1, STAT3, IL23R, IL12B, TLR9 and claudin-12	oxidative stress, inflammation, gut barrier	CD patients	(Laing et al. 2013)
Chinese cabbage		N.R.	N.R., self-reported consumption	N.R., self-reported consumption	N.R., self-reported consumption	IL23R and claudin-12	inflammation, gut barrier	CD patients	(Laing et al. 2013)
Cauliflower		SFN	cooked	36.4% (w/w)	14 days	Bacteroidota, Bacillota, Actinobacteria	Prebiotic	Healthy participants	(Li et al. 2009)
		GSL	Frozen and steamed	84 g	2 wks	microbial diversity	Prebiotic	Healthy participants	(Kellingray et al. 2017)

Table 1.3 continued

		N.R.	N.R., self-reported consumption	N.R., self-reported consumption	N.R., self-reported consumption	GPX3, TNFSF15, cadherin-29, Janus kinase-2, NOD2 and OCTN1/2	oxidative stress, inflammation, gut barrier	CD patients	(Laing et al. 2013)
Horseradish		N.R.	N.R., self-reported consumption	N.R., self-reported consumption	N.R., self-reported consumption	OCTN1/2	gut barrier	CD patients	(Laing et al. 2013)
Mustard green		N.R.	Powder and sauce	N.R., self-reported consumption	N.R., self-reported consumption	IL6, ICAM1, NOD2, GPX3, GPX2, IL12B, NOD2, TNFSF15 and OCTN2	oxidative stress, inflammation, gut barrier	CD patients	(Laing et al. 2013)
Radish sprouts		SFN	raw	4% (w/w)	14 days	Bacteroidota, Bacillota, Actinobacteria	Prebiotic	Healthy participants	(Li et al. 2009)

Table 1.3 continued

Rocket arugula		N.R.	N.R., self-reported consumption	N.R., self-reported consumption	N.R., self-reported consumption	IL6, ICAM1, GPX3 and claudin-2	oxidative stress, inflammation, gut barrier	CD patients	(Laing et al. 2013)

This review suggests that the effects of cruciferous vegetables depend on the preparation and presence of bioactive compounds, which can alleviate inflammation, oxidative stress, dysbiosis, and gut barrier damage pathways during clinical inflammatory bowel disease studies. NA means not available. N.R means data not reported in the article.

CHAPTER 2

EFFECT OF CRUCIFEROUS CONSUMPTION ON HEALTHY EATING INDEX AND OVERALL NUTRITIONAL QUALITY IN DIET HISTORY ASSESSMENT OF HEALTHY AND SELF-REPORTED IBD SUBJECTS.

2.1 Abstract

Vegetables, fibers, and fruits are major food components that can increase the healthy eating index score, microbiome diversity and produce beneficial metabolites in the human gut. Glucosinolates are plant metabolites derived from cruciferous vegetables, such as broccoli, from which *Bacteroides thetaiotaomicron* in th4 mammalian gut can hydrolyze through at least one gene of the *BT2160-2156* operons to produce isothiocyanates, a microbial-derived metabolite, with anti-inflammatory and antioxidation activities. In this study, we explored the role of self-reported fiber, dark green vegetables, and fruits on healthy eating index, based on daily guidelines for Americans, and the effect of steamed broccoli sprouts intervention on the expression of BT genes in humans. These results show increased individualized diet-microbiome interaction before, at the end and after the broccoli sprouts intervention with upregulated BT genes in young and middle-aged persons who self-reported gut problems and consumed prior daily values of fibers, dark green vegetables, and fruits above the daily guidelines for Americans' recommendation. Our work provides valuable information about that compliance to healthy eating guidelines and individualized diet-microbial gene responsiveness as measures for future research to explore the benefits of steamed broccoli sprouts and other dark green vegetables, such as crucifers as potential nutritional management for persons with IBD conditions.

2.2 Introduction

Dietary intake is a primary determinant of individuals' well-being and the probability of pathological development (World Health Organization 2003; Conlon and Bird 2014; National Academy of Sciences and Inst...). The type, amount, and diversity of food choices are some of the main factors associated with health quality and human security (Edelstein 2022; National Research Council et al. 2006). Nutritional surveys reported associations between Inflammatory Bowel Diseases (IBD) risk and high consumption of sugar, fat, and meat protein diets (Han et al. 2020; Xu et al. 2021; Talebi et al. 2023; Basson et al. 2020), as these may alter the chemical and microbial activity in the gut and act as triggers for people who also have genetic or other environmental risk factors for IBD. According to a report, consuming animal protein such as red and white meat had a 40% positive correlation with IBD development (Talebi et al. 2023). A similar correlation between energy drinks, particularly regular soda, has been reported in adults (Han et al. 2020; Xu et al. 2021). Other reports revealed a positive correlation between the consumption of diets rich in fat, dairy products, and sugar among teenagers and young adults (Li et al. 2020; Tayyem et al. 2021; Khorshidi et al. 2020; Rashvand et al. 2018). High consumption of dietary saturated fats has been implicated in microbial dysbiosis and mucosal inflammation via TLRs and NFkB pro-inflammatory stimulation during chronic bowel disease (Statovci et al. 2017; Basson et al. 2020). Meanwhile, developing a healthy dietary lifestyle with regular consumption of food rich in vegetables, fibers, and fruits could promote cognitive function (Bigman et al. 2023), provide a healthy gut via beneficial microbial byproducts (Visconti et al. 2019), and reduce the risk of IBD development (Reddavide et al. 2018; Lopes et al. 2022) due to the anti-inflammatory and antioxidative phytochemicals (Deng et al. 2023; Alaba et al. 2024).

Recent nutritional research has highlighted vegetables, particularly crucifers and broccoli, as beneficial against IBD in both the young and adult population (Manski et al. 2024; Deas et al. 2024; Shen et al. 2023; Yanaka 2018). Our team and other researchers have reported cruciferous vegetables, consisting of diverse plant species such as broccoli, cauliflower, cabbage, kale, bok choy, brussels sprouts, collards, turnips, and radishes, could promote healthy gut (Yanaka 2018), due to the presence of bioactive phytochemicals such as isothiocyanates, polyphenols, flavonoids and SCFAs (Alaba et al. 2024; Holman et al. 2023; Holcomb et al. 2023) and protect against chronic inflammation, oxidative stress, dysbiosis and IBD risks (Hernandez-Ledesma and Martinez-Villal...; Cicio et al. 2022). Therefore, incorporating crucifers such as broccoli sprouts into the dietary guidelines for healthy and IBD populations might prevent and alleviate bowel diseases. However, many factors could impact people's ability to eat healthily. An individual's choice of food could be influenced by location, residential neighborhood, closeness to farming areas, affordability, workplace, social class, and palatability (Ghenadenik et al. 2018; Townshend and Lake 2017; Lam et al. 2024; Leng et al. 2017). Diets rich in fat and sugar are more appealing and desirable among younger adults than vegetables and fruits due to taste perception (Avena 2015; Montmayeur and le Coutre 2009; Murimi et al. 2016). Food affordability and local agricultural production determine access to organic and fresh vegetables (Segal and Demos 2016; Evans et al. 2015; Ver Ploeg 2010; Clark and Tilman 2017).

Nutritional research utilizes diet scores as one of the primary tools for understanding the role of food choices and individual dietary components on health and nutritional qualities (de Jesus et al. 2024; National Academies of Sciences, Engin...). Diet scores examine dietary patterns and nutrient intake (Van Den Heuvel et al. 2021) to provide relevant information about potential disease development or prevention (Hou et al. 2022; Cohen et al. 2013). A systematic review

revealed three categories of pattern-based dietary scores: (1) dietary variety-based scores, (2) scores derived from the assessment of concordance with nutrient- or food-related dietary guidance, and (3) scores based on an assessment of Mediterranean diet characteristics (Kant 2004). The healthy eating index (HEI) utilizes the 2nd category of dietary score to grade the nutritional components of an individual in compliance with the dietary guidelines for Americans (DGA) recommendation. The USDA has recommended the dietary history questionnaires III (DHQ3) as an online tool for researchers to collect nutritional data through surveys (DHQ3). The DHQ3 generates reports and information, such as health index and nutritional quality, as data on participants responding to the study, which researchers could use to investigate how healthy or poor the diet of individuals or populations corresponds to potential health or pathological status. The results of such assessment could be used to encourage the consumption of healthy food among participants and serve as a recommended dietary intervention to prevent or alleviate pathological conditions (Ruiz Esparza Cisneros et al. 2020; Pool-Zobel et al. 1997; McCullough et al. 2002).

Chronic diseases are associated with poor food quality and a low HEI calculated based on the DGA recommendation. For instance, adults in the US with poor diet quality and low diet scores are at risk of developing chronic diseases, such as obesity, diabetes, and cardiovascular diseases (Fanelli et al. 2020; Ghazaryan et al. 2021; Venci and Lee 2018; Vaccaro and Huffman 2017). Older men are less likely to develop chronic diseases due to a higher HEI (Chen et al. 2011) than women (Vaccaro and Huffman 2017; Weaver and Fasel 2018; Jun et al. 2020). The choice to reduce fatty food and increase dietary fiber has been proven to improve diet scores, improve the HEI, and reduce disease risks, especially among adults (López-Cepero et al. 2023). Awareness about the relationship between healthy food choices and an individual's well-being may guide them to incorporate healthy diets for nutritional management of diseases.

Despite the reports on food choices and their essential role in disease onset, progression, or prevention, there are limited human and clinical studies investigating the importance of vegetables such as broccoli as dietary recommendations for IBD. Cruciferous vegetables, such as broccoli, have been reported to contain phytochemicals that could alleviate inflammation, oxidative stress, dysbiosis, and IBD symptoms in mostly *in vivo* and *in vitro* studies (Alaba et al. 2024). Glucosinolates-derived metabolites, primarily isothiocyanates, have been attributed to the cruciferous diet's antioxidant and anti-inflammatory effects, particularly broccoli (Li et al. 2022; Holman et al. 2023). Upon crucifer consumption, commensal bacteria such as *Bacteroides* spp. can metabolize glucosinolates to produce isothiocyanates for gut health and beneficial activities. *Bacteroides thetaiotaomicron* (*B. theta*) utilizes these five gene codes to form several enzymes. Liou et al. reported that at least one *BT2160-2156* operon is required for *Bacteroides* to hydrolyze glucosinolates into isothiocyanates (Liou et al., 2020). The exact DNA sequence of these five genes is only found together in *B. theta*, although a closely related species, *B. ovatus*, has 2 of the genes but cannot convert glucoraphanin (Liou et al. 2019). However, there is a need for more nutritional research to translate these results into the clinical use of cruciferous and broccoli diets to alleviate human gut diseases such as IBD. Therefore, this study aims to assess the effect of regular self-reported food choices, including vegetables and fibers, on individuals' HEI, nutritional quality, and potential well-being. We used DHQ3 and Qualtrics online survey tools to collect 24-hour recall data from healthy individuals with self-reported IBD or inflammatory bowel syndrome (IBS) symptoms. Also, we examined individual's responsiveness to the benefit of self-reported fiber, fruits and vegetables diet, particularly by quantifying the presence of *Bacteroides thetaiotaomicron, BT 2160-2156* genes, to measure the bacterial hydrolysis of glucosinolates for antioxidant, anti-inflammatory, and gut protective effect of metabolites in participants that

regularly consumed high portions of these foods. We hypothesized that self-reported high consumption of dark green such as cruciferous vegetables or broccoli sprouts would increase dietary HEI score and *Bacteroides thetaiotaomicron* genes in the gut.

2.3 Methods

2.3.1 Participant recruitment

This study was conducted at the University of Maine in Orono, Maine, USA. All the study's protocols, recruitment process, and human handling were approved by the University of Maine's Institutional Review Board and Biosafety Committee (IRB #2022-08-01 and IBC #SI080122). We recruited study participants as healthy adults 18 years old and above by posting fliers at stores, on social media, and local news. Inclusion criteria are abilities to provide information about food consumed in the last year and stool samples. We excluded subjects taking oral antibiotics for two weeks or less before the study, allergic to broccoli sprouts, and pregnant or lactating. Our initial goal was to recruit healthy participants, but some subjects voluntarily reported having pre-existing conditions of IBS, ulcerative colitis, or Crohn's without flare-up symptoms during the study. The participants' first visit to our lab involved the discussion of the consent forms (supplementary file 1), the study's goal and objectives, how to use the tools for the diet history survey and sample stool collection, and the potential benefits and drawbacks of the study.

2.3.2 Study design

This study utilized steamed broccoli sprouts as a potential dietary intervention for healthy individuals with self-reported IBD/IBS symptoms. The preparation of a steamed broccoli sprout diet has been previously described and reported that inactivating plant enzyme, myrosinase, will prevent the hydrolysis of GLR to SFN, making gut microbes the only source of metabolism (Holman et al. 2023). We performed a randomized parallel study from June 2023 to May 2024 for

a preliminary investigation on the effect of self-reported cruciferous consumption on HEI and nutritional quality. Also, we investigated if steamed broccoli sprouts as a dietary intervention could increase the presence of bacteria genes, *BT 2160-2156* operon, which encode enzymes that can convert GLR to SFN (Liou et al. 2019; Holman et al. 2023).

On Day 0 of the study, we obtained dietary recall data from the subjects for the past year using Qualtrics and fecal samples for microbial analysis, as a baseline for dietary and bacteria activities. Then we gave them one or half a box of broccoli sprouts (Jonahan's Sprouts, Massachusetts, sourced through local grocery stores) to steam for 10 minutes and added to their regular meals every day for 28 days. Then, we collected fecal samples again from subjects on Day 28 to assess the effect of the broccoli sprout diet on gut bacteria genes. On Day 35, after seven days without the broccoli sprouts (treatment washout), we obtained information on how they consumed steamed sprouts for palatability, and fecal samples from subjects to observe the benefits of broccoli sprouts on bacteria genes persisted. We collected dietary recall for the past year using diet history questionnaire version 3 (DHQ3) during the study, after previously using a modified version of the DHQ3 from which we could not obtain HEI, which DHQ3 is programmed to calculate and report automatically.

2.3.3 Dietary data collection

The diet history information was obtained through electronic surveys on Qualtrics (Qualtrics Software, 2023, Provo, UT, USA), which hosted a modified version of the National Cancer Institute's Diet History Questionnaire version 3 (NCI DHQ3 (National Cancer Institute 2023)) to simplify diet history survey questions. Qualtrics generated weighted points for the frequency at which an individual consumed nutrients per year, i.e., yearly frequency. However, due to the lack of mass portions in the Qualtrics questionnaire, we asked the participants to utilize

the DHQ3 software (DHQ3) for healthy eating and nutritional quality reports based on USDA's HEI by comparing the weight and portions of classes of food consumed in mass and frequency, according to the 2020-2025 DGA recommendations for each individual. The DHQ3 software calculated and generated the HEI score for each subject based on 100 points. The HEI score is a crucial metric that provides comprehensive information about an individual's diet quality and adherence to the nutritional guidelines (Shams-White et al. 2023; Pope and Nizielski 2021). A score of <50 is a poor or unhealthy diet, 51-80 needs improvement, and >80 is a good or healthy diet (Shams-White et al. 2023). Also, healthy eating was examined with the nutrients consumed by an individual compared with their DGA recommendation, and the percentage difference was used as compliance difference. DHQ3 provides information about the daily values of each food group in grams or cups to assess the relationship between food intake and HEI score, informing of the HEI component represented in a spider plot. It was essential to use the DHQ3 because the HEI score tells us if the participants were healthy eaters or not. Meanwhile, Qualtrics generated the food frequency according to their classes plotted in a food cluster pie to observe the diversity of food consumed by all the participants. Since age and gender are associated with dietary patterns, HEI, and the development of chronic diseases, we calculated the mean age and HEI within our population. We also categorized our participants as young adults (18-39), mid-aged adults (40-59), and older adults (60+), and by gender to calculate the HEI components of each age and gender group (Artegoitia et al. 2021; Shang et al. 2023; Ma et al. 2022; Weng et al. 2024; Rahmani et al. 2019).

2.3.4 Stool collection, DNA extraction and quantitative PCR for BT2160-2156

During the participants' first visit to our lab, we provided them with instructions on how to label materials and collect feces (supplementary file B.2) using flushable fecal collection materials (Protocult, from Therapak, now Avantor), fecal occult blood cards (Hemoccult II® SENSA® Fecal Occult cards, Beckman-Coulter), and jars filled with RNA later. We obtained samples from participants on Days 0, 28, and 35 within 24 hours of defecation, and stored them at -80℃ until processed. *Bacteroides thetaiotaomicron* is one of the primary gut bacteria involved in GLR metabolism, with the expression of its *BT2160-2156* genes that are known to code for enzymes that convert GLR to SFN for anti-inflammatory and gut protective effects (Holman et al. 2023; Li et al. 2013; Zhang et al. 2023; Liou et al. 2019). The fecal DNA was extracted for quantitative PCR and analyzed for the expression of *BT2160-2156,* as previously reported (Marissa Kinney, Johanna Holman, Alexi...; Holman et al. 2023; Holcomb et al. 2023)**.**

2.3.5 Statistical analysis

The primary data from this study were analyzed using R, DisplayR, and Prism (v10). Sample demographics were summarized using descriptive statistics, and the HEI scores were obtained from HEI to examine whether our participants ate healthily. We performed a Spearman's correlation (*rs*) with the food nutrients and percentage difference of compliance to DGA recommendation (±). The individual's responsiveness to the broccoli sprouts diet was assessed by performing one-way ANOVA to quantify the *Bacteroides thetaiotaomicron*, *BT2160-2156* gene expression between high or low consumers, with or without gut problems. Statistical differences between consumption of nutrients were expressed as mean±SD.

2.4 Results

2.4.1 Participants Demographics and HEI score

The mean demographics of the twenty participants involved in this study are presented in Table 2.1. Three participants withdrew and did not complete the study. Four of seventeen participants completed the study but did not fill out the DHQ3 survey. Therefore, twenty subjects began the study and filled out the Qualtrics surveys, seventeen completed the research and provided fecal samples for BT gene expression, and thirteen completed the DHQ3 survey (Table 2.1). The mean age of the subjects was 43±14 years, and the mean HEI score was 70.8±8.7. As shown in Figure 2.1a, the majority of participants identified as female (*n*=13, 65%) with a higher HEI mean score of 74.3±5.2, as shown in Figure 2.1b, while males were minority (*n*=7, 35%) with an HEI score of 67.0±10.9. The young adults comprised (*n*=9, 45%) of our subjects with HEI mean score of 72.6±5.7, middle-aged adults were (*n*=5, 25%) with mean HEI of 65.5±12.8, and older adults were (*n*=6, 30%) of the subjects with mean HEI score of 74±6.6.

Table 2.1: The demographics of the participants and the healthy eating index score of those who filled out the diet history questionaries.

Variable	Total participants, N=20		Completed DHQ3, N=13
Age(years), Mean±SD	43.4±14.4	HEI (100), Mean±SD	70.8±8.4
Age Group, *n* (%)			
Young Adult	(9, 45)		(4,72)
Middle Adult	(5, 25)		(4,65)
Older Adult	(6, 30)		(5,74)
Sex, *n* (%)			

Table 2.1 continued

Female	(13, 65)	(7, 74)
Male	(7, 35)	(6, 67)

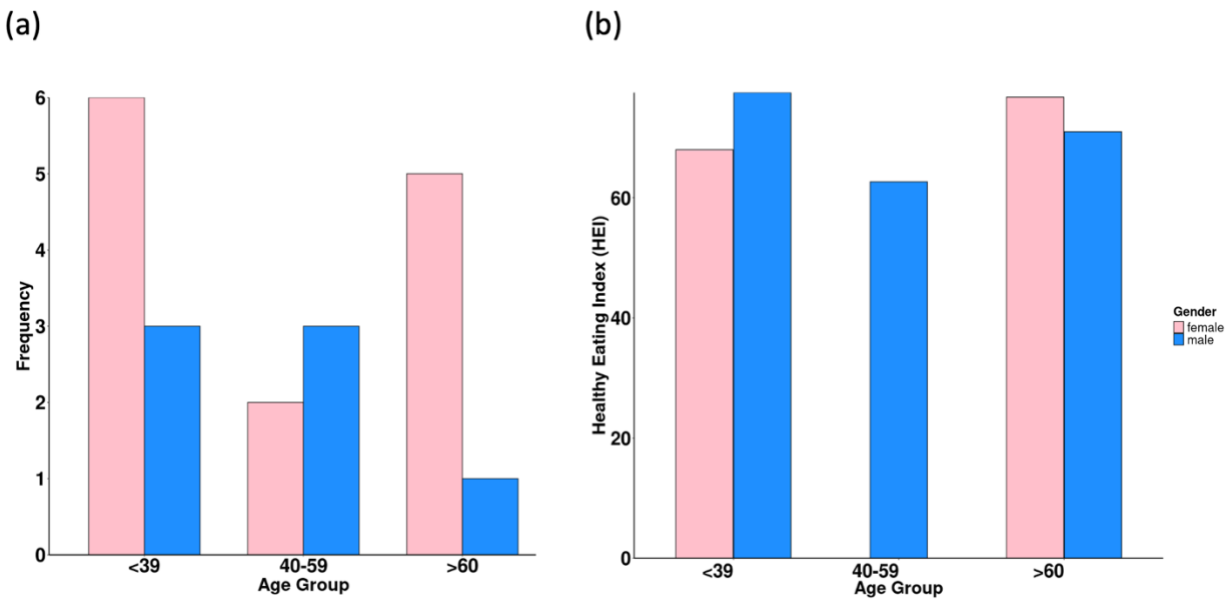
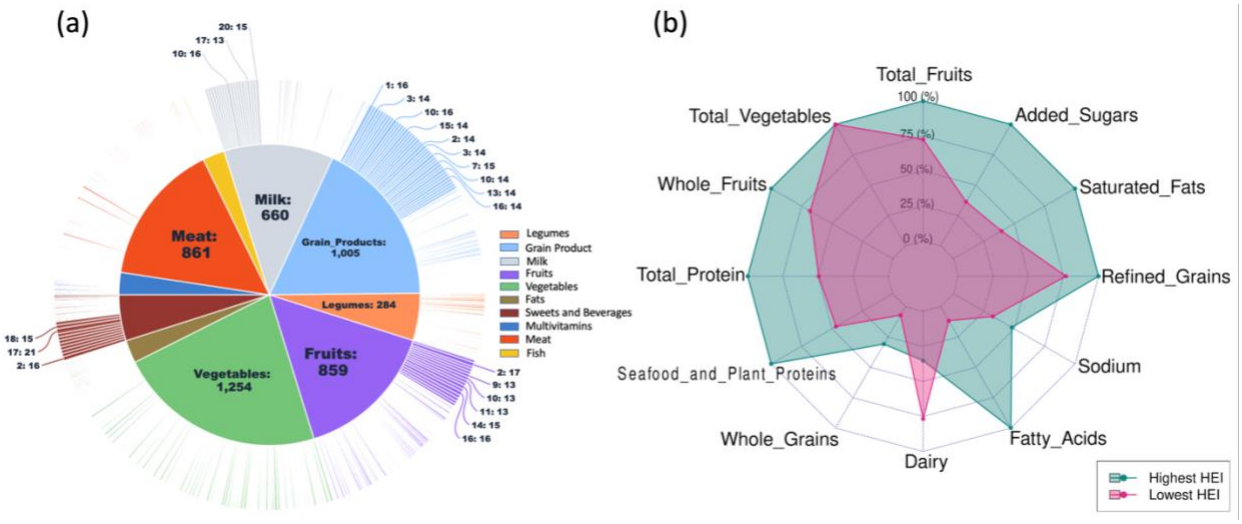


Figure 2.1: Bar graphs graphically display the demographics and healthy eating index distribution of participants. (a) The frequency of the twenty participants involved in the diet study according to sex and age groups classified according to the three stages of adult life, and (b) the healthy eating index distribution of the thirteen participants that filled the DHQ3 survey by age and gender.

2.4.2 Dietary diversity and HEI components

The nutrients consumed over the last year by our participants who completed the DHQ3 are visualized in Figure 2.2a, in which group clusters are weighed by the amount and frequency of consumption. The pie chart shows that vegetables (green) and grain products (blue) had the highest consumption rate among our subjects' population. However, sweet beverages, fruits, grain products, and milk are the most frequently consumed foods. For instance, as indicated in

the figure, subject 17 had the highest frequency of sweet and beverage consumption with a yearly weight of 21 points, and subject 2 had the highest frequency of fruit consumption with an annual weight of 17 points. Yearly food weight is a point allocated by Qualtrics based on the frequency by which an individual ate a particular food group. The spider plots in Figures 2.2b-f show the HEI components that map our participants' dietary patterns based on age and sex groups in alignment with the 2020-2025 DGA recommendation (Shams-White et al. 2023). The HEI components of the participants with the highest and lowest HEI values are illustrated in Figures 2.2b. A general comparison of our results across all ages and genders showed that the diet of older females (Figure 2.2c) aligned with HEI components except in food categories such as saturated fats, sodium, and dairy. Middle-aged adult men had the lowest alignment components except in whole grains, sodium, and vegetables, while older men had the lowest with total vegetables and saturated fat components. Comparison between different age groups (Figure 2.2d) showed the highest alignment in the older adults' HEI components and the lowest in the middle-aged adult group. Gender comparison within each group (Figures 2.2d-f) showed the highest alignment of women across all genders in HEI components of total vegetables and first, except in young adult women's vegetable consumption (Figure 2.2f).



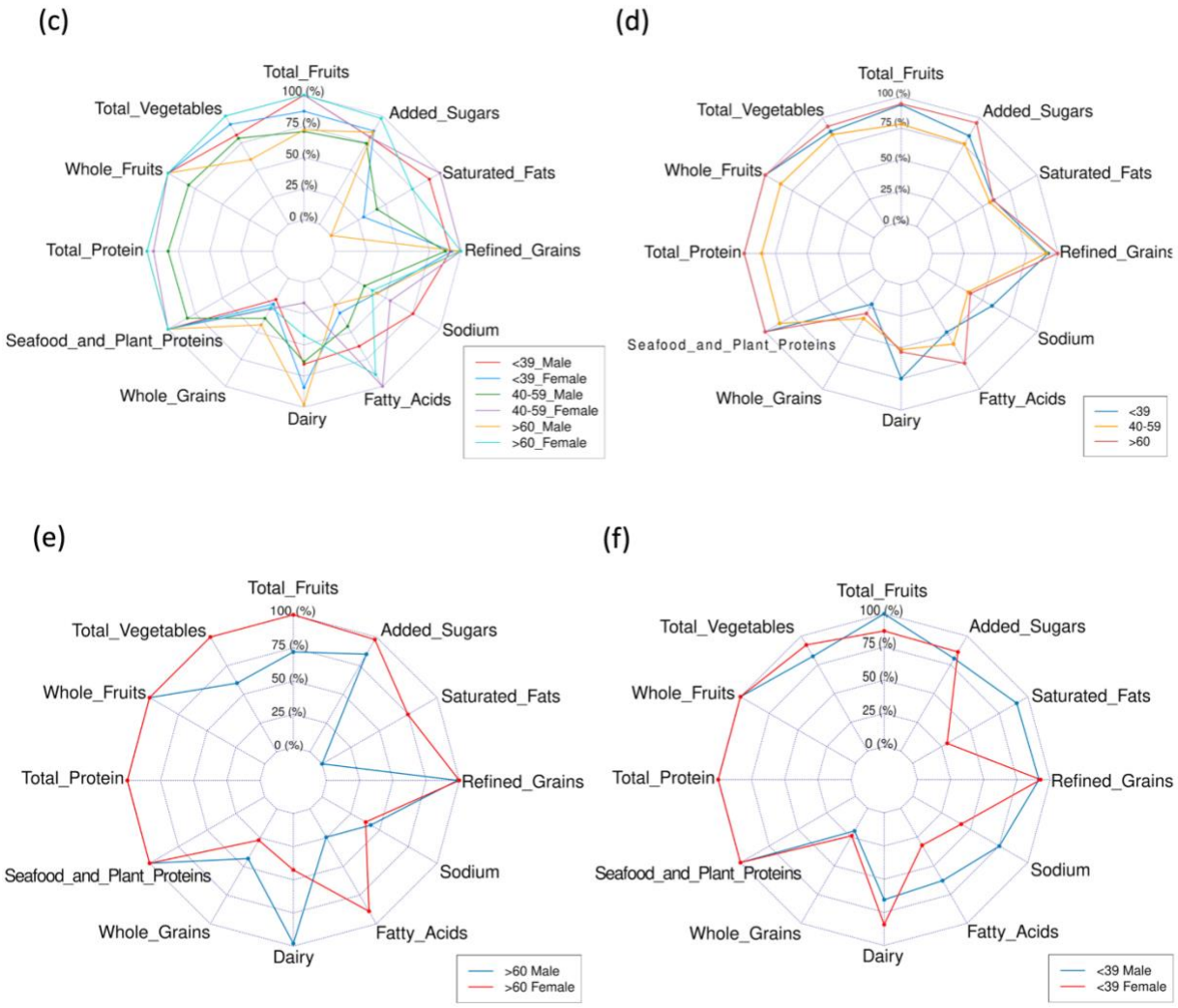


Figure 2.2: Food nutrients and healthy eating index differed among participants by age and sex. (a) Cluster pie of food nutrients in yearly weight of consumption frequency per individual represented by the thickness of the lines (Qualtrics survey); (b-f) Percentage food components that contribute to the healthy eating index of participants represented in spider plots based on age and sex groups (DHQ3 survey).

Since total vegetables and fruits aligned maximally as the major HEI components in women compared to men in this study, we compared the difference between the daily values of fibers, vegetables, and fruits based on gender. Women had a trend of higher daily consumption of these food categories when compared with men (Figure 2.3). Although the dietary gender-

based differences between their daily consumption of fiber, vegetables, and fruits were insignificant, the trends were consistently higher in women than men.

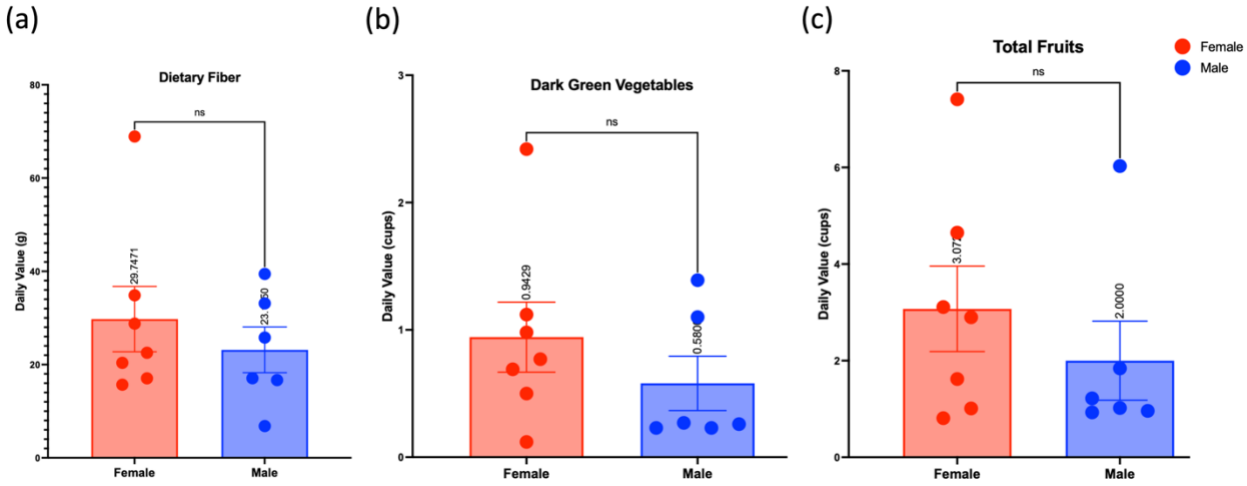


Figure 2.3: Bar charts of dietary fiber, dark green vegetables, and total fruit intake comparing female and male participants that filled the DHQ3 survey. There is a positive trend of increased (a) dietary fiber in grams, (b) dark green vegetables in cups, and (c) total fruit consumption in cups among female participants (red) compared with males (blue). Abbreviations: g=grams

2.4.3 Correlation between participants' HEI scores, nutrients, and compliance to the DGA

The mean HEI scores of all participants and nutrition values compared with the DGA recommendation are shown in Table 2.2. We calculated the percentage difference for individuals' daily nutrients to assess their compliance with DGA per nutrients (HEI scores for Americans; Drewnowski et al. 2019). We normalized the individual nutrition values for participants with the recommendation per food by calculating the percentage differences for the analysis baseline. The daily value of fiber intake (g) of all the participants had the highest positive correlation with the HEI score, although the compliance difference (%) was more correlated (Figure 2.4). Similarly, the daily value of saturated fat intake (g) of all the participants had the highest negative correlation

with the HEI score. However, the daily dietary difference of compliance to DGA (%) was significantly correlated with HEI, while the daily values were not (Table 2.2).

Table 2.2: The correlation values of participants' healthy eating index scores with their daily nutrition values in grams and compliance differences compared with dietary guidelines for Americans in percentages.

Variable	N=13	
	Correlation Analysis	
HEI Score 2020 (100 points)	Daily nutrition values (g)	Daily compliance difference (%)
Fibers	0.35	0.53*
Carbohydrate	0.19	0.1
Protein	-0.02	-0.08
Saturated Fat	-0.51	-0.9***
Sugar	-0.35	-0.22
Salts	-0.32	-0.32

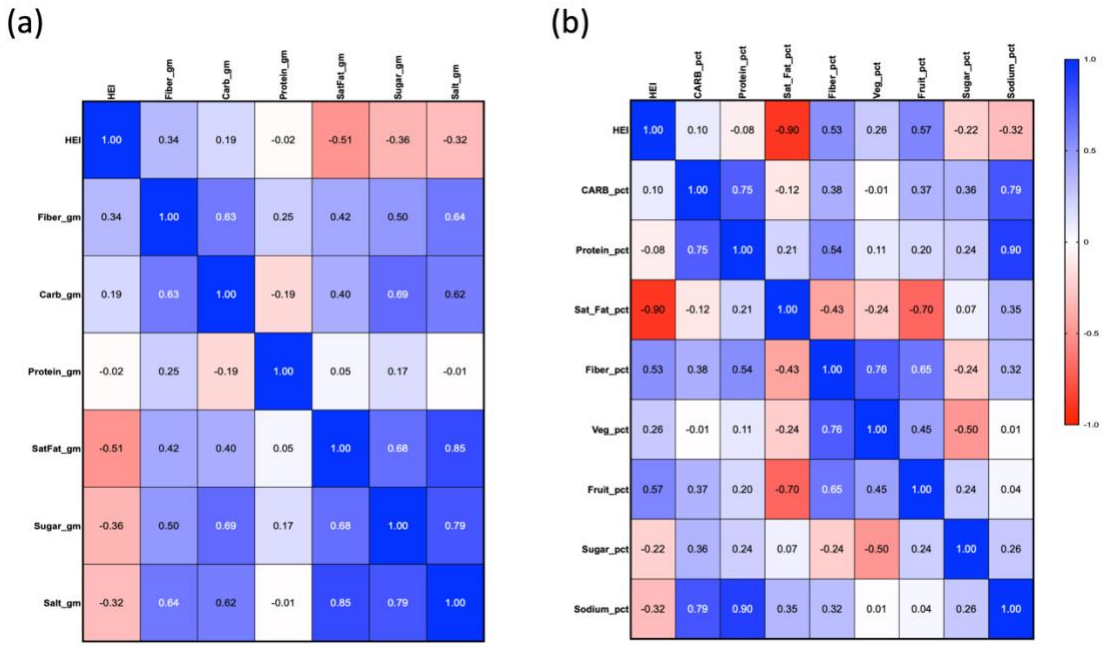


Figure 2.4: Heat map of Spearman correlation of HEI with fiber, vegetables, saturated fats, sugar, and salt. (a) The data of HEI scores compared with daily nutrient values in grams, and (b) HEI scores compared with daily nutrient compliance difference from the dietary guidelines for Americans in percentages. Correlations colored blue are positively, and red are negatively associated. Abbreviations: HEI=healthy Eating Index-2020, gm=grams, and pct=percentage.

2.4.4 Personalized expression of fecal BT2160-2156 genes with broccoli sprouts diet and high consumption of fibers, vegetables, and fruits

The expression of BT genes responsible for converting glucosinolates to isothiocyanates were not significantly different before and after broccoli sprout diet or between high and low consumers of fibers, vegetables, and fruits in this study (Figure 2.5). However, there was a trend of personalized response in individuals with self-reported high daily values of dietary fiber, vegetables, and fruit before and days after the broccoli sprout diet, particularly among the young- and middle-adult participants with self-reported pre-existing gut conditions in both genders. For instance, subject 13, a middle-aged woman, had the highest expression of BT gene combinations with at least three copies before, at the end, and days after the steamed broccoli sprout diet. Subject

3, a young adult man, had high expression and combinations of BT genes days after the steamed broccoli sprout diet. Meanwhile, subjects 20 and 2, older adult women with self-reported high and middle daily values of dietary fiber, vegetables, and fruit, respectively, both with preexisting conditions, did not show significant high BT gene expression before and after the steamed broccoli sprout diet. Subject 19, with the self-reported high yearly frequency of cruciferous diets and a low daily combination of dietary fiber, vegetables, and fruit with no gut conditions, had high BT gene expression at the end and days after the steamed broccoli sprout diet (Figure 2.5b&c). Subjects 10, with low self-reported dietary fiber, vegetables, and fruit consumption, had high expressions of *the BT2158* gene at the end and days after the steamed broccoli sprout diet. Subjects 14, with a moderate yearly frequency of cruciferous diet, had high expressions of *BT2158-2157* gene at the end and *BT2159-2157* days after the steamed broccoli sprout diet.

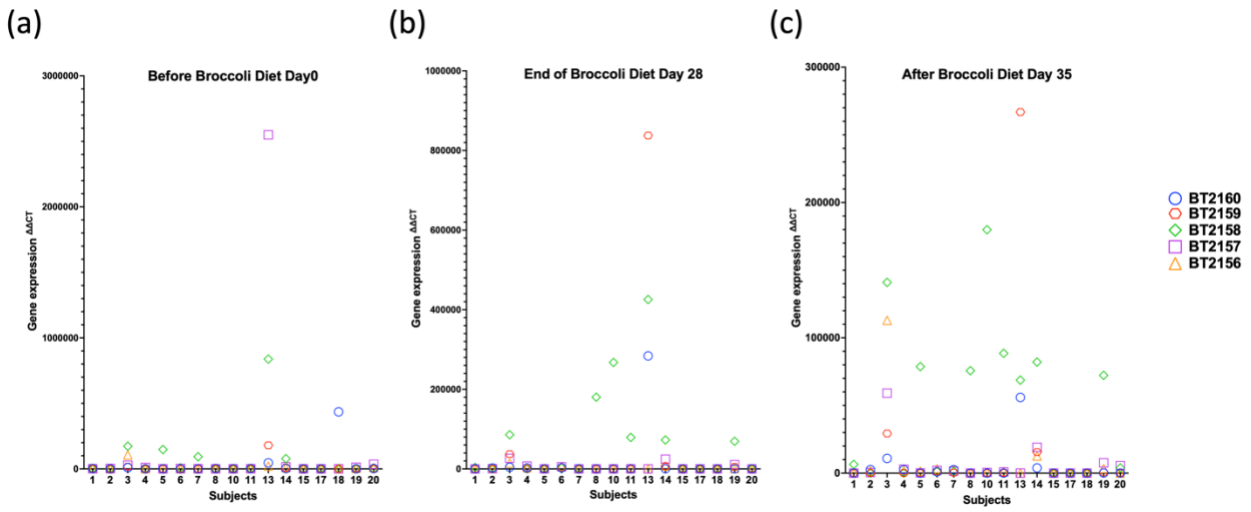


Figure 2.5: Scattered bars illustrate the expression of *BT2160-2156* genes in response to broccoli diet among the twenty participants. Individualized response is based on prior fiber, dark vegetables, fruit intake, and self-reported gut conditions, primarily in young and mid-aged adults. (a-c) The *BT2160-2156* gene expression per participant before, at the end, and seven days after the steamed broccoli sprouts intervention. Abbreviation: BT= *Bacteroides thetaiotaomicron.*

2.5 Discussion

The result from this study indicates that our participants had an overall healthier diet than average Americans based on a mean HEI score of 70.8 compared with 56.6 of the general adult population (Shams-White et al. 2023; Vaudin et al. 2021). The female population in this study showed a higher mean HEI score of 74.3 than males of 67.0 which supports the report that adult men are generally at risk of poor diet and associated chronic diseases such as colon cancer (Deierlein et al. 2023; Fanelli et al. 2020; Boustany et al. 2023). The older adults seem to eat healthier (74) than young (72.6) and mid-aged (65.5) adults, which supports the report that older adults may eat healthier food as a measure of healthy living at late stages of life (Xu et al. 2021; Vaudin et al. 2021).

Nutrient clustering is a critical analysis tool to examine food intake as nutritional patterns of individuals in a given population (O'Hara et al. 2022). Our results showed that food categories of vegetables, fruits, and grain products constitute about 50% of the yearly food frequency weight consumed by subjects in this study. These findings support the reports that high consumption of fibers in vegetables, fruits, and unrefined grains correlates with high HEI scores due to the micronutrients that could improve quality of life (Wright et al. 2020; López-González et al. 2021). The HEI components score further reflects our participants' dietary pattern of food categories in alignment with DGA 2020-2025 by specific age and gender groups (Shams-White et al. 2023). Our data set confirms that dietary patterns differ with age and gender and may predict disease risk factors (Artegoitia et al. 2021). The older adults aligned more with the HEI components than younger and middle adults and women compared to men across all age groups. Older women tend to eat a healthier diet with HEI components maximally aligned with total vegetables, total fruits,

whole fruits, total proteins, seafood, and plant proteins, and minimum alignment with saturated fats, sodium, and dairy in this study.

Meanwhile, men had a lower alignment with the majority of the HEI components, especially middle-aged men, putting them at risk of poor diet-associated diseases. Although there was no significant difference between the amount of daily fiber, dark green vegetables, and total fruits consumed by women and men in this study, there were positive trends of increased daily values in women compared to men. These combinations of HEI components may benefit older women who consume less sodium, saturated fats, and sugar, and more vegetables, fruits, and fibers may be protected against pathological conditions (Kang et al. 2019; Gao et al. 2022; Weisman et al. 2023). These results support the findings that men tend to overwork into the 60s with little cognition of their diet and are at risk for long-term diseases, such as IBD, while older women tend to retire early and focus on their well-being (Jacobs et al. 2020; Leso et al. 2015; Giovannucci et al. 1994).

The consumption of food daily values that align with the DGA recommendation has been reported to lower disease risk (HEI scores for Americans; Drewnowski et al. 2019; Jessri et al. 2016). Our results showed that high fiber consumption and low saturated fat are significantly associated with HEI, which may reduce the risk associated with gut conditions (Eynard and Lopez 2003; Fanelli et al. 2020). The percentage difference in the subjects' compliance with the DGA daily food weight recommendations showed a stronger correlation with HEI than the food nutrition values. The consumption of fibers, vegetables, and fruits above the daily recommended amount had a positive correlation with the population's HEI score and a negative correlation with saturated fats intake. These results suggest that the percentage difference in the subjects' compliance with

DGA recommendations may better represent association with HEI than the daily values. Consuming higher fiber, vegetables, and fruits may provide micronutrients such as glucosinolates, flavonoids, and short-chain fatty acids within the gut against inflammatory bowel diseases (Alaba et al. 2024). The participant with the highest HEI score of 81 consumed more than 50% of the amount of recommended fiber, 300% of the vegetables, 100% of the fruits, and 50% less saturated fats than the DGA recommendation. Meanwhile, the participants with the lowest HEI consumed about 50% of the recommended amount of fiber and 25% less than the recommended amount of vegetables.

Our results showed that young and middle-aged adults may benefit more from their personalized responses to BT genes than older adults. Subjects with self-reported gut conditions in their middle and young adulthood had increased gene expression. For instance, subject 13, a 59-year old woman with a preexisting IBS and adherence to a vegan diet, had the highest total and combinations of BT genes at different times. She had a combination of highly expressed BT2159-2157 before the steamed broccoli sprout diet intervention (day 0). This result may be due to the high regular consumption of dietary fiber, dark green vegetables, and total fruits. According to the HEI report, her values were the highest for all daily values for these nutrients. At the end of the steamed broccoli sprout diet (day 28), she maintained the highest total and combinations of BT genes, with BT2160-2158 genes. After seven days of the steamed broccoli sprout diet (day 35), she retained the BT2160-2158 genes at lower expressions than day 28. Similarly, subject 3, a young adult man with a preexisting gut problem, had increased expressions of BT2158 and 2156 at day, with a switch to higher expression of BT2159-2158 genes at day 28 and increased expression of all the five genes at day 35. Meanwhile, subjects 20 and 2, older women with self-reported high and middle daily values of dietary fiber, vegetables, and fruit, respectively, both with

preexisting conditions, did not show a significant increase in BT gene expression before and after the steamed broccoli sprout diet. Subject 20, a 61-year old woman with a preexisting Crohn's disease which was controlled with diet and without medication, had a slightly upregulated BT2157 at days 0 and 35. These suggest that the older population may not respond to BT gene expression during the steamed broccoli sprout diet intervention. This pattern is similar to a report that individuals with prior high daily fiber diets may not benefit sufficiently from prebiotic diet intervention (Holmes et al. 2022). These results also support the report that the younger IBD population in mice benefited from the steamed broccoli sprout diet than older adults (Holcomb et al. 2023). It has also been reported that gut microbiota differ among young and older adults. The expression of *Bacteroides* is unique among young adults, while *Parabacteroides* co-exist more in older adults (Ragonnaud and Biragyn 2021; Jeffery et al. 2016). The expression of fecal *BT2160-2156* genes is essential for the hydrolysis of glucosinolates to isothiocyanates by *Bacteroides spp.* for broccoli benefits against IBD information and oxidative stress (Liou et al. 2020; Holman et al. 2023). *Bacteroides thetaiotaomicron* (*B. theta*) utilizes at least one of these five gene codes to form several enzymes, and multiple combinations of these enzymes may reflect a high conversion of isothiocyanates-from glucosinolates-rich diet (Liou et al. 2019). Therefore, young and adult subjects with high daily consumption of fibers, vegetables, and fruits may respond better to the BT gene expression and benefit from the steamed broccoli sprout diet intervention than old adults with IBD conditions. The low expression of *Bacteroides* with aging may be associated with less molecular response to dietary anti-inflammatory metabolites (Macia et al. 2015), such as isothiocyanates, polyphenols, and short-chain fatty chains from broccoli. A similar pattern to subject 20 was observed with subject 5 in response to BT2158 at days 0 and 35, a 52-year old woman with no preexisting gut problem and a high yearly frequency of cruciferous diets. She had

slightly expressed BT2158 before the broccoli sprouts diet, less expression at the end of the study, and more excellent expression days after the survey. Meanwhile, Subject 19, a 42-year old man with self-reported high yearly frequency of cruciferous diets and low combination of fibers, vegetables, and fruits, with no gut conditions, has increased BT gene expression at the end and days after the broccoli sprouts diet. We observed a similar pattern of increased expressions of *the BT2158-2157* gene at the end and days after the broccoli sprouts diet with subject 10, a 34-year old man with low-self-reported consumption of dietary fiber, vegetables, and fruit, and subject 14, a 35-year old woman who reported a moderate yearly frequency of cruciferous diets. In addition, subject 14 slightly increased *BT2159* days after the broccoli sprouts diet. The expression of fecal BT genes was sustained up to seven days after the broccoli sprout diet, particularly in highly responsive individuals in the study. These data support the report that gut *Bacteroides thetaiotaomicron* were colonized with increased and sustained genetic expression for six weeks and high polysaccharide utilization loci in mice for effective dietary metabolism of complex glucose (Kennedy et al. 2023). These results further establish that response to probiotic interventions such as broccoli sprout diets is individualized and may be favorable to persons with prior diet history of low fiber, vegetables, and fruits (Holmes et al. 2022; Li 2023; Ticinesi et al. 2023; Johnson et al. 2019) and depends on age, preexisting gut problems and other multi-level factors in subjects. One participant reported regular consumption of sulforaphane supplements but did not have high gene copies of BT genes.

2.6 Conclusion, limitations and future direction.

This study is the first to provide evidence on the individualized response of humans to BT gene expression before and after broccoli intervention. Young and middle-aged adults who regularly consume high amounts of fiber, fruits, and dark green vegetables tend to respond better

to broccoli dietary intervention, with sustained upregulated multiple copies of *Bacteroides thetaiotaomicron* genes, especially BT2159-2158 genes. Such responses were observed among individuals who self-reported that they have been eating fibers, fruits and vegetables due to preexisting gut problems. These individuals have HEI scores mostly in the 70s and amount of daily nutrients values above DGA recommendation. Some young and middle-aged adults who ate low or moderate fiber and dark green vegetables had a greater upregulated expression of at least BT2158 gene, seven days after the steamed broccoli sprouts intervention. Therefore, the regular consumption of steamed broccoli sprouts with high consumption of daily amounts of fiber, dark green vegetables and fruits may benefit young and middle-aged adults with IBD or IBS symptoms. Some individuals, especially older adults, did not respond with more copies of BT genes, with either broccoli sprouts intervention or high consumption of these nutrients. Early life intervention with a broccoli diet and incorporating dark green vegetables and fruits as daily diet may benefit the nutritional management of IBD via individualized *Bacteroides thetaiotaomicron* response in young and middle-aged adults.

There are a number of factors that interplay in the cause of an individuals' lifetime that determine their response to microbiota such as BT genes (Wu et al. 2021). The limitations of this work include the repeat of DHQ3 after some participants have already started or finished the study which may introduce bias as subjects may change their initial response collected at the beginning of the study. Also, a multi-factorial approach to broccoli intervention in persons with IBD may reflect comprehensive benefit and microbial response. Future research would consider incorporating other factors beyond diet into consideration for effective assessment of microbial response to dietary intervention. Factors that could influence dietary intervention include smoking, BMI, hormonal changes and therapy (Bostanci et al. 2021; Dominianni et al. 2015; Brettle et al.

2022), which were not obtained during this research. A larger cohort of participants who are healthy without gut problems and those diagnosed with IBD conditions might better reflect broccoli intervention and microbial responsiveness.

CHAPTER 3

STEAMED BROCCOLI SPROUTS DIET ALLEVIATES INFLAMMATORY BOWEL DISEASE BY INCREASING ANTI-INFLAMMATORY, ANTIOXIDANT AND GUT PROTECTIVE METABOLITES IN DSS-MICE

3.1 Abstract

Broccoli is a cruciferous vegetable rich in bioactive compounds that are beneficial against inflammatory bowel disease through antioxidative, anti-inflammatory, and gut-protective activities. However, an in-depth annotation and understanding of the metabolites and microbial interactions associated with the broccoli sprouts diet is needed. This study investigated the effects of a steamed broccoli sprouts diet on the concentrations of metabolites and associated bacterial communities with antioxidant, anti-inflammatory, and gut-protective effects against ulcerative colitis in mice. Our data showed that steamed broccoli sprouts intervention significantly increased the concentrations of beneficial metabolites, such as sulforaphane, short-chain fatty acids, tryptophan, indoles, glutamic and polyphenolic metabolites, with positive correlations with commensal bacteria, B*acteroides spp.*, *Intestinimonas*, *Oscillibacter* and *Lachnospiraceae* in the colon, cecal and jejunum regions of the gut. These data suggest that as a nutritional intervention, broccoli sprouts may confer region-specific metabolites and microbial interactions for antioxidants, anti-inflammatory, and gut-protective benefits during inflammatory bowel diseases.

3.2 Introduction

Broccoli, *Brassica oleracea var. italica*, is a type of cruciferous vegetable in the *Brassicaceae* family, with nutrients and bioactive compounds beneficial to maintain a healthy gut, alleviate bowel diseases and other associated systemic conditions (Alaba et al. 2024; Syed et al.

2023; Kumar et al. 2022). Broccoli is a non-fatty vegetable with insignificant amounts of fatty acids and low calories. It is rich in dietary amines, carbohydrates, micronutrients, fibers, and vitamins, with anti-inflammatory and antioxidant benefits (Li et al. 2022). Broccoli sprouts, in particular, have been reported to possess more nutritional and beneficial phytochemicals than mature broccoli heads (Chen et al. 2018; Bhandari et al. 2019; Gao et al. 2014). Diets rich in broccoli sprouts could contribute to overall well-being in healthy and disease states (Yanaka 2018; Atwell et al. 2015; Charron et al. 2020). Regular consumption of such a broccoli diet could exert nutritional benefits against cancer (Das and Ghosh 2021; Li et al. 2013), oxidative stress (Tian et al. 2022; Hou et al. 2023), inflammation (Holman et al. 2023; Holcomb et al. 2023; Holman et al. 2023), obesity (Zandani et al. 2021; Taiwo et al. 2024), diabetes (Mohammed and Mohammed 2023; Suresh et al. 2016), liver damage (Mao et al. 2023; Yoshida et al. 2015), renal dysfunction (Hashem and Malak 2024; Rubattu et al. 2015), cardiovascular diseases (Pereyra et al. 2020; Mukherjee et al. 2008), neurodegeneration (Vega-Galvez et al. 2023; Rhee et al. 2020), gut dysbiosis (Zhang et al. 2023; Holman et al. 2023) and maintenance intestinal damage (Wu et al. 2023; Lynn et al. 2015; Ferruzza et al. 2016).

Inflammatory bowel disease (IBD) is one of the major intestinal pathological conditions with a rapid rate of occurrence and global concern (Ye et al. 2015). The disease is difficult to treat due to the complex interplay between multiple pathological pathways such as inflammation, antioxidants, dysbiosis, and gut damage (Larabi et al. 2020; Alaba et al. 2024). Meanwhile, our studies have shown that various broccoli diets, including steamed broccoli sprouts, could alleviate inflammation by decreasing inflammatory oxidative stress while promoting healthy gut microbial diversity and intestinal structures (Alaba et al. 2024; Zhang et al. 2023). Broccoli treatment increased the antioxidants-scavenging activities of ORAC, HSAC, DPPH and ABTS, the

production of SCFA, healthy gut microbes communities, Bacteroidota/Bacillota ratio, and anti-inflammatory cytokines, IL10 and CD36, and it decreased inflammatory cytokines, IL1β, iNOS, COX-2, CCLs and CXCCLs, in macrophages and monocytes media (Pal and Konkimalla 2016; Deng et al. 2017; Choe et al. 2018). Similarly, steamed and raw broccoli sprouts increased antioxidants, NRF2, GPX2 and GSTM1, and tight junction proteins, ZO-1, occludin and claudin-2 against colon damage, decreased cytokines IL6, IL1β and TNFα, increased expression of PGC1α/ NFR2 pathway and microbial richness in mouse models of ulcerative colitis (dextran sodium sulfate (DSS) induced) and Crohn's disease (microbially induced in interleukin 10 knockouts (IL10 KO) (Holman et al. 2023; Holcomb et al. 2023; Lippmann et al. 2014).

The nutritional benefits mentioned above may be associated with the hydrolysis effect of some gut bacteria on dietary metabolites from broccoli, such as glucosinolates (GSL), amines, polyphenols, and fibers. Gut microbes can hydrolyze these classes of plant metabolites into isothiocyanates (ITC), conjugates of flavonoids, phenolic acids, short-chain fatty acids (SCFA), and vitamins (Alaba et al. 2024; Bhandari et al. 2019; Choe et al. 2018). For instance, consuming cooked or raw broccoli for four or more days could increase the abundance of myrosinase-like microbial hydrolases, *Escherichia coli, Lactobacillus spp., Bifidobacterium spp.* and *Bacteroides spp.*, to metabolize GSLs, such as GLR, glucoerucin and sinigrin, into ITCs, nitriles, erucin and indole conjugates to upregulate antioxidant and anti-inflammatory genes, such as GSH, NFR2, NQ1 and IL10, and gut mucosal barrier to alleviate IBD pathologies (Bouranis et al. 2021; Liu et al. 2017; Kaczmarek et al. 2019; Cebeci 2017; Liang et al. 2018). Similarly, flavanol and polyphenols such as curcumin, ferulic acids, and quercetin can be metabolized by *Clostridium, Bacteroides, Ruminococcus spp.* and *Lactobacillus spp.* with the ability to decrease proinflammatory cells, increase Treg cells, antioxidants (SOD) and barrier protection such as ZO-

1, occludin (Xu et al. 2021; Catalkaya et al. 2020; Peterson et al. 2018; Guo et al. 2022). Enterocytes have been reported to contain phenolic cleaving bacteria such as *Aldercreutzia equolifaciens* and *Flavonifractor plautii* and enzymes such as b-glucuronidase, *sulfotransferases* (SULT) and *catechol-O-methyltransferases* (COMT) (Murota et al. 2018; Takagaki et al. 2014). Microbiota such as *Bacteroides, Lactobacillus,* and *Eubacterium* can hydrolyze dietary fibers into SCFAs such as butyrate, propionate and acetate to stimulate anti-inflammatory responses of Treg cells to release IL10 and protect epithelial tight junction from damage (Ananthakrishnan et al. 2013; Caballero-Flores et al. 2023; Parada Venegas et al. 2019). Broccoli could change intestinal microbial population for tryptophan hydrolysis by *Clostridium sporogenes* and *Peptostreptococcus spp.* to increase indole metabolites with anti-inflammatory effects via inhibition of COX2 and stimulation of NRF2 pathways (Wlodarska et al. 2017; Liu et al. 2022; Duan et al. 2023; Nisha et al. 2022). Phenylalanine, a dietary amino acid from broccoli, can be metabolized by *Enterococcus* and *Lactobacillus* spp. to increase anti-inflammatory and neuroprotective metabolites, which decreased cytokines such as IL (Portune et al. 2016; Hou et al. 2023; Hubbard et al. 2017; Liu et al. 2014). Despite the reports that steamed studies could improve IBD pathologies with decreased inflammatory markers such as IL6, TNFα, and IL1β and maintained microbial richness along the gut biogeography (Zhang et al. 2023; Holman et al. 2023), there is limited information about the role of microbiome-derived metabolites modulating these IBD pathways into healthy conditions. It is essential to analyze the metabolomic intensity of compounds with antioxidant, anti-inflammatory, and gut-protective properties during IBD management of broccoli.

In this study, we aim to assess the effect of steamed broccoli sprouts on dietary and microbial metabolites using a comprehensive panel of untargeted metabolomics (Clish 2015). We

hypothesized that steamed broccoli sprouts would increase the concentration of dietary and microbial anti-inflammatory, antioxidant, prebiotic, and gut-protective compounds against IBD. We used fecal samples from a previously published study which demonstrated that a 10% steamed broccoli sprout diet would prevent inflammation and changes to gut microbiota which otherwise occur during chemically induced ulcerative colitis (Holman et al. 2023). To further explore broccoli's nutraceutical effect against IBD, we compare the peak intensity of fecal metabolites in mice fed steamed broccoli diets alone with steamed broccoli diets in induced IBD conditions. This research used advanced untargeted metabolomic approaches to identify novel dietary and microbial anti-inflammatory, antioxidant, prebiotic, and gut-protective metabolites associated with steamed broccoli that are effective against IBD pathologies. These findings provide relevant information on the potential nutritional management of IBD by using steamed broccoli sprouts as a nutrition intervention.

3.3 Methods

3.3.1 Diet preparation and experimental design

We purchased broccoli sprouts from a local grocery store (Shaw's, ME), steamed them for 10 minutes over boiling water, and freeze-dried and ground them into fine powder. The powder was mixed with purified AIN93G powder (ScottPharma Solutions, Marlborough, MA) to make a 10% (w/w) steamed broccoli diet according to our previous studies, which reported adequate conversion of GLS to ITN (Holman et al. 2023; Zhang et al. 2023). We have documented the materials for this experiment in Holman's article (Holman et al. 2023). Specific pathogen-free C57BL/6 mice were purchased from Jackson Laboratory at six weeks of age, acclimated for seven days, and distributed into cages based on four treatment groups as reported in this previous study (Holman et al. 2023). Treatments consisted of 1) Control mice fed with AIN93G diet; 2) 2.5% DSS mice

fed with DSS in water to induce IBD; 3) 10% SS mice fed with 10% steamed broccoli sprouts diet; 4) 2.5% DSS + 10% SS mice fed with both DSS and steamed broccoli sprouts (SS) diets. After the first seven days of adaption to diets, mice were placed on 2.5% DSS treatment in drinking water for five days, then a recovery period of five days. After two more cycles of DSS treatment and recovery, fecal samples were collected on day 30 for metabolomics analysis. Mice remained on their diets for the duration of the study.

3.3.2 Untargeted metabolomics data acquisition and statistical analyses

Frozen fecal samples of 50 mg were sonicated in 200 µl of water and mixed with 800 µl of cold buffer (1:1 of MeCN:MeOH). The homogenate solution was vortexed for 30 seconds and centrifuged at 14,000 g for 5 mins at 4°C. A supernatant of 900 µl was collected into a fresh tube and centrifuged as aforementioned. A supernatant of 800 µl was collected into another tube, then centrivap dried. Metabolites were reconstituted in a cold 400 µl buffer (1:1 of water + 0.1 % formic acid:MeCN) for metabolomics analysis. Liquid chromatography with a high-resolution mass spectrometry (LC-HRMS) data acquisition was performed using Waters Synapt G2, time-of-flight (TOF) XEVO machine at the mass spectrometry facility of the University of California, Irvine, according to the previous report (Hosseinkhani et al. 2021). This earlier report is a significant reference in the field and validates our methodology. We transferred 250 µl of each sample in the column for 10 µl injection (C18 column, 7 x 40 mm) at liquid phase conditions as shown in Table 3.1 for metabolite analysis in an Acquity iClass UPLC and Synapt G2 system (Waters Corp., Manchester, UK) (Helm et al. 2014). These tools are widely used and trusted in the field, ensuring the reliability of our results. We processed the LCMS metadata in MassLynx, Progenesis QI, mzMine, and Sirius tools with built-in search engines to obtain the retention time, mass charge

ratio, molecular weight, peak intensity, metabolites name, and formula using several libraries (Esperanza et al. 2020; Peng et al. 2021).

Table 3.1: Liquid Chromatography conditions for metabolomics.

Time (min)	A(%)	B(%)
0.00	98.0	2.0
1.00	98.0	2.0
15.00	2.0	98.0
17.00	2.0	98.0
18.10	98.0	2.0
20.00	98.0	2.0

A: 95:5 water: acetonitrile with 0.1% formic acid

B: acetonitrile with 0.1% formic acid. Flow rate of 3 uL/min.

The metadata was pre-processed in R to remove duplicate metabolite calls and correct for peak intensity errors before importing into MetaboAnalyst 5.0 (http://www.metaboanalyst.ca) (Pang et al. 2022). We normalized the data by log transformation and performed univariate One-way ANOVA analysis at $p< 0.05$ in a scatter plot in MetaboAnalyst and Prism with Fisher's post-hoc test to annotate specific metabolites of interest that are statistically different across the experimental groups in bar plots. We performed unsupervised and supervised discriminant

analysis for sample and group variation. The unsupervised principal component analysis (PCA) made the 2-way comparison of variability and sample cluster. We performed Hierarchical clusters with heatmaps to observe the correlation between the experimental groups and metabolite peak intensity. To identify potential relationships between bacteria involved in the hydrolysis of dietary metabolites from broccoli, we further performed Spearman correlation analyses with screening for p values 0.05 or less between some metabolites of interest and previously published microbiome richness, taxa diversity, and *B. thetaiotaomicron* genes, *BT2160-2156* operon (Holman et al. 2023).

3.4 Results

3.4.1 Univariate ANOVA of metabolites, discriminant analysis, and hierarchical clustering of samples in MetaboAnalyst 6.0

The partial least squares-discriminant analysis (PLS-DA) of samples showed significant differences and variations between the control, 2.5% DSS, 10% SS treated, and 2.5% DSS + 10%SS groups (Figure 3.1a). Additional analysis with K-mean clustering of the PCA plot identified inter-cluster similarity between the 10% SS treated and 2.5% DSS + 10% SS groups, while control and 2.5% DSS clustered within their groups (Figure 3.1b). Hierarchical clusters of samples confirmed the separation of samples into their treatment groups (Figure 3.1c). A total of 12,313 metabolites were observed based on metabolomics analysis, about 7000 were filtered out by quality control analysis in MetaboAnalyst, and 3,103 have significantly increased or decreased concentrations across the four treatment groups, as shown in the scatter plot (Figure 3.1d).

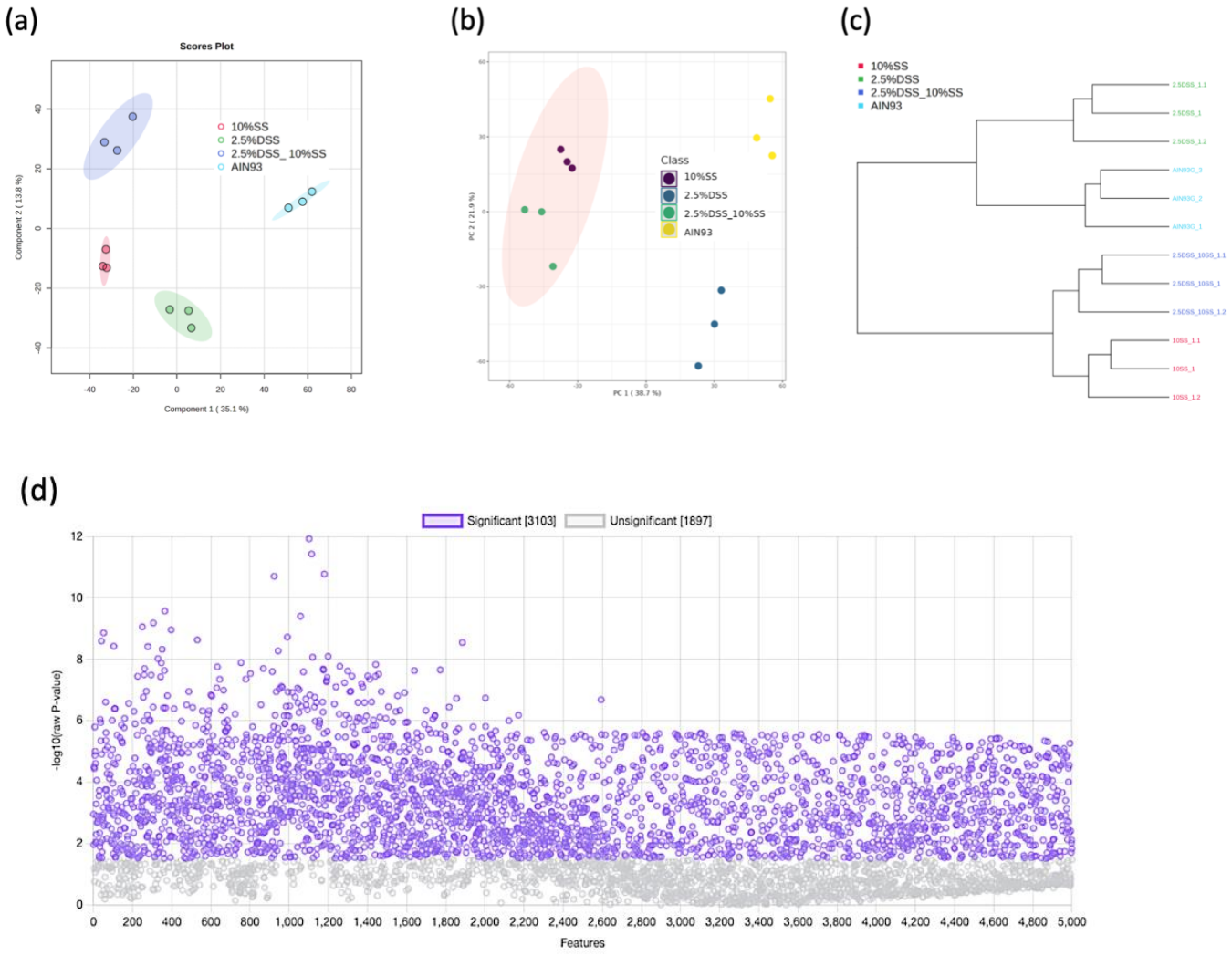


Figure 3.1: Fecal metabolites were differentially present by steamed broccoli sprout diet and DSS treatment. (a) PCA plots of samples with component 1 at 13.8% and component 2 at 35.1%; (b) K-mean plots with PC 1 at 21.9% and PC 2 at 38.7%; (c) Hierarchical cluster of experimental groups shown in dendrogram trees. (d) The univariate one-way ANOVA at $p< 0.05$ highlighted 3,103 metabolites differentially expressed across the experimental groups. Abbreviation: DSS= Dextran Sulfate Sodium.

3.4.2 Hierarchical clustering of differentially expressed fecal metabolites across the four treatment groups in heatmaps

The heat maps show the effect of steamed broccoli sprouts on metabolite clusters contributing to the differential impact of the broccoli diet against IBD across all the treatment groups in Figure 3.2a and specific metabolites of interest highlighted in Figure 3.2b. The

microbiota-derived metabolites of interest with differences in concentrations with broccoli diet or DSS treatment are isothiocyanates, tryptophan metabolites, indole derivatives of tryptophan, amines derivatives of glutamine and phenylalanine, prolines, and SCFAs.

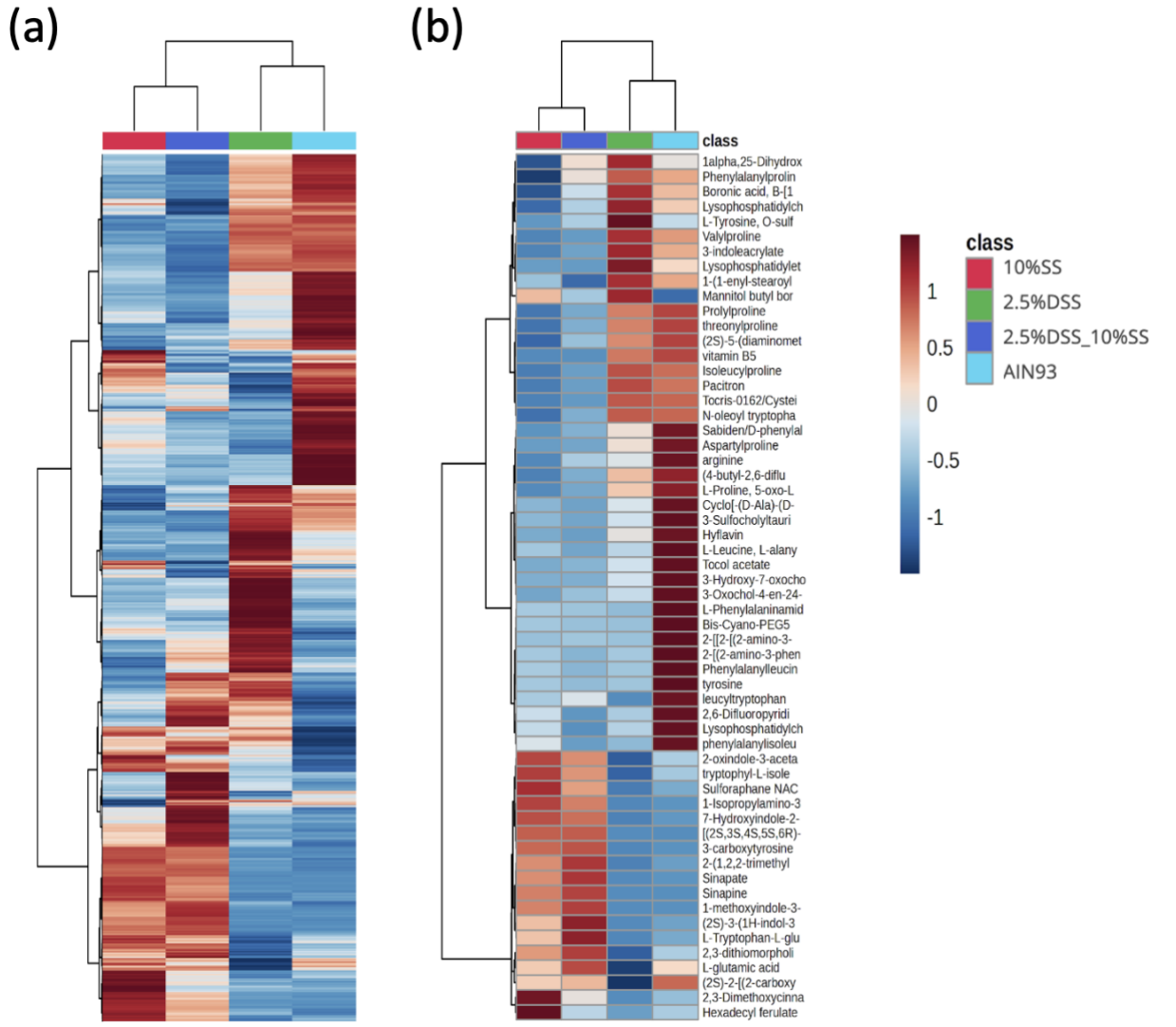
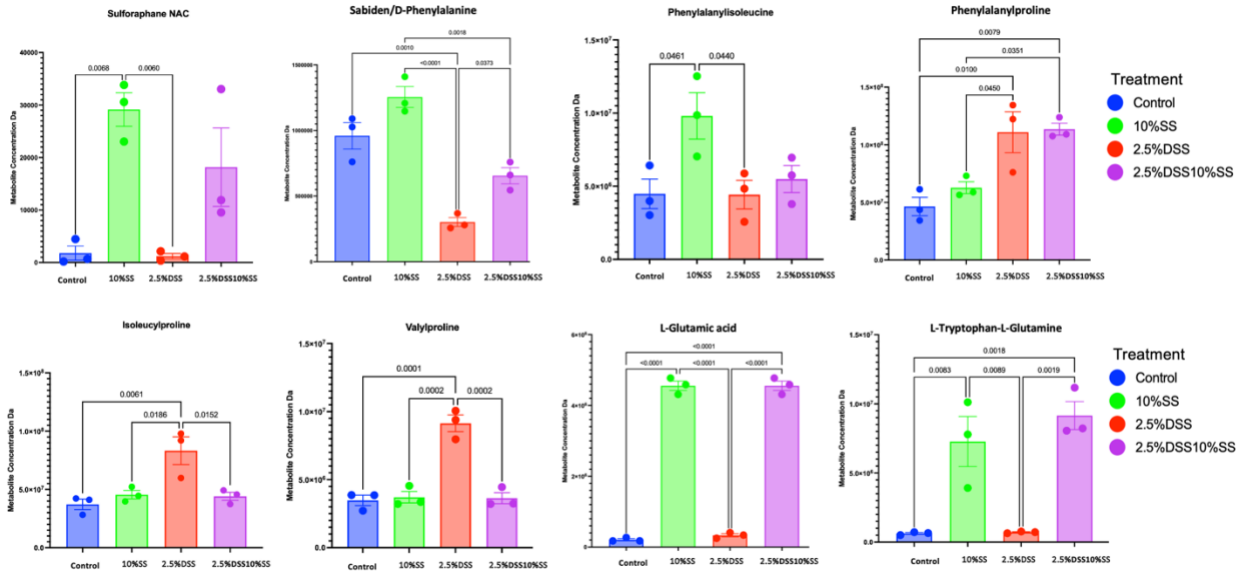
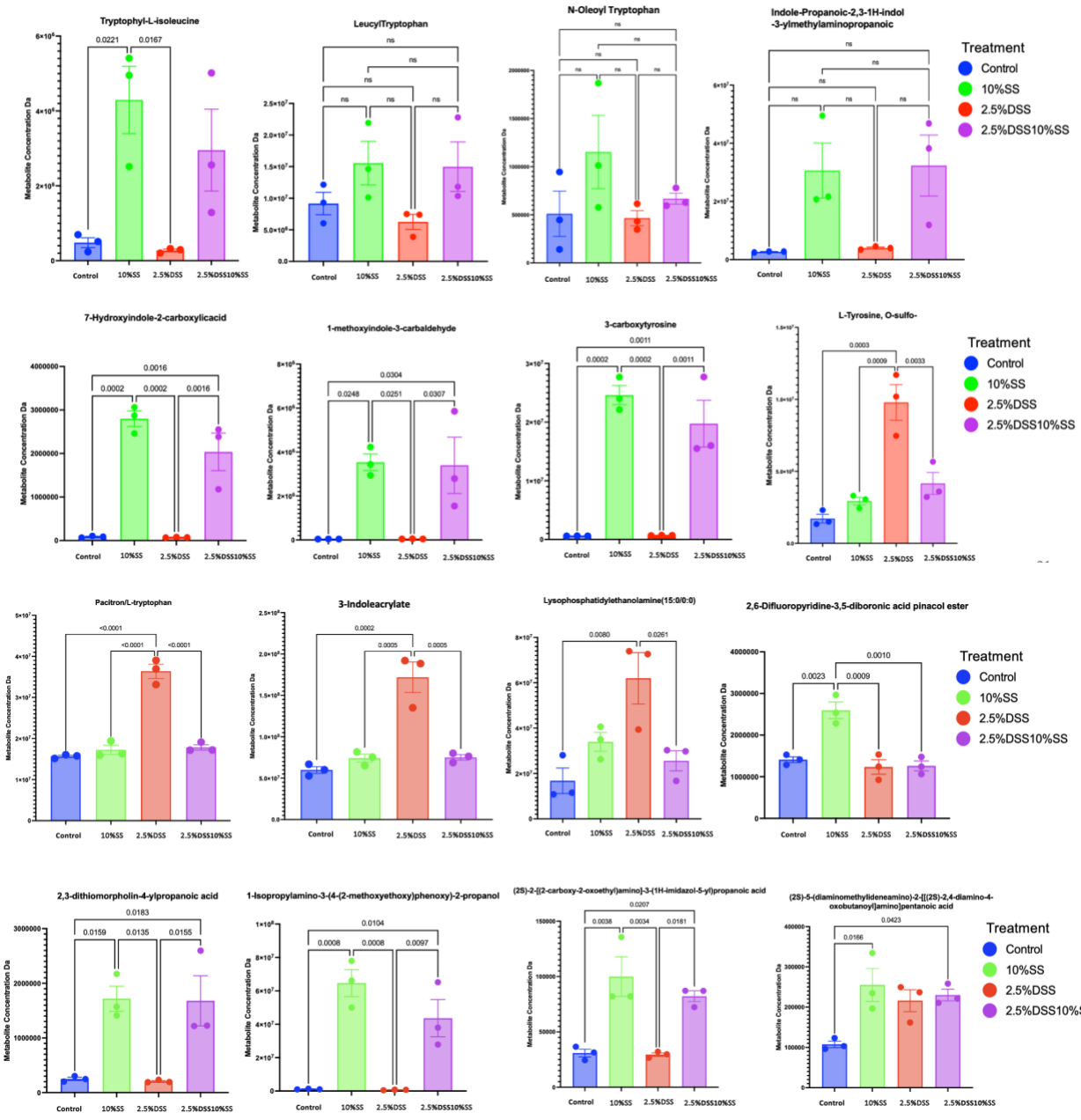


Figure 3.2: Hierarchical clusters analysis comparing the metabolites among the treatment groups (10% SS, 2.5%DSS, and 2.5%DSS_10%SS) and the control (AIN93). The heatmaps showed that (a) all differentially changed fecal metabolites and (b) specific differentially changed fecal metabolites of interest between the groups. The red color denotes an increase in metabolite concentration, while the blue color shows a decrease. Abbreviations: DSS= Dextran Sulfate Sodium and SS= steamed broccoli sprouts.

3.4.3 The effect of steamed broccoli sprouts on metabolite concentration

To identify the effect of steamed broccoli sprouts, we performed one-way ANOVA at $p < 0.05$ using the metabolite concentrations. The microbiota-derived metabolites of interest with positive significant concentrations in mice that ate broccoli are sulforaphane N-acetyl cysteine (SFN-NAC), D-phenylalanine (sabiden), phenylalanyl isoleucine, L-glutamic acid, L-tryptophan-L-glutamine, tryptophyl-L-isoleucine, indoles, 3-carboxytyrosine, sinapic acid (polyphenol), derivatives of boronic acid, propanoic acid and acetic acid. We observed positive trends of polyphenolic metabolites, hexadecyl ferulate, and 2,3-Dimethoxycinnamic acid in the feces of mice that ate broccoli, but those differences were not significant. Likewise, tryptophan metabolites N-oleoyl tryptophan, leucyl tryptophan, and indole propanoic acid showed a positive trend in mice that ate broccoli without any considerable difference. Meanwhile, L-tryptophan, indole acrylate, O-sulfotyrosine, cysteine-s-sulfate, pentanoic acid, and proline metabolites had positive significant differences with the DSS treatment as shown in Figure 3.3. Open source information about the metabolites of interest are reported in Table 3.2




Tryptophyl-L-isoleucine
LeucylTryptophan
N-Oleoyl Tryptophan
Indole-Propanoic-2,3-1H-indol
-3-ylmethylaminopropanoic
7-Hydroxyindole-2-carboxylicacid
1-methoxyindole-3-carbaldehyde
3-carboxytyrosine
L-Tyrosine, O-sulfo-
Pacitron/L-tryptophan
3-Indoleacrylate
Lysophosphatidylethanolamine(15:0/0:0)
2,6-Difluoropyridine-3,5-diboronic acid pinacol ester
2,3-dithiomorpholin-4-ylpropanoic acid
1-Isopropylamino-3-(4-(2-methoxyethoxy)phenoxy)-2-propanol
(2S)-2-{[2-carboxy-2-oxoethyl]amino}-3-(1H-imidazol-5-yl)propanoic acid
(2S)-5-(diaminomethylideneamino)-2-[[(2S)-2,4-diamino-4-oxobutanoyl]amino]pentanoic acid
Metabolite Concentration Da
Control
10%SS
2.5%DSS
2.5%DSS10%SS
Treatment

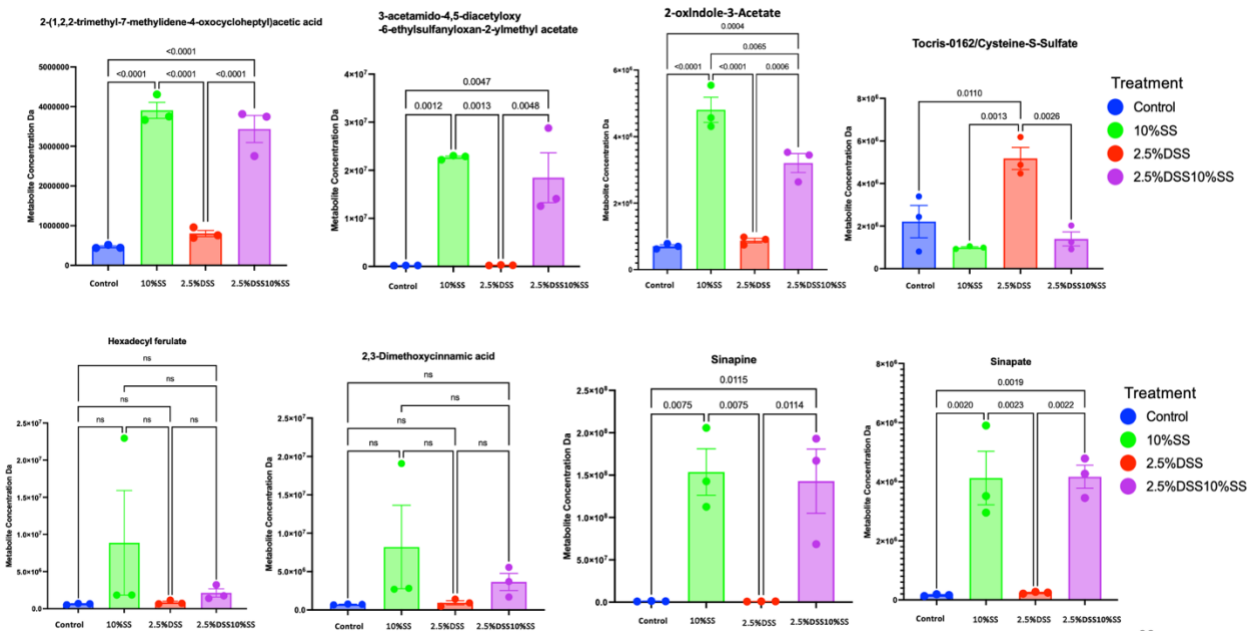


Figure 3.3: Bar plots showing the concentrations of fecal metabolites with significant effects in mice fed with a 10%SS diet, 2.5%DSS treatment, 2.5%DSS10%SS, compared with control groups (n=3 mice/group). Selected beneficial metabolites from steamed broccoli diet can potentially alleviate or are related to a specific metabolic pathway in inflammatory bowel disease. Numbers topping the box indicate significance levels of a post-hoc test following a one-way analysis of variance. Test that were not significant at an alpha level of 0.05 were indicated with ns. Abbreviations: DSS= Dextran Sulfate Sodium and SS= steamed broccoli sprouts.

Table 3.2: Open source information about annotated fecal metabolites of interest, Human Metabolomics Database or PubChem identification number, chemical formula, average molecular weight and CAS number.

S/N	Name	HMDB ID or PubChem CID	Chemical Formula	Average Molecular Weight	CAS Registry number
1	Sulforaphane-N-acetyl-cysteine	HMDB0240561	C11H20N2O4S3	340.47	NA
2	Sabiden/ D-Phenylalanine	HMDB0250791	C9H11NO2	165.1891	NA
3	Phenylalanyl Isoleucine	HMDB0028998	C15H22N2O3	278.352	22951-94-6
4	Phenylalanyl Proline	HMDB0011177	C14H18N2O3	262.309	7669-65-0

Table 3.2 continued

5	Isoleucyl Proline	HMDB0011174	C11H20N2O3	228.292	37462-92-3
6	Valyl Proline	HMDB0029135	C10H18N2O3	214.265	20488-27-1
7	N-(N-(4-(((2,4-Diamino-6-pteridinyl)methyl)methylamino)benzoyl)glycyl)-L-glutamic acid	PubChem CID 326464	C22H25N9O6	511.5	71177-43-0
8	L-Tryptophan-L-Glutamine	HMDB0029081	C16H20N4O4	332.3544	NA
9	Tryptophyl-L-Isoleucine	HMDB0029086	C17H23N3O3	317.3828	NA
10	Leucyl- Tryptophan	HMDB0028940	C17H23N3O3	317.3828	NA
11	N-oleoyl tryptophan	HMDB0241968	C29H44N2O3	NA	NA
12	Indole-Propanoic OR 2,3-1H-indol-3-ylmethyl amino)propanoic	PubChem CID 24802222	C20H19N3O2	333.4	149724-31-2
13	3-Indoleacrylate	HMDB0000734	C11H9NO2	187.198	29953-71-7
14	Pacitron/L-tryptophan	HMDB0000929	C11H12N2O2	204.2252	73-22-3
15	7-Hydroxyindole-2-carboxylic acid	PubChem CID 20039897	C9H7NO3	177	84639-84-9
16	1-Meoxyindole-3-carbaldehyde	HMDB0040972	C10H9NO2	175.184	67282-55-7
17	L-Tyrosine, O-Sulfo-	HMDB0155722	C9H11NO6S	261.252	956-46-7
18	3-carboxy tyrosine	PubChem CID 53728607	C10H11NO5	225.2	NA
19	2,6-Difluoropyridine-3,5-diboronic acid pinacol ester	PubChem SID 458703181	C17H25B2F2NO4	366.2	1204333-58-3
20	Lysophosphatidylethanolamine (15:0/0:0)	HMDB0011502	C20H42NO7P	439.523	NA

Table 3.2 continued

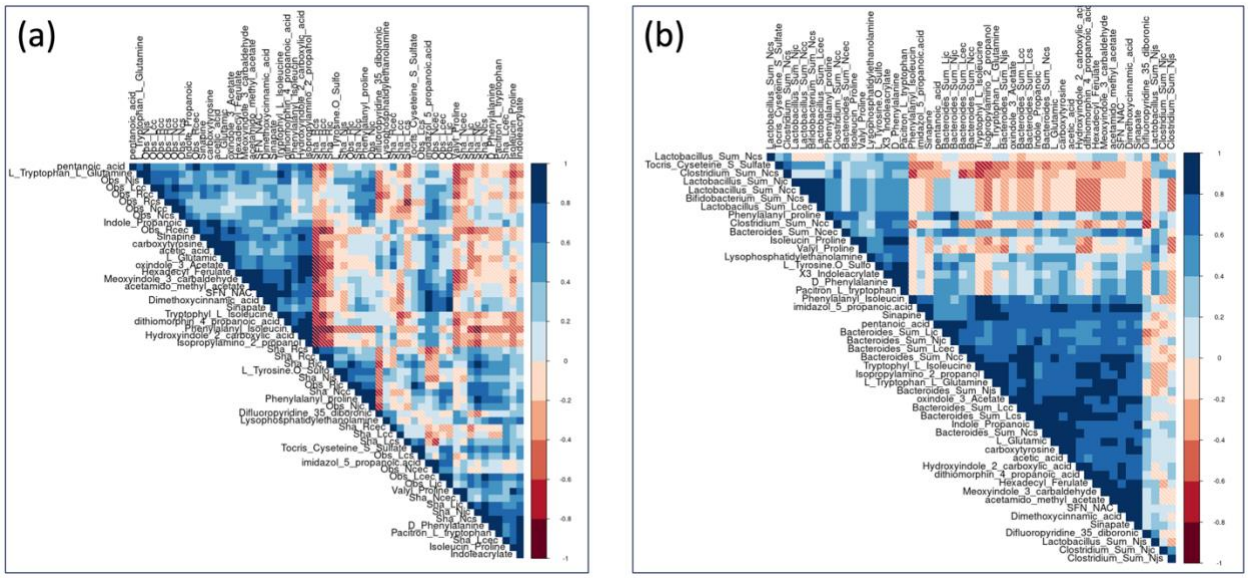
21	2,3-dithiomorphin-4-ylpropanoic acid	ChemWhat Code 997648	C11H20N2O2S2	276.42	691410-93-2
22	1-Isopropylamino-3-(4-(2-methoxyethoxy)phenoxy)-2-propanol	PubChem CID 193122	C15H25NO4	283.36	30311-37-6
23	(2S)-2-[(2-carboxy-2-oxoethyl)amino]-3-(1H-imidazol-5-yl)propanoic acid	HMBD0002271	C6H8N2O2	140.058	1074-59-5
24	(2S)-5 (diaminomethylideneamino)-2-[[(2S)-2,4-diamino-4-oxobutanoyl]amino]pentanoic acid	PubChem CID 92266784	C10H20N6O4	288.3	NA
25	2-(1,2,2-trimethyl-7-methylidene-4-oxocycloheptyl)acetic acid	PubChem CID 16720154	C13H20O3	225.1	NA
26	[(2S,2S,4S,5S,6R)-3-acetamido-4,5-diacetyloxy-6-ethylsulfanyloxan-2-yl]methyl acetate	PubChem CID 4347027	C16H25NO8S	391.4	49810-41-5
27	Hexadecyl Ferulate	HMDB0039317	C26H42O4	418.609	158306-36-6
28	2,3Dimethoxy cinnamic acid	HMDB0034315	C11H12O4	208.211	14737-89-4
29	Sinapine	HMDB0029379	C16H24NO5	310.365	84123-22-8
30	Sinapate	HMDB0032616	C11H12O5	224.51	7362-37-0
31	2-oxindole-3-Acetate	HMDB0035514	C10H9NO3	191.058	2971-31-5
32	Tocris-0162/ Cysteine-S-Sulfate	HMDB0000731	C3H7NO5S2	201.221	1637-71-4

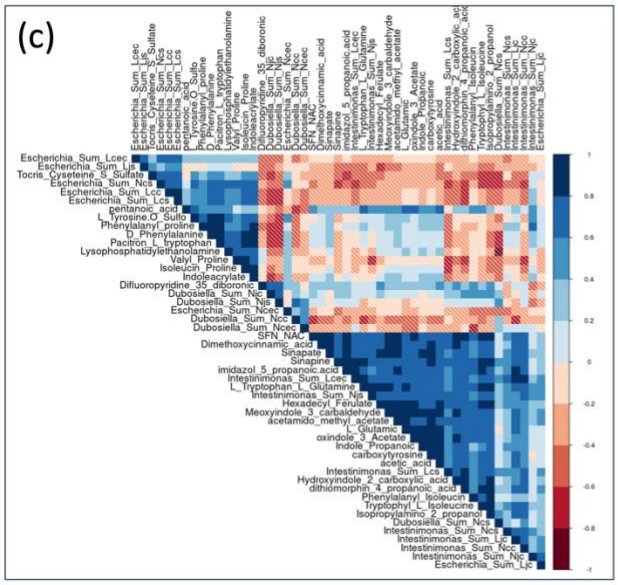
3.4 Correlation analysis between fecal metabolites, microbial richness, bacterial taxa, and *B. thetaiotaomicron*, *BT2160-2156* genes.

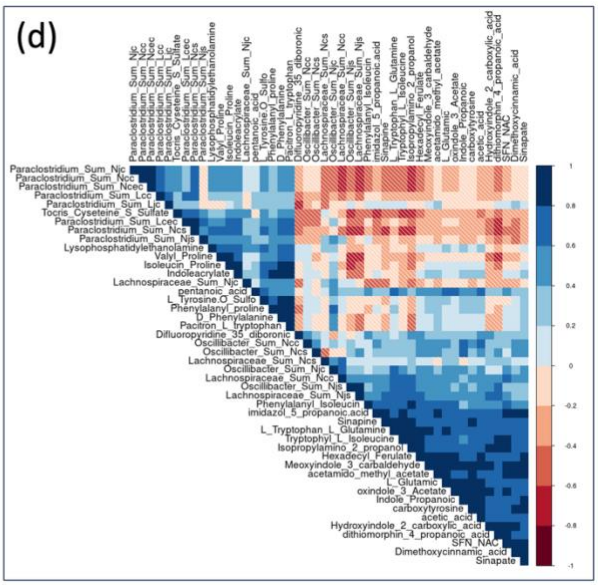
We further performed correlation analyses between the metabolites of interest and previously published data from this experiment on microbial richness, bacteria taxa, and *B.thetaiotaomicron BT2160-2156* genes (Holman et al. 2023). We observed strong relationships between metabolites and a selection of the hydrolyzing microbial populations, further highlighting the importance of microbially derived metabolites during broccoli dietary management of IBD, as shown in Figure 3.4. Cecal microbial richness had positive correlations with broccoli-increased beneficial metabolites, SFN-NAC, phenylalanyl-isoleucine, L-glutamic acid, tryptophyl-L-isoleucine, indoles, 3-carboxy tyrosine, sinapic acid, derivatives of propanoic acid and acetic acid, hexadecyl ferulate, and 2,3-Dimethoxycinnamic acid. Meanwhile, cecal microbial richness had zero or negative correlation with the DSS-increased metabolites such as L-tryptophan, indole acrylate, O-sulfotyrosine, cysteine-s-sulfate, and prolines. Colon scraping and content have similar strong positive correlation with broccoli beneficial metabolites such as L-glutamic (strongest correlation), L-tryptophan-L-glutamic, trsptophyl-L-isoleucine, indoles, 3-carboxytyrosine, sinapic acid, derivatives of propanoic acids, boronic acid and acetic acids, hexadecyl ferulate, and 2,3-Dimethoxycinnamic acid.

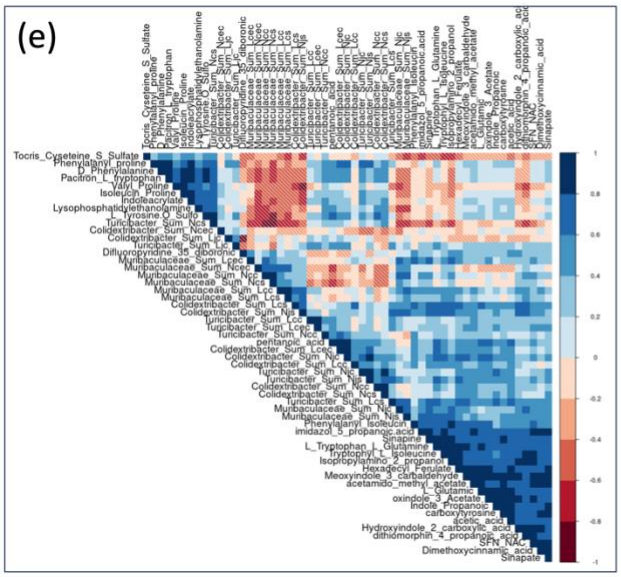
Metabolites SFN-NAC, phenylalanyl-isoleucine, L-glutamic, L-tryptophan-L-glutamic, tryptophyl-L-isoleucine, indoles, 3-carboxytyrosine, sinapic acid, derivatives of propanoic acids, boronic acid and acetic acids, hexadecyl ferulate, and 2,3-Dimethoxycinnamic acid showed positive relationships with *Bacteroides spp.* in the gut, especially from jejunum scrapings, colon content, and scrapings, *Intestinimonas* in jejunum scraping, colon, cecal contents and scrapings, and *Lachnospiraceae* in jejunum scraping, colon scrapings and cecal contents. There were

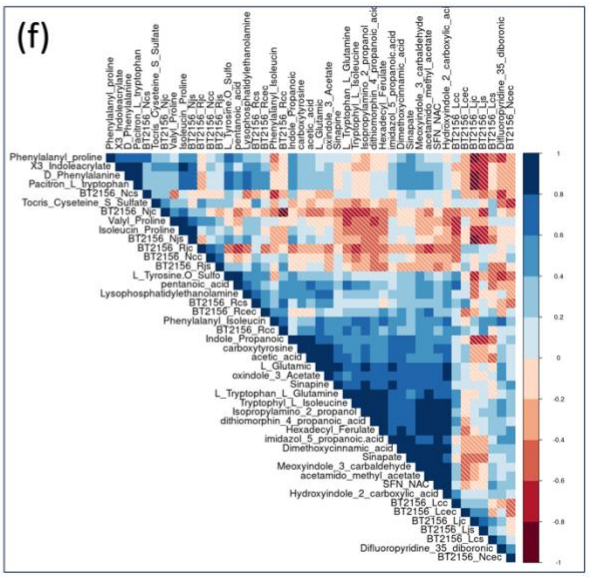
negative correlations between these metabolites and *Paraclostridium* in the cecal contents and colon scrapings, *Dubosiella* in the colon and cecal contents, *Escherichia* in the jejunum and colon scrapings and cecal content. Positive correlations were observed between D-Phenylalanine, L-tryptophan, indole acrylate, O-sulfotyrosine, cysteine-s-sulfate, and prolines and *Lactobacillus* in jejunum and colon scrapings, cecal and jejunum contents, *Paraclostridium* in the cecal contents and colon scrapings. Similar to *Bacteroides* in the gut, most of the broccoli-increased metabolites, SFN-NAC, phenylalanyl-isoleucine, L-glutamic, L-tryptophan-L-glutamic, tryptophyl-L-isoleucine, indoles, 3-carboxytyrosine, sinapic acid, derivatives of propanoic acids, boronic acid and acetic acids, hexadecyl ferulate, and 2,3-Dimethoxycinnamic acid positively correlated with *BT 2156* in the cecal content, *BT 2157-2158* in the cecal content and jejunum scrapings, *BT 2159* in cecal content, and *BT 2160* in the jejunum scraping.




(c)


(d)


(e)


(f)

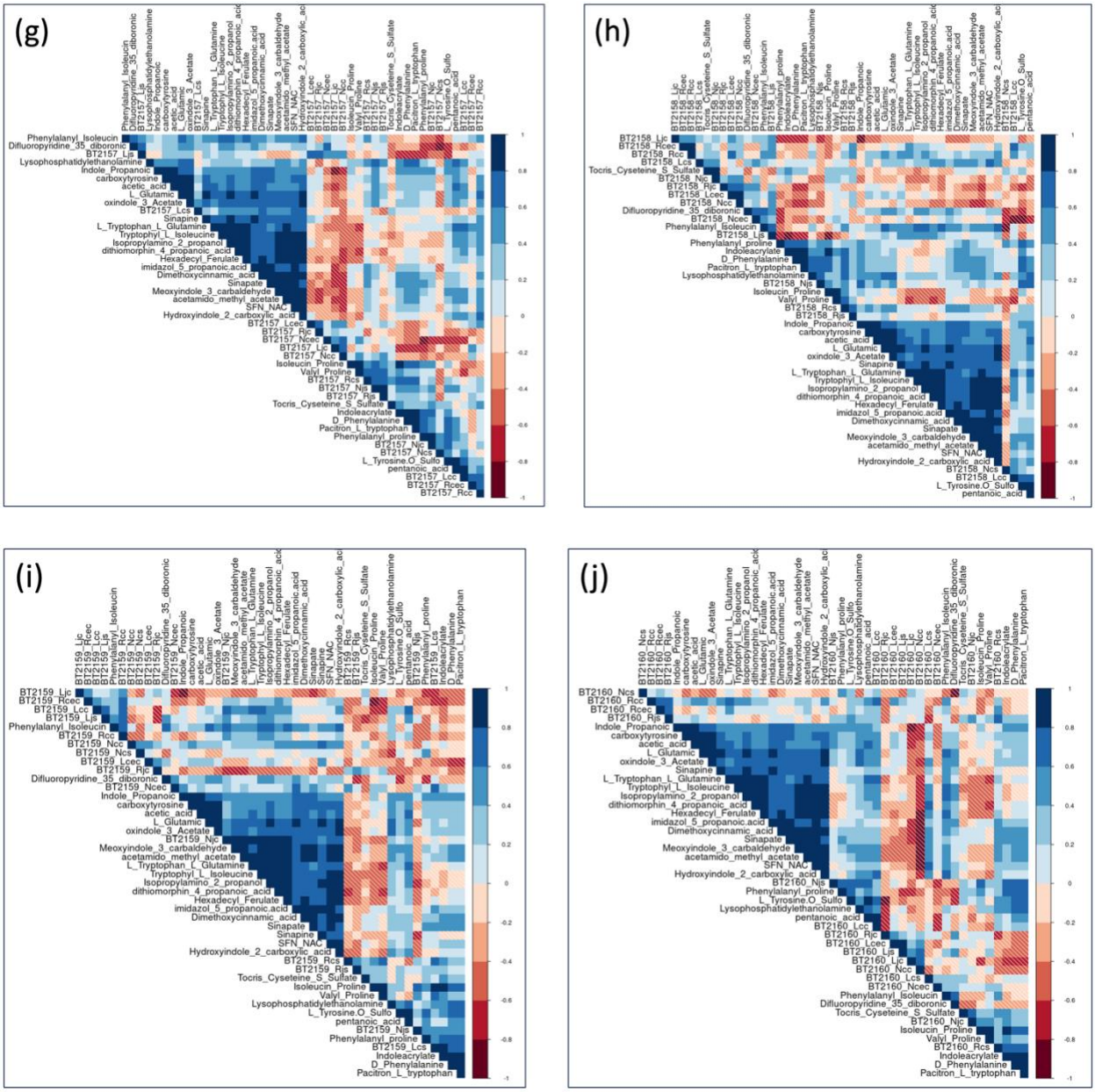


Figure 3.4: Correlogram of fecal metabolites, bacterial diversity metrics, IBD-associated bacterial taxa, and B. thetaiotaomicron genes (BT 2160-2156). The color scale by the plot illustrates the strength and direction of the pairwise association, which is determined by the correlation coefficient between each pair of variables at p values 0.05 or less.

3.5 Discussion

Broccoli sprouts diets have become of interest to nutritional scientists as a potential option for IBD management due to the presence of beneficial phytochemicals such as glucosinolates,

polyphenols, flavonoids, and fibers (Nagraj et al. 2020; Yanaka 2018; Bouranis et al. 2024). These dietary metabolites can be hydrolyzed by certain gut bacteria to increase the production of microbial-derived bioactive compounds with the capacity to modulate and alleviate IBD-induced inflammatory, oxidation, dysbiosis, and gut cellular damage pathological pathways(Liu et al. 2017; Catalkaya et al. 2020; Parada Venegas et al. 2019; Duan et al. 2023; Roager and Dragsted 2019). We have suggested that broccoli and other cruciferous vegetables could serve as potential nutritional options for IBD management by increasing microbial diversity and modulatory bioactive compounds with antioxidants, anti-inflammatories, and gut protective properties to alleviate IBD pathologies (Alaba et al. 2024). As part of this study, we have previously reported the beneficial effect of a 10% SS diet to restore IBD-related weight loss, decrease disease activity index and inflammatory markers, increase the metabolism of GLR to anti-inflammatory SFN and preserve healthy biogeographical patterns of diverse bacteria in the gut (Holman et al. 2023; Zhang et al. 2023). Although many studies have reported SFN as the active compound related to broccoli's nutritional benefit, other vital metabolites and the gut bacteria involved in their metabolisms still remain understudied. Therefore, this research reports the novel dietary and microbially hydrolyzed metabolites with specific anti-inflammatory, antioxidative, and gut protective effects and their associated bacteria communities after mice were fed with steamed broccoli sprouts diets as nutritional management for IBD in the DSS-mice model.

This study revealed a significantly increased SFN-NAC concentration in the 10% SS diet compared to the control and 2.5% DSS groups, which is expected as the base mouse diet does not contain GSLs, and bacteria are not known to spontaneously produce SFN conjugates. However, the administration of the diet did not significantly increase SFN-NAC in the 2.5% DSS + 10% SS group, though there was a trend of increase similar to the 10% SS group, which likely indicates

that GLR-converting bacteria were harmed or inactivated by the presence of DSS. Previous research indicated that high concentrations of NaCl can inactivate myrosinase (Guo et al. 2013), thus DSS treatment may perhaps affect bacterial myrosinase-like enzymes as well. This data is consistent with the previous report that suggests that SFN-NAC is upregulated in the fecal samples through microbial hydrolysis of glucoraphanin (Wu et al. 2023; Bouranis et al. 2024). SFN-NAC metabolite possesses antioxidant and anti-inflammatory properties in the gut with the ability to transform proinflammatory monocytes into macrophages, upregulate Treg cells to release IL-10 and modulate NFkB, STAT3 inflammatory, and activate NRF oxidative pathways (Liang et al. 2018; Fernandez-Prades et al. 2023; He et al. 2022; Negi et al. 2011). The concentrations of polyphenolic metabolites, sinapate, and sinapine were enormously significant in the fecal samples of mice that ate 10% SS and 2.5% DSS + 10% SS groups compared with control and DSS groups. This finding supports the importance of sinapic metabolites as one of the significant crucifer-derived phenolic compounds reported in the literature regarding the antioxidant and anti-inflammatory benefits of the broccoli diet (Olszewska et al. 2020). Sinapic acid possesses the ability to upregulate GSH and decrease MDA, NFkB, IL1β, IL-6, TNFα, iNOS, COX-2, and MCP-1 gene expression (Ayaz et al. 2008; Choi et al. 2016). It can also protect gut barriers against damage via I-CAM expression and mitigate psychological stress during IBD (Park et al. 2017; Kim et al. 2021; Choi et al. 2016). Hexadecyl ferulate and 2,3-Dimethoxycinnamic acid are phenolic metabolites with antioxidative and anti-inflammatory effects against COX-2 and NFkB pathways (Jayaprakasam et al. 2006; Atta et al. 2017; Murad et al. 2016).

Amines, D-phenylalanine, phenylalanyl isoleucine, 3-carboxy tyrosine, and L-glutamine were significantly increased in the 10%SS when compared with the control and 2.5%DSS, and in the 2.5%DSS10%SS mice when compared with the 2.5%DSS mice. Phenylalanine and tyrosine

metabolites can be beneficial or detrimental during IBD, depending on the conjugates and pathways engaged (Montenegro-Burke et al. 2021; Uranga et al. 2016). For instance, O-sulfotyrosine, upregulated in our DSS mice, has been implicated in leukocyte cell adhesion for inflammatory invasion (Stewart and Ronald 2022). Meanwhile, conjugating a carboxyl group and tyrosine is associated with COX-2 inhibition, which was revealed as upregulated 3-carboxytyrosine in mice that ate broccoli with or without DSS (Levina et al. 2018). In our data set, D-phenylalanine (sabiden) and phenylalanyl isoleucine increased in the 2.5%DSS10%SS and 10%SS groups, which could alleviate oxidative stress and recruit Tregs into the gut against inflammation (Wang et al. 2020; Guan et al. 2020). Glutamic metabolite was increased with diet with and without DSS. This metabolite possesses the ability to protect the mucosal and cellular layer of the gut from cytokines, NFkB, TNFα, and nitric oxidative stress damage and increase epithelial proliferation to maintain tight junction and prevent pathologic translocation of bacteria into the gut (Lee et al. 2022; Xu et al. 2021; Santos et al. 2014; Mondello et al. 2010). Similarly, our data identified increased concentrations of tryptophan metabolites and indoles, L-Tryptophan-L-glutamine, tryptophyl-L-isoleucine, 7-dihydroxyindole-2-carboxylic acid, 1-methoxyindole-3-carbaldehyde with antioxidative, anti-inflammatory and gut protective benefits, after broccoli sprouts diet (Zhang et al. 2021; Geng et al. 2018; Vega-Galvez et al. 2023). Thus, these results confirm that tryptophan microbial metabolism may play a significant role in the beneficial effects of broccoli on IBD management. We observed an increased level of L-tryptophan with DSS treatment when compared with the control and broccoli diet, which could be due to the activities of tryptophan to recruit T-cells to the site of damage in response to the presence of cytokines during IBD (Ding et al. 2020; Esperanza et al. 2020; Sikalidis 2015). The role of tryptophan and glutamine conjugate, L-Tryptophan-L-glutamine, increased with 10%SS and 2.5%DSS10%SS, which

buttress the role of glutamate rings in tryptophan/kynurenine metabolic pathway (Andrade et al. 2015; Santos et al. 2014; Moroni 1999). The broccoli diet is fiber-rich, which serves as a substrate for microbial production of SCFAs. The metabolites identified in our data were mainly propanoic/propionic, boronic acid and acetates, such as 2-oxindole-3-acetate, 1-isopropylamino-3-(4-(2-methoxyethoxy)phenoxy)-2-propanol, 2,6-difluoropyridine-3,5-diboronic acid pinacol ester, 2,3-dithiomorpholin-4-ylpropanoic acid and 2-(1,2,2-trimethyl-7-methylidene-4-oxocycloheptyl) acetic acid with broccoli sprouts diet suggesting the gut protective, anti-inflammatory and antioxidative and microbial diversity effects of SCFAs in benefits of broccoli diet against IBD (Yan et al. 2023; Gao et al. 2018; Yang et al. 2021).

The production of microbially-derived metabolites in the gut depends mainly on the bacteria communities engaged during the hydrolysis of dietary compounds from broccoli sprouts. We have reported that steamed broccoli sprout diet supports communities' richness, especially *Bacteroides spp.*, which are involved in GLS hydrolysis to SFN (Holman et al. 2023; Zhang et al. 2023). We also reported biogeographical patterns of bacteria richness in the gut, with higher *BT 2159-2156* genes in jejunum scrapings, cecal contents and colon scrapings after the broccoli diet with and without DSS. The *Bacteroides spp.* and *BT2160-2156* operon were increased in cecal contents after the broccoli diet with DSS (Holman et al. 2023). To identify bacteria communities and taxa patterns associated with microbially-derived metabolites, we performed correlation analysis between fecal metabolites, microbial richness, bacterial taxa, and *B. thetaiotaomicron, BT 2160-2156* genes. Our results revealed a positive relationship between beneficial metabolites upregulated with the broccoli sprouts, such as SFN-NAC, L-glutamic, L-tryptophan-L-glutamic, tryptophyl-L-isoleucine, indoles, 3-carboxy tyrosine, sinapate, derivatives of propanoic acids, boronic acid and acetic acids, hexadecyl ferulate, and 2,3-Dimethoxycinnamic, and observed

richness, commensal bacteria taxa, *Bacteroides spp.*, *Intestinimonas*, *Oscillibacter* and *Lachnospiraceae,* mainly in the colon, cecal and jejunum regions of the gut. These findings support the report that GLS hydrolysis to SFN occurs majorly in the colon and cecum (Zhang et al. 2023; Lai et al. 2010), primarily due to *Bacteroides spp.* enriched in the colon (Liou et al. 2020; Frank et al. 2007). *Bacteroides thetaiotaomicron, BT 2160-2156* genes are required for GLS hydrolysis in the gut (Liou et al. 2020), and our finding showed a positive relationship between beneficial metabolites and *BT 2160-2156* genes in the colon and cecum. *Intestinimonas* are involved in the microbial hydrolysis of amine metabolites and SCFAs production as anti-inflammatory mechanisms and protection against intestinal oxidative stress and gut damage (Du et al. 2020; Riaz Rajoka et al. 2021; Bui et al. 2020). Similarly, *Lachnospiraceae* has been reported to protect against the risk of Crohn's disease in the ileocolic region and ulcerative colitis rectosigmoiditis in the gut by hydrolysis dietary fibers for SCFA and amines for phenolic acids production (Li et al. 2023; Zhang et al. 2021; Frank et al. 2007; Zaplana et al. 2023; Vita et al. 2024). The *Oscillibacter* has been associated with microbial metabolism of polyphenols, decreased hyperlipidemia, recovery from colitis and increased gut protection (Liu et al. 2022; Zhang et al. 2022; Haiou et al. 2024; Shi et al. 2022). Meanwhile, our data showed positive correlations between cecal and colonic-located *Lactobacillus* and *Paraclostridium* with broccoli-enriched metabolite, D-Phenylalanine, and DSS enriched metabolites, L-tryptophan, indole acrylate, O-sulfotyrosine, cysteine-s-sulfate, and prolines, (Vita et al. 2024). Proline residues and tryptophan are targets for *Lactobacillus* hydrolysis to increase aryl hydrocarbon receptor sensitivity and decrease gut inflammation (Hou et al. 2023; Geng et al. 2018). *Paraclostridium* is associated with pathological development and severity of ulcerative colitis, such as intestinal barrier damage, colon rupture, and bloody stool (Kutsuna et al. 2019; Kutsuna et al. 2018). Bacterial diversity and metabolic

activities prominent in the colon and cecum may be associated with less pH in regions favorable for hydrolysis of dietary metabolites (Press et al. 1998; Nugent et al. 2001; Newmark and Lupton 1990).

3.6 Conclusion, limitations and future direction

Steamed broccoli sprouts demonstrate significant potential as a nutritional intervention for managing inflammatory bowel disease (IBD) by modulating fecal metabolites, such as sulforaphane N-acetyl cysteine, amines, indoles, phenolic metabolites and short-chain fatty acids, associated with anti-inflammatory, antioxidative, and gut-protective effects. This study elucidates novel microbially derived metabolites and their associations with specific bacterial communities, such as *Bacteroides spp., Intestinimonas, Oscillibacter*, *Lachnospiraceae, and Bacteroides thetaiotaomicron, BT 2160-2156* genes, shedding light on the intricate mechanisms underlying the beneficial effects of broccoli in IBD management. Additionally, identifying increased concentrations of crucial metabolites such as sulforaphane N-acetyl cysteine (SFN-NAC) and sinapic acid highlights their potential as critical mediators of the observed therapeutic effects. We highlighted these specific regions of metabolites and microbial activities in the gut. Further research should focus on elucidating the targeted nutritional interventions with broccoli-derived metabolites that interact with the gut microbiota to mitigate IBD pathology in diverse age groups. Additionally, investigations should include translating these findings into human trials to validate the efficacy of steamed broccoli sprouts as a viable dietary approach for clinical IBD management.

CHAPTER 4

BROCCOLI SPROUTS ALLEVIATE ULCERATIVE COLITIS IN MICE BY INCREASING DIETARY AND MICROBIAL METABOLITES: DIFFERENTIAL EFFECTS IN YOUNG AND ADULT MALE AND FEMALE MICE

4.1 Abstract

Inflammatory bowel disease is a chronic condition with a higher risk and disease severity in older adults, especially women. Raw broccoli sprouts diet may cover benefits in early life through the anti-inflammatory bioactive compounds and increase microbial diversity. Still, these benefits have not been explored with differential diet preparation, gender, and age groups. Therefore, we assessed the potential effect of steamed and mildly heated broccoli sprouts on plasma metabolite concentrations in young and adult and female and male mice with ulcerative colitis type of inflammatory bowel disease. Our data revealed protective effects of a steamed broccoli sprouts diet in adult female mice against colon damage, weight loss, and disease activity, with increased plasma concentrations of dietary metabolites, such as glucoraphanin and glucoerucin, and microbial metabolites such as iso-ferulic acid, flavanone-4-glucuronide, and denticulaflavonol. Meanwhile, adult male and younger mice had similar anti-colitis benefits with mildly heated broccoli sprout intervention with increased concentrations of dietary and microbial-derived metabolites such as catechin, proanthocyanidins, short-chain fatty acids, and glutathione derivatives. This research generates novel information for future targeted applications of different broccoli sprouts diets as dietary interventions for the nutritional management of ulcerative colitis in specific age groups and genders.

4.2 Introduction

Inflammatory bowel disease (IBD) is a gastrointestinal tract pathological condition categorized into two main presentations: ulcerative colitis (UC) and Crohn's disease (CD). IBDs affect millions of people worldwide (Seyedian et al. 2019; Tian et al. 2017). In the United States, over 3 million have been diagnosed with IBD (Han et al. 2020; Xu et al. 2021), and the prevalence of IBD has increased substantially in recent years, causing a significant burden to healthcare systems (Ng et al. 2017; Jairath and Feagan 2020). Chronic colon inflammation, dysbiosis, and intestinal damage are some of the characteristics of both presentations, with nuances in severity and location of symptoms by disease and age. Onset is often in children and young adults, although it can take years to be formally diagnosed, but 10-year longitudinal study revealed that about 45% of patients (both CD and UC) are adults between 30-59 years and about 48% are patients older than 60 years (Lewis et al. 2023). Similarly, the risk of IBD is also high among female adults over 50 years of age (8,9), which is attributed to immune and microbial interactions with estrogens (Jacenik et al. 2019).

The primary cause of IBD is still unknown, and there are multiple triggers. Age and particularly puberty are linked to disease onset (Papa et al. 2012; Kowalska-Duplaga et al. 2019), but stress, environmental conditions, and diet are some of the factors linked to the high incidence of IBD (Goodman et al. 2020; Ge et al. 2022). As diet is a source of anti-inflammatories, antioxidants, and a strong modifier of gut microbial communities, it has long been a strategy for mitigating IBD symptoms. Although there was no significant difference between the dietary habits of adults with and without IBD, some nutritional surveys have revealed interesting associations between IBD risk and diet - particularly unhealthy consumption of diets high in sugar, fat, and meat protein (Han et al. 2020; Xu et al. 2021). Specifically, dietary total meat consumption

increases the risk of IBD by almost 40% (Talebi et al. 2023), as does more energy drinks, as well as regular soda, than adults without IBD (Han et al. 2020; Xu et al. 2021). So-called "Western diets", which include contain highly processed foods which tend to contain high fats, red meat or processed protein, and simple carbohydrates, may promote IBD pathogenesis (Mithul Aravind et al. 2021; Catalkaya et al. 2020; Lin et al. 2017; Shen et al. 2014), although dairy proteins which are also high in these diets are protective against IBD (Talebi et al. 2023). Thus, specific foods may play less of a role in disease onset than the overall pro/anti- oxidative or inflammatory potential of a diet.

Consuming diets loaded with fiber, antioxidants, and phytochemicals have been shown to provide anti-inflammatory benefits, promote a healthy gut, aid digestion, and lower the risk of IBD (Deng et al. 2023; Alaba et al. 2024). Cruciferous vegetables are a diverse group of economically important plant species, including broccoli, cauliflower, cabbage, kale, bok choy, brussels sprouts, collards, turnips, and radishes. Cruciferous vegetables are representative of a healthy diet that promotes good health and well-being (Statovci et al. 2017; Kassem et al. 2023), and contain numerous bioactive phytochemicals with anti-oxidant and anti-inflammatory properties (Alaba et al. 2024). Previous studies have linked broccoli consumption to protection against dysbiosis, chronic gut inflammation, and IBD risks, however; little research is available on the processing methods that confer these protective benefits, as preparation can affect the location of benefits in the gut.

The methods of broccoli preparation significantly impact the presence of dietary metabolites (Wu et al. 2016; Wang et al. 2018; Zhang et al. 2023; Charron et al. 2020; Yuan et al. 2009), as mild heat treat preserves broccoli-sourced myrosinase but inactivates epithiospecifier protein which results in high dietary anti-inflammatories which are absorbed in the stomach and

duodenum, while heat treatment inactivates both enzymes and preserves dietary glucosinolates which instigate microbially derived metabolites in the large intestines (Holcomb et al. 2023; Zhang et al. 2023; Holman et al. 2023; Li et al. 2013). For instance, a blanched broccoli diet decreased SFN metabolites and promoted microbial interconversion into erucin metabolites in healthy human subjects (Saha et al. 2012), while steamed broccoli diet and juice increased SFN levels with decreased inflammatory markers and dysbiosis in DSS-mice (Samuel et al. 2015; Zhang et al. 2023; Holman et al. 2023; Li Y, Zhang T, Holman J, et al. 2022). Another study compared the disparate availability of SFN and erucin metabolites in extraintestinal tissues after female hairless mice consumed steamed, raw, or mildly heated broccoli sprouts. These researchers reported mildly heated broccoli preparation with the most abundant presence of the microbially derived SFN and erucin metabolites (Bricker et al. 2014). Since younger CD patients experience more small intestine-based symptoms which older CD patients experience more colitis, and since puberty and immune development modulates microbial communities, age and immune development may make microbial communities more or less receptive to change (Holcomb et al. 2023), additional investigation is required to identify the best broccoli processing strategies that can provide the most significant benefits to create targeted treatment plans for different age and sex groups of people with IBD.

In this study, we aim to assess the effect of steamed and mildly heated broccoli preparations using a comprehensive panel of untargeted metabolomics (Clish 2015) to annotate the concentrations of dietary and microbial metabolites. We hypothesized that steamed and mildly heated broccoli sprouts may differentially increase the protective properties of broccoli with age (young and adult) and sex (male and female)-specific benefits against IBD. To explore the potential benefits of broccoli sprouts against IBD, the metabolites from steamed (ST) and mildly

heated (MH) broccoli in male and female, young and adult, were compared with dextran sodium sulfate-induced ulcerative colitis mice. Advanced untargeted metabolomic approaches have rarely been used to compare different preparations of broccoli and their effectiveness in producing microbial metabolites with bioactivity on different age and sex groups. Our research identified the differentially expressed metabolites between different sexes and age groups that may be beneficial against IBD risk. These findings provide relevant information on personalized IBD nutritional management for adults and women at highest risk of IBD (Lewis et al. 2023; Weisman et al. 2023) through using broccoli sprout preparation as part of an IBD nutrition intervention.

4.3 Methods

4.3.1 Diet preparation

We purchased raw broccoli sprouts from a local grocery store (Shaw's, ME) and then steamed them for 10 minutes over boiling water or mildly heated them at 60 °C in a water bath for 10 minutes, after which both preparations were freeze-dried and ground into fine powder. The steamed and mildly heated broccoli sprout powders were mixed individually with purified AIN93G powder (ScottPharma Solutions, Marlborough, MA) to make either a 10% (w/w) steamed diet or 10% (w/w) mildly heated broccoli diet.

4.3.2 Animals and experimental design

The animal protocol was approved by the Binghamton University IACUC (protocol number 882-22). Male and female (n = 4 of each per treatment group), young (four weeks old) and mature (eight weeks old) Specific Pathogen Free (SPF) C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, Maine). After acclimation to the facility, mice were randomized into 12 experimental groups based on broccoli diet preparation, age, and sex, as shown in Table 1. Mice

were housed in individual cages in a pathogen-free, humidity- and temperature-controlled room under a 12:12 hour light: dark cycle at the School of Pharmacy and Pharmaceutical Sciences, SUNY Binghamton University (Johnson City, New York). The mice in the broccoli groups were fed for 7 days, after which colitis was induced for five days in all the mice with 2% DSS added to the drinking water, followed by a five day recovery period in which mice received the broccoli diet without DSS (Zhang et al. 2023; Holman et al. 2023).

Table 4.1: Classification of experimental groups based on broccoli diet, age and sex.

Cage #	**Experimental group**	**Cage #**	**Experimental group**
Cage 1	4 week old (young) male control with DSS	Cage 7	8 week old (mature) male control with DSS
Cage 2	4 week old (young) female control with DSS	Cage 8	8 week old (mature) female control with DSS
Cage 3	4 week old (young) male MH with DSS	Cage 9	8 week old (mature) male MH with DSS
Cage 4	4 week old (young) female MH with DSS	Cage 10	8 week old (mature) female MH with DSS
Cage 5	4 week old (young) male ST with DSS	Cage 11	8 week old (mature) male ST with DSS

Table 4.1 continued

Cage 6	4 week old (young) female ST with DSS	Cage 12	8 week old (mature) female ST with DSS

DSS- dextran sodium sulfate; ST- steamed broccoli diet; MH- mildly heated broccoli diet.

4.3.3 Assessment of colitis and tissue harvest

Fresh fecal samples were collected daily. Body weight (BW), fecal occult blood, activity levels, and appearance were monitored daily for colitis assessment. Disease activity index (DAI) scores were calculated daily based on the sum of the weight loss, the rate of diarrhea, and the hematochezia. Weight loss percentage was scored as 0 for <1%, 1 for 1-5%, 2 for 6-10%, 4 for >15%. Stool consistency was scored 0 for normal, 2 for loose stool, and 4 for diarrhea. Hematochezia scored 0 for absence and 2 for presence. After five days of DSS and five days of recovery, mice were sacrificed for tissue harvest. Blood samples were obtained by direct cardiac puncture and collected in heparinized tubes. Colon tissues were collected and measured for length. Statistical analysis was performed for BW, DAI scores, and colon length differences among treatment groups based on age and sex using One-way ANOVA at $p<0.05$ with Tukey's post-hoc test.

4.3.4 Metabolomics analysis

Plasma was collected from blood samples via centrifuge at 3000 rpm for 10 minutes at 4 °C and stored at -80°C for metabolomics analysis. Aliquots of 10 µl serum samples were added to 70 ul of extraction buffer (40% acetonitrile, 25% methanol, and 35% water). The solution was incubated on ice for 10 minutes and centrifuged at 13000 rpm for 20 mins at 4°C. The precipitated metabolites were aliquoted into fresh tubes, vacuum dried and reconstituted into 50%

acetonitrile/water for analysis. Liquid chromatography-mass spectrometry (LC-MS) data acquisition and processing were performed using trapped ion mobility spectrometry time-of-flight machines (timsTOF), according to the previous report (Alsoud et al. 2022). The extracted proteins were analyzed using a UHPLC system (Bruker Intensity Solo 2 C18 column, 2.1mm x 100 mm; MS system: Bruker timsTOF Pro 2, San Jose, CA) with 3 µl of injection of each sample and blanks per column at liquid phase conditions shown in Table 2. Metabolites were identified from LCMS data preprocessed with an in-built Bruker's MetaboScape 2023b and MetaboAnalyst 5.0 (http://www.metaboanalyst.ca) data analysis software (Pang et al. 2022) to obtain the retention time, mass charge ratio, molecular weight and peak intensity, metabolites name and formula for each sample using several libraries (Esperanza et al. 2020; Peng et al. 2021).

Table 4.2: Liquid Chromatography conditions for metabolomics.

Time (min)	**A (%)**	**B (%)**
0.00	98.0	2.0
1.00	98.0	2.0
15.00	2.0	98.0
17.00	2.0	98.0
18.10	98.0	2.0
20.00	98.0	2.0

A: 95:5 water:acetonitrile with 0.1% formic acid

B: acetonitrile with 0.1% formic acid. Flow rate of 0.3 mL/min

4.3.5 Metadata and statistical analyses

The metadata was pre-processed in R to remove duplicate metabolite calls and correct for peak intensity errors before importing into MetaboAnalyst 5.0 (Pang et al. 2022). After that, the data were normalized by log transformation. Univariate One-way ANOVA analysis was performed at $p< 0.05$ in a scatter plot with Fisher's post-hoc test to annotate specific metabolites of interest that are statistically different across the experimental groups in box plots. We performed unsupervised and supervised discriminant analysis for sample and group variation. The unsupervised principal component analysis (PCA) made the 2-way comparison, while multigroup supervised partial least squares discriminant analysis (PLS-DA) and sparse PLS-DA (sPLS-DA) to reduce variability and improve the estimate. Hierarchical clusters were performed with heatmaps to observe the correlation between the experimental groups and metabolite concentration. Quantitative enrichment analysis was conducted to identify metabolic patterns compared with KEGG databases represented as bubble plots and enrichment ratio graphs. The differential expression within the treatment groups reflects the metabolic pathways associated with the metabolites based on broccoli diets, age, and sex.

4.4 Results

4.4.1 Body Weight, DAI scores and Colon length

The results from this experiment showed that MH and ST broccoli diets differentially decreased the severity of DSS-induced colitis based on age and sex. The ST diet restored weight loss in young male and adult female mice, while MH restored BW in young female and adult male mice(Figure 4.1a). Both the MH and ST diets significantly decrease DAI scores in young female and male, and adult male mice, while only the ST diet decreased DAI in adult female mice (Figure 4.1b). The MH and ST diets significantly restored colon and rectum length in young male and

adult female mice, while none of colon and rectum length in adult males were restored (Figure 4.1c).

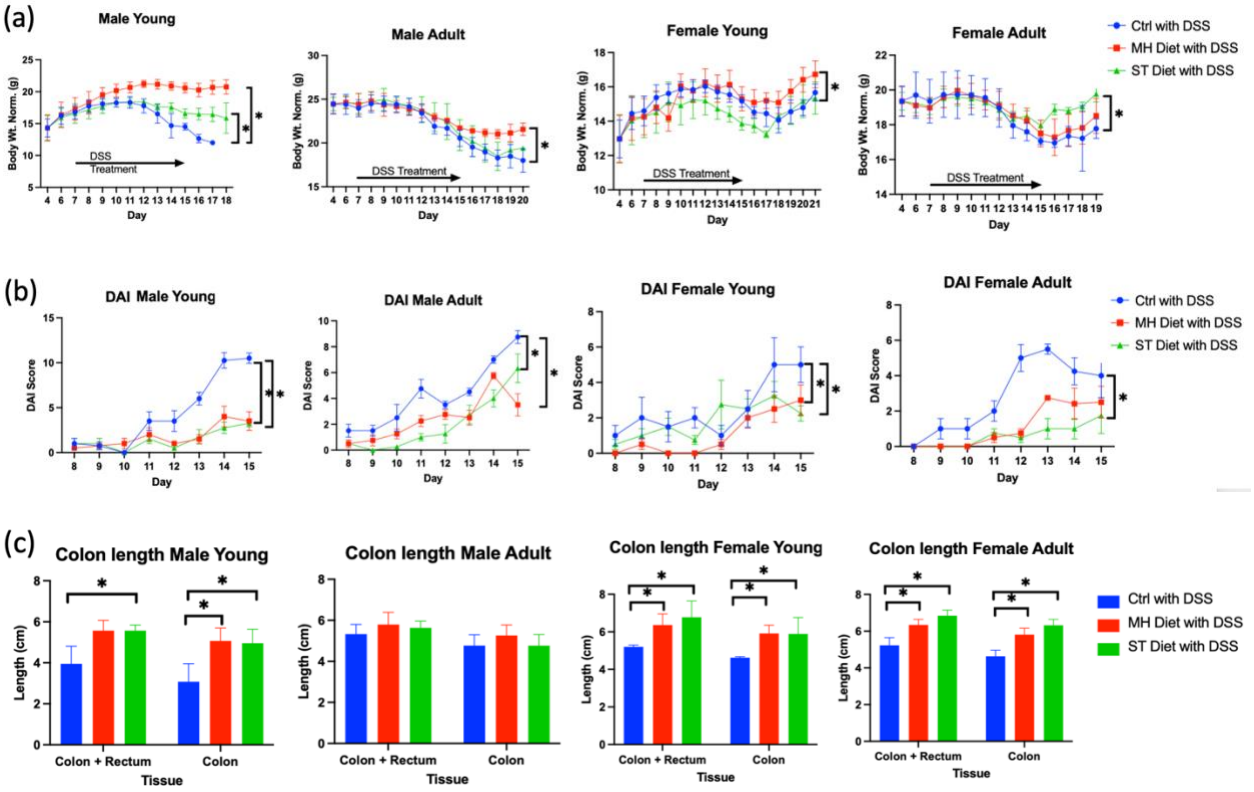


Figure 4.1: Mildly heated or steamed broccoli sprout diets improve weight gain and colon growth and reduce disease activity index by age and sex of mice with chemically induced colitis. One-way ANOVA ($p<0.05$) graphs of (a) body weight difference; (b) DAI score difference, and (c) colon length difference of male and female young, male and female adult groups. Abbreviations: DAI=disease activity index, g=grams, and cm=centimeter.

4.4.2 Univariate ANOVA and discriminant analyses of metabolites in MetaboAnalyst 5.0

Further analysis with supervised PLS-DA showed intergroup clustering of the data set, with PC 1 at 19.9% and PC 2 at 11.8% (Figure 4.2a). Further analysis with supervised PLS-DA plots showed the data set's intergroup clustering, except for the young male groups, with PC 1 at 9.6% and PC 2 at 11.5% (Figure 4.2b). We conducted sPLS-DA, which separated the young F

control and young F MH groups from other groups, and the result showed the inter-clustering with component 1 at 10% and component 2 at 10.1% (Figure 4.2c).

The untargeted metabolites analysis we conducted identified 2572 metabolites. After filtering the data in R, this was reduced to 848 metabolites imported in MetaboAnalyst for univariate one-way ANOVA at $p< 0.05$. The result showed 476 metabolites with significant increased or decreased concentrations across the twelve experimental groups in a scatter plot (Figure 4.2d). These differential concentrations are glucosinolates, flavonoids, polyphenols, short-chain fatty acids, amino acids, tryptophan, carnitine derivatives and organic acid metabolites. The post-Hoc test shown in box plots (supplementary Figure A.1) highlight some significant metabolites of interest such as glucoerucin, glucoraphanin, isoferulic acid, denticulaflavonol, equol 4'-O-glucuronide, propionic acid, ethyl heptafluorobutyrylacetate, dilauryl thiodipropionate, 3-hydroxydodecanoic acid, 2-oxo-tetradecanoic acid, acetylcysteine, taurine, indolelactic acid, gamma-CEHC, alpha-tocopherol succinate, hippuric acid, nicotine glucuronide and uridine triphosphate glucose associated age, diet preparation and sex difference across the groups. The ST diet increased glucosinolate and flavonoid metabolites such as glucoerucin, glucoraphanin, denticulaflavonol and 4',5-dihydroxy-3,3'-dimethoxy-6, 7-methylenedioxyflavone-4'-glucuronide in the adults and young female mice. Mainly, glucoraphanin, denticulaflavonol, and 4',5-dihydroxy-3,3'-dimethoxy-6, 7-methylenedioxyflavone-4'-glucuronide had the highest concentrations in the adult female ST group, while isoferulic acid 3-sulfate was highest concentrations in adult female MH group. Meanwhile, propionic acid, dilauryl thiodipropionate, ethyl heptafluorobutyrylacetate, 5-(3',4',5'-trihydroxyphenyl)-gamma-valerolactone-3'-O-sulphate and 3'-O-methyl-(-)-epicatechin were highest in young female MH compared with the ST group. Similarly, L-Palmitoylcarnitine, linoleyl carnitine and heptadecanoyl carnitine were highest in

young female MH. Acetylcysteine and 2-oxo-tetradecanoic acid were highest in young males and female broccoli diet groups, respectively.

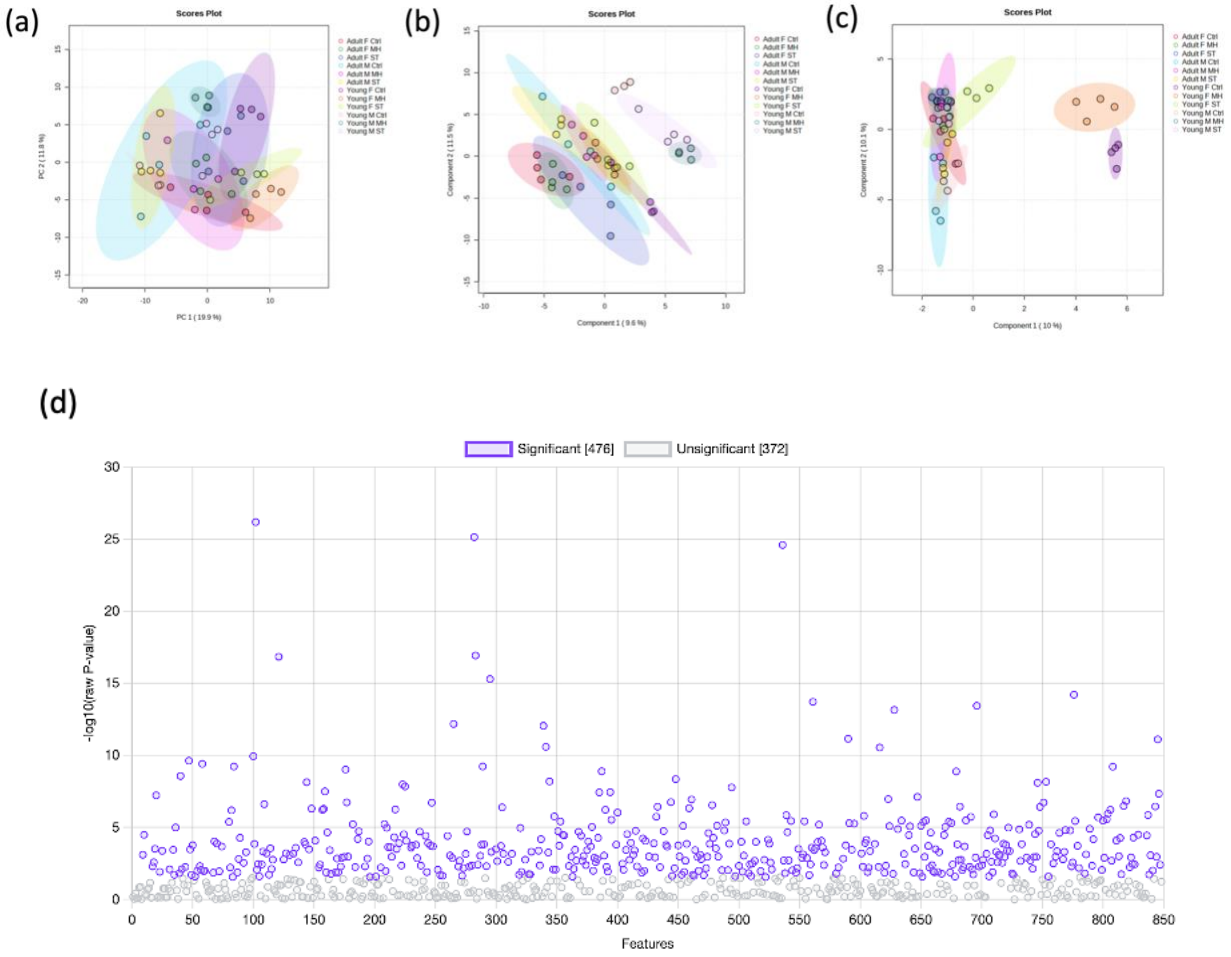


Figure 4.2: Serum metabolites were differentially present in mice of different ages and sexes, and the preparation of broccoli sprouts. (a) PCA plots with PC 1 at 19.9% and PC 2 at 11.8%; (b) PLSDA plots with PC 1 at 9.6% and PC 2 at 11.5%; (c) further analysis with sPLSDA with component 1 at 10% and component 2 at 10.1%. (d) The univariate ANOVA analysis at $p< 0.05$ showed 476 metabolites differentially expressed across the experimental groups. Abbreviations: PCA= principal component analysis, PLSDA= partial least squares discriminant analysis, sPLSDA=supervised PLSDA, PC= Principal Component.

4.4.3 Hierarchical clustering of differentially expressed plasma metabolites in heatmaps

The heat maps show the metabolites clusters contributing to the beneficial effect of the broccoli sprouts intervention across all the treatment groups in Figure 4.3a. The top 150

metabolites include glucosinolates, flavonoids, polyphenols, tryptophan, carnitine and amino acid derivatives with antioxidative and anti-inflammatory activities as shown in Figure 4.3b. The specific metabolites of interest that were increased and decreased between the experimental groups with treatment preparation, age, and sex are reported in supplementary Figure A.2. We compared the differential effect of diet preparation on metabolite concentration with age and sex. The ST diet increased glucoerucin mostly in young females, while carnitine derivatives were increased with the MH diet in young males. The ST diet increased glucoerucin in adult male and female mice and decreased leukotriene D4 in males compared to MH.

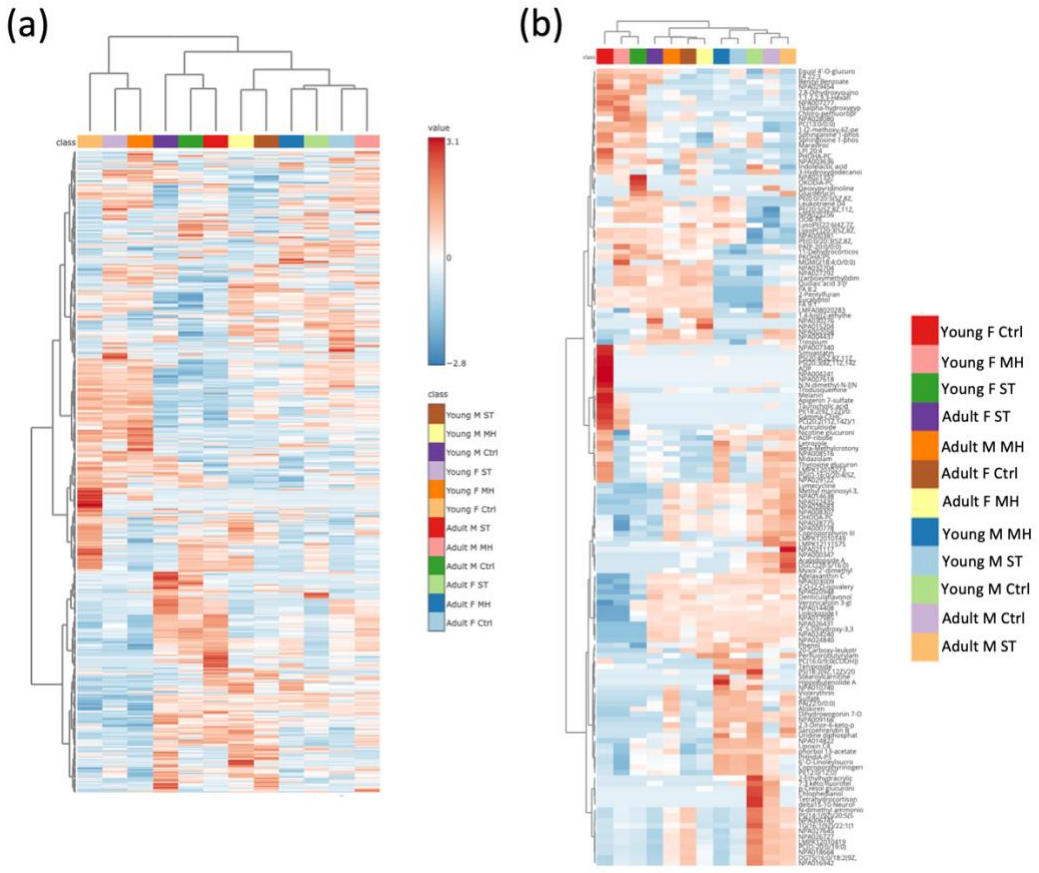


Figure 4.3: Serum metabolites were differentially abundant in mice according to age and sex, and preparation of the broccoli sprouts. (a) Hierarchical clusters of the 476 significant metabolites and (b) the top 150 metabolites are differentially abundant across the experimental groups. The red color intensity denotes an increase in metabolite concentration, while the blue color shows a decrease.

4.4.4 Enrichment analysis mapped plasma metabolites with metabolic pathways in bubble plots and bar graphs

The quantitative enrichment analysis reported in bubble plots and bar graphs (supplementary Figure A.3) revealed arachidonic acids, fatty acids, glycolysis, leucine and isoleucine, amino sugar and nucleotide sugar, taurine, propanoate and tyrosine, and drugs metabolism among the topmost metabolic pathways associated with the metabolites different between the experimental groups. We compared the differential effect of diet preparation on metabolic enrichment and pathway with age and sex. Glycolysis/gluconeogenesis metabolism, fructose and mannose metabolism, arachidonic acid metabolism, taurine and hypotaurine metabolism, amino sugar, and nucleotide sugar metabolism were enriched with ST and MH diet in young female mice. Steroid hormone metabolism, arachidonic acid metabolism, ether lipid metabolism, valine, leucine, and isoleucine metabolism were enriched with ST and MH diet in young male mice. Arachidonic acid metabolism, fatty acid metabolism, elongation, fatty acid degradation, tyrosine metabolism, and purine metabolism were enriched with ST and MH diet in adult female mice. Ether lipid metabolism, propanoate metabolism, fructose and mannose metabolism, arachidonic acid metabolism, taurine and hypotaurine metabolism, amino sugar, and nucleotide sugar metabolism were enriched with ST and MH diet in adult male mice.

4.5 Discussion

Recent surveys have reported adults, especially women, as a high-risk population for IBD (Xu et al. 2021; Weisman et al. 2023). The use of vegetables for intestinal health has been established, particularly broccoli sprouts against IBD pathogenesis such as inflammation, dysbiosis, and gut damage (Zandani et al. 2021; Holman et al. 2023; Zhang et al. 2023). The ameliorative effects have been associated primarily with gut microbial metabolites of

glucosinolates, flavonoids, polyphenols, and amino and fatty acids (Cicio et al. 2022; Martins et al. 2023; Ortega Hernández and Others). However, broccoli preparation could influence its benefits against IBD (Charron et al. 2020; Wu et al. 2016; Wang et al. 2018) in different populations by modulating the kinetics of bioavailability and resulting isothiocyanate concentrations in different body tissues (Alaba et al. 2024; Li et al. 2013; Vermeulen et al. 2008). This pilot study is the first to report the metabolic difference associated with the differential benefits of broccoli sprout preparation (ST and MH), including in a disease model of colitis. Our data showed that adult females may benefit more from the ST diet. In contrast, adult males and young females may benefit more from the MH diet intervention by increasing the concentration of beneficial metabolites against ulcerative colitis in the DSS-mice model of IBD.

The DSS model of IBD is an effective method to assess the UC type of IBD characterized by clinical symptoms similar to UC patients, such as weight loss and DAI score of stool consistency and fecal blood. Our result revealed that only ST broccoli diets could restore colitis-induced weight loss and decrease colitis severity as DAI scores, and both ST and MH restored colon length in adult female mice, suggesting that the anti-colitis effect of ST diet may benefit the adult female population more than the MH diet. Meanwhile, both diets restored BW and decreased the severity of colitis in young female and all male mice. Still, none of the diets restored column length in adult males, suggesting the anti-colitis benefits of the MH diet may benefit a younger population than adults. Similar results have been documented in IL-10-knockout CD mice, with a more significant decrease in BW and fecal blood in younger mice after a raw broccoli diet (Holcomb et al. 2023).

Our data indicate that the anti-colitis impact of the ST diet in the adult female population may be associated with the high concentrations of glucosinolates and flavonoid metabolites in the

group, while the anti-colitis benefit of the MH diet may be due to the high concentrations of polyphenol and SCFA metabolites in adult male and younger groups (supplementary Figure A.1). Gut microbes could hydrolyze dietary glucosinolates into isothiocyanates such as SFN and erucin metabolites to promote a healthy gut (Bouranis et al. 2021; Liu et al. 2017). Likewise, flavonoids and polyphenols confer gut protective benefits as primary or microbial metabolites (Makarewicz et al. 2021; Baky et al. 2022). The major glucosinolates expressed in our plasma samples are glucoraphanin and glucoerucin which could be metabolized microbially by bacteria such as *Bifidobacterium* species and glucosidases (Wu et al. 2023; Cebeci 2017; Radünz et al. 2021) to confer anti-inflammatory, antioxidant and gut protective benefits (Holman et al. 2023; Wu et al. 2019; Holman et al. 2023) particularly in adult female mice after consumption of ST diet. These results supported the previous claim that a broccoli diet could contribute to the presence of glucosinolates-derived plasma metabolites to prevent intestinal damage (Charron et al. 2020; Martins et al. 2022).

Dietary metabolites, denticulaflavonol and microbially derived 4',5-Dihydroxy-3,3'-dimethoxy-6,7-methylenedioxyflavone-4'-glucuronide possess similar anti-inflammatory and antioxidant benefits against IBD (Ortega Hernández and Others ; Choi et al. 2023; Anastasaki et al. 2017; Adnan et al. 2021; Anastasaki et al. 2017). Although these flavonoids increased in all the adult males and females and young male treatment groups, denticulaflavonol had a higher intensity in young male ST and adult female ST groups. These results further suggest the potential benefits of ST diet for adult females and that both age and sex are significant factors to consider during broccoli preparation for IBD intervention. Isoferulic acid-3-sulfate, generated by sulfotransferases hydrolysis of ferulic acid (Serreli and Deiana 2019), was significantly increased in all adult and young male treatment groups but had a higher concentration in adult female and young male MH

groups. The results suggest that MH broccoli may increase polyphenolic metabolites to prevent IBD via its anti-inflammatory and antioxidant properties (Serreli et al. 2021; Zhao and Jiang 2021; Stevens et al. 2022; Kaulmann and Bohn 2016), predominantly in adult female and young male populations. Microbial metabolites from polyphenols, 3'-O-methyl-(-)-epicatechin derived from catechin and 5-(3',4',5'-Trihydroxyphenyl)-gamma-valerolactone-3'-O-sulphate derived from of proanthocyanidins were increased in young female MH than the ST group and may exhibit anti-inflammatory and antioxidant effect against colitis (Shay et al. 2015; Fan et al. 2017; Dryden et al. 2013; Vasconcelos et al. 2012) and prevent immune cells adhesion to gut mucosal (Angelino et al. 2019; Márquez Campos et al. 2019; Catalkaya et al. 2020).

Gut bacteria could metabolize fatty acids and amino acids to produce beneficial SCFAs and tryptophan metabolites (Russo et al. 2019). Amino acids, tryptophan, and phenylalanine are essential for promoting protein synthesis, which could also confer ameliorative effects against IBD (He et al. 2018; Lai et al. 2019). Major SCFA microbially derived from vegetables include acetic acids, butyric acid propionic acids, and their metabolites are crucial anti-inflammatory markers and prebiotics to promote gut homeostasis (Parada Venegas et al. 2019; Gonçalves et al. 2018) and alleviate dysbiotic IBD (Chen et al. 2023; Ashaolu et al. 2021). Our broccoli diet increased the concentration of SCFAs, such as propionic acid, dilauryl thiodipropionate, ethyl heptafluorobutyrylacetate, L-palmitoylcarnitine, linoleyl carnitine, and heptadecanoyl carnitine, while indoxyl sulfate, an inflammatory tryptophan metabolite, was decreased in young females with MH than in the ST group. The results suggest that the MH diet may benefit the young female IBD population by increasing SCFA metabolites against pathogenic bacteria, proinflammatory monocyte polarity and immune cell adhesion to gut mucosal (Wu et al. 2023; Rivière et al. 2016;

Recharla et al. 2023), increased carnitine derivatives and decreased indoxyl sulfate metabolites to prevent intestinal inflammation and protect gut barrier (Zhu et al. 2020; Semba et al. 2017).

Acetylcysteine, a substrate for glutathione, and 2-oxo-tetradecanoic acid, a precursor of microbially derived lipids, were differentially increased in young males and females fed with both diets, suggesting the sex differential effect of the broccoli diet. Acetylcysteine may protect the young population via antioxidative and anti-inflammatory effects against NFkB pathways and ROS production in the gut (Frye and Berk 2018; Uraz et al. 2013; Wang et al. 2022), while 2-oxo-tetradecanoic acid may protect against *Helicobacter pylori* gastric infection to promote intestinal barrier homeostasis (Morozumi et al. 2022; Hu et al. 2023; Matsui et al. 2017). Valeric conjugates derived from leucine could promote IBD by recruiting proinflammatory cells, mitochondria oxidation (Liu et al. 2019; Tummolo et al. 2022; Mavilio et al. 2016), and epithelial damage (Folz et al. 2023; Martin et al. 2017; Li et al. 2020). Broccoli diet decreased alpha-ketoisovaleric acid in young males MH and more significantly in the ST group, compared with the young male DSS group, suggesting the differential effect on age and sex during anti-inflammatory and antioxidant benefits of broccoli. However, the diets were not significantly different in the adult and young female groups. Taurocholic acid, a metabolite of taurine, was decreased by MH and ST diet only in the young female group to prevent inflammation and colitis caused by bacteria metabolism of bile (Thomas et al. 2022; Kumar et al. 2022; Zhou et al. 2023). The pathway analysis compared each diet preparation against the DSS control, age, or sex group to annotate potential metabolic pathways associated with the nutritional benefits of diets and the pathology of IBD. Arachidonic acid, fatty acid and phenylalanine, tryptophan, and tyrosine metabolism are major pathways enriched by ST and MH diets in adult male and female groups. Arachidonic and fatty acid pathways may be associated with IBD generating pro-inflammatory and adhesive markers in the

gut (Coquenlorge et al. 2016; Huang et al. 2021), while fatty acid, phenylalanine, tryptophan, and tyrosine could be associated with bacterial hydrolysis of fat and amino acids to generate metabolites with anti-inflammation, antioxidation and barrier protection (Asakura and Kitahora 2018; Elbarbry et al. 2014; Das 2016). Steroid hormone, fatty acid, arachidonic acid, ether lipids, arginine biosynthesis, propanoate, valine, leucine, and isoleucine metabolism are major pathways enriched by ST and MH diets in young male groups. Fatty acid and arachidonic acid could impact the production of steroid hormones in young people, and nutrition may be a significant factor influencing the steroidal hormones in the younger male with IBD to protect against gut inflammation (Wiese et al. 2016; Bhathena and Velasquez 2002; Calder et al. 2009). Ether lipids metabolism is associated with IBD development (Ferru-Clément et al. 2023; Liu et al. 2023), while nutritional intervention such as broccoli diet could promote arginine biosynthesis (Li et al. 2023), propanoate, valine, leucine, and isoleucine may protect against IBD in the young male population (Basson et al. 2016; Forsman 2022). Glycolysis/gluconeogenesis, fructose and mannose, amino sugar and nucleotide sugar, pentose phosphate, sphingolipids, and arachidonic acid are major pathways ST and MH diets enrich young female groups. Glycolysis/gluconeogenesis, fructose, mannose and pentose phosphate pathways may generate substrates for microbial hydrolysis of phytochemicals (Hou et al. 2023; Hagihara et al. 2019) against IBD in young female population, while sphingolipids and arachidonic acid may be associated with pro-inflammatory and adhesion markers during IBD development (Coquenlorge et al. 2016; Huang et al. 2021).

4.6. Conclusion, limitations and future direction

To the best of our knowledge, this study is the first to report that different preparations of broccoli sprouts differentially benefit females and males, as well as young and adult mice, as a potential dietary intervention for IBD conditions. Adult female mice benefited more from steamed

broccoli sprouts intervention than mildly heated with significant recovery from IBD-induced weight loss and colon damage, decreased disease activity index, and increased concentration of antioxidant, anti-inflammatory, and gut-protective metabolites. Meanwhile, adult male and younger mice benefited more from mildly heated broccoli sprouts. The increased concentrations of dietary-derived glucosinolates and microbially derived flavanols in the adult female mice fed steamed broccoli sprouts points to the potential use of such anti-inflammatory and antioxidant metabolites as a late-life dietary intervention for the adult female population, who are more at risk of IBD. Meanwhile, early-life use of broccoli sprouts as a dietary intervention may benefit the younger population by using mildly heated broccoli sprouts with microbially derived polyphenols, short-chain fatty acids, and glutathione substrates. The limitation of this study is the need for funds, a larger sample size, and tissues for multiple analyses, as only plasma metabolites were tested in the cohort of mice. There needed to be sufficient housing equipment so the mice would not be housed in groups, making it challenging to generate enough fecal samples per mouse. Future research may use the preliminary information from this study to develop a robust approach to the differential benefit of broccoli sprout preparation across different stages of human life.

REFERENCES

Abeles SR, Jones MB, Santiago-Rodriguez TM, Ly M, Klitgord N, Yooseph S, Nelson KE, Pride DT. 2016. Microbial diversity in individuals and their household contacts following typical antibiotic courses. Microbiome 4:39.

Adnan M, Siddiqui AJ, Jamal A, Hamadou W, Awadelkareem AM, Sachidanandan M, Patel M. Evidence-based medicinal potential and possible role of Selaginella in the prevention of modern chronic diseases: Ethnopharmacological and ethnobotanical perspective. Rec Nat Prod 2021

Agus A, Denizot J, Thévenot J, Martinez-Medina M, Massier S, Sauvanet P, Bernalier-Donadille A, Denis S, Hofman P, Bonnet R, Billard E, Barnich N. 2016. Western diet induces a shift in microbiota composition enhancing susceptibility to Adherent-Invasive E. coli infection and intestinal inflammation. Sci Rep 8:19032.

Ahern PP, Schiering C, Buonocore S, McGeachy MJ, Cua DJ, Maloy KJ, Powrie F. 2010. Interleukin-23 Drives Intestinal Inflammation through Direct Activity on T Cells. Immunity 33:279–288.

Alaba, Tolu E., Johanna M. Holman, Suzanne L. Ishaq, and Yanyan Li. 2024. "Current Knowledge on the Preparation and Benefits of Cruciferous Vegetables as Relates to In Vitro, In Vivo, and Clinical Models of Inflammatory Bowel Disease." Current Developments in Nutrition 8 (5): 102160.

Aldars-García L, Chaparro M, Gisbert JP. 2021. Systematic Review: The Gut Microbiome and Its Potential Clinical Application in Inflammatory Bowel Disease. Microorganisms 30:977.

Alsoud LO, Soares NC, Al-Hroub HM, Mousa M, Kasabri V, Bulatova N, Suyagh M, Alzoubi KH, El-Huneidi W, Abu-Irmaileh B, et al. Identification of Insulin Resistance Biomarkers in Metabolic Syndrome Detected by UHPLC-ESI-QTOF-MS. Metabolites 2022;12.

Amron NA, Konsue N. 2018. Antioxidant capacity and nitrosation inhibition of cruciferous vegetable extracts. Food Chem Toxicol 25:65-73.

Ananthakrishnan, Ashwin N., Hamed Khalili, Gauree G. Konijeti, Leslie M. Higuchi, Punyanganie de Silva, Joshua R. Korzenik, Charles S. Fuchs, Walter C. Willett, James M. Richter, and Andrew T. Chan. 2013. "A Prospective Study of Long-Term Intake of Dietary Fiber and Risk of Crohn's Disease and Ulcerative Colitis." Gastroenterology 145 (5): 970–77.

Anastasaki E, Zoumpopoulou G, Astraka K, Kampoli E, Skoumpi G, Papadimitriou K, Tsakalidou E, Polissiou M. Phytochemical analysis and evaluation of the antioxidant and antimicrobial properties of selected herbs cultivated in Greece. Ind Crops Prod 2017;108:616–28.

Andrade, Maria Emília Rabelo, Raquel Silva Araújo, Patrícia Aparecida Vieira de Barros, Anne Danieli Nascimento Soares, Fernanda Alves Abrantes, Simone de Vasconcelos Generoso, Simone Odília Antunes Fernandes, and Valbert Nascimento Cardoso. 2015. "The Role of Immunomodulators on Intestinal Barrier Homeostasis in Experimental Models." Clinical Nutrition 34 (6): 1080–87.

Angelino D, Carregosa D, Domenech-Coca C, Savi M, Figueira I, Brindani N, Jang S, Lakshman S, Molokin A, Urban JF, et al. 5-(Hydroxyphenyl)-γ-Valerolactone-Sulfate, a Key Microbial Metabolite of Flavan-3-ols, Is Able to Reach the Brain: Evidence from Different in Silico, In Vitro and In Vivo Experimental Models. Nutrients Multidisciplinary Digital Publishing Institute; 2019 [cited 2024 Feb 20];11:2678.

Artegoitia, Virginia M., Sridevi Krishnan, Ellen L. Bonnel, Charles B. Stephensen, Nancy L. Keim, and John W. Newman. 2021. "Healthy Eating Index Patterns in Adults by Sex and Age Predict Cardiometabolic Risk Factors in a Cross-Sectional Study." BMC Nutrition 7 (1): 30.

Asakura H, Kitahora T. Chapter 23 - Antioxidants and Polyphenols in Inflammatory Bowel Disease: Ulcerative Colitis and Crohn Disease. In: Watson RR, Preedy VR, Zibadi S, editors. Polyphenols: Prevention and Treatment of Human Disease (Second Edition). Academic Press; 2018. p. 279–92.

Ashaolu TJ, Ashaolu JO, Adeyeye SAO. Fermentation of prebiotics by human colonic microbiota in vitro and short-chain fatty acids production: a critical review. J Appl Microbiol Oxford Academic; 2021 [cited 2024 Feb 21];130:677–87.

Atta, Attia H., Hamdy Soufy, Soad M. Nasr, Ahmed M. Soliman, Somia A. Nassar, A. Maweri, Tamer H. Abd El-Aziz, H. M. Desouky, and A. M. Abdalla. 2017. "Hepatoprotective and Antioxidant Effects of Methanol Extractof Moringa Oleifera Leaves in Rats." Wulfenia 24 (3): 249–68.

Atwell L, Clarke J, Hsu A, Bella D, Choi J, Stevens J, Pereira C, Jiang Y, Dashwood R, Williams D, Ho E. 2015. Plasma metabolomic profiles in healthy adults following consumption of broccoli sprouts. FASEB J 29:S1.

Atwell LL, Hsu A, Wong CP, Stevens JF, Bella D, Yu T-W, Pereira CB, Löhr CV, Christensen JM, Dashwood RH, Williams DE, Shannon J, Ho E. 2015. Absorption and chemopreventive targets of sulforaphane in humans following consumption of broccoli sprouts or a myrosinase-treated broccoli sprout extract. Mol Nutr Food Res 59:424–433.

Atwell, Lauren, John Clarke, Anna Hsu, Deborah Bella, Jaewoo Choi, Jan Stevens, Cliff Pereira, et al. 2015. "Plasma Metabolomic Profiles in Healthy Adults Following Consumption of Broccoli Sprouts." FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology 29 (S1): S1.

Avena, Nicole M. 2015. Hedonic Eating: How the Pleasure of Food Affects Our Brains and Behavior. Oxford University Press.

Ayaz, Faik Ahmet, Sema Hayırlıoglu-Ayaz, Sengul Alpay-Karaoglu, Jiři Grúz, Kateřina Valentová, Jitka Ulrichová, and Miroslav Strnad. 2008. “Phenolic Acid Contents of Kale (Brassica Oleraceae L. Var. Acephala DC.) Extracts and Their Antioxidant and Antibacterial Activities.” Food Chemistry 107 (1): 19–25.

Baky MH, Elshahed M, Wessjohann L, Farag MA. Interactions between dietary flavonoids and the gut microbiome: a comprehensive review. Br J Nutr 2022;128:577–91.

Barberio B, Zamani M, Black CJ, Savarino EV, Ford AC. 2021. Prevalence of symptoms of anxiety and depression in patients with inflammatory bowel disease: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol 6:359–370.

Barnett M, Young W, Cooney J, Roy N. 2014. Metabolomics and Proteomics, and What to Do with All These “Omes”: Insights from Nutrigenomic Investigations in New Zealand. J Nutrigenet Nutrigenomics 7:274-282.

Barton LL, Ritz NL, Fauque GD, Lin HC. 2017. Sulfur Cycling and the Intestinal Microbiome. Dig Dis Sci 62:2241–2257.

Basson A, Trotter A, Rodriguez-Palacios A, Cominelli F. Mucosal Interactions between Genetics, Diet, and Microbiome in Inflammatory Bowel Disease. Front Immunol 2016;7:290.

Basson, Abigail R., Christy Chen, Filip Sagl, Ashley Trotter, Ilya Bederman, Adrian Gomez-Nguyen, Mark S. Sundrud, Sanja Ilic, Fabio Cominelli, and Alex Rodriguez-Palacios. 2020. “Regulation of Intestinal Inflammation by Dietary Fats.” Frontiers in Immunology 11: 604989.

Benchimol EI, Kaplan GG, Otley AR, Nguyen GC, Underwood FE, Guttmann A, Jones JL, Potter BK, Catley CA, Nugent ZJ, Cui Y, Tanyingoh D, Mojaverian N, Bitton A, Carroll MW, deBruyn J, Dummer TJB, El-Matary W, Griffiths AM, Jacobson K, Kuenzig ME, Leddin D, Lix LM, Mack DR, Murthy SK, Sánchez JNP, Singh H, Targownik LE, Vutcovici M, Bernstein CN. 2017. Rural and Urban Residence During Early Life is Associated with Risk of Inflammatory Bowel Disease: A Population-Based Inception and Birth Cohort Study. Am J Gastroenterol 112:1412–1422.

Bhandari, Shiva Ram, Jung-Ho Kwak, Jung Su Jo, and Jun Gu Lee. 2019. “Changes in Phytochemical Content and Antioxidant Activity during Inflorescence Development in Broccoli.” Chilean Journal of Agricultural Research 79 (1): 36–47.

Bhathena SJ, Velasquez MT. Beneficial role of dietary phytoestrogens in obesity and diabetes. Am J Clin Nutr 2002;76:1191–201.

Bhatt S, Singh B, Gupta M. 2020. Antioxidant and prebiotic potential of Murraya koenigii and Brassica oleracea var. botrytis leaves as food ingredient. Journal of agriculture and food research 2:100069.

Bian Y, Dong Y, Sun J, Sun M, Hou Q, Lai Y Zhang B. 2020. Protective Effect of Kaempferol on LPS-Induced Inflammation and Barrier Dysfunction in a Coculture Model of Intestinal Epithelial Cells and Intestinal Microvascular Endothelial Cells. J Agric Food Chem. 68:160-167.

Bian Y, Liu P, Zhong J, Hu Y, Zhuang S, Fan K, Liu Z. 2018. Quercetin Attenuates Adhesion Molecule Expression in Intestinal Microvascular Endothelial Cells by Modulating Multiple Pathways. Dig Dis Sci 63:3297-3304.

Bigman, G., K. Shea, Marius Emil Rusu, and A. Ryan. 2023. "Intake of Dark Green Vegetables May Benefit Specific Cognitive Domains in US Men and Women Aged 60 Years or Older." Nutrition and Healthy Aging, May.

Borrelli A, Bonelli P, Tuccillo FM, Goldfine ID, Evans JL, Buonaguro FM, Mancini A. 2018. Role of oxidative stress, gut microbiota and derived metabolites in the etiology and progression of nonalcoholic fatty liver disease. Redox Experimental Medicine 15:467–479.

Bostanci, Nagihan, Maria Christine Krog, Luisa W. Hugerth, Zahra Bashir, Emma Fransson, Fredrik Boulund, Georgios N. Belibasakis, et al. 2021. "Dysbiosis of the Human Oral Microbiome During the Menstrual Cycle and Vulnerability to the External Exposures of Smoking and Dietary Sugar." Frontiers in Cellular and Infection Microbiology 11 (March): 625229.

Bouranis JA, Beaver LM, Choi J, Wong CP, Jiang D, Sharpton TJ, Stevens JF, Ho E. Composition of the Gut Microbiome Influences Production of Sulforaphane-Nitrile and Iberin-Nitrile from Glucosinolates in Broccoli Sprouts. Nutrients 2021;13.

Bouranis JA, Beaver LM, Jiang D, Choi J, Wong CP, Davis EW, Williams DE, Sharpton TJ, Stevens JF, Ho E. 2022. Interplay between Cruciferous Vegetables and the Gut Microbiome: A Multi-Omic Approach. Nutrients 15:42.

Bouranis, John A., Laura M. Beaver, and Emily Ho. 2021. "Metabolic Fate of Dietary Glucosinolates and Their Metabolites: A Role for the Microbiome." Frontiers in Nutrition 8 (September): 748433.

Bouranis, John A., Laura M. Beaver, Carmen P. Wong, Jaewoo Choi, Sean Hamer, Ed W. Davis, Kevin S. Brown, et al. 2024. "Sulforaphane and Sulforaphane-Nitrile Metabolism in Humans Following Broccoli Sprout Consumption: Inter-Individual Variation, Association with Gut Microbiome Composition, and Differential Bioactivity." Molecular Nutrition & Food Research 68 (4): e2300286.

Boustany, Antoine, Romy Rahhal, Jad Mitri, Somtochukwu Onwuzo, Hadi Khaled Abou Zeid, and Imad Asaad. 2023. “INCREASED RISK OF COLORECTAL CANCER IN PATIENTS WITH CHRONIC TOPHACEOUS GOUT: A POPULATION-BASED STUDY.” Arquivos de Gastroenterologia 60 (3): 339–44.

Brettle, Holly, Vivian Tran, Grant R. Drummond, Ashley E. Franks, Steve Petrovski, Antony Vinh, and Maria Jelinic. 2022. “Sex Hormones, Intestinal Inflammation, and the Gut Microbiome: Major Influencers of the Sexual Dimorphisms in Obesity.” Frontiers in Immunology 13 (September): 971048.

Bricker GV, Riedl KM, Ralston RA, Tober KL, Oberyszyn TM, Schwartz SJ. Isothiocyanate metabolism, distribution, and interconversion in mice following consumption of thermally processed broccoli sprouts or purified sulforaphane. Mol Nutr Food Res 2014;58:1991–2000.

Bui, Thi Phuong Nam, Antonio Dario Troise, Bart Nijsse, Giovanni N. Roviello, Vincenzo Fogliano, and Willem M. de Vos. 2020. “Intestinimonas-like Bacteria Are Important Butyrate Producers That Utilize Nε-Fructosyllysine and Lysine in Formula-Fed Infants and Adults.” Journal of Functional Foods 70 (July): 103974.

Busbee Pb Nagarkatti M Nagarkatti. 2017. Indole-3-carbinol ameliorates murine colitis symptoms through alterations in gut microbial composition and metabolomic pathways, particularly through decreasing disease-associated Bacteroides acidifaciens species. The Journal of immunology (1950) 198:218.

Caballero-Flores, Gustavo, Joseph M. Pickard, and Gabriel Núñez. 2023. “Microbiota-Mediated Colonization Resistance: Mechanisms and Regulation.” Nature Reviews. Microbiology 21 (6): 347–60.

Caban M, Lewandowska U. 2023. Encapsulation of Polyphenolic Compounds Based on Hemicelluloses to Enhance Treatment of Inflammatory Bowel Diseases and Colorectal Cancer. Molecules 28:4189.

Calder PC, Albers R, Antoine J-M, Blum S, Bourdet-Sicard R, Ferns GA, Folkerts G, Friedmann PS, Frost GS, Guarner F, et al. Inflammatory disease processes and interactions with nutrition. Br J Nutr 2009;101 Suppl 1:S1–45.

Caparrós E, Wiest R, Scharl M, Rogler G, Gutiérrez Casbas A, Yilmaz B, Wawrzyniak M, Francés R. 2021. Dysbiotic microbiota interactions in Crohn’s disease. Gut Microbes 13:1949096.

Cartea ME, Francisco M, Soengas P, Velasco P. 2010. Phenolic compounds in Brassica vegetables. Molecules 16:251-280.

Catalkaya G, Venema K, Lucini L, Rocchetti G, Delmas D, Daglia M, De Filippis A, Xiao H, Quiles JL, Xiao J, et al. Interaction of dietary polyphenols and gut microbiota: Microbial

metabolism of polyphenols, influence on the gut microbiota, and implications on host health. Food Front Wiley; 2020;1:109–33.

Catalkaya, Gizem, Koen Venema, Luigi Lucini, Gabriele Rocchetti, Dominique Delmas, Maria Daglia, Anna De Filippis, et al. 2020. “Interaction of Dietary Polyphenols and Gut Microbiota: Microbial Metabolism of Polyphenols, Influence on the Gut Microbiota, and Implications on Host Health.” Food Frontiers 1 (2): 109–33.

Caviglia GP, Garrone A, Bertolino C, Vanni R, Bretto E, Poshnjari A, Tribocco E, Frara S, Armandi A, Astegiano M, Saracco GM, Bertolusso L, Ribaldone DG. 2023. Epidemiology of Inflammatory Bowel Diseases: A Population Study in a Healthcare District of North-West Italy. J Clin Med Res 12:641.

Cebeci F. The metabolism of plant glucosinolates by gut bacteria [doctoral]. University of East Anglia; 2017 [cited 2024 Feb 27].

Cebeci, Fatma. 2017. “The Metabolism of Plant Glucosinolates by Gut Bacteria.” Doctoral, University of East Anglia.

Charron CS, Vinyard BT, Jeffery EH, Ross SA, Seifried HE, Novotny JA. 2020. BMI Is Associated With Increased Plasma and Urine Appearance of Glucosinolate Metabolites After Consumption of Cooked Broccoli. Front Nutr 7:575092.

Charron CS, Vinyard BT, Jeffery EH, Ross SA, Seifried HE, Novotny JA. BMI Is Associated With Increased Plasma and Urine Appearance of Glucosinolate Metabolites After Consumption of Cooked Broccoli. Front Nutr 2020;7:575092.

Charron, Craig S., Bryan T. Vinyard, Elizabeth H. Jeffery, Sharon A. Ross, Harold E. Seifried, and Janet A. Novotny. 2020. “BMI Is Associated With Increased Plasma and Urine Appearance of Glucosinolate Metabolites After Consumption of Cooked Broccoli.” Frontiers in Nutrition 7 (September): 575092.

Chen W, Tan D, Yang Z, Tang J, Bai W, Tian L. Fermentation patterns of prebiotics fructooligosaccharides-SCFA esters inoculated with fecal microbiota from ulcerative colitis patients. Food Chem Toxicol 2023;180:114009.

Chen, Lin, Glenna Jue Tong Tan, Xinyi Pang, Wenqian Yuan, Shaojuan Lai, and Hongshun Yang. 2018. “Energy Regulated Nutritive and Antioxidant Properties during the Germination and Sprouting of Broccoli Sprouts (Brassica Oleracea Var. Italica).” Journal of Agricultural and Food Chemistry 66 (27): 6975–85.

Chen, Xiaoli, Lawrence J. Cheskin, Leiyu Shi, and Youfa Wang. 2011. “Americans with Diet-Related Chronic Diseases Report Higher Diet Quality than Those without These Diseases.” The Journal of Nutrition 141 (8): 1543–51.

Choe U, Li Y, Gao B, Yu L, Wang TTY, Sun J, Chen P, Liu J, Yu L. 2018. Chemical Compositions of Cold-Pressed Broccoli, Carrot, and Cucumber Seed Flours and Their in Vitro Gut Microbiota Modulatory, Anti-inflammatory, and Free Radical Scavenging Properties. J Agric Food Chem 66:9309–9317.

Choe, Uyory, Yanfang Li, Boyan Gao, Lu Yu, Thomas T. Y. Wang, Jianghao Sun, Pei Chen, Jie Liu, and Liangli Yu. 2018. "Chemical Compositions of Cold-Pressed Broccoli, Carrot, and Cucumber Seed Flours and Their in Vitro Gut Microbiota Modulatory, Anti-Inflammatory, and Free Radical Scavenging Properties." Journal of Agricultural and Food Chemistry 66 (35): 9309–17.

Choi K-C, Cho S-W, Kook S-H, Chun S-R, Bhattarai G, Poudel SB, Kim M-K, Lee K-Y, Lee J-C. 2016. Intestinal anti-inflammatory activity of the seeds of Raphanus sativus L. in experimental ulcerative colitis models. J Ethnopharmacol 179:55–65.

Choi YJ, Lee SY, Son S-R, Park JY, Jang DS, Lee S. The Effects of Flavonol and Flavone Glucuronides from Potentilla chinensis Leaves on TNF-α-Exposed Normal Human Dermal Fibroblasts. Antioxidants (Basel) 2023;12.

Choi, Ki-Choon, Seong-Wan Cho, Sung-Ho Kook, Sa-Ra Chun, Govinda Bhattarai, Sher Bahadur Poudel, Min-Kook Kim, Kyung-Yeol Lee, and Jeong-Chae Lee. 2016. "Intestinal Anti-Inflammatory Activity of the Seeds of Raphanus Sativus L. in Experimental Ulcerative Colitis Models." Journal of Ethnopharmacology 179 (February): 55–65.

Christ A, Günther P, Lauterbach MAR, Duewell P, Biswas D, Pelka K, Scholz CJ, Oosting M, Haendler K, Baßler K, Klee K, Schulte-Schrepping J, Ulas T, Moorlag SJCF, Kumar V, Park MH, Joosten LAB, Groh LA, Riksen NP, Terje Espevik 10 AS, Li Y, Fitzgerald ML, Netea MG, Schultze JL, Latz E. 2018. Western Diet Triggers NLRP3-Dependent Innate Immune Reprogramming. Cell 172:162–175.e14.

Cicio, Adele, Rosa Serio, and Maria Grazia Zizzo. 2022. "Anti-Inflammatory Potential of Brassicaceae-Derived Phytochemicals: In Vitro and In Vivo Evidence for a Putative Role in the Prevention and Treatment of IBD." Nutrients 15 (1).

Clark A, Mach N. 2017. The Crosstalk between the Gut Microbiota and Mitochondria during Exercise. Front Physiol 8:319.

Clark, Michael, and David Tilman. 2017. "Comparative Analysis of Environmental Impacts of Agricultural Production Systems, Agricultural Input Efficiency, and Food Choice." Environmental Research Letters: ERL [Web Site] 12 (6): 064016.

Clarke JD, Hsu A, Riedl K, Bella D, Schwartz SJ, Stevens JF, Ho E. 2011. Bioavailability and inter-conversion of sulforaphane and erucin in human subjects consuming broccoli sprouts or broccoli supplement in a cross-over study design. Pharmacol Res 64:456–463.

Clish CB. Metabolomics: an emerging but powerful tool for precision medicine. Cold Spring Harb Mol Case Stud 2015;1:a000588.

Clish, Clary B. 2015. “Metabolomics: An Emerging but Powerful Tool for Precision Medicine.” Cold Spring Harbor Molecular Case Studies 1 (1): a000588.

Cohen, Aaron B., Dale Lee, Millie D. Long, Michael D. Kappelman, Christopher F. Martin, Robert S. Sandler, and James D. Lewis. 2013. “Dietary Patterns and Self-Reported Associations of Diet with Symptoms of Inflammatory Bowel Disease.” Digestive Diseases and Sciences 58 (5): 1322–28.

Conlon, Michael A., and Anthony R. Bird. 2014. “The Impact of Diet and Lifestyle on Gut Microbiota and Human Health.” Nutrients 7 (1): 17–44.

Coquenlorge S, Van Landeghem L, Jaulin J, Cenac N, Vergnolle N, Duchalais E, Neunlist M, Rolli-Derkinderen M. The arachidonic acid metabolite 11β-ProstaglandinF2α controls intestinal epithelial healing: deficiency in patients with Crohn’s disease. Sci Rep 2016;6:25203.

Cruz-Muñoz JR, Barrios-García T, Valdez-Morales EE, Durán-Vazquez MF, Méndez-Rodríguez KB, Barajas-Espinosa A, Ochoa-Cortes F, Martínez-Saldaña MC, Gómez-Aguirre YA, Alba RG. 2022. Ethanolic extract from Lepidium virginicum L. ameliorates DNBS-induced colitis in rats. J Ethnopharmacol 289:115056.

da Rocha IMG, Torrinhas R, Fonseca D, de Oliveira Lyra C, de Sousa Alves Neri JL, Balmant BD, Callado L, Charlton K, Queiroz N, Waitzberg DL. 2023. Pro-Inflammatory Diet Is Correlated with High Veillonella rogosae, Gut Inflammation and Clinical Relapse of Inflammatory Bowel Disease. Nutrients 15:4148.

Dahlhamer JM, Zammitti EP, Ward BW, Wheaton AG, Croft JB. 2016. Prevalence of Inflammatory Bowel Disease Among Adults Aged ≥18 Years — United States, 2015. MMWR Surveill Summ 65:1166–1169.

Damas OM, Estes D, Avalos D, Quintero MA, Morillo D, Caraballo F, Lopez J, Deshpande AR, Kerman D, McCauley JL, Palacio A, Abreu MT, Schwartz SJ. 2018. Hispanics Coming to the US Adopt US Cultural Behaviors and Eat Less Healthy: Implications for Development of Inflammatory Bowel Disease. Dig Dis Sci 63:3058–3066.

Das TK, Ganesh BP. 2023. Interlink between the gut microbiota and inflammation in the context of oxidative stress in Alzheimer’s disease progression. Gut Microbes 15:2206504.

Das UN. Inflammatory bowel disease as a disorder of an imbalance between pro- and anti-inflammatory molecules and deficiency of resolution bioactive lipids. Lipids Health Dis 2016;15:11.

Das, Poulomi, and D. Ghosh. 2021. “Yours Truly Broccoli.” Science and Culture 87 (April): 109–14.

David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Sloan Devlin A, Varma Y, Fischbach MA, Biddinger SB, Rachel J. Dutton & Peter J. Turnbaugh. 2014. Diet rapidly and reproducibly alters the human gut microbiome. Nature 505:559–563.

De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proceedings of the National Academy of Sciences 107:14691–14696.

Deas, Jessica, Neha D. Shah, Gauree G. Konijeti, Abigail Lundin, Olivia Lanser, Pooja Magavi, and Sabina Ali. 2024. “Dietary Therapies for Adult and Pediatric Inflammatory Bowel Disease.” Nutrition in Clinical Practice: Official Publication of the American Society for Parenteral and Enteral Nutrition, March.

Deban L, Correale C, Vetrano S, Malesci A, Danese S. 2008. Multiple Pathogenic Roles of Microvasculature in Inflammatory Bowel Disease: A Jack of All Trades. Am J Pathol 172:1457-1466.

Deierlein, Andrea L., Jaqueline Litvak, Chang Liu, and Cheryl R. Stein. 2023. “Diet Quality, Diet-Related Factors and Disability Status among Male Adults of Reproductive Age in the USA.” Public Health Nutrition 26 (10): 1976–85.

Deng M, Dan L, Ye S, Chen X, Fu T, Wang X, Chen J. Higher dietary fibre intake is associated with lower risk of inflammatory bowel disease: prospective cohort study. Aliment Pharmacol Ther 2023;58:516–25.

Deng Z, Rong Y, Teng Y, Mu J, Zhuang X, Tseng M, Samykutty A, Zhang L, Yan J, Miller D, Suttles J, Zhang H-G. 2017. Broccoli-Derived Nanoparticle Inhibits Mouse Colitis by Activating Dendritic Cell AMP-Activated Protein Kinase. Mol Ther 25:1641–1654.

Deng, Zhongbin, Yuan Rong, Yun Teng, Jingyao Mu, Xiaoying Zhuang, Michael Tseng, Abhilash Samykutty, et al. 2017. “Broccoli-Derived Nanoparticle Inhibits Mouse Colitis by Activating Dendritic Cell AMP-Activated Protein Kinase.” Molecular Therapy: The Journal of the American Society of Gene Therapy 25 (7): 1641–54.

DHQ3.” n.d. Accessed June 1, 2024. https://www.dhq3.org.

Dill-McFarland KA, Tang Z-Z, Kemis JH, Kerby RL, Chen G, Palloni A, Sorenson T, Rey FE, Herd P. 2019. Close social relationships correlate with human gut microbiota composition. Sci Rep 9:703.

Ding, Xueyan, Peng Bin, Wenwen Wu, Yajie Chang, and Guoqiang Zhu. 2020. “Tryptophan Metabolism, Regulatory T Cells, and Inflammatory Bowel Disease: A Mini Review.” Mediators of Inflammation 2020 (June): 9706140.

Dolan KT, Chang EB. 2017. Diet, gut microbes, and the pathogenesis of inflammatory bowel diseases. Mol Nutr Food Res 61:1600129.

Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, Knight R. 2010. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proceedings of the National Academy of Sciences 107:11971–11975.

Dominianni, Christine, Rashmi Sinha, James J. Goedert, Zhiheng Pei, Liying Yang, Richard B. Hayes, and Jiyoung Ahn. 2015. “Sex, Body Mass Index, and Dietary Fiber Intake Influence the Human Gut Microbiome.” PloS One 10 (4): e0124599.

Drewnowski, Adam, Johanna Dwyer, Janet C. King, and Connie M. Weaver. 2019. “A Proposed Nutrient Density Score That Includes Food Groups and Nutrients to Better Align with Dietary Guidance.” Nutrition Reviews 77 (6): 404–16.

Dryden GW, Lam A, Beatty K, Qazzaz HH, McClain CJ. A pilot study to evaluate the safety and efficacy of an oral dose of (-)-epigallocatechin-3-gallate-rich polyphenon E in patients with mild to moderate ulcerative colitis. Inflamm Bowel Dis 2013;19:1904–12.

Du, Gang, Wei Dong, Qing Yang, Xueying Yu, Jinghong Ma, Weihong Gu, and Yue Huang. 2020. “Altered Gut Microbiota Related to Inflammatory Responses in Patients With Huntington’s Disease.” Frontiers in Immunology 11: 603594.

Duan, Tianchi, Xiaoyuan Wang, Xinyue Dong, Chennan Wang, Lu Wang, Xingbin Yang, and Ting Li. 2023. “Broccoli-Derived Exosome-like Nanoparticles Alleviate Loperamide-Induced Constipation, in Correlation with Regulation on Gut Microbiota and Tryptophan Metabolism.” Journal of Agricultural and Food Chemistry 71 (44): 16568–80.

Edelstein, Sari. 2022. Community and Public Health Nutrition. Jones & Bartlett Learning.

El Amrousy D, El-Afify D, Elsawy A, Elsheikh M, Donia A, Nassar M. 2022. Lactoferrin for iron-deficiency anemia in children with inflammatory bowel disease: a clinical trial. Pediatr Res 92:762–766.

Elbarbry F, Vermehren-Schmaedick A, Balkowiec A. Modulation of arachidonic Acid metabolism in the rat kidney by sulforaphane: implications for regulation of blood pressure. ISRN Pharmacol 2014;2014:683508.

Esperanza MG, Wrobel K, Ojeda AG, Garay-Sevilla ME, Escobosa ARC, Barrientos EY, Wrobel K. Liquid chromatography-mass spectrometry untargeted metabolomics reveals increased levels of tryptophan indole metabolites in urine of metabolic syndrome patients. Eur J Mass Spectrom SAGE Publications; 2020;26:379–87.

Esperanza, Moises Guerrero, Katarzyna Wrobel, Armando Gomez Ojeda, Ma Eugenia Garay-Sevilla, Alma Rosa Corrales Escobosa, Eunice Yanez Barrientos, and Kazimierz Wrobel. 2020. “Liquid Chromatography-Mass Spectrometry Untargeted Metabolomics Reveals Increased Levels of Tryptophan Indole Metabolites in Urine of Metabolic Syndrome Patients.” European Journal of Mass Spectrometry 26 (6): 379–87.

Evans, Alexandra, Karen Banks, Rose Jennings, Eileen Nehme, Cori Nemec, Shreela Sharma, Aliya Hussaini, and Amy Yaroch. 2015. “Increasing Access to Healthful Foods: A Qualitative Study with Residents of Low-Income Communities.” The International Journal of Behavioral Nutrition and Physical Activity 12 Suppl 1 (Suppl 1): S5.

Eynard, Aldo R., and Cristina B. Lopez. 2003. “Conjugated Linoleic Acid (CLA) versus Saturated Fats/cholesterol: Their Proportion in Fatty and Lean Meats May Affect the Risk of Developing Colon Cancer.” Lipids in Health and Disease 2 (August): 6.

Facchin S, Vitulo N, Calgaro M, Buda A, Romualdi C, Pohl D, Perini B, Lorenzon G, Marinelli C, D’Incà R, Sturniolo GC, Savarino EV. 2020. Microbiota changes induced by microencapsulated sodium butyrate in patients with inflammatory bowel disease. Neurogastroenterol Motil 32:e13914.

Fan F-Y, Sang L-X, Jiang M. Catechins and Their Therapeutic Benefits to Inflammatory Bowel Disease. Molecules 2017;22.

Fanelli, Stephanie M., Satya S. Jonnalagadda, Janell L. Pisegna, Owen J. Kelly, Jessica L. Krok-Schoen, and Christopher A. Taylor. 2020. “Poorer Diet Quality Observed Among US Adults With a Greater Number of Clinical Chronic Disease Risk Factors.” Journal of Primary Care & Community Health 11: 2150132720945898.

Fernandez-Prades, Laura, Mariano Brasal-Prieto, Gonzalo Alba, Victoria Martin, Sergio Montserrat-de la Paz, Marta Cejudo-Guillen, Consuelo Santa-Maria, et al. 2023. “Sulforaphane Reduces the Chronic Inflammatory Immune Response of Human Dendritic Cells.” Nutrients 15 (15): 3405.

Ferru-Clément R, Boucher G, Forest A, Bouchard B, Bitton A, Lesage S, Schumm P, Lazarev M, Brant S, Duerr RH, et al. Serum Lipidomic Screen Identifies Key Metabolites, Pathways, and Disease Classifiers in Crohn’s Disease. Inflamm Bowel Dis 2023;29:1024–37.

Ferruzza, Simonetta, Fausta Natella, Giulia Ranaldi, Chiara Murgia, Carlotta Rossi, Kajetan Trošt, Fulvio Mattivi, et al. 2016. “Nutraceutical Improvement Increases the Protective Activity of Broccoli Sprout Juice in a Human Intestinal Cell Model of Gut Inflammation.” Pharmaceuticals 9 (3).

Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. 2012. Microbial degradation of complex carbohydrates in the gut. Gut Microbes 3:289-306.

Folz J, Culver RN, Morales JM, Grembi J, Triadafilopoulos G, Relman DA, Huang KC, Shalon D, Fiehn O. Human metabolome variation along the upper intestinal tract. Nat Metab 2023;5:777–88.

Forsman H. Targeted and non-targeted approaches to ImprUV the nutritional profile of greenhouse crops. diva-portal.org; 2022.

Francis KL, Alonge KM, Pacheco MC, Hu SJ, Krutzsch CA, Morton GJ, Schwartz MW, Scarlett JM. 2023. Diabetes exacerbates inflammatory bowel disease in mice with diet-induced obesity. World J Gastroenterol 29:4991–5004.

Frank, Daniel N., Allison L. St Amand, Robert A. Feldman, Edgar C. Boedeker, Noam Harpaz, and Norman R. Pace. 2007. "Molecular-Phylogenetic Characterization of Microbial Community Imbalances in Human Inflammatory Bowel Diseases." Proceedings of the National Academy of Sciences 104 (34): 13780–85.

Fritsch J, Garces L, Quintero MA, Pignac-Kobinger J, Santander AM, Fernández I, Ban YJ, Kwon D, Phillips MC, Knight K, Mao Q, Santaolalla R, Chen XS, Maruthamuthu M, Solis N, Damas OM, Kerman DH, Deshpande AR, Lewis JE, Chen C, Abreu MT. 2021. Low-Fat, High-Fiber Diet Reduces Markers of Inflammation and Dysbiosis and Improves Quality of Life in Patients With Ulcerative Colitis. Clin Gastroenterol Hepatol 19:1189–1199.e30.

Frye RE, Berk M. The Therapeutic Use of N-Acetylcysteine (NAC) in Medicine. Springer; 2018. 394 p.

Gallagher K, Catesson A, Griffin JL, Holmes E, Williams HRT. 2021. Metabolomic Analysis in Inflammatory Bowel Disease: A Systematic Review. J Crohns Colitis 15:813–826.

Gao, Jing, Kang Xu, Hongnan Liu, Gang Liu, Miaomiao Bai, Can Peng, Tiejun Li, and Yulong Yin. 2018. "Impact of the Gut Microbiota on Intestinal Immunity Mediated by Tryptophan Metabolism." Frontiers in Cellular and Infection Microbiology 8 (February): 13.

Gao, Jinjun, Xinxin Yu, Fengming Ma, and Jing Li. 2014. "RNA-Seq Analysis of Transcriptome and Glucosinolate Metabolism in Seeds and Sprouts of Broccoli (Brassica Oleracea Var. Italic)." PloS One 9 (2): e88804.

Gao, Yasheen, Doratha A. Byrd, Anna Prizment, Deann Lazovich, and Roberd M. Bostick. 2022. "Associations of Novel Lifestyle- and Whole Foods-Based Inflammation Scores with Incident Colorectal Cancer Among Women." Nutrition and Cancer 74 (4): 1356–69.

Gates TJ, Yuan C, Shetty M, Kaiser T, Nelson AC, Chauhan A, Starr TK, Staley C, Subramanian S. 2023. Fecal Microbiota Restoration Modulates the Microbiome in Inflammation-Driven Colorectal Cancer. Cancers 15:2260.

Ge L, Liu S, Li S, Yang J, Hu G, Xu C, Song W. Psychological stress in inflammatory bowel disease: Psychoneuroimmunological insights into bidirectional gut–brain communications. Front Immunol 2022;13.

Geertsema S, Bourgonje AR, Fagundes RR, Gacesa R, Weersma RK, van Goor H, Mann GE, Dijkstra G, Faber KN. 2023. The NRF2/Keap1 pathway as a therapeutic target in inflammatory bowel disease. Trends Mol Med 29:830–842.

Geng, Shijie, Saisai Cheng, Yuan Li, Zhengshun Wen, Xin Ma, Xuemei Jiang, Yizhen Wang, and Xinyan Han. 2018. "Faecal Microbiota Transplantation Reduces Susceptibility to Epithelial Injury and Modulates Tryptophan Metabolism of the Microbial Community in a Piglet Model." Journal of Crohn's & Colitis 12 (11): 1359–74.

Ghazaryan, Armen, Andrea Carlson, Alana Y. Rhone, and Kakoli Roy. 2021. "Association between the Nutritional Quality of Household At-Home Food Purchases and Chronic Diseases and Risk Factors in the United States, 2015." Nutrients 13 (9).

Ghenadenik, A. E., L. Kakinami, A. Van Hulst, M. Henderson, and T. A. Barnett. 2018. "Neighbourhoods and Obesity: A Prospective Study of Characteristics of the Built Environment and Their Association with Adiposity Outcomes in Children in Montreal, Canada." Preventive Medicine 111 (June): 35–40.

Giovannucci, E., E. B. Rimm, M. J. Stampfer, G. A. Colditz, A. Ascherio, and W. C. Willett. 1994. "Intake of Fat, Meat, and Fiber in Relation to Risk of Colon Cancer in Men." Cancer Research 54 (9): 2390–97.

Gonçalves P, Araújo J, Di Santo JD. A cross-talk between Microbiota-derived short-chain fatty acids and the host mucosal immune system regulates intestinal homeostasis and inflammatory bowel disease. Inflamm Bowel Dis 2018;24:558–72.

Goodman WA, Erkkila IP, Pizarro TT. Sex matters: impact on pathogenesis, presentation and treatment of inflammatory bowel disease. Nat Rev Gastroenterol Hepatol 2020;17:740–54.

Groussin M, Poyet M, Sistiaga A, Kearney SM, Moniz K, Noel M, Hooker J, Gibbons SM, Segurel L, Froment A, Mohamed RS, Fezeu A, Juimo VA, Lafosse S, Tabe FE, Girard C, Iqaluk D, Nguyen LTT, Jesse Shapiro B, Lehtimäki J, Alm EJ. 2021. Elevated rates of horizontal gene transfer in the industrialized human microbiome. Cell 184:2053–2067.e18.

Gu P, Theiss A, Han J, Feagins LA. 2017. Increased Cell Adhesion Molecules, PECAM-1, ICAM-3, or VCAM-1, Predict Increased Risk for Flare in Patients With Quiescent Inflammatory Bowel Disease. J Clin Gastroenterol 51:522–527.

Guan, Yuge, Wenzhong Hu, Aili Jiang, Yongping Xu, Jiaoxue Yu, Manru Zhao, Yaru Ji, Ke Feng, Sarengaowa, and Xiaozhe Yang. 2020. "Influence of Cut Type on Quality, Antioxidant Substances and Antioxidant Activity of Fresh-cut Broccoli." International Journal of Food Science & Technology 55 (8): 3019–30.

Guo X, Xu Y, Geng R, Qiu J, He X. 2022. Curcumin Alleviates Dextran Sulfate Sodium-Induced Colitis in Mice Through Regulating Gut Microbiota. Mol Nutr Food Res 60:e2100943.

Guo, Rong-Fang, Gao-Feng Yuan, and Qiao-Mei Wang. 2013. “Effect of NaCl Treatments on Glucosinolate Metabolism in Broccoli Sprouts.” Journal of Zhejiang University. Science. B 14 (2): 124–31.

Guo, Xiaoxuan, Ye Xu, Ruixuan Geng, Jing Qiu, and Xiaoyun He. 2022. “Curcumin Alleviates Dextran Sulfate Sodium-Induced Colitis in Mice Through Regulating Gut Microbiota.” Molecular Nutrition & Food Research 66 (8): e2100943.

Hagihara M, Yamashita R, Matsumoto A, Mori T, Inagaki T, Nonogaki T, Kuroki Y, Higashi S, Oka K, Takahashi M, et al. The impact of probiotic Clostridium butyricum MIYAIRI 588 on murine gut metabolic alterations. J Infect Chemother 2019;25:571–7.

Han, Moon K., Raeda Anderson, Emilie Viennois, and Didier Merlin. 2020. “Examination of Food Consumption in United States Adults and the Prevalence of Inflammatory Bowel Disease Using National Health Interview Survey 2015.” PloS One 15 (4): e0232157.

Hansen R, Russell RK, Reiff C, Louis P, McIntosh F, Berry SH, Mukhopadhya I, Michael Bisset W, Barclay AR, Bishop J, Flynn DM, McGrogan P, Loganathan S, Mahdi G, Flint HJ, El-Omar EM, Hold GL. 2012. Microbiota of De-Novo Pediatric IBD: Increased Faecalibacterium Prausnitzii and Reduced Bacterial Diversity in Crohn’s But Not in Ulcerative Colitis. Am J Gastroenterol 107:1913–1922.

Hashem, Heba, and Hany Abdel Malak. 2024. “Broccoli Extract Protects against Induced Renal Cortical Damage by Hemolytic Anemia in Adult Male Rats.” The Egyptian Journal of Anatomy, April.

Hauder J, Winkler S, Bub A, Rüfer CE, Pignitter M, Somoza V. 2011. LC-MS/MS quantification of sulforaphane and indole-3-carbinol metabolites in human plasma and urine after dietary intake of selenium-fortified broccoli. J Agric Food Chem 59:8047–8057.

He F, Wu C, Li P, Li N, Zhang D, Zhu Q, Ren W, Peng Y. Functions and Signaling Pathways of Amino Acids in Intestinal Inflammation. Biomed Res Int 2018;2018:9171905.

He, Canxia, Mingfei Gao, Xiaohong Zhang, Peng Lei, Haitao Yang, Yanping Qing, and Lina Zhang. 2022. “The Protective Effect of Sulforaphane on Dextran Sulfate Sodium-Induced Colitis Depends on Gut Microbial and Nrf2-Related Mechanism.” Frontiers in Nutrition 9 (9:893344-893344.): 893344.

HEI Scores for Americans.” n.d. Accessed June 1, 2024. https://www.fns.usda.gov/cnpp/hei-scores-americans.

Heinken A, Hertel J, Thiele I. 2021. Metabolic modelling reveals broad changes in gut microbial metabolism in inflammatory bowel disease patients with dysbiosis. NPJ systems biology and applications 7:19.

Helm, Dominic, Johannes P. C. Vissers, Christopher J. Hughes, Hannes Hahne, Benjamin Ruprecht, Fiona Pachl, Arkadiusz Grzyb, et al. 2014. “Ion Mobility Tandem Mass Spectrometry Enhances Performance of Bottom-up Proteomics.” Molecular & Cellular Proteomics: MCP 13 (12): 3709–15.

Hernandez-Ledesma, Blanca, and Cristina Martinez-Villaluenga. 2021. Current Advances for Development of Functional Foods Modulating Inflammation and Oxidative Stress. Academic Press.

Ho S-M, Lewis JD, Mayer EA, Plevy SE, Chuang E, Rappaport SM, Croitoru K, Korzenik JR, Krischer J, Hyams JS, Judson R, Kellis M, Jerrett M, Miller GW, Grant ML, Shtraizent N, Honig G, Hurtado-Lorenzo A, Wu GD. 2019. Challenges in IBD Research: Environmental Triggers. Inflamm Bowel Dis 25:S13–S23.

Hoarau G, Mukherjee PK, Gower-Rousseau C, Hager C, Chandra J, Retuerto MA, Neut C, Vermeire S, Clemente J, Colombel JF, Fujioka H, Poulain D, Sendid B, Ghannoum MA. 2016. Bacteriome and Mycobiome Interactions Underscore Microbial Dysbiosis in Familial Crohn’s Disease. MBio 7:e01250–16.

Holcomb L, Holman JM, Hurd M, Lavoie B, Colucci L, Hunt B, Hunt T, Kinney M, Pathak J, Mawe GM, Moses PL, Perry E, Stratigakis A, Zhang T, Chen G, Ishaq SL, Li Y. 2023. Early life exposure to broccoli sprouts confers stronger protection against enterocolitis development in an immunological mouse model of inflammatory bowel disease. mSystems 8:e0068823.

Holcomb, Lola, Grace Chen, Louisa Colucci, Johanna Holman, Molly Hurd, Sue Ishaq, Brigitte Lavoie, et al. 2023. “P20-021-23 Early Life Exposure to Broccoli Sprouts Confers Stronger Protection Against Enterocolitis Development in an Immunological Mouse Model of Inflammatory Bowel Disease.” Current Developments in Nutrition 7 (July): 101526.

Holman J, Colucci L, Baudewyns D, Balkan J, Hunt T, Hunt B, Kinney M, Holcomb L, Stratigakis A, Chen G, et al. Steamed broccoli sprouts alleviate DSS-induced inflammation and retain gut microbial biogeography in mice. mSystems 2023;e00532–23.

Holman J, Hurd M, Moses P, Mawe G, Zhang T, Ishaq SL, Li Y. Interplay of Broccoli/Broccoli Sprout Bioactives with Gut Microbiota in Reducing Inflammation in Inflammatory Bowel Diseases. J Nutr Biochem 2023;113:109238.

Holman J, Hurd M, Moses PL, Mawe GM, Zhang T, Ishaq SL, Li Y. 2023. Interplay of broccoli/broccoli sprout bioactives with gut microbiota in reducing inflammation in inflammatory bowel diseases. J Nutr Biochem 113:109238.

Holman JM, Colucci L, Baudewyns D, Balkan J, Hunt T, Hunt B, Kinney M, Holcomb L, Stratigakis A, Chen G, Moses PL, Mawe GM, Zhang T, Li Y, Ishaq SL. 2023. Steamed broccoli sprouts alleviate DSS-induced inflammation and retain gut microbial biogeography in mice. mSystems e0053223.

Holman, Johanna, Molly Hurd, Peter L. Moses, Gary M. Mawe, Tao Zhang, Suzanne L. Ishaq, and Yanyan Li. 2023. "Interplay of Broccoli/broccoli Sprout Bioactives with Gut Microbiota in Reducing Inflammation in Inflammatory Bowel Diseases." The Journal of Nutritional Biochemistry 113 (March): 109238.

Holmes, Zachary C., Max M. Villa, Heather K. Durand, Sharon Jiang, Eric P. Dallow, Brianna L. Petrone, Justin D. Silverman, Pao-Hwa Lin, and Lawrence A. David. 2022. "Microbiota Responses to Different Prebiotics Are Conserved within Individuals and Associated with Habitual Fiber Intake." Microbiome 10 (1): 114.

Hossain MN, De Leo V, Tamborra R, Laselva O, Ingrosso C, Daniello V, Catucci L, Losito I, Sollitto F, Loizzi D, Conese M, Di Gioia S. 2022. Characterization of anti-proliferative and anti-oxidant effects of nano-sized vesicles from Brassica oleracea L. (Broccoli). Sci Rep 12:14362.

Hosseinkhani, Farideh, Anne-Charlotte Dubbelman, Naama Karu, Amy C. Harms, and Thomas Hankemeier. 2021. "Towards Standards for Human Fecal Sample Preparation in Targeted and Untargeted LC-HRMS Studies." Metabolites 11 (6).

Hou F Cai Y Wang. 2023. Antioxidant Capacity Changes and Untargeted Metabolite Profile of Broccoli during Lactic Acid Bacteria Fermentation. Fermentation 9:474.

Hou F, Cai Y, Wang J. Antioxidant Capacity Changes and Untargeted Metabolite Profile of Broccoli during Lactic Acid Bacteria Fermentation. Fermentation Multidisciplinary Digital Publishing Institute; 2023;9:474.

Hou, Feixiang, Yanxue Cai, and Jihui Wang. 2023. "Antioxidant Capacity Changes and Untargeted Metabolite Profile of Broccoli during Lactic Acid Bacteria Fermentation." Fermentation 9 (5): 474.

Hou, Jun-Jie, A-Huo Ma, and Yue-Hua Qin. 2023. "Activation of the Aryl Hydrocarbon Receptor in Inflammatory Bowel Disease: Insights from Gut Microbiota." Frontiers in Cellular and Infection Microbiology 13 (October): 1279172.

Hou, Yue, Sai-Feng Wang, Ke Zhou, and Shi-Xue Dai. 2022. "Comparison and Recommendation of Dietary Patterns Based on Nutrients for Eastern and Western Patients with Inflammatory Bowel Disease." Frontiers in Nutrition 9: 1066252.

Housley L, Magana AA, Hsu A, Beaver LM, Wong CP, Stevens JF, Choi J, Jiang Y, Bella D, Williams DE, Maier CS, Shannon J, Dashwood RH, Ho E. 2018. Untargeted Metabolomic

Screen Reveals Changes in Human Plasma Metabolite Profiles Following Consumption of Fresh Broccoli Sprouts. Mol Nutr Food Res 62:e1700665.

Hu J, Chen J, Xu X, Hou Q, Ren J, Yan X. Correction: Gut microbiota-derived 3-phenylpropionic acid promotes intestinal epithelial barrier function via AhR signaling. Microbiome 2023;11:113.

Huang N, Wang M, Peng J, Wei H. Role of arachidonic acid-derived eicosanoids in intestinal innate immunity. Crit Rev Food Sci Nutr 2021;61:2399–410.

Hubbard TD, Murray IA, Nichols RG, Cassel K, Podolsky M, Kuzu G, Tian Y, Smith P, Kennett MJ, Patterson AD, Perdew GH. 2017. Dietary broccoli impacts microbial community structure and attenuates chemically induced colitis in mice in an Ah receptor dependent manner. J Funct Foods 37:685-698.

Hudesman DP, Chakravarty SD, Emond B, Ellis LA, Lefebvre P, Sadik K, Scher JU. 2020. Healthcare resource utilization and costs associated with inflammatory bowel disease among patients with chronic inflammatory diseases: a retrospective cohort study. BMC rheumatology 4:16-16.

Humblot C, Bruneau A, Sutren M, Lhoste EF, Doré J, Andrieux C, Rabot S. 2005. Brussels sprouts, inulin and fermented milk alter the faecal microbiota of human microbiota-associated rats as shown by PCR-temporal temperature gradient gel electrophoresis using universal, Lactobacillus and Bifidobacterium 16S rRNA gene primers. Br J Nutr 93:677–684.

Humblot C, Lhoste E, Knasmüller S, Gloux K, Bruneau A, Bensaada M, Durao J, Rabot S, Andrieux C, Kassie F. 2004. Protective effects of Brussels sprouts, oligosaccharides and fermented milk towards 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-induced genotoxicity in the human flora associated F344 rat: role of xenobiotic metabolising enzymes and intestinal microflora. J Chromatogr B Analyt Technol Biomed Life Sci 802:231–237.

Jacenik D, Cygankiewicz AI, Mokrowiecka A, Małecka-Panas E, Fichna J, Krajewska WM. Sex- and Age-Related Estrogen Signaling Alteration in Inflammatory Bowel Diseases: Modulatory Role of Estrogen Receptors. Int J Mol Sci 2019;20.

Jacobs, Karen, Miranda Hellman, Jacqueline Markowitz, and Ellen Wuest. 2020. “Workload.” In Encyclopedia of Behavioral Medicine, edited by Marc D. Gellman, 2356–2356. Cham: Springer International Publishing.

Jairath V, Feagan BG. Global burden of inflammatory bowel disease. The lancet. Gastroenterology & hepatology. 2020. p. 2–3.

Jayaprakasam, Bolleddula, Mulabagal Vanisree, Yanjun Zhang, David L. Dewitt, and Muraleedharan G. Nair. 2006. “Impact of Alkyl Esters of Caffeic and Ferulic Acids on Tumor Cell Proliferation, Cyclooxygenase Enzyme, and Lipid Peroxidation.” Journal of Agricultural and Food Chemistry 54 (15): 5375–81.

Jeffery, Ian B., Denise B. Lynch, and Paul W. O'Toole. 2016. "Composition and Temporal Stability of the Gut Microbiota in Older Persons." The ISME Journal 10 (1): 170–82.

Jeon E-J, Davaatseren M, Hwang J-T, Park JH, Hur HJ, Lee AS, Sung MJ. 2016. Effect of Oral Administration of 3,3′-Diindolylmethane on Dextran Sodium Sulfate-Induced Acute Colitis in Mice. J Agric Food Chem 64:7702–7709.

Jeong S, Kim HM, Lee M, Yang JE, Park HW. 2023. Use of Vegetable Waste as a Culture Medium Ingredient Improves the Antimicrobial and Immunomodulatory Activities of Lactiplantibacillus plantarum WiKim0125 Isolated from Kimchi. J Microbiol Biotechnol 33:75-82.

Jessri, Mahsa, Wendy Y. Lou, and Mary R. L'Abbé. 2016. "The 2015 Dietary Guidelines for Americans Is Associated with a More Nutrient-Dense Diet and a Lower Risk of Obesity." The American Journal of Clinical Nutrition 104 (5): 1378–92.

Jesus, Janet M. de, Eve E. Stoody, Dana M. DeSilva, Julia B. Quam, Julie E. Obbagy, Dennis Anderson-Villaluz, Elizabeth B. Rahavi, Meghan E. Adler, Tessa A. Lasswell, and Kara A. Beckman. 2024. "Addressing Misinformation about the Dietary Guidelines for Americans." The American Journal of Clinical Nutrition, March.

Jo SG, Noh EJ, Lee JY, Kim G, Choi JH, Lee ME, Song JH, Chang JY, Park JH. 2016. Lactobacillus curvatus WiKim38 isolated from kimchi induces IL-10 production in dendritic cells and alleviates DSS-induced colitis in mice. J Microbiol 54:503–509.

Johnson, Abigail J., Pajau Vangay, Gabriel A. Al-Ghalith, Benjamin M. Hillmann, Tonya L. Ward, Robin R. Shields-Cutler, Austin D. Kim, et al. 2019. "Daily Sampling Reveals Personalized Diet-Microbiome Associations in Humans." Cell Host & Microbe 25 (6): 789–802.e5.

Jun, Shinyoung, Alexandra E. Cowan, Anindya Bhadra, Kevin W. Dodd, Johanna T. Dwyer, Heather A. Eicher-Miller, Jaime J. Gahche, et al. 2020. "Older Adults with Obesity Have Higher Risks of Some Micronutrient Inadequacies and Lower Overall Dietary Quality Compared to Peers with a Healthy Weight, National Health and Nutrition Examination Surveys (NHANES), 2011-2014." Public Health Nutrition 23 (13): 2268–79.

Kaczmarek JL, Liu X, Charron CS, Novotny JA, Jeffery EH, Seifried HE, Ross SA, Miller MJ, Swanson KS, Holscher HD. 2019. Broccoli consumption affects the human gastrointestinal microbiota. J Nutr Biochem 63:27–34.

Kaczmarek, Jennifer L., Xiaoji Liu, Craig S. Charron, Janet A. Novotny, Elizabeth H. Jeffery, Harold E. Seifried, Sharon A. Ross, Michael J. Miller, Kelly S. Swanson, and Hannah D. Holscher. 2019. "Broccoli Consumption Affects the Human Gastrointestinal Microbiota." The Journal of Nutritional Biochemistry 63 (January): 27–34.

Kaisar MMM, Pelgrom LR, van der Ham AJ, Yazdanbakhsh M, Everts B. 2017. Butyrate Conditions Human Dendritic Cells to Prime Type 1 Regulatory T Cells via both Histone Deacetylase Inhibition and G Protein-Coupled Receptor 109A Signaling. Front Immunol 8:1429.

Kang J, Choi S, Jang J, Ramalingam P, Ko YT, Kim SY, Oh SH. 2017. Wasabia japonica is a potential functional food to prevent colitis via inhibiting the NF-κB signaling pathway. Food Funct 8:2865-2874.

Kang S, Denman SE, Morrison M, Yu Z, Dore J, Leclerc M, McSweeney CS. 2010. Dysbiosis of fecal microbiota in Crohn's disease patients as revealed by a custom phylogenetic microarray. Inflamm Bowel Dis 16:2034–2042.

Kang, Minji, Song-Yi Park, Yurii B. Shvetsov, Lynne R. Wilkens, Loïc Le Marchand, Carol J. Boushey, and Hee-Young Paik. 2019. "Sex Differences in Sociodemographic and Lifestyle Factors Associated with Diet Quality in a Multiethnic Population." Nutrition 66 (October): 147–52.

Kant, Ashima K. 2004. "Dietary Patterns and Health Outcomes." Journal of the American Dietetic Association 104 (4): 615–35.

Kaplan GG, Ng SC. 2017. Understanding and Preventing the Global Increase of Inflammatory Bowel Disease. Gastroenterology 152:313–321.e2.

Kaplan GG. 2015. The global burden of IBD: from 2015 to 2025. Nat Rev Gastroenterol Hepatol 12:720–727.

Kassem NM, Abdelmegid YA, El-Sayed MK, Sayed RS, Abdel-Aalla MH, Kassem HA. Nutrigenomics and microbiome shaping the future of personalized medicine: a review article. J Genet Eng Biotechnol 2023;21:134.

Kaulmann A, Bohn T. Bioactivity of Polyphenols: Preventive and Adjuvant Strategies toward Reducing Inflammatory Bowel Diseases—Promises, Perspectives, and Pitfalls. Oxid Med Cell Longev Hindawi; 2016 [cited 2024 Feb 20];2016.

Kaulmann A, Planchon S, Renaut J, Schneider Y, Hoffmann L, Bohn T. 2016. Proteomic response of inflammatory stimulated intestinal epithelial cells to in vitro digested plums and cabbages rich in carotenoids and polyphenols. Food Funct 7:4388-4399.

Keewan E, Narasimhulu CA, Rohr M, Hamid S, Parthasarathy S. 2020. Are Fried Foods Unhealthy? The Dietary Peroxidized Fatty Acid, 13-HPODE, Induces Intestinal Inflammation In Vitro and In Vivo. Antioxid Redox Signal 9:926.

Kellingray L, Tapp HS, Saha S, Doleman JF, Narbad A, Mithen RF. 2017. Consumption of a diet rich in Brassica vegetables is associated with a reduced abundance of sulphate-reducing bacteria: A randomised crossover study. Mol Nutr Food Res 61:1600992.

Kennedy, Megan S., Manjing Zhang, Orlando DeLeon, Jacie Bissell, Florian Trigodet, Karen Lolans, Sara Temelkova, et al. 2023. “Dynamic Genetic Adaptation of Bacteroides Thetaiotaomicron during Murine Gut Colonization.” Cell Reports 42 (8): 113009.

Khalili H, Chan SSM, Lochhead P, Ananthakrishnan AN, Hart AR, Chan AT. 2018. The role of diet in the aetiopathogenesis of inflammatory bowel disease. Nat Rev Gastroenterol Hepatol 15:525–535.

Khorshidi, Masoud, Kurosh Djafarian, Elham Aghayei, and Sakineh Shab-Bidar. 2020. “A Posteriori Dietary Patterns and Risk of Inflammatory Bowel Disease: A Meta-Analysis of Observational Studies.” International Journal for Vitamin and Nutrition Research. Internationale Zeitschrift Fur Vitamin- Und Ernahrungsforschung. Journal International de Vitaminologie et de Nutrition 90 (3-4): 376–84.

Kim G, Jang M, Hwang I, Cho J, Kim S. 2021. Radish sprout alleviates DSS-induced colitis via regulation of NF-kB signaling pathway and modifying gut microbiota. Biomed Pharmacother 144:112365.

Kim J, Ahn M, Choi Y, Kang T, Kim J, Lee NH, Kim GO, Shin T. 2020. Alpha-Linolenic Acid Alleviates Dextran Sulfate Sodium-Induced Ulcerative Colitis in Mice. Inflammation 43:1876–1883.

Kim Y-J, Ju J, Song J-L, Yang S-G, Park K-Y. 2018. Anti-Colitic Effect of Purple Carrot on Dextran Sulfate Sodium (DSS)-Induced Colitis in C57BL/6J Mice. Preventive nutrition and food science 23:77–83.

Knoop KA, Gustafsson JK, McDonald KG, Kulkarni DH, Coughlin PE, McCrate S, Kim D, Hsieh C-S, Hogan SP, Elson CO, Tarr PI, Newberry RD. 2017. Microbial antigen encounter during a preweaning interval is critical for tolerance to gut bacteria. Sci Immunol 2:eaao1314.

Kowalska-Duplaga K, Gosiewski T, Kapusta P, Sroka-Oleksiak A, Wędrychowicz A, Pieczarkowski S, Ludwig-Słomczyńska AH, Wołkow PP, Fyderek K. Differences in the intestinal microbiome of healthy children and patients with newly diagnosed Crohn’s disease. Sci Rep Nature Publishing Group; 2019 [cited 2023 Jun 15];9:1–11.

Kruidenier L, Kuiper I, van Duijn W, Mieremet-Ooms MA, van Hogezand RA, Lamers CB, Verspaget HW. 2003. Imbalanced secondary mucosal antioxidant response in inflammatory bowel disease. J Pathol 201:17-27.

Kumar A, Al-Hassi HO, Steed H, Phipps O, Brookes MJ. Bile Acids and the Microbiome: Making Sense of This Dynamic Relationship in Their Role and Management in Crohn’s Disease. Can J Gastroenterol Hepatol 2022;2022:8416578.

Kumar S, Sharma S, Kumar V, Sharma R, Minhas A, Boddu R. 2022. Chapter 20 - Cruciferous vegetables: a mine of phytonutrients for functional and nutraceutical enrichment. In Hernández-Ledesma, B, Martínez-Villaluenga, C (eds.). Current Advances for Development of Functional Foods Modulating Inflammation and Oxidative Stress. Academic Press p. 401–426.

Kutsuna, Ryo, Junko Tomida, Yuji Morita, and Yoshiaki Kawamura. 2018. "Paraclostridium Bifermentans Exacerbates Pathosis in a Mouse Model of Ulcerative Colitis." PloS One 13 (5): e0197668.

Kutsuna, Ryo, Tohru Miyoshi-Akiyama, Koji Mori, Masahiro Hayashi, Junko Tomida, Yuji Morita, Kaori Tanaka, and Yoshiaki Kawamura. 2019. "Description of Paraclostridium Bifermentans Subsp. Muricolitidis Subsp. Nov., Emended Description of Paraclostridium Bifermentans (Sasi Jyothsna et Al., 2016), and Creation of Paraclostridium Bifermentans Subsp. Bifermentans Subsp. Nov." Microbiology and Immunology 63 (1): 1–10.

Lai Y, Xue J, Liu C-W, Gao B, Chi L, Tu P, Lu K, Ru H. Serum Metabolomics Identifies Altered Bioenergetics, Signaling Cascades in Parallel with Exposome Markers in Crohn's Disease. Molecules 2019;24.

Lai, Ren-Hau, Michael J. Miller, and Elizabeth Jeffery. 2010. "Glucoraphanin Hydrolysis by Microbiota in the Rat Cecum Results in Sulforaphane Absorption." Food & Function 1 (2): 161–66.

Laing B, Han DY, Ferguson LR. 2013. Candidate genes involved in beneficial or adverse responses to commonly eaten brassica vegetables in a New Zealand Crohn's disease cohort. Nutrients 5:5046–5064.

Lam, Thao Minh, Nicolette R. den Braver, Haykanush Ohanyan, Alfred J. Wagtendonk, Ilonca Vaartjes, Joline Wj Beulens, and Jeroen Lakerveld. 2024. "The Neighourhood Obesogenic Built Environment Characteristics (OBCT) Index: Practice versus Theory." Environmental Research 251 (Pt 1): 118625.

Larabi, Anaïs, Nicolas Barnich, and Hang Thi Thu Nguyen. 2020. "New Insights into the Interplay between Autophagy, Gut Microbiota and Inflammatory Responses in IBD." Autophagy 16 (1): 38–51.

Lee JY, Cevallos SA, Byndloss MX, Tiffany CR, Olsan EE, Butler BP, Young BM, Rogers AWL, Nguyen H, Kim K, Choi SW, Bae E, Lee JH, Min UG, Lee D-C, Bäumler AJ. 2020. High-Fat Diet and Antibiotics Cooperatively Impair Mitochondrial Bioenergetics to Trigger Dysbiosis that Exacerbates Pre-inflammatory Bowel Disease. Cell Host Microbe 28:273–284.e6.

Lee, Hana, Kwang Bo Jung, Ohman Kwon, Ye Seul Son, Eunho Choi, Won Dong Yu, Naeun Son, et al. 2022. "Limosilactobacillus Reuteri DS0384 Promotes Intestinal Epithelial Maturation via the Postbiotic Effect in Human Intestinal Organoids and Infant Mice." Gut Microbes 14 (1): 2121580.

Leng, Gareth, Roger A. H. Adan, Michele Belot, Jeffrey M. Brunstrom, Kees de Graaf, Suzanne L. Dickson, Todd Hare, et al. 2017. “The Determinants of Food Choice.” The Proceedings of the Nutrition Society 76 (3): 316–27.

Leso, V., W. Ricciardi, and I. Iavicoli. 2015. “Occupational Risk Factors in Inflammatory Bowel Disease.” European Review for Medical and Pharmacological Sciences 19 (15): 2838–51.

Levina, Elena O., Nikita V. Penkov, Natalia N. Rodionova, Sergey A. Tarasov, Daria V. Barykina, and Mikhail V. Vener. 2018. “Hydration of the Carboxylate Group in Anti-Inflammatory Drugs: ATR-IR and Computational Studies of Aqueous Solution of Sodium Diclofenac.” ACS Omega 3 (1): 302–13.

Lewis JD, Parlett LE, Jonsson Funk ML, Brensinger C, Pate V, Wu Q, Dawwas GK, Weiss A, Constant BD, McCauley M, et al. Incidence, Prevalence, and Racial and Ethnic Distribution of Inflammatory Bowel Disease in the United States. Gastroenterology 2023;165:1197–205.e2.

Lewis JD, Scott FI, Brensinger CM, Roy JA, Osterman MT, Mamtani R, Bewtra M, Chen L, Yun H, Xie F, Curtis JR. 2018. Increased Mortality Rates With Prolonged Corticosteroid Therapy When Compared With Antitumor Necrosis Factor-α-Directed Therapy for Inflammatory Bowel Disease. Am J Gastroenterol 113:405–417.

Li F, Hullar MAJ, Schwarz Y, Lampe JW. 2009. Human Gut Bacterial Communities Are Altered by Addition of Cruciferous Vegetables to a Controlled Fruit- and Vegetable-Free Diet. J Nutr 139:1685–1691.

Li F, Liu X, Wang W, Zhang D. 2015. Consumption of vegetables and fruit and the risk of inflammatory bowel disease: a meta-analysis. Eur J Gastroenterol Hepatol 27:623-630.

Li G, Lin J, Gao X, Su H, Lin R, Gao H, Feng Z, Wu H, Feng B, Zuo K, Li Y, Wu W, Fang L, Liu Z. 2023. Intestinal epithelial pH-sensing receptor GPR65 maintains mucosal homeostasis via regulating antimicrobial defense and restrains gut inflammation in inflammatory bowel disease. Gut Microbes 12:2257269.

Li H, Christman LM, Li R, Gu L. 2020. Synergic interactions between polyphenols and gut microbiota in mitigating inflammatory bowel diseases. Food Funct 11:4878-4891.

Li J-Y, Guo Y-C, Zhou H-F, Yue T-T, Wang F-X, Sun F, Wang W-Z. Arginine metabolism regulates the pathogenesis of inflammatory bowel disease. Nutr Rev 2023;81:578–86.

Li Q-J, Wang Z-G, Xie Y, Liu Q, Hu H-L, Gao Y-X. Mechanistic evaluation of gastro-protective effects of KangFuXinYe on indomethacin-induced gastric damage in rats. Chin J Nat Med 2020;18:47–56.

Li Y, Zhang T, Holman J, McKinstry D, Trindade BC, Mendoza-Castrejon J, Ho S, Sun D, Wells E, Chen G. 2022. A Specific Broccoli Sprout Preparation Reduces Chemically-Induced Colitis Via Gut Microbiota. Curr. Dev. Nutr. 6:307.

Li Y, Zhang T, Li X, Zou P, Schwartz SJ, Sun D. 2013. Kinetics of sulforaphane in mice after consumption of sulforaphane-enriched broccoli sprout preparation. Mol Nutr Food Res 57:2128-2136.

Li, Cheng. 2023. "Understanding Interactions among Diet, Host and Gut Microbiota for Personalized Nutrition." Life Sciences 312 (January): 121265.

Li, Fan, Chanjiao Yu, Qi Zhao, Zhaodi Wang, Zhi Wang, Yu Chang, Zifeng Xu, et al. 2023. "Exploring the Intestinal Ecosystem: From Gut Microbiota to Associations with Subtypes of Inflammatory Bowel Disease." Frontiers in Cellular and Infection Microbiology 13: 1304858.

Li, Hang, Yu Xia, Hong-Yan Liu, Huan Guo, Xiao-Qin He, Yi Liu, Ding-Tao Wu, et al. 2022. "Nutritional Values, Beneficial Effects, and Food Applications of Broccoli (Brassica Oleracea Var. Italica Plenck)." Trends in Food Science & Technology 119 (January): 288–308.

Li, Tong, Yun Qiu, Hong Sheng Yang, Man Ying Li, Xiao Jun Zhuang, Sheng Hong Zhang, Rui Feng, et al. 2020. "Systematic Review and Meta-Analysis: Association of a Pre-Illness Western Dietary Pattern with the Risk of Developing Inflammatory Bowel Disease." Journal of Digestive Diseases 21 (7): 362–71.

Li, Yanyan, Tao Zhang, Xiaoqin Li, Peng Zou, Steven J. Schwartz, and Duxin Sun. 2013. "Kinetics of Sulforaphane in Mice after Consumption of Sulforaphane-Enriched Broccoli Sprout Preparation." Molecular Nutrition & Food Research 57 (12): 2128–36.

Liang, Jie, Beate Jahraus, Emre Balta, Jacqueline D. Ziegler, Katrin Hübner, Norbert Blank, Beate Niesler, Guido H. Wabnitz, and Yvonne Samstag. 2018. "Sulforaphane Inhibits Inflammatory Responses of Primary Human T-Cells by Increasing ROS and Depleting Glutathione." Frontiers in Immunology 9 (November): 2584.

Lin R, Liu W, Piao M, Zhu H. A review of the relationship between the gut microbiota and amino acid metabolism. Amino Acids 2017;49:2083–90.

Liou, Catherine S., Shannon J. Sirk, Camil A. C. Diaz, Andrew P. Klein, Curt R. Fischer, Steven K. Higginbottom, Amir Erez, Mohamed S. Donia, Justin L. Sonnenburg, and Elizabeth S. Sattely. 2020. "A Metabolic Pathway for Activation of Dietary Glucosinolates by a Human Gut Symbiont." Cell 180 (4): 717–28.e19.

Lippmann, Doris, Carsten Lehmann, Simone Florian, Gitte Barknowitz, Michael Haack, Inga Mewis, Melanie Wiesner, et al. 2014. "Glucosinolates from Pak Choi and Broccoli Induce

Enzymes and Inhibit Inflammation and Colon Cancer Differently." Food & Function 5 (6): 1073–81.

Liu JZ, van Sommeren S, Huang H, Ng SC, Alberts R, Takahashi A, Ripke S, Lee JC, Jostins L, Shah T, Abedian S, Cheon JH, Cho J, Dayani NE, Franke L, Fuyuno Y, Hart A, Juyal RC, Juyal G, Kim WH, Morris AP, Poustchi H, Newman WG, Midha V, Orchard TR, Vahedi H, Sood A, Sung JY, Malekzadeh R, Westra H-J, Yamazaki K, Yang S-K, International Multiple Sclerosis Genetics Consortium, International IBD Genetics Consortium, Barrett JC, Alizadeh BZ, Parkes M, Bk T, Daly MJ, Kubo M, Anderson CA, Weersma RK. 2015. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. Nat Genet 47:979–986.

Liu X, Wang Y, Hoeflinger JL, Neme BP, Jeffery EH, Miller MJ. Dietary Broccoli Alters Rat Cecal Microbiota to Improve Glucoraphanin Hydrolysis to Bioactive Isothiocyanates. Nutrients 2017;9.

Liu X, Zheng H, Lu R, Huang H, Zhu H, Yin C, Mo Y, Wu J, Liu X, Deng M, et al. Intervening Effects of Total Alkaloids of Corydalis saxicola Bunting on Rats With Antibiotic-Induced Gut Microbiota Dysbiosis Based on 16S rRNA Gene Sequencing and Untargeted Metabolomics Analyses. Front Microbiol 2019;10:1151.

Liu Y, Jiao C, Zhang T, Li X, Li P, Lu M, Ye Z, Du Y, Du R, Zhang W, et al. Early-Life Gut Microbiota Governs Susceptibility to Colitis via Microbial-Derived Ether Lipids. Research 2023;6:0037.

Liu, Fang, Wenjuan Xu, Lihui Du, Daoying Wang, Yongzhi Zhu, Zhiming Geng, Muhan Zhang, and Weimin Xu. 2014. "Heterologous Expression and Characterization of Tyrosine Decarboxylase from Enterococcus Faecalis R612Z1 and Enterococcus Faecium R615Z1." Journal of Food Protection 77 (4): 592–98.

Liu, Xiaoji, Yanling Wang, Jennifer L. Hoeflinger, Bárbara P. Neme, Elizabeth H. Jeffery, and Michael J. Miller. 2017. "Dietary Broccoli Alters Rat Cecal Microbiota to Improve Glucoraphanin Hydrolysis to Bioactive Isothiocyanates." Nutrients 9 (3).

Liu, Yuanyuan, Haoqing Chen, William Van Treuren, Bi-Huei Hou, Steven K. Higginbottom, and Dylan Dodd. 2022. "Clostridium Sporogenes Uses Reductive Stickland Metabolism in the Gut to Generate ATP and Produce Circulating Metabolites." Nature Microbiology 7 (5): 695–706.

Lomer MCE, Cahill O, Baschali A, Partha Sarathy P, Sarantidou M, Mantzaris GJ, Gaya DR, Katsanos K, Christodoulou DK, Gerasimidis K. 2019. A multicentre Study of Nutrition Risk Assessment in Adult Patients with Inflammatory Bowel Disease Attending Outpatient Clinics. Ann Nutr Metab 74:18–23.

Lopes, Emily W., Simon S. M. Chan, Mingyang Song, Jonas F. Ludvigsson, Niclas Håkansson, Paul Lochhead, Allan Clark, et al. 2022. "Lifestyle Factors for the Prevention of Inflammatory Bowel Disease." Gut, December.

López-González, Leyre, Nerea Becerra-Tomás, Nancy Babio, Miguel Ángel Martínez-González, Andrés Díaz-López, Dolores Corella, Albert Goday, et al. 2021. “Variety in Fruits and Vegetables, Diet Quality and Lifestyle in an Older Adult Mediterranean Population.” Clinical Nutrition 40 (4): 1510–18.

Lynn, Anthony, Zoë Fuller, Andrew R. Collins, and Brian Ratcliffe. 2015. “Comparison of the Effect of Raw and Blanched-Frozen Broccoli on DNA Damage in Colonocytes.” Cell Biochemistry and Function 33 (5): 266–76.

Ma L, Hou C, Yang H, Chen Q, Lyu W, Wang Z, Wang J, Xiao Y. 2023. Multi-omics analysis reveals the interaction of gut microbiome and host microRNAs in ulcerative colitis. Annals of medicine 55:2261477.

Ma, Teng-Chi, Jing Zhou, Chen-Xi Wang, Zhi-Zhi Lin, and Feng Gao. 2022. “Associations between the Healthy Eating Index-2015 and S-Klotho Plasma Levels: A Cross-Sectional Analysis in Middle-to-Older Aged Adults.” Frontiers in Nutrition 9: 904745.

Machiels K, Joossens M, Sabino J, De Preter V, Arijs I, Eeckhaut V, Ballet V, Claes K, Van Immerseel F, Verbeke K, Ferrante M, Verhaegen J, Rutgeerts P, Vermeire S. 2014. A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. Gut 63:1275–1283.

Macia, Laurence, Jian Tan, Angelica T. Vieira, Katie Leach, Dragana Stanley, Suzanne Luong, Mikako Maruya, et al. 2015. “Metabolite-Sensing Receptors GPR43 and GPR109A Facilitate Dietary Fibre-Induced Gut Homeostasis through Regulation of the Inflammasome.” Nature Communications 6 (April): 6734.

Makarewicz M, Drożdż I, Tarko T, Duda-Chodak A. The Interactions between Polyphenols and Microorganisms, Especially Gut Microbiota. Antioxidants (Basel) 2021;10.

Manski, Scott, Nicholas Noverati, Tatiana Policarpo, Emily Rubin, and Raina Shivashankar. 2024. “Diet and Nutrition in Inflammatory Bowel Disease: A Review of the Literature.” Crohn’s & Colitis 360 6 (1): otad077.

Mao, Bingyong, Baojing Ren, Jiaying Wu, Xin Tang, Qiuxiang Zhang, Jianxin Zhao, Le Zhang, Wei Chen, and Shumao Cui. 2023. “The Protective Effect of Broccoli Seed Extract against Lipopolysaccharide-Induced Acute Liver Injury via Gut Microbiota Modulation and Sulforaphane Production in Mice.” Foods (Basel, Switzerland) 12 (14).

Mardinoglu A, Shoaie S, Bergentall M, Ghaffari P, Zhang C, Larsson E, Bäckhed F, Nielsen J. 2015. The gut microbiota modulates host amino acid and glutathione metabolism in mice. Mol Syst Biol 11:834.

Marissa Kinney, Johanna Holman, Alexis Kirkendall, Emelia Tremblay, Mazie Gordon. n.d. "Using Steamed Broccoli Sprouts to Better Understand Bacterial Glucosinolate Metabolism." UMaine Student Research Symposium, Orono Maine.

Márquez Campos E, Stehle P, Simon M-C. Microbial Metabolites of Flavan-3-Ols and Their Biological Activity. Nutrients 2019;11.

Martin F-P, Su M-M, Xie G-X, Guiraud SP, Kussmann M, Godin J-P, Jia W, Nydegger A. Urinary metabolic insights into host-gut microbial interactions in healthy and IBD children. World J Gastroenterol 2017;23:3643–54.

Martins T, Ferreira T, Colaço B, Medeiros-Fonseca B, Pinto M de L, Barros AN, Venâncio C, Rosa E, Antunes LM, Oliveira PA, et al. Absorption and Excretion of Glucosinolates and Isothiocyanates after Ingestion of Broccoli (Brassica oleracea L. var italica) Leaf Flour in Mice: A Preliminary Study. Nutraceuticals Multidisciplinary Digital Publishing Institute; 2023 [cited 2024 Feb 16];3:540–55.

Martins T, Oliveira PA, Pires MJ, Neuparth MJ, Lanzarin G, Félix L, Venâncio C, Pinto M de L, Ferreira J, Gaivão I, et al. Effect of a Sub-Chronic Oral Exposure of Broccoli (Brassica oleracea L. Var. Italica) By-Products Flour on the Physiological Parameters of FVB/N Mice: A Pilot Study. Foods 2022;11.

Matsui H, Takahashi T, Murayama SY, Kawaguchi M, Matsuo K, Nakamura M. Protective efficacy of a hydroxy fatty acid against gastric Helicobacter infections. Helicobacter 2017;22.

Mavilio M, Marchetti V, Fabrizi M, Stöhr R, Marino A, Casagrande V, Fiorentino L, Cardellini M, Kappel B, Monteleone I, et al. A Role for Timp3 in Microbiota-Driven Hepatic Steatosis and Metabolic Dysfunction. Cell Rep 2016;16:2269.

McCullough, Marjorie L., Diane Feskanich, Meir J. Stampfer, Edward L. Giovannucci, Eric B. Rimm, Frank B. Hu, Donna Spiegelman, David J. Hunter, Graham A. Colditz, and Walter C. Willett. 2002. "Diet Quality and Major Chronic Disease Risk in Men and Women: Moving toward Improved Dietary Guidance." The American Journal of Clinical Nutrition 76 (6): 1261–71.

Mithul Aravind S, Wichienchot S, Tsao R, Ramakrishnan S, Chakkaravarthi S. Role of dietary polyphenols on gut microbiota, their metabolites and health benefits. Food Res Int 2021;142:110189.

Miyatani Y KT. 2023. De-escalation of Therapy in Patients with Quiescent Inflammatory Bowel Disease. Gut Liver 17:181–189.

Mohammed, Aminu, and Hafsat Abdullahi Mohammed. 2023. "Beneficial Role of Broccoli and Its Active Ingredient, Sulforaphane in the Treatment of Diabetes." Phytomedicine Plus 3 (2): 100431.

Mondello, Stefania, Maria Galuppo, Emanuela Mazzon, Italiano Domenico, Patrizia Mondello, Aloisi Carmela, and Salvatore Cuzzocrea. 2010. “Glutamine Treatment Attenuates the Development of Ischaemia/reperfusion Injury of the Gut.” European Journal of Pharmacology 643 (2-3): 304–15.

Montenegro-Burke, J. Rafael, Bernard P. Kok, Carlos Guijas, Xavier Domingo-Almenara, Clara Moon, Andrea Galmozzi, Seiya Kitamura, et al. 2021. “Metabolomics Activity Screening of T Cell-Induced Colitis Reveals Anti-Inflammatory Metabolites.” Science Signaling 14 (702): eabf6584.

Montmayeur, Jean-Pierre, and Johannes le Coutre. 2009. Fat Detection: Taste, Texture, and Post Ingestive Effects. CRC Press.

Moroni, F. 1999. “Tryptophan Metabolism and Brain Function: Focus on Kynurenine and Other Indole Metabolites.” European Journal of Pharmacology 375 (1-3): 87–100.

Morozumi S, Ueda M, Okahashi N, Arita M. Structures and functions of the gut microbial lipidome. Biochim Biophys Acta Mol Cell Biol Lipids 2022;1867:159110.

Moura FA, Goulart MOF, Campos SBG, da Paz Martins AS. 2020. The Close Interplay of Nitro-Oxidative Stress, Advanced Glycation end Products and Inflammation in Inflammatory Bowel Diseases. Curr Med Chem 27:2059–2076.

Mueller K, Blum NM, Mueller AS. 2013. Examination of the Anti-Inflammatory, Antioxidant, and Xenobiotic-Inducing Potential of Broccoli Extract and Various Essential Oils during a Mild DSS-Induced Colitis in Rats. ISRN Gastroenterol 2013:710856.

Mukherjee, Subhendu, Hiranmoy Gangopadhyay, and Dipak K. Das. 2008. “Broccoli: A Unique Vegetable That Protects Mammalian Hearts through the Redox Cycling of the Thioredoxin Superfamily.” Journal of Agricultural and Food Chemistry 56 (2): 609–17.

Murad, Hussam A. S., Hossam M. Abdallah, and Soad S. Ali. 2016. “Mentha Longifolia Protects against Acetic-Acid Induced Colitis in Rats.” Journal of Ethnopharmacology 190 (August): 354–61.

Murimi, M., M. Chrisman, R. Heather, David L. McCollum, and Olevia Mcdonald. 2016. “A Qualitative Study on Factors That Influence Students ’ Food Choices.” The Journal of Nutrition.

Murota, Kaeko, Yoshimasa Nakamura, and Mariko Uehara. 2018. “Flavonoid Metabolism: The Interaction of Metabolites and Gut Microbiota.” Bioscience, Biotechnology, and Biochemistry 82 (4): 600–610.

Nagraj, G. S., A. Chouksey, and S. Jaiswal. 2020. “Broccoli.” Nutritional Composition and. https://www.sciencedirect.com/science/article/pii/B9780128127803000015.

National Academies of Sciences, Engineering, and Medicine, Health and Medicine Division, Food and Nutrition Board, and Committee to Review the Process to Update the Dietary Guidelines for Americans. 2017. Optimizing the Process for Establishing the Dietary Guidelines for Americans: The Selection Process. National Academies Press.

National Academy of Sciences, and Institute of Medicine. 1992. Eat for Life: The Food and Nutrition Board's Guide to Reducing Your Risk of Chronic Disease. National Academies Press.

National Research Council, Division of Behavioral and Social Sciences and Education, Committee on National Statistics, and Panel to Review the U.S Department of Agriculture's Measurement of Food Insecurity and Hunger. 2006. Food Insecurity and Hunger in the United States: An Assessment of the Measure. National Academies Press.

Negi, Geeta, Ashutosh Kumar, and Shyam S. Sharma. 2011. "Nrf2 and NF-κB Modulation by Sulforaphane Counteracts Multiple Manifestations of Diabetic Neuropathy in Rats and High Glucose-Induced Changes." Current Neurovascular Research 8 (4): 294–304.

Newmark, H. L., and J. R. Lupton. 1990. "Determinants and Consequences of Colonic Luminal pH: Implications for Colon Cancer." Nutrition and Cancer 14 (3-4): 161–73.

Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, Panaccione R, Ghosh S, Wu JCY, Chan FKL, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet Elsevier; 2017;390:2769–78.

Nielsen OH, Gubatan JM, Juhl CB, Streett SE, Maxwell C. 2022. Biologics for Inflammatory Bowel Disease and Their Safety in Pregnancy: A Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol 20:74–87.e3.

Nisha, Snigdha Singh, Neera Sharma, and Ramesh Chandra. 2022. "The Indole Nucleus as a Selective COX-2 Inhibitor and Anti-Inflammatory Agent (2011–2022)." Organic Chemistry Frontiers 9 (13): 3624–39.

Nishino K, Nishida A, Inoue R, Kawada Y, Ohno M, Sakai S, Inatomi O, Bamba S, Sugimoto M, Kawahara M, Naito Y, Andoh A. 2018. Analysis of endoscopic brush samples identified mucosa-associated dysbiosis in inflammatory bowel disease. J Gastroenterol 95:95–106.

Nugent, S. G., D. Kumar, D. S. Rampton, and D. F. Evans. 2001. "Intestinal Luminal pH in Inflammatory Bowel Disease: Possible Determinants and Implications for Therapy with Aminosalicylates and Other Drugs." Gut 48 (4): 571–77.

Nuñez P, García Mateo S, Quera R, Gomollón F. 2021. Inflammatory bowel disease and the risk of cardiovascular diseases. Gastroenterol Hepatol 44:236–242.

O'Hara, Cathal, Aifric O'Sullivan, and Eileen R. Gibney. 2022. "A Clustering Approach to Meal-Based Analysis of Dietary Intakes Applied to Population and Individual Data." The Journal of Nutrition 152 (10): 2297–2308.

Okunade O, Niranjan K, Ghawi SK, Kuhnle G, Methven L. 2018. Supplementation of the Diet by Exogenous Myrosinase via Mustard Seeds to Increase the Bioavailability of Sulforaphane in Healthy Human Subjects after the Consumption of Cooked Broccoli. Mol Nutr Food Res 62:e1700980.

Olszewska, M., S. Granica, J. Kołodziejczyk-Czepas, A. Magiera, M. Czerwińska, P. Nowak, M. Rutkowska, Piotr Wasiński, and A. Owczarek. 2020. "Variability of Sinapic Acid Derivatives during Germination and Their Contribution to Antioxidant and Anti-Inflammatory Effects of Broccoli Sprouts on Human Plasma and Human Peripheral Blood Mononuclear Cells." Food & Function, August.

Ortega Hernández E, Others. Effect of osmotic conditions and germination time on the content and profile of glucosinolates, carotenoids and phenolic compounds in kale, and their antioxidant and anti-inflammatory properties. Instituto Tecnológico y de Estudios Superiores de Monterrey.

Pal S, Konkimalla VB. 2016. Sulforaphane regulates phenotypic and functional switching of both induced and spontaneously differentiating human monocytes. Int Immunopharmacol 35:85-98.

Pang, Zhiqiang, Guangyan Zhou, Jessica Ewald, Le Chang, Orcun Hacariz, Niladri Basu, and Jianguo Xia. 2022. "Using MetaboAnalyst 5.0 for LC–HRMS Spectra Processing, Multi-Omics Integration and Covariate Adjustment of Global Metabolomics Data." Nature Protocols 17 (8): 1735–61.

Papa E, Docktor M, Smillie C, Weber S, Preheim SP, Gevers D, Giannoukos G, Ciulla D, Tabbaa D, Ingram J, et al. Non-invasive mapping of the gastrointestinal microbiota identifies children with inflammatory bowel disease. PLoS One 2012;7:e39242.

Parada Venegas, Daniela, Marjorie K. De la Fuente, Glauben Landskron, María Julieta González, Rodrigo Quera, Gerard Dijkstra, Hermie J. M. Harmsen, Klaas Nico Faber, and Marcela A. Hermoso. 2019. "Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases." Frontiers in Immunology 10 (March): 277.

Park B, Chun E, Choi JJ, Shin Y, Kho YT, Oh SH, Kim SY, Lee TH, Kim T, Shin E, Do S, Jin M. 2017. Administration of Wasabia koreana Ameliorates Irritable Bowel Syndrome-Like Symptoms in a Zymosan-Induced Mouse Model. J Med Food 20:474-484.

Park H, Song M. 2017. Leaves of Raphanus sativus L. Shows Anti-Inflammatory Activity in LPS-Stimulated Macrophages via Suppression of COX-2 and iNOS Expression. Prev Nutr Food Sci 22:50-55.

Paturi G, Ph.D, Mandimika T, Ph.D, Butts CA, Ph.D, Zhu S, Ph.D, Roy NC, Ph.D, McNabb WC, Ph.D, Ansell J, D.Phil. 2012. Influence of dietary blueberry and broccoli on cecal microbiota activity and colon morphology in mdr1a−/− mice, a model of inflammatory bowel diseases. Nutrition 28:324-330.

Peng, X., H. M. Heyman, M. Szesny, and S. Hess. 2021. "Metabolomic Profiling of Human Urine Samples Using LC-TIMS-QTOF Mass Spectrometry." Journal of the ACS Publications.

Pereira C, Grácio D, Teixeira JP, Magro F. 2015. Oxidative Stress and DNA Damage: Implications in Inflammatory Bowel Disease. Inflamm Bowel Dis 21:2403–2417.

Pereyra, Katherin V., David C. Andrade, Camilo Toledo, Karla G. Schwarz, Atenea Uribe-Ojeda, Angélica P. Ríos-Gallardo, Rodrigo A. Quintanilla, Samuel Contreras, Andrea Mahn, and Rodrigo Del Rio. 2020. "Dietary Supplementation of a Sulforaphane-Enriched Broccoli Extract Protects the Heart from Acute Cardiac Stress." Journal of Functional Foods 75 (December): 104267.

Petagna L, Antonelli A, Ganini C, Bellato V, Campanelli M, Divizia A, Efrati C, Franceschilli M, Guida AM, Ingallinella S, Montagnese F, Sensi B, Siragusa L, Sica GS. 2020. Pathophysiology of Crohn's disease inflammation and recurrence. Biol Direct 15:23.

Peterson, Christine T., Alexandra R. Vaughn, Vandana Sharma, Deepak Chopra, Paul J. Mills, Scott N. Peterson, and Raja K. Sivamani. 2018. "Effects of Turmeric and Curcumin Dietary Supplementation on Human Gut Microbiota: A Double-Blind, Randomized, Placebo-Controlled Pilot Study." Journal of Evidence-Based Integrative Medicine 23: 2515690X18790725.

Piechota-Polanczyk A, Fichna J. 2014. Review article: the role of oxidative stress in pathogenesis and treatment of inflammatory bowel diseases. Naunyn Schmiedebergs Arch Pharmacol 387:605–620s

Pool-Zobel, B. L., A. Bub, H. Müller, I. Wollowski, and G. Rechkemmer. 1997. "Consumption of Vegetables Reduces Genetic Damage in Humans: First Results of a Human Intervention Trial with Carotenoid-Rich Foods." Carcinogenesis 18 (9): 1847–50.

Pope, Jamie, and Steven Nizielski. 2021. Scientific American Nutrition for a Changing World: Dietary Guidelines for Americans 2020-2025 & Digital Update. Macmillan Higher Education.

Portune, Kevin J., Martin Beaumont, Anne-Marie Davila, Daniel Tomé, François Blachier, and Yolanda Sanz. 2016. "Gut Microbiota Role in Dietary Protein Metabolism and Health-Related Outcomes: The Two Sides of the Coin." Trends in Food Science & Technology 57 (November): 213–32.

Press, A. G., I. A. Hauptmann, L. Hauptmann, B. Fuchs, M. Fuchs, K. Ewe, and G. Ramadori. 1998. “Gastrointestinal pH Profiles in Patients with Inflammatory Bowel Disease.” Alimentary Pharmacology & Therapeutics 12 (7): 673–78.

Quick V, McWilliams R, Byrd-Bredbenner C. 2015. A case-control study of current psychological well-being and weight-teasing history in young adults with and without bowel conditions. J Hum Nutr Diet 28:28–36.

Radünz M, Mota Camargo T, Dos Santos Hackbart HC, Blank JP, Hoffmann JF, Moro Stefanello F, da Rosa Zavareze E. Encapsulation of broccoli extract by electrospraying: Influence of in vitro simulated digestion on phenolic and glucosinolate contents, and on antioxidant and antihyperglycemic activities. Food Chem 2021;339:128075.

Ragonnaud, Emeline, and Arya Biragyn. 2021. “Gut Microbiota as the Key Controllers of ‘healthy’ Aging of Elderly People.” Immunity & Ageing: I & A 18 (1): 2.

Rahmani J, Kord-Varkaneh H, Ryan PM, Rashvand S, Clark C, Day AS, Hekmatdoost A. 2019. Dietary total antioxidant capacity and risk of ulcerative colitis: A case-control study. J Dig Dis 20:636–641.

Rahmani, Jamal, Hamed Kord Varkaneh, Paul M. Ryan, Meysam Zarezadeh, Samaneh Rashvand, Cain Clark, Andrew S. Day, and Azita Hekmatdoost. 2019. “Healthy Eating Index-2015 as a Predictor of Ulcerative Colitis Risk in a Case-Control Cohort.” Journal of Digestive Diseases 20 (12): 649–55.

Rajca S, Grondin V, Louis E, Vernier-Massouille G, Grimaud J-C, Bouhnik Y, Laharie D, Dupas J-L, Pillant H, Picon L, Veyrac M, Flamant M, Savoye G, Jian R, Devos M, Paintaud G, Piver E, Allez M, Mary JY, Sokol H, Colombel J-F, Seksik P. 2014. Alterations in the intestinal microbiome (dysbiosis) as a predictor of relapse after infliximab withdrawal in Crohn’s disease. Inflamm Bowel Dis 20:978–986.

Rajilić-Stojanović M, Shanahan F, Guarner F, de Vos WM. 2013. Phylogenetic analysis of dysbiosis in ulcerative colitis during remission. Inflamm Bowel Dis 19:481–488.

Rana SV, Sharma S, Prasad KK, Sinha SK, Singh K. 2014. Role of oxidative stress & antioxidant defence in ulcerative colitis patients from north India. Indian J Med Res 139:568–571.

Rashvand, S., M. Behrooz, M. Samsamikor, K. Jacobson, and A. Hekmatdoost. 2018. “Dietary Patterns and Risk of Ulcerative Colitis: A Case-Control Study.” Journal of Human Nutrition and Dietetics: The Official Journal of the British Dietetic Association 31 (3): 408–12.

Raychaudhuri S, Shahinozzaman M, Subedi U, Fan S, Ogedengbe O, Obanda DN. 2023. The Vegetable “Kale” Protects against Dextran-Sulfate-Sodium-Induced Acute Inflammation through Moderating the Ratio of Proinflammatory and Anti-Inflammatory LPS-Producing Bacterial Taxa and Augmenting the Gut Barrier in C57BL6 Mice. Nutrients 15:3222.

Recharla N, Geesala R, Shi X-Z. Gut Microbial Metabolite Butyrate and Its Therapeutic Role in Inflammatory Bowel Disease: A Literature Review. Nutrients 2023;15.

Reddavide, Rosa, Ornella Rotolo, Maria Gabriella Caruso, Elisa Stasi, Maria Notarnicola, Chiara Miraglia, Antonio Nouvenne, et al. 2018. "The Role of Diet in the Prevention and Treatment of Inflammatory Bowel Diseases." Acta Bio-Medica: Atenei Parmensis 89 (9-S): 60–75.

Rhee, J. S., Y. B. Jang, G. S. Choi, and J. J. Choung. 2020. "Neuroprotective Effects of Extract of Broccoli, Cultivated in Desalinated Magma Seawater, on Neuron-like SH-SY5Y Cells." Journal of the Korean.

Riaz Rajoka, Muhammad Shahid, Rohit Thirumdas, Hafiza Mahreen Mehwish, Muhammad Umair, Mohsin Khurshid, Hafiz Fakhar Hayat, Yuthana Phimolsiripol, Noelia Pallarés, Francisco J. Martí-Quijal, and Francisco J. Barba. 2021. "Role of Food Antioxidants in Modulating Gut Microbial Communities: Novel Understandings in Intestinal Oxidative Stress Damage and Their Impact on Host Health." Antioxidants (Basel, Switzerland) 10 (10).

Rivière A, Selak M, Lantin D, Leroy F, De Vuyst L. Bifidobacteria and Butyrate-Producing Colon Bacteria: Importance and Strategies for Their Stimulation in the Human Gut. Front Microbiol 2016;7:979.

Roager, H. M., and L. O. Dragsted. 2019. "Diet-derived Microbial Metabolites in Health and Disease." Nutrition Bulletin / BNF 44 (3): 216–27.

Roberts CL, Keita AV, Duncan SH, O'Kennedy N, Söderholm JD, Rhodes JM, Campbell BJ. 2010. Translocation of Crohn's disease Escherichia coli across M-cells: contrasting effects of soluble plant fibres and emulsifiers. Gut 59:1331–1339.

Rom O, Liu Y, Liu Z, Zhao Y, Wu J, Ghrayeb A, Villacorta L, Fan Y, Chang L, Wang L, Liu C, Yang D, Song J, Rech JC, Guo Y, Wang H, Zhao G, Liang W, Koike Y, Lu H, Koike T, Hayek T, Pennathur S, Xi C, Wen B, Sun D, Garcia-Barrio MT, Aviram M, Gottlieb E, Mor I, Liu W, Zhang J, Chen YE. 2020. Glycine-based treatment ameliorates NAFLD by modulating fatty acid oxidation, glutathione synthesis, and the gut microbiome. Sci Transl Med 12:572.

Rubattu, Speranza, Sara Di Castro, Maria Cotugno, Franca Bianchi, Roberto Mattioli, Simona Baima, Rosita Stanzione, et al. 2015. "Protective Effects of Brassica Oleracea Sprouts Extract toward Renal Damage in High-Salt-Fed SHRSP: Role of AMPK/PPARα/UCP2 Axis." Journal of Hypertension 33 (7): 1465–79.

Ruiz Esparza Cisneros, Josefina, Javier J. Vasconcelos-Ulloa, Daniel González-Mendoza, Guillermo Beltrán-González, and Raúl Díaz-Molina. 2020. "Effect of Dietary Intervention with a Legume-Based Food Product on Malondialdehyde Levels, HOMA Index, and Lipid Profile." Endocrinologia, Diabetes Y Nutricion 67 (4): 235–44.

Russo E, Giudici F, Fiorindi C, Ficari F, Scaringi S, Amedei A. Immunomodulating Activity and Therapeutic Effects of Short Chain Fatty Acids and Tryptophan Post-biotics in Inflammatory Bowel Disease. Front Immunol 2019;10:2754.

Saha S, Hollands W, Teucher B, Needs PW, Narbad A, Ortori CA, Barrett DA, Rossiter JT, Mithen RF, Kroon PA. 2012. Isothiocyanate concentrations and interconversion of sulforaphane to erucin in human subjects after consumption of commercial frozen broccoli compared to fresh broccoli. Mol Nutr Food Res 56:1906–1916.

Saha S, Hollands W, Teucher B, Needs PW, Narbad A, Ortori CA, Barrett DA, Rossiter JT, Mithen RF, Kroon PA. Isothiocyanate concentrations and interconversion of sulforaphane to erucin in human subjects after consumption of commercial frozen broccoli compared to fresh broccoli. Mol Nutr Food Res2012;56:1906–16.

Saint-Georges-Chaumet Y, Edeas M. 2015. Microbiota–mitochondria inter-talk: consequence for microbiota–host interaction. Pathog Dis 74:ftv096.

Samuel LM, Francisca AS, Khiong K. 2015. Effect of Steamed Broccoli Juice (Brassica oleraceae L. var. italica) to the Serum Interleukin 8 Level in Colitis Murine Model. The Indonesian Journal of Gastroenterology, Hepatology, and Digestive Endoscopy 16:148–152.

Sandborn WJ, Ghosh S, Panes J, Schreiber S, D'Haens G, Tanida S, Siffledeen J, Enejosa J, Zhou W, Othman AA, Huang B, Higgins PDR. 2020. Efficacy of Upadacitinib in a Randomized Trial of Patients With Active Ulcerative Colitis. Gastroenterology 158:2139–2149.e14.

Santos, Rosana G. C., Iara E. P. Quirino, Mirelle L. Viana, Simone V. Generoso, Jacques R. Nicoli, Flaviano S. Martins, José A. Nogueira-Machado, Rosa M. E. Arantes, Maria I. T. D. Correia, and Valbert N. Cardoso. 2014. "Effects of Nitric Oxide Synthase Inhibition on Glutamine Action in a Bacterial Translocation Model." The British Journal of Nutrition 111 (1): 93–100.

Saroli Palumbo C, Restellini S, Chao C-Y, Aruljothy A, Lemieux C, Wild G, Afif W, Lakatos PL, Bitton A, Cocciolillo S, Ghali P, Bessissow T, Sebastiani G. 2019. Screening for Nonalcoholic Fatty Liver Disease in Inflammatory Bowel Diseases: A Cohort Study Using Transient Elastography. Inflamm Bowel Dis 25:124–133.

Scarano A, Butelli E, De Santis S, Cavalcanti E, Hill L, De Angelis M, Giovinazzo G, Chieppa M Martin C, Santino A. 2018. Combined Dietary Anthocyanins, Flavonols, and Stilbenoids Alleviate Inflammatory Bowel Disease Symptoms in Mice. Front Nutr 4:75.

Schaubeck M, Clavel T, Calasan J, Lagkouvardos I, Haange SB, Jehmlich N, Basic M, Dupont A, Hornef M, von Bergen M, Bleich A, Haller D. 2016. Dysbiotic gut microbiota causes transmissible Crohn's disease-like ileitis independent of failure in antimicrobial defence. Gut 65:225–237.

Schirbel A, Kessler S, Rieder F, West G, Rebert N, Asosingh K, McDonald C, Fiocchi AC. 2013. Pro-angiogenic activity of TLRs and NLRs: a novel link between gut microbiota and intestinal angiogenesis. Gastroenterology (New York, NY 1943) 144:613–623.e9.

Schulzke JD, Ploeger S, Amasheh M, Fromm A, Zeissig S, Troeger H, Richter J, Bojarski C, Schumann M, Fromm M. 2009. Epithelial tight junctions in intestinal inflammation. Ann N Y Acad Sci 1165:294–300.

Segal, Marcia Texler, and Vasilikie (vicky) Demos. 2016. Gender and Food: From Production to Consumption and After. Emerald Group Publishing.

Selma-Royo M, Calatayud Arroyo M, García-Mantrana I, Parra-Llorca A, Escuriet R, Martínez-Costa C, Collado MC. 2020. Perinatal environment shapes microbiota colonization and infant growth: impact on host response and intestinal function. Microbiome 167:8.

Selma-Royo M, García-Mantrana I, Calatayud M, Parra-Llorca A, Martínez-Costa C, Collado MC. 2021. Maternal diet during pregnancy and intestinal markers are associated with early gut microbiota. Eur J Nutr 60:1429–1442.

Semba RD, Trehan I, Li X, Moaddel R, Ordiz MI, Maleta KM, Kraemer K, Shardell M, Ferrucci L, Manary M. Environmental Enteric Dysfunction is Associated with Carnitine Deficiency and Altered Fatty Acid Oxidation. EBioMedicine 2017;17:57–66.

Serreli G, Deiana M. In vivo formed metabolites of polyphenols and their biological efficacy. Food Funct 2019;10:6999–7021.

Serreli G, Naitza MR, Zodio S, Leoni VP, Spada M, Melis MP, Boronat A, Deiana M. Ferulic Acid Metabolites Attenuate LPS-Induced Inflammatory Response in Enterocyte-like Cells. Nutrients 2021;13.

Seyedian SS, Nokhostin F, Malamir MD. 2019. A review of the diagnosis, prevention, and treatment methods of inflammatory bowel disease. J Med Life 12:113–122.

Shams-White, Marissa M., Tusarebecca E. Pannucci, Jennifer L. Lerman, Kirsten A. Herrick, Meghan Zimmer, Kevin Meyers Mathieu, Eve E. Stoody, and Jill Reedy. 2023. “Healthy Eating Index-2020: Review and Update Process to Reflect the Dietary Guidelines for Americans,2020-2025.” Journal of the Academy of Nutrition and Dietetics 123 (9): 1280–88.

Shang J, Yang S, Tang Z, Chen Y, Duan B, Meng X. 2022. Bifidobacterium bifidum H3-R2 and Its Molecular Communication within the Context of Ulcerative Colitis. J Agric Food Chem 70:11678–11688.

Shang, Xianwen, Jiahao Liu, Zhuoting Zhu, Xueli Zhang, Yu Huang, Shunming Liu, Wei Wang, et al. 2023. “Healthy Dietary Patterns and the Risk of Individual Chronic Diseases in Community-Dwelling Adults.” Nature Communications 14 (1): 6704.

Shaw KA, Bertha M, Hofmekler T, Chopra P, Vatanen T, Srivatsa A, Prince J, Kumar A, Sauer C, Zwick ME, Satten GA, Kostic AD, Mulle JG, Xavier RJ, Kugathasan S. 2016. Dysbiosis, inflammation, and response to treatment: a longitudinal study of pediatric subjects with newly diagnosed inflammatory bowel disease. Genome Medicine 8:75.

Shay J, Elbaz HA, Lee I, Zielske SP, Malek MH, Hüttemann M. Molecular Mechanisms and Therapeutic Effects of (-)-Epicatechin and Other Polyphenols in Cancer, Inflammation, Diabetes, and Neurodegeneration. Oxid Med Cell Longev 2015;2015:181260.

Shen W, Gaskins HR, McIntosh MK. Influence of dietary fat on intestinal microbes, inflammation, barrier function and metabolic outcomes. J Nutr Biochem 2014;25:270–80.

Shen, Yu, Mingming Song, Shihao Wu, Hongbo Zhao, and Yu Zhang. 2023. "Plant-Based Dietary Fibers and Polysaccharides as Modulators of Gut Microbiota in Intestinal and Lung Inflammation: Current State and Challenges." Nutrients 15 (15).

Shon W-J, Jung MH, Kim Y, Kang GH, Choi EY, Shin D-M. 2023. Sugar-sweetened beverages exacerbate high-fat diet-induced inflammatory bowel disease by altering the gut microbiome. J Nutr Biochem 113:109254.

Siegel RL, Miller KD, Fuchs HE, Jemal A. 2021. Cancer Statistics, 2021. CA Cancer J Clin 71:7–33.

Sikalidis, Angelos K. 2015. "Amino Acids and Immune Response: A Role for Cysteine, Glutamine, Phenylalanine, Tryptophan and Arginine in T-Cell Function and Cancer?" Pathology Oncology Research: POR 21 (1): 9–17.

Song Y, He Y, Rong L, Wang Z, Ma Y, Zhang N, Wang B. 2023. "Platelet-coated bullets" biomimetic nanoparticles to ameliorate experimental colitis by targeting endothelial cells. Biomaterials Advances 148:213378-213378.

Statovci D, Aguilera M, MacSharry J, Melgar S. The Impact of Western Diet and Nutrients on the Microbiota and Immune Response at Mucosal Interfaces. Front Immunol 2017;8:838.

Statovci, Donjete, Mònica Aguilera, John MacSharry, and Silvia Melgar. 2017. "The Impact of Western Diet and Nutrients on the Microbiota and Immune Response at Mucosal Interfaces." Frontiers in Immunology 8 (July): 838.

Stevens Y, de Bie T, Pinheiro I, Elizalde M, Masclee A, Jonkers D. The effects of citrus flavonoids and their metabolites on immune-mediated intestinal barrier disruption using an in vitro co-culture model. Br J Nutr 2022;128:1917–26.

Stewart, Valley, and Pamela C. Ronald. 2022. "Sulfotyrosine Residues: Interaction Specificity Determinants for Extracellular Protein–protein Interactions." The Journal of Biological Chemistry 298 (8).

Sun J, Charron CS, Novotny JA, Peng B, Yu L, Chen P. 2020. Profiling glucosinolate metabolites in human urine and plasma after broccoli consumption using non-targeted and targeted metabolomic analyses. Food Chem 309:125660.

Sun Y, Tang J, Li C, Liu J, Liu H. 2022. Sulforaphane attenuates dextran sodium sulphate induced intestinal inflammation via IL-10/STAT3 signaling mediated macrophage phenotype switching. Food Sci. Hum. Wellness 11:129-142.

Suresh, Sithara, Mostafa I. Waly, Nejib Guizani, and Mohammad Shafiur Rahman. 2016. "Broccoli (Brassica Oleracea) Extract Combats Streptozotocin-induced Diabetes and Oxidative Stress in Rats." FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology 30 (S1).

Syed, Rahamat Unissa, Sivakumar Sivagurunathan Moni, Mohammed Khaled Bin Break, Weam M. A. Khojali, Mohammed Jafar, Maali D. Alshammari, Karim Abdelsalam, et al. 2023. "Broccoli: A Multi-Faceted Vegetable for Health: An In-Depth Review of Its Nutritional Attributes, Antimicrobial Abilities, and Anti-Inflammatory Properties." Antibiotics (Basel, Switzerland) 12 (7).

Taiwo, Bankole, Ma Tianzhou, Arora Itika, Lei Zhentian, Raju Murugesan, Li Zhenhai, and Li Yuanyuan. 2024. "The Effect of Broccoli Glucoraphanin Supplementation on Ameliorating High-Fat-Diet-Induced Obesity through the Gut Microbiome and Metabolome Interface." Molecular Nutrition & Food Research 68 (9): e2300856.

Takagaki, Akiko, Yuko Kato, and Fumio Nanjo. 2014. "Isolation and Characterization of Rat Intestinal Bacteria Involved in Biotransformation of (-)-Epigallocatechin." Archives of Microbiology 196 (10): 681–95.

Talebi S, Zeraattalab-Motlagh S, Rahimlou M, Naeini F, Ranjbar M, Talebi A, Mohammadi H. The Association between Total Protein, Animal Protein, and Animal Protein Sources with Risk of Inflammatory Bowel Diseases: A Systematic Review and Meta-Analysis of Cohort Studies. Adv Nutr 2023;14:752–61.

Tayyem RF, Qalqili TR, Ajeen R, Rayyan YM. 2021. Dietary Patterns and the Risk of Inflammatory Bowel Disease: Findings from a Case-Control Study. Nutrients 13:1889.

Tayyem, Reema F., Tamara R. Qalqili, Rawan Ajeen, and Yaser M. Rayyan. 2021. "Dietary Patterns and the Risk of Inflammatory Bowel Disease: Findings from a Case-Control Study." Nutrients 13 (6).

The Lancet Gastroenterology Hepatology. 2023. The economic burden of inflammatory bowel disease. Lancet Gastroenterol Hepatol 8:391.

Thomas JP, Modos D, Rushbrook SM, Powell N, Korcsmaros T. The Emerging Role of Bile Acids in the Pathogenesis of Inflammatory Bowel Disease. Front Immunol 2022;13:829525.

Tian Q, Xu Z, Sun Q, Iniguez AB, Du M, Zhu M. 2022. Broccoli-Derived Glucoraphanin Activates AMPK/PGC1α/NRF2 Pathway and Ameliorates Dextran-Sulphate-Sodium-Induced Colitis in Mice. Antioxidants 11:2404.

Tian S, Liu X, Lei P, Zhang X, Shan Y. 2018. Microbiota: a mediator to transform glucosinolate precursors in cruciferous vegetables to the active isothiocyanates. J Sci Food Agric 98:1255-1260.

Tian T, Wang Z, Zhang J. 2017. Pathomechanisms of Oxidative Stress in Inflammatory Bowel Disease and Potential Antioxidant Therapies. Oxid Med Cell Longev 2017:4535194.

Tian, Qiyu, Zhixin Xu, Qi Sun, Alejandro Bravo Iniguez, Min Du, and Mei-Jun Zhu. 2022. "Broccoli-Derived Glucoraphanin Activates AMPK/PGC1α/NRF2 Pathway and Ameliorates Dextran-Sulphate-Sodium-Induced Colitis in Mice." Antioxidants (Basel, Switzerland) 11 (12): 2404.

Ticinesi, Andrea, Angela Guerra, Antonio Nouvenne, Tiziana Meschi, and Stefania Maggi. 2023. "Disentangling the Complexity of Nutrition, Frailty and Gut Microbial Pathways during Aging: A Focus on Hippuric Acid." Nutrients 15 (5).

Tiffany CR, Lee J-Y, Rogers AWL, Olsan EE, Morales P, Faber F, Bäumler AJ. 2021. The metabolic footprint of Clostridia and Erysipelotrichia reveals their role in depleting sugar alcohols in the cecum. Microbiome 9:174.

Townshend, Tim, and Amelia Lake. 2017. "Obesogenic Environments: Current Evidence of the Built and Food Environments." Perspectives in Public Health 137 (1): 38–44.

Truyens M, Lobatón T, Ferrante M, Bossuyt P, Vermeire S, Pouillon L, Dewint P, Cremer A, Peeters H, Lambrecht G, Louis E, Rahier J, Dewit O, Muls V, Holvoet T, Vandermeulen L, Peeters A, Gonzales GB, Bos S, Laukens D, De Vos, M. 2022. Effect of 5-Hydroxytryptophan on Fatigue in Quiescent Inflammatory Bowel Disease: A Randomized Controlled Trial. Gastroenterology 163:1294-1305.e3.

Tummolo A, Leone P, Tolomeo M, Solito R, Mattiuzzo M, Lepri FR, Lorè T, Cardinali R, De Giovanni D, Simonetti S, et al. Combined isobutyryl-CoA and multiple acyl-CoA dehydrogenase deficiency in a boy with altered riboflavin homeostasis. JIMD Rep Wiley; 2022;63:276–91.

Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. 2007. The human microbiome project. Nature 449:804–810.

Uranga, José Antonio, Visitación López-Miranda, Felipe Lombó, and Raquel Abalo. 2016. "Food, Nutrients and Nutraceuticals Affecting the Course of Inflammatory Bowel Disease." Pharmacological Reports: PR 68 (4): 816–26.

Uraz S, Tahan G, Aytekin H, Tahan V. N-acetylcysteine expresses powerful anti-inflammatory and antioxidant activities resulting in complete improvement of acetic acid-induced colitis in rats. Scand J Clin Lab Invest 2013;73:61–6.

Vaccaro, Joan A., and Fatma G. Huffman. 2017. "Sex and Race/ethnic Disparities in Food Security and Chronic Diseases in U.s. Older Adults." Gerontology & Geriatric Medicine 3 (January): 2333721417718344.

Van Den Heuvel, Ellen G. H. M., Lisette Cornelia Petronella Gerardina Maria De Groot, Monique Maria Raats, Jan Steijns, Edith Feskens, and Inge Tetens. 2021. Food-Based Dietary Guidelines: The Relevance of Nutrient Density and a Healthy Diet Score. Frontiers Media SA.

Vasconcelos PC de P, Seito LN, Di Stasi LC, Akiko Hiruma-Lima C, Pellizzon CH. Epicatechin used in the treatment of intestinal inflammatory disease: an analysis by experimental models. Evid Based Complement Alternat Med 2012;2012:508902.

Vaudin, Anna, Edwina Wambogo, Alanna Moshfegh, and Nadine R. Sahyoun. 2021. "Awareness and Use of Nutrition Information Predict Measured and Self-Rated Diet Quality of Older Adults in the USA." Public Health Nutrition 24 (7): 1687–97.

Vega-Galvez, Antonio, Elsa Uribe, Alexis Pasten, Javiera Camus, Luis S. Gomez-Perez, Nicol Mejias, René L. Vidal, Felipe Grunenwald, Lorgio E. Aguilera, and Gabriela Valenzuela-Barra. 2023. "Comprehensive Evaluation of the Bioactive Composition and Neuroprotective and Antimicrobial Properties of Vacuum-Dried Broccoli (Brassica Oleracea Var. Italica) Powder and Its Antioxidants." Molecules 28 (2).

Vega-Galvez, Antonio, Elsa Uribe, Alexis Pasten, Javiera Camus, Michelle Rojas, Vivian Garcia, Michael Araya, Gabriela Valenzuela-Barra, Angara Zambrano, and Maria Gabriela Goñi. 2023. "Low-Temperature Vacuum Drying on Broccoli: Enhanced Anti-Inflammatory and Anti-Proliferative Properties Regarding Other Drying Methods." Foods (Basel, Switzerland) 12 (17).

Venci, Brittany J., and Seung-Yeon Lee. 2018. "Functional Limitation and Chronic Diseases Are Associated with Food Insecurity among U.S. Adults." Annals of Epidemiology 28 (3): 182–88.

Ver Ploeg, Michele. 2010. Access to Affordable and Nutritious Food: Measuring and Understanding Food Deserts and Their Consequences: Report to Congress. DIANE Publishing.

Vermeulen M, Klöpping-Ketelaars IWAA, van den Berg R, Vaes WHJ. Bioavailability and kinetics of sulforaphane in humans after consumption of cooked versus raw broccoli. J Agric Food Chem 2008;56:10505–9.

Vita, Alexandra Adorno, Kristen M. Roberts, Anders Gundersen, Yuliya Farris, Heather Zwickey, Ryan Bradley, and Tiffany L. Weir. 2024. “Relationships between Habitual Polyphenol Consumption and Gut Microbiota in the INCLD Health Cohort.” Nutrients 16 (6).

Walker AW, Sanderson JD, Churcher C, Parkes GC, Hudspith BN, Rayment N, Brostoff J, Parkhill J, Dougan G, Petrovska L. 2011. High-throughput clone library analysis of the mucosa-associated microbiota reveals dysbiosis and differences between inflamed and non-inflamed regions of the intestine in inflammatory bowel disease. BMC Microbiol 11:7.

Wang J, Liu Z, Dou J, Lv J, Jin N, Jin L, Li Z, Zhang B, Tang Z, Yu J. 2022. A Comparative Study on the Nutrients, Mineral Elements, and Antioxidant Compounds in Different Types of Cruciferous Vegetables. Agronomy (Basel) 12:3121.

Wang L, Ji T, Yuan Y, Fu H, Wang Y, Tian S, Hu J, Wang L, Wang Z. High-fructose corn syrup promotes proinflammatory Macrophage activation via ROS-mediated NF-κB signaling and exacerbates colitis in mice. Int Immunopharmacol 2022;109:108814.

Wang Y, Jeffery EH, Miller MJ, Wallig MA, Wu Y. 2018. Lightly Cooked Broccoli Is as Effective as Raw Broccoli in Mitigating Dextran Sulfate Sodium-Induced Colitis in Mice. Nutrients 10:748.

Wang Y, Jeffery EH, Miller MJ, Wallig MA, Wu Y. Lightly Cooked Broccoli Is as Effective as Raw Broccoli in Mitigating Dextran Sulfate Sodium-Induced Colitis in Mice. Nutrients 2018;10:748.

Wang, Haiping, Chun-Hao Lu, and Ping-Chih Ho. 2020. “Metabolic Adaptation Orchestrates Tissue Context-Dependent Behavior in Regulatory T Cells.” Immunological Reviews 295 (1): 126–39.

Weaver, Lesley Jo, and Connor B. Fasel. 2018. “A Systematic Review of the Literature on the Relationships between Chronic Diseases and Food Insecurity.” Food and Nutrition Sciences 09 (05): 519–41.

Wei L, Zhang J, Zheng L, Chen Y. 2022. The functional role of sulforaphane in intestinal inflammation: a review. Food Funct 13:514-529.

Weisman MH, Oleg Stens, Seok Kim H, Hou JK, Miller FW, Dillon CF. Inflammatory Bowel Disease Prevalence: Surveillance data from the U.S. National Health and Nutrition Examination Survey. Preventive Medicine Reports 2023;33:102173.

Weisman, Michael H., Oleg Stens, Hyun Seok Kim, Jason K. Hou, Frederick W. Miller, and Charles F. Dillon. 2023. “Inflammatory Bowel Disease Prevalence: Surveillance Data from the U.S. National Health and Nutrition Examination Survey.” Preventive Medicine Reports 33 (June): 102173.

Weng, Jiayi, Yukang Mao, Qiyang Xie, Kangyun Sun, and Xiangqing Kong. 2024. “Gender Differences in the Association between Healthy Eating Index-2015 and Hypertension in the US Population: Evidence from NHANES 1999–2018.” BMC Public Health 24 (1): 330.

Wiese DM, Horst SN, Brown CT, Allaman MM, Hodges ME, Slaughter JC, Druce JP, Beaulieu DB, Schwartz DA, Wilson KT, et al. Serum Fatty Acids Are Correlated with Inflammatory Cytokines in Ulcerative Colitis. PLoS One 2016;11:e0156387.

Wlodarska, Marta, Chengwei Luo, Raivo Kolde, Eva d’Hennezel, John W. Annand, Cortney E. Heim, Philipp Krastel, et al. 2017. “Indoleacrylic Acid Produced by Commensal Peptostreptococcus Species Suppresses Inflammation.” Cell Host & Microbe 22 (1): 25–37.e6.

World Health Organization. 2003. Diet, Nutrition, and the Prevention of Chronic Diseases: Report of a Joint WHO/FAO Expert Consultation. World Health Organization.

Wright, Breanne N., Janet A. Tooze, Regan L. Bailey, Yibin Liu, Rebecca L. Rivera, Lacey McCormack, Suzanne Stluka, et al. 2020. “Dietary Quality and Usual Intake of Underconsumed Nutrients and Related Food Groups Differ by Food Security Status for Rural, Midwestern Food Pantry Clients.” Journal of the Academy of Nutrition and Dietetics 120 (9): 1457–68.

Wu J, Cui S, Tang X, Zhang Q, Jin Y, Zhao J, Mao B, Zhang H. 2023. Bifidobacterium longum CCFM1206 Promotes the Biotransformation of Glucoraphanin to Sulforaphane That Contributes to Amelioration of Dextran-Sulfate-Sodium-Induced Colitis in Mice. J Agric Food Chem 71:1100-1112.

Wu J, Cui S, Tang X, Zhang Q, Jin Y, Zhao J, Mao B, Zhang H. Bifidobacterium longum CCFM1206 promotes the biotransformation of glucoraphanin to sulforaphane that contributes to amelioration of dextran-sulfate-sodium-induced colitis in mice. J Agric Food Chem American Chemical Society (ACS); 2023;71:1100–12.

Wu J, Guo W, Cui S, Tang X, Zhang Q, Lu W, Jin Y, Zhao J, Mao B, Chen W. 2023. Broccoli seed extract rich in polysaccharides and glucoraphanin ameliorates DSS-induced colitis via intestinal barrier protection and gut microbiota modulation in mice. J Sci Food Agric 103:1749–1760.

Wu M, Luo Q, Nie R, Yang X, Tang Z, Chen H. 2021. Potential implications of polyphenols on aging considering oxidative stress, inflammation, autophagy, and gut microbiota. Crit Rev Food Sci Nutr 61:2175-2193.

Wu X, Zhao Y, Bhagwat S, Haytowitz DB, Harnly JM, Pehrsson PR, Chen P. Calculation of retention factors for flavonoids in cooked broccoli. FASEB J Wiley; 2016;30.

Wu Y, Pham Q, Wang Y, Huang H, Jiang X, Li RW, Yu L, Luo Y, Wang J, Wang TTY. 2023. Red cabbage microgreen modulation of gut microbiota is associated with attenuation of diet-induced obesity risk factors in a mouse model. Food Funct 14:6654-6664.

Wu Y, Shen Y, Zhu Y, Mupunga J, Zou L, Liu C, Liu S, Mao J. 2019. Broccoli ingestion increases the glucosinolate hydrolysis activity of microbiota in the mouse gut. Int J Food Sci Nutr 70:585-594.

Wu Y, Shen Y, Zhu Y, Mupunga J, Zou L, Liu C, Liu S, Mao J. Broccoli ingestion increases the glucosinolate hydrolysis activity of microbiota in the mouse gut. Int J Food Sci Nutr 2019;70:585–94.

Wu Z, He J, Zhang Z, Li J, Zou H, Tan X, Wang Y, Yao Y, Xiong W. Propionic Acid Driven by the Lactobacillus johnsonii Culture Supernatant Alleviates Colitis by Inhibiting M1 Macrophage Polarization by Modulating the MAPK Pathway in Mice. J Agric Food Chem American Chemical Society; 2023;71:14951–66.

Wu, Jiaying, Shumao Cui, Xin Tang, Qiuxiang Zhang, Yan Jin, Jianxin Zhao, Bingyong Mao, and Hao Zhang. 2023. "Bifidobacterium Longum CCFM1206 Promotes the Biotransformation of Glucoraphanin to Sulforaphane That Contributes to Amelioration of Dextran-Sulfate-Sodium-Induced Colitis in Mice." Journal of Agricultural and Food Chemistry 71 (2): 1100–1112.

Wu, Jiaying, Weiling Guo, Shumao Cui, Xin Tang, Qiuxiang Zhang, Wenwei Lu, Yan Jin, Jianxin Zhao, Bingyong Mao, and Wei Chen. 2023. "Broccoli Seed Extract Rich in Polysaccharides and Glucoraphanin Ameliorates DSS-Induced Colitis via Intestinal Barrier Protection and Gut Microbiota Modulation in Mice." Journal of the Science of Food and Agriculture 103 (4): 1749–60.

Wu, Yong-Lin, Jun Xu, Xing-Yu Rong, Feifei Wang, Hui-Jing Wang, and Chao Zhao. 2021. "Gut Microbiota Alterations and Health Status in Aging Adults: From Correlation to Causation." Aging Medicine (Milton (N.S.W)) 4 (3): 206–13.

Wullaert A. 2010. Role of NF-κB activation in intestinal immune homeostasis. Int J Med Microbiol 300:49–56.

Xu F, Carlson SA, Liu Y, Greenlund KJ. Prevalence of Inflammatory Bowel Disease Among Medicare Fee-For-Service Beneficiaries - United States, 2001-2018. MMWR Morb Mortal Wkly Rep 2021;70:698–701.

Xu F, Park S, Liu Y, Greenlund KJ. Dietary intake patterns among adults with inflammatory bowel disease in the United States, 2015. PLoS One 2021;16:e0250441.

Xu, Baoyang, Wenxia Qin, Yunzheng Xu, Wenbo Yang, Yuwen Chen, Juncheng Huang, Jianan Zhao, and Libao Ma. 2021. "Dietary Quercetin Supplementation Attenuates Diarrhea and

Intestinal Damage by Regulating Gut Microbiota in Weanling Piglets." Oxidative Medicine and Cellular Longevity 2021 (December): 6221012.

Xu, Baoyang, Yiqin Yan, Boqi Yin, Le Zhang, Wenxia Qin, Yaorong Niu, Yimei Tang, Shuyi Zhou, Xianghua Yan, and Libao Ma. 2021. "Dietary Glycyl-Glutamine Supplementation Ameliorates Intestinal Integrity, Inflammatory Response, and Oxidative Status in Association with the Gut Microbiota in LPS-Challenged Piglets." Food & Function 12 (8): 3539–51.

Xu, Fang, Sohyun Park, Yong Liu, and Kurt J. Greenlund. 2021. "Dietary Intake Patterns among Adults with Inflammatory Bowel Disease in the United States, 2015." PloS One 16 (4): e0250441.

Yan, Tao, Lin Shi, Tianqi Liu, Xiangnan Zhang, Minmin Yang, Wen Peng, Xiaomin Sun, Lijing Yan, Xiaoshuang Dai, and Xinbing Yang. 2023. "Diet-Rich in Wheat Bran Modulates Tryptophan Metabolism and AhR/IL-22 Signalling Mediated Metabolic Health and Gut Dysbacteriosis: A Novel Prebiotic-like Activity of Wheat Bran." Food Research International 163 (January): 112179.

Yanaka, Akinori. 2018. "Daily Intake of Broccoli Sprouts Normalizes Bowel Habits in Human Healthy Subjects." Journal of Clinical Biochemistry and Nutrition 62 (1): 75–82.

Yanaka, Akinori. 2018. "Daily Intake of Broccoli Sprouts Normalizes Bowel Habits in Human Healthy Subjects." Journal of Clinical Biochemistry and Nutrition 62 (1): 75–82.

Yang, Chengcheng, Yao Du, Daoyuan Ren, Xingbin Yang, and Yan Zhao. 2021. "Gut Microbiota-Dependent Catabolites of Tryptophan Play a Predominant Role in the Protective Effects of Turmeric Polysaccharides against DSS-Induced Ulcerative Colitis." Food & Function 12 (20): 9793–9807.

Ye, Yulan, Zhi Pang, Weichang Chen, Songwen Ju, and Chunli Zhou. 2015. "The Epidemiology and Risk Factors of Inflammatory Bowel Disease." International Journal of Clinical and Experimental Medicine 8 (12): 22529–42.

Yoshida, Kazutaka, Yusuke Ushida, Tomoko Ishijima, Hiroyuki Suganuma, Takahiro Inakuma, Nobuhiro Yajima, Keiko Abe, and Yuji Nakai. 2015. "Broccoli Sprout Extract Induces Detoxification-Related Gene Expression and Attenuates Acute Liver Injury." World Journal of Gastroenterology: WJG 21 (35): 10091–103.

Zandani G, Kaftori-Sandler N, Sela N, Nyska A, Madar Z. Dietary broccoli improves markers associated with glucose and lipid metabolism through modulation of gut microbiota in mice. Nutrition 2021;90:111240.

Zandani, Gil, Sarit Anavi-Cohen, Nina Tsybina-Shimshilashvili, Noa Sela, Abraham Nyska, and Zecharia Madar. 2021. "Broccoli Florets Supplementation Improves Insulin Sensitivity and Alters Gut Microbiome Population-A Steatosis Mice Model Induced by High-Fat Diet." Frontiers in Nutrition 8 (July): 680241.

Zaplana, Tom, Solange Miele, and Andrew C. Tolonen. 2023. “Lachnospiraceae Are Emerging Industrial Biocatalysts and Biotherapeutics.” Frontiers in Bioengineering and Biotechnology 11: 1324396.

Zhang T, Holman J, McKinstry D, Trindade BC, Eaton KA, Mendoza-Castrejon J, Ho S, Wells E, Yuan H, Wen B, Sun D, Chen GY, Li Y. 2023. A steamed broccoli sprout diet preparation that reduces colitis via the gut microbiota. J Nutr Biochem 112:109215.

Zhang Z, Bergan R, Shannon J, Slatore CG, Bobe G, Takata Y. 2018. The Role of Cruciferous Vegetables and Isothiocyanates for Lung Cancer Prevention: Current Status, Challenges, and Future Research Directions. Mol Nutr Food Res 62:e1700936.

Zhang, Jie, Shengwei Zhu, Ning Ma, Lee J. Johnston, Chaodong Wu, and Xi Ma. 2021. “Metabolites of Microbiota Response to Tryptophan and Intestinal Mucosal Immunity: A Therapeutic Target to Control Intestinal Inflammation.” Medicinal Research Reviews 41 (2): 1061–88.

Zhang, Lin, Chudan Liu, Qingyan Jiang, and Yulong Yin. 2021. “Butyrate in Energy Metabolism: There Is Still More to Learn.” Trends in Endocrinology and Metabolism: TEM 32 (3): 159–69.

Zhao Y, Jiang Q. Roles of the Polyphenol-Gut Microbiota Interaction in Alleviating Colitis and Preventing Colitis-Associated Colorectal Cancer. Adv Nutr 2021;12:546–65.

Zhou C, Wang Y, Li C, Xie Z, Dai L. Amelioration of Colitis by a Gut Bacterial Consortium Producing Anti-Inflammatory Secondary Bile Acids. Microbiol Spectr 2023;11:e0333022.

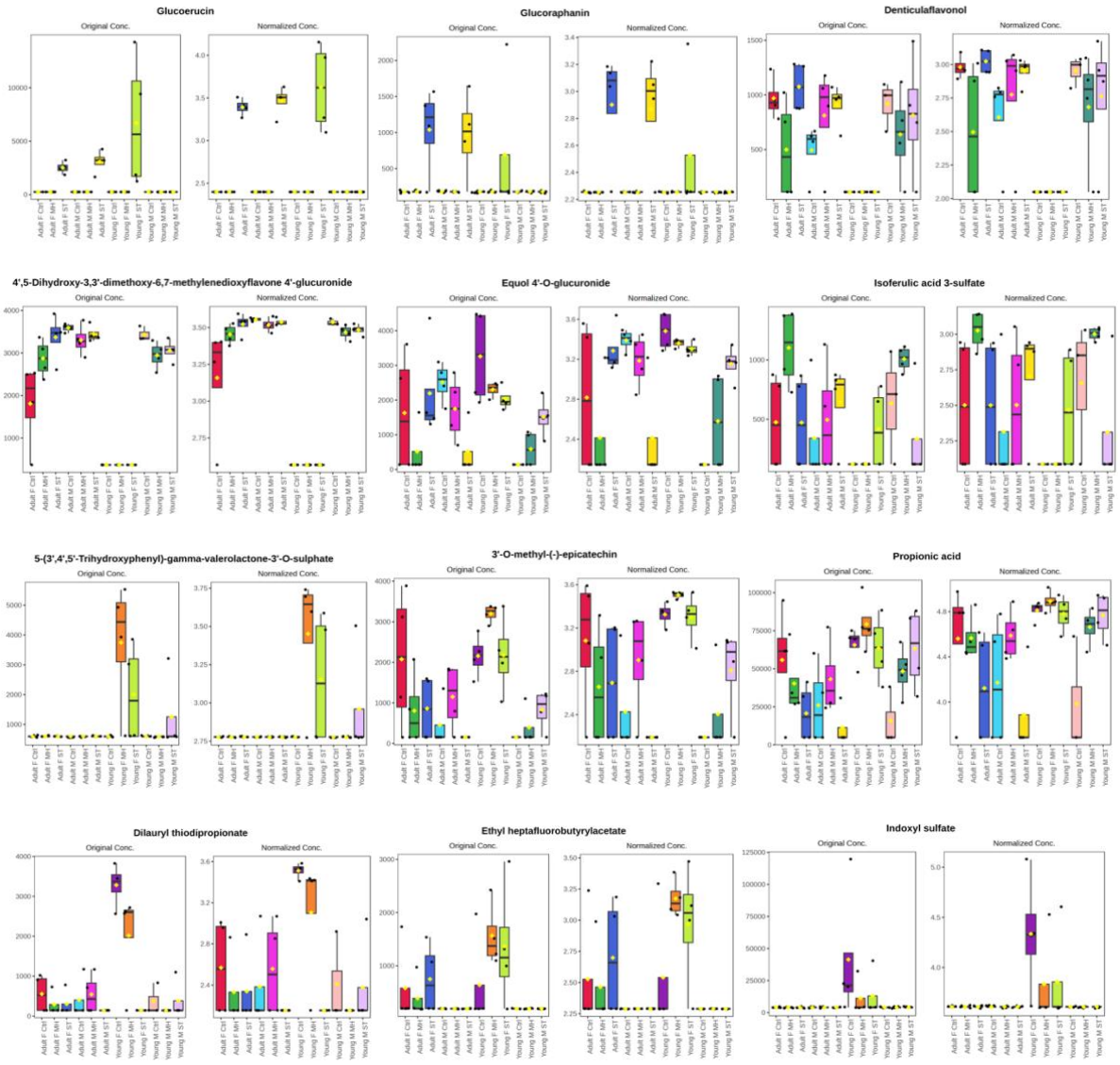
Zhu C, Sawrey-Kubicek L, Beals E, Rhodes CH, Houts HE, Sacchi R, Zivkovic AM. Human gut microbiome composition and tryptophan metabolites were changed differently by fast food and Mediterranean diet in 4 days: a pilot study. Nutr Res 2020;77:62–72.

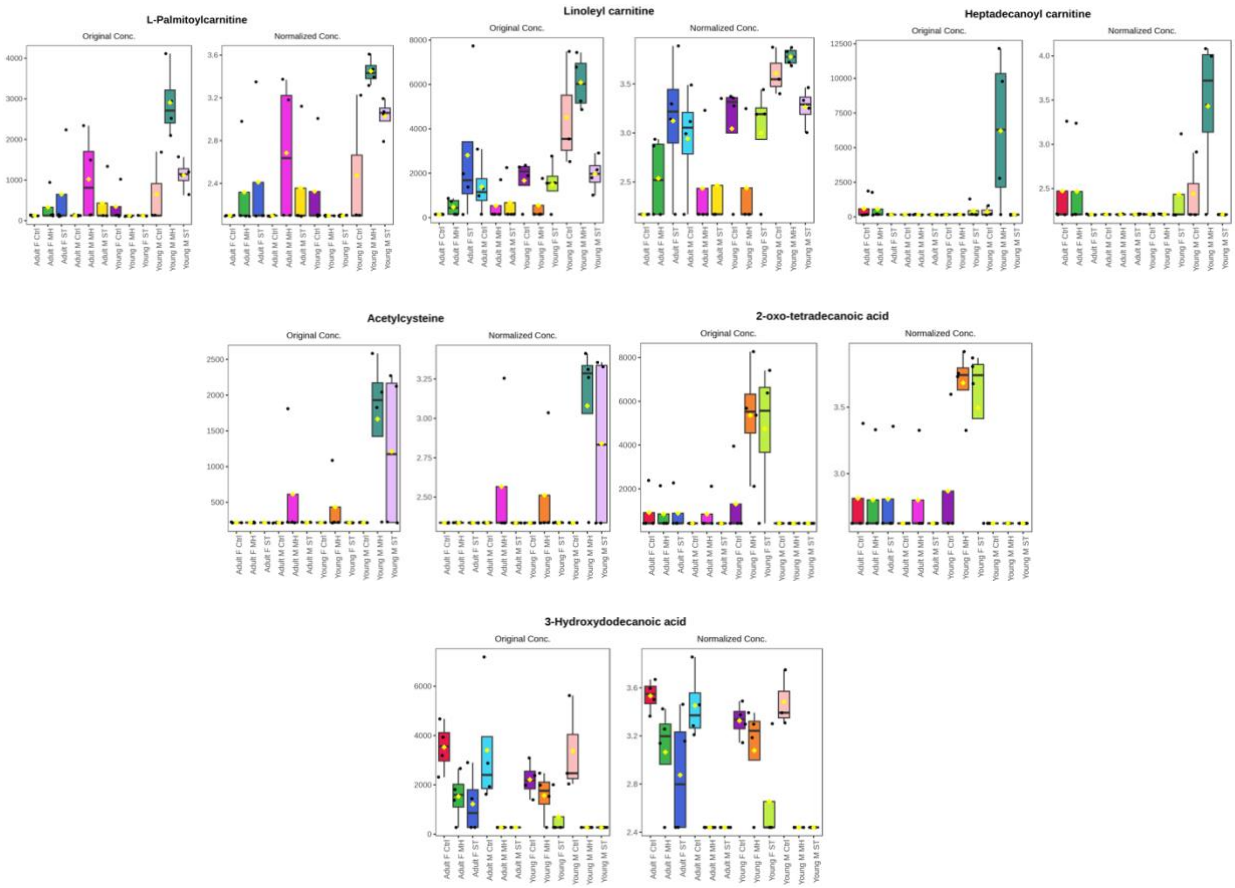
Zhuang M, Shang W, Ma Q, Strappe P, Zhou Z. 2019. Abundance of probiotics and butyrate-production microbiome manages constipation via short-chain fatty acids production and hormones secretion. Mol Nutr Food Res 63:e1801187.

Zuo T, Ng SC. 2018. The Gut Microbiota in the Pathogenesis and Therapeutics of Inflammatory Bowel Disease. Front Microbiol 9:2247.

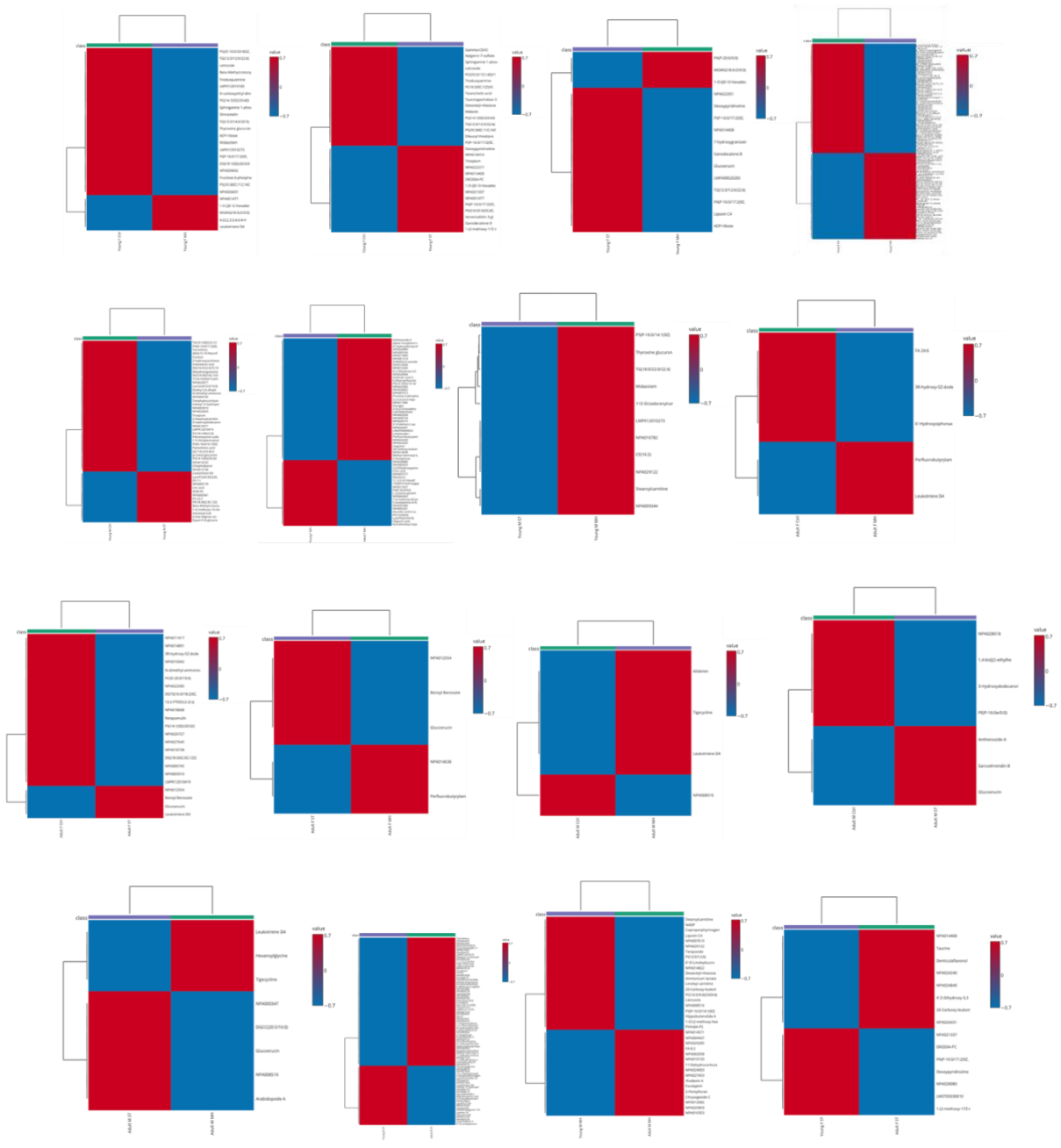
APPENDICES

Appendix A. Supplementary Figures

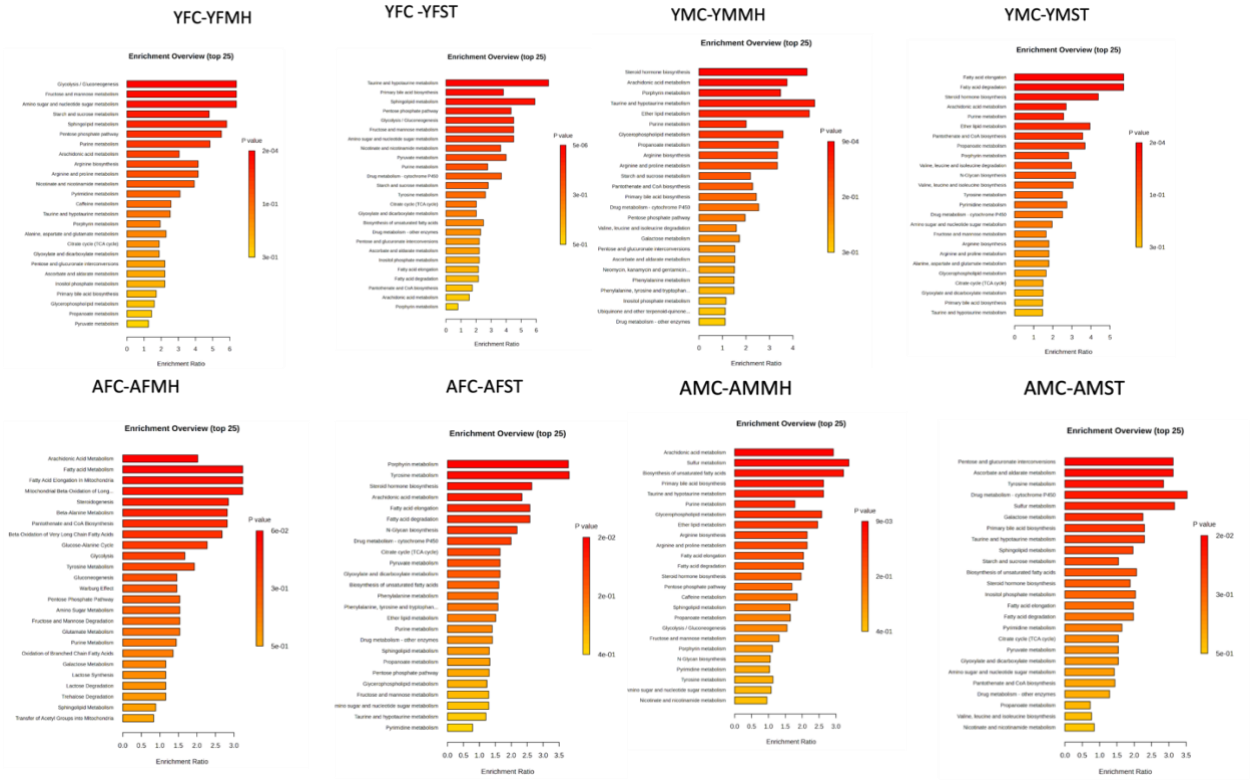




A.1 Boxplots show differences in the concentration of metabolites across the experimental groups. Selected metabolites can potentially alleviate IBD or are related to a specific metabolic pathway in IBD.



A.2 Heatmaps illustrate differentially regulated metabolites between the experimental groups with diet preparation, age, and sex. Selected metabolites can potentially alleviate or are related to a specific metabolic pathway involved in inflammatory bowel disease.



A.3 The top 25 metabolic pathways associated with broccoli intervention or inflammatory bowel disease among the experimental groups were based on the diet preparations, age, and sex. Bar charts show the enrichment ratio of the associated pathways.

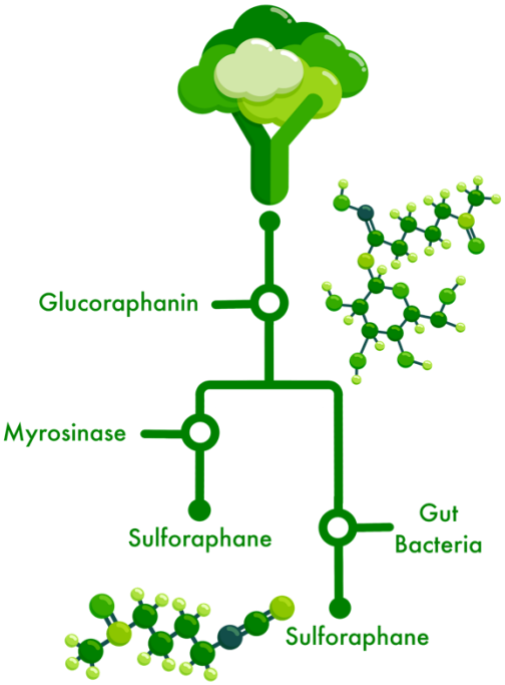
Appendix B. Supplementary Files

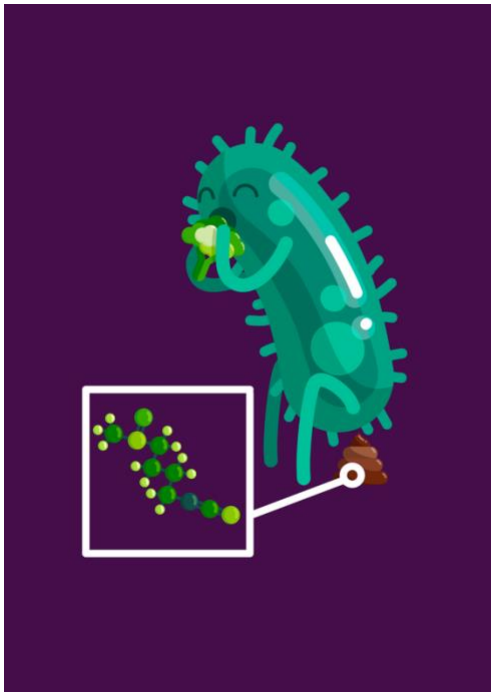
B.1 Consent form:

Welcome to the Broccoli Sprout Project!

You are invited to participate in a research project being conducted by two Principal Investigators, Dr. Sue Ishaq and Dr. Yanyan Li, who are Assistant Professors in the School of Food and Agriculture. Johanna Holman, a graduate student in the School of Food and Agriculture at the University of Maine is the lead student researcher (co-investigator) of this study.

Why are we running this study?
We want to understand how adding steamed broccoli sprouts to your diet will influence your gut microbiota and whether some microbes produce anti-inflammatory effects, in healthy individuals.





You can participate if you are…	You cannot participate if you are…
✓ At least 18 years of age	X Allergic to broccoli sprouts
✓ In good general health	X Pregnant
	X Taking oral antibiotics in the previous 2 weeks. Please notify us if you start taking oral antibiotics after starting the study: you can continue and receive compensation.

What will you be asked to do?

- You will be asked to eat steamed broccoli sprouts along with your regular diet every day for 28 days. You will eat *either* 1 serving of broccoli sprouts daily for four weeks, *or* 1 serving of broccoli sprouts every other day for four weeks, depending on which group you are assigned to on the day you visit the University of Maine to get your study materials. You will be provided with fresh broccoli sprouts each week. You have the option to get them delivered to where you live or pick them up from the lab.
- You will be asked to fill out surveys on your diet and on how you feel about participating in the study.
- You will be asked to give us three fecal samples during the study: 1 before you start eating sprouts, 1 after you have been eating sprouts for 4 weeks, and 1 after you have stopped eating sprouts for a week.
- We expect the length of time you will participate in this study will be approximately 52 days: 14 days before eating sprouts, 28 days of eating sprouts, and then 7 days after you stop eating sprouts. On average it may take one hour each day to participate (details on the time for each activity are below).

Contact Information: If you have any questions about this study, please contact:

- Johanna Holman (co-investigator and lead student researcher) at johanna.holman@maine.edu or 207-558-2634, who is in charge of ordering sprouts for participants and setting up visits.
- Lead researchers: sue.ishaq@maine.edu (Dr. Sue Ishaq) or yanyan.li@maine.edu (Dr. Yanyan Li).
- If you have any questions about your rights as a research participant, please contact the Office of Research Compliance, University of Maine, 207-581-2657 or umric@maine.edu.

Benefits. What are the potential benefits if I participate?

There may be no direct benefits to you, because not everyone reacts to the broccoli sprouts.

- You will be provided with broccoli sprouts and learn how to incorporate this healthy food to your diet.
- Your digestive health may benefit from participating in this study.
- This research will help us learn more about the influence of broccoli sprouts on the gut bacteria and anti-inflammatory effects in healthy humans, allowing us to develop dietary strategies for prevention of IBD.

- You will receive a copy of the results from your samples, and a copy of the results from the whole study.

Compensation. How will I be compensated for my time and effort?
You will receive $70 as an Amazon e-gift card for participating in the first 4 weeks of this study and then an additional $70 as an Amazon e-gift card for the remaining part of the study, for a total of $140. The first part of this study includes 2 study visits, diet history survey, cruciferous veggie survey, 3 days of food diaries, 1 stool sample, 2 sprouts samples, and 4 check-in phone calls. The second part of this study includes 2 study visits, completing cruciferous veggie survey again, completing 6 days of food diaries, 2 stool samples, 1 sprout sample, 3 check-in phone calls, and an end-of-study questionnaire.

Voluntary. Can I quit at any time? Yes! Participation is voluntary. Participation is voluntary. If you choose to take part in this study, you may stop at any time. If you choose to withdraw from the study, you will be compensated only for the activities that you participated in prior to your withdrawal. You may skip any questions you do not wish to answer.

Risk. What are the potential risks if I participate? There are minimal risks to you from participating in this study.
- You may feel uncomfortable about some activities (e.g., collection of stool samples).
- The survey questions ask you about your dietary habits and gut health. You may skip any questions you do not wish to answer in the questionnaires. Example questions from the surveys include: How often did you eat lettuce salads (with or without other vegetables)? On a number scale from 1 to 5, how difficult was it for you to eat the required amount of broccoli sprouts, 1 being very easy and 5 being very difficult? Was there anything we could have done differently in our study to make the diet easier for you to eat? How would you describe your overall gut health today? Is it normal, better, or worse to how you normally feel?
- Ingestion of broccoli sprouts may cause bloating, gas and other stomach discomfort, though steamed sprouts may cause less issues as compared to fresh sprouts. You can spread out the broccoli sprouts you need to eat throughout the day, in small quantities at a time, to reduce the discomfort. You may withdraw from the study at any time if the discomfort persists or becomes more severe.

Confidentiality. How will my privacy be protected in this study?
- *A code name will be used on samples/surveys.* Your name will not be on any of the data, the samples, or included in any publication or report. *A consent form that you sign will link your name to your code name.* This form will be kept separate from the data, in the PI's locked office and destroyed by May 2025.
- *Only the researchers who are named in this protocol and trained in data confidentiality have access to data and samples.* To further protect your privacy: some members interact with you and bring your samples to the laboratory, and different team members work with your samples.
- All electronic data (surveys and any data collected during visits or phone calls) will be stored on a password-protected computer. If the surveys or study data are collected on paper, these will be stored in a locked file cabinet in the lead researcher's office. Survey and sample data

are retained indefinitely for record keeping purposes and potential future research. All stool samples will be stored in a locked -80℃ freezer in PI's lab and destroyed by May 2028.

- If you leave the study before day 14, your data will not be included in the data analysis and will be destroyed by May 2024. If you leave between day 14 and completion of the study, your data will be included in the data analysis unless you request that your data not be saved.

_____(Signature) **_____(Date)**

Your signature indicates that you have read the above information and agree to participate. You will receive a copy of this form.

Schedule for Code Name: _____ who will eat _____ box of sprouts each day

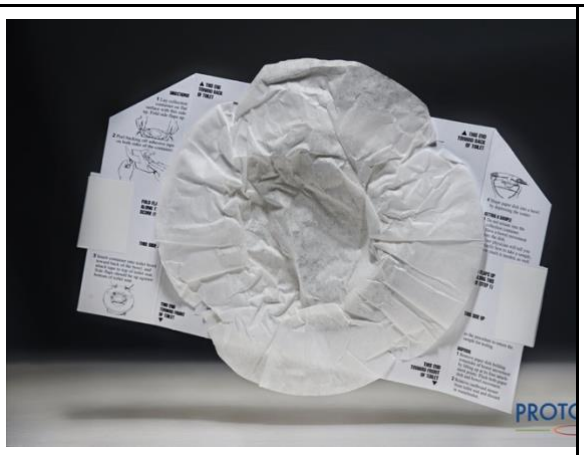
B.2 Fecal collection instructions

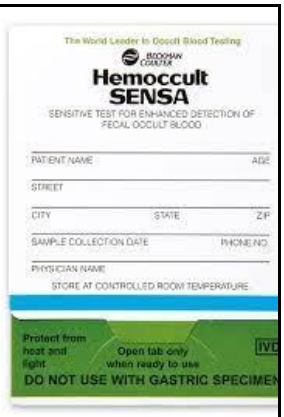
Stool Collection Kit Instructions for the Broccoli Sprout Project

Research Coordinator: Johanna Holman (johanna.holman@ maine.edu, 207-558-2760)
Lead Researchers: Sue Ishaq (sue.ishaq@maine.edu, 207-581-2770) and Yanyan Li (yanyan.li@maine.edu, 207-581-3134)

Fecal samples should be collected on
1) the day before or the morning of beginning the steamed broccoli sprout diet, D0;
2) the day you finish 4 weeks of eating the steamed broccoli sprout diet, D28;
3) 7 days after you stop eating the steamed sprout diet, D35.

Materials needed for each collection date:

<table>
<tr>
<td> 
A Protocult brand collection device</td>
<td> 
2 plastic collection tubes, filled with preservatives, and the plastic bags the tubes were in.

We use one jar (marked R) to identify gut bacteria, and one jar (marked E) to identify the compounds gut microbes are making with the sprouts.</td>
<td> 

1 FOBT smear card, and a biohazard bag from the FOBT envelope.

We'll use a chemical compound that will change color if there is any blood in your sample which could indicate inflammation.</td>
</tr>
<tr>
<td>A pair of latex gloves</td>
<td>A sharpie or pen</td>
<td>A fecal collection survey</td>
</tr>
</table>

	Step 1: Gather the materials you'll need. Make sure the two plastic collection tubes and the FOBT smear card all have the same time point written on them (D0, D28, D35). Use a sharpie or pen to put your code name and the date on two plastic collection tubes and one FOBT smear card. Open the FOBT card flap.
	**Step 2: Unfold the Protocult collection device and attach it to the toilet seat.** Put on the nitrile gloves provided. Lay one of the white stool collection pouches on a flat surface with the instructions facing upwards. Fold the outer flaps upwards. Peel the backing off of the adhesive tape on both sides of the pouch. Attach the pouch over the toilet bowl and stick the tape to the toilet seat.
	Step 3: Empty your bowels directly onto the collection device. A golf ball sized amount is plenty.

	**Step 4: Use the spoon from one of the jars to create a thin smear of feces on panels A and B inside the card.** Close the FOBT card flap, and place the card inside one of the plastic “biohazard” bags.
	Step 5: Use the spoons to put 1 teaspoon of feces into each jar. Keeping the tubes to avoid spilling the preservative. If accidental spilling occurs, do not add water or any other liquid to the tubes. Close the jars firmly and put them in their plastic bags.
 	**Step 6: Cleanup.** The remaining feces can be left in the collection pouch. Detach the centerpiece from the paper frame, and flush the feces and centerpiece down the toilet. The remaining portion of the stool collection pouch can be discarded in the trash. **Return the samples to the lab within 1 - 2 days**, at the University of Maine in 111 Rogers Hall, Orono. Rogers Hall is next to the library and the student union. The samples can be kept at room temperature.

BIOGRAPHY OF THE AUTHOR

Tolu Esther Alaba was born and raised in Kwara State, Nigeria on October 14, 1987. She graduated in 2011 with a B.Tech degree in Human Physiology from the Ladoke Akintola University of Technology Ogbomosho. In 2015, she obtained a Master's degree in Human Physiology at the prestigious University of Ibadan, Nigeria. Thereafter, She continued to work as a researcher at the University of Ibadan, under the mentorship of Professor Fasanmade at the for Cardiopulmonary Laboratory. Thereafter, she joined the School of Biology and Ecology at the University of Maine (United States) in 2019 and transferred to the Graduate School of Biomedical Science and Engineering in 2020. Tolu is a candidate for the Doctor of Philosophy degree in Biological Sciences from the University of Maine in August 2024.