BIOENGINEERED WHEAT ARABINOXYLAN – FOSTERING NEXT-GENERATION PREBIOTICS TARGETING GUT MICROBIOME AND DEPRESSION INVERSELY-LINKED MICROBES.

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Thesis submitted to the University of Ottawa
in partial Fulfillment of the requirements for the
Master's degree in Interdisciplinary Health Sciences

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ABSTRACT

Various disorders closely linked to gut dysbiosis have been associated with poor dietary patterns. Dietary prebiotic fibers play an essential role in modulating the gut microbiome by enhancing the abundance of beneficial microorganisms and improving the production of short-chain fatty acids. Arabinoxylan (AX) is a major component of most dietary fibers and has been shown to exhibit potential prebiotic properties and modulate gut microbiome composition. This study aimed to investigate the *in vitro* impact of bioengineered wheat arabinoxylan on depression-inversely linked gut microbes and human gut microbiome diversity and metabolism. This study demonstrates the ability of bioengineered AX to stimulate the growth of depression-inversely linked gut bacterial species (*Faecalibacterium prausnitzii* and *Lacticaseibacillus rhamnosus* LGG). On the microbiome composition, the bioengineered AX induced an increased abundance of beneficial bacterial taxa (*Bacteroides, Bifidobacterium, Anaerofustis, and Eubacterium*) compared to the control and native AX. These effects on microbes translated into significant metabolic activity and produced primary SCFAs (acetate, butyrate, and propionate). The findings from this study suggest that bioengineered wheat arabinoxylan could be considered a promising strategy for fostering next-generation prebiotics targeting depression-inversely linked gut microbes and also supports the structure-function relationship between AX and the human gut microbiome.

Keywords: depression-inversely linked microbes, enzymatic bioengineering, wheat arabinoxylan, prebiotic effect, gut microbiome, short-chain fatty acids, *in vitro* fermentation

RÉSUMÉ

Divers troubles étroitement liés à la dysbiose intestinale ont été associés à de mauvaises habitudes alimentaires. Les fibres prébiotiques alimentaires jouent un rôle essentiel dans la modulation du microbiome intestinal en augmentant l'abondance des micro-organismes bénéfiques et en améliorant la production d'acides gras à chaîne courte. L'arabinoxylane (AX) est un composant majeur de la plupart des fibres alimentaires et il a été démontré qu'il présentait des propriétés prébiotiques potentielles et modulait la composition du microbiome intestinal. Cette étude visait à examiner l'impact in vitro de l'arabinoxylane de blé issu de la bioingénierie sur les microbes intestinaux liés à la dépression ainsi que sur la diversité et le métabolisme du microbiome intestinal humain. Cette étude démontre la capacité de l'AX structuralement modifié à stimuler la croissance des espèces bactériennes intestinales inversement liées à la dépression (*Faecalibacterium prausnitzii* et *Lacticaseibacillus rhamnosus* LGG). En ce qui concerne la composition du microbiome, l'AX modifié a induit une abondance accrue de taxons bactériens bénéfiques (*Bacteroides*, *Bifidobacterium*, *Anaerofustis* et *Eubacterium*) par rapport au contrôle et à l'AX natif. Ces effets sur les microbes se sont traduits par une activité métabolique significative et ont produit une quantité supérieure d'AGCC primaires (acétate, butyrate et propionate). Les résultats de cette étude suggèrent que l'arabinoxylane de blé issu de la bio-ingénierie pourrait être considéré comme une stratégie prometteuse pour favoriser la prochaine génération de prébiotiques ciblant les microbes intestinaux inversement liés à la dépression et soutient également la relation structure-fonction entre l'AX et le microbiome intestinal humain.

Mots clés: microbes liés à la dépression, bioingénierie enzymatique, arabinoxylane de blé, effet prébiotique, microbiome intestinal, acides gras à chaîne courte, fermentation *in vitro*.

ACKNOWLEDGMENTS

My profound and sincere gratitude to my supervisor, Dr. Riadh Hammami, for his tutelage, support, advice, and guidance throughout this research. His guidance during the laboratory work and thesis writing was very instrumental to the success of this thesis.

I would also like to thank the members of my thesis advisory committee, Dr. Nicolas Bordenave, Dr. Marie-Claude Audet, and Dr. Frédérique Tesson, for the constructive feedback and helpful suggestions.

I will not fail to thank the members of the Hammami lab, Dr. Walid Mottawea, for his tutoring and guidance from the beginning of my research up until the end, and to other members of the lab, Dr. Hebatoallah Hassan, Dr. Basit Yousuf, Yasmina Ait Chait, Mariem Chiba, and Nour Elhouda Bouhlel for their support and assistance during my research.

I sincerely appreciate my friends Ogadinma Okagu, Precious Agboinghale, Chinonye Udechukwu, Joy Obeme-Nmom, Joel Leviton and Natalie Amato for their support.

I am grateful to the University of Ottawa for the Nutrition and Mental Health Master's Scholarship I received throughout my study and research.

Finally, without the unfathomable love shown to me by my family, this work wouldn't have been successful. With a heart filled with joy and gratitude, I thank my parents, Mr. Benson and Mrs. Charity, and my siblings, Adaobi and Chiamaka, my uncle Mr. Emmanuel Idoko for their financial, moral, and spiritual support. Also, to Dr. Chibuike and Dr. Ogo Udenigwe for their encouragement and support from the day I arrived in Canada.

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GENERAL INTRODUCTION

The human intestinal microbiome is a habitat for numerous and diverse populations of microorganisms and contains approximately 100 times as many genes as the human genome (Demuth et al., 2021; Qin et al., 2010). Microbial diversity in the human gut contributes to host immunity, metabolism, and nutrition (Fehlbaum et al., 2018a). Diet, including other factors, such as geography, ethnicity, and mode of child delivery, are essential elements that can affect the composition of an individual's gut microbiome (Bokulich et al., 2016; Schnorr, 2015; Yatsunenko et al., 2012). For example, dietary fiber consumption is vital for a healthy diet and gut microbiome modulation, thereby preventing gut dysbiosis (Swann et al., 2020). Gut dysbiosis is defined as a change in the composition of the human gut microbiota from a generally diversified and commensal microbial community to a more maladaptive and pathogenic profile (Keightley et al., 2015; Patterson et al., 2014). Appreciable evidence from human and animal studies revealed that gut microbiome plays a significant role in the pathogenesis of diseases, including obesity, bowel diseases, and anxiety- and depressive-like disorders (Karlsson et al., 2012; Png et al., 2010; Rajilić–Stojanović et al., 2011; Sanz Herranz, n.d.; Tilg & Moschen, 2014). Some gut resident microbes, including *Lactobacillaceae* (Dong et al., 2022), *Faecalibacterium prausnitzii* (Jiang et al., 2015; Leylabadlo et al., 2020), *Bacteroides* (Carlson et al., 2018), *Bifidobacterium breve* (Tian et al., 2019, 2022), and *Akkermansia muciniphila* (Cheng et al., 2022) have been reported to be associated with improved overall well-being and a reduction in depressive-like and anxiety-like symptoms. These microorganisms produce essential bioactive compounds as metabolic products, mainly short-chain fatty acids and neurotransmitters such as gamma-aminobutyric acid (GABA). The microbially produced metabolites could potentially induce positive effects on host health because of their psychobiotic capabilities (Evrensel & Ceylan, 2019; Ngo & Vo, 2019). For example, butyrate and GABA have been reported to possess psychobiotic potentials (de Noronha et al., 2017; Hao et al., 2019; Ngo & Vo, 2019).

Dietary fibers are structurally complex carbohydrates that can alter the gut microbiome (Benítez-Páez et al., 2016). They include, but are not limited to, non-starch polysaccharides, fructooligosaccharides (FOS), galactooligosaccharides (GOS), celluloses, arabinoxylans (AX), arabinogalactans, pectins, and fructans (Caprita et al., 2010; Deehan et al., 2017). Several *in vivo, in vitro,* and *ex vivo* studies have demonstrated the efficacy of dietary fiber as a tool for managing dysbiosis and modulating gut microbiome composition (Demuth et al., 2021; Duque et al., 2021; Mottawea et al., 2020; Paesani et al., 2019). In addition, dietary fibers serve as the primary substrate for colonic microbes, and their fermentation in the gut results in the production of beneficial metabolites, mainly short-chain fatty acids (SCFAs) (Evans et al., 2013; Ríos-Covián et al., 2016). SCFAs offer essential benefits to host and microbial communities (Mondal et al., 2022; Ríos-Covián et al., 2016).

Arabinoxylan is an important dietary fiber and a major component of most cereals. Previous studies have demonstrated that slight structural differences in prebiotic dietary fibers can have an impact on the gut microbiome (Deehan et al., 2017; Tuncil et al., 2020), and these structural variations may target certain microbial species (Cantu-Jungles & Hamaker, 2020; Louis, 2017), thus affecting the production of SCFAs. In addition, arabinoxylans have attracted considerable attention because of their potential prebiotic characteristics, which can be affected by their complex structural features (Sun et al., 2019). However, to the best of our knowledge, research focusing on the effects of enzymatic modification on the physicochemical properties of wheat arabinoxylan on gut microbes and gut microbiota is yet to be fully explored.

The purpose of the present study was to investigate (i) the *in vitro* effect of molar mass and changes in the chemical structure of wheat arabinoxylan fibers on the prebiotic growth and metabolism of selected depression-inversely linked bacterial strains encountered in the human gut and (ii) the impact of enzymatic-structural modification of wheat arabinoxylan on the composition, diversity, and metabolism of the human gut microbiome using a batch culture fermentation model.

CHAPTER 1: MOODULATING THE GUT MICROBIOME: THE ROLE OF PSYCHOBIOTIC DIETARY FIBERS – AN UPDATE

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Abstract

The gut microbiota is crucial in maintaining human health, and its composition can be influenced by diet, environment, and health status, among other factors. Diet remains an effective strategy for enhancing the composition and metabolism of the gut microbiome, whose alterations or dysbiosis have been linked to several psychiatric disorders such as depression and anxiety. There is renewed interest in the use of dietary prebiotic fibers and prebiotics to modulate the composition and metabolism of complex gut microbiota to prevent gut-related diseases and improve health. When ingested, they serve as the primary fermentation substrate for gut microbes, enabling the production of bioactive metabolites, such as short-chain fatty acids and neurotransmitters, which can elicit positive health benefits for the host's psychiatric and neurological functions. This article outlines and discusses the current knowledge from *in vivo*, *in vitro,* and *ex vivo* studies on the prebiotic and psychobiotic characteristics of dietary fibers and their impact on the gut microbiome. Similarly, we highlighted that the effects of dietary prebiotics on gut microbes inversely correlated with mental health illnesses.

Keywords: Prebiotics, dietary fibers, gut microbiome, mental health, depression-inversely linked gut microbes.

1. Introduction

Increasing evidence has highlighted that the gut microbiome, via the gut-brain axis, plays a vital role in brain functions related to behavior and emotions, with important implications in several neurological and psychiatric illnesses (Burokas et al., 2015; Dinan & Cryan, 2012). For instance, a study that analyzed the fecal microbiome of over 2,000 individuals found that specific bacterial genera were more common in individuals with depression, highlighting the potential role of gut microbes as disease biomarkers (Radjabzadeh et al., 2022). According to the same study, increased depressive symptoms were associated with a decrease in certain bacterial taxa, such as *Coprococcus, Eubacterium ventricose, Subdoligranulum,* and *Ruminococcaceae* (Radjabzadeh et al., 2022). These bacterial taxa are important for gut health as they produce short-chain fatty acids, primarily butyrate (Chassard et al., 2014; Shang et al., 2016; Valles-Colomer et al., 2019). Likewise, Liang et al. (2023) found that specific types of bacteria are related to depressive symptoms and identified metabolic pathways that play a role in the connection between gut bacteria and mental health. For example, it was revealed that the group less prone to depressive symptoms had a higher abundance of butyrate-producing bacteria, such as *Faecalibacterium* and *Oscillospirace*ae, and fewer inflammatory bacteria *Escherichia-Shigella* (Liang et al., 2023). Factors such as diet, genetics, drugs, and stress can alter the composition and metabolism of the gut microbiome (Hasan & Yang, 2019). Indeed, most available pharmaceutical treatments in the form of antidepressants for the treatment and management of major depressive disorder (MDD) often exhibit metabolic side effects and can lead to dysbiosis in the gut (Almohammed et al., 2022; Olguner Eker et al., 2017). Ait Chait and coauthors showed that some commonly prescribed antidepressants exhibit antimicrobial properties and can alter gut microbiome composition. Hence, preventive and adjuvant nutritional therapies are considered sustainable and effective means of maintaining or alleviating negatively altered gut microbiome diversity and metabolism (Ait Chait et al., 2021).

Although the importance of prebiotic fiber consumption is known, only a few studies have focused on the effects of prebiotics on mood, behavior (Tarr et al., 2015), and the central nervous system (Savignac et al., 2016; Williams et al., 2016). For instance, dietary prebiotic interventions have proven effective in modulating the gut microbiome in humans and mice (Meijer et al., 2010; Tachon et al., 2013). In addition, various studies have shown that the fermentation of prebiotic fibers in the colon by gut microbes produces important metabolites that greatly benefit host health (Zmora et al., 2019). For example, as shown in figure 1, dietary fiber consumption can improve gut health and impact overall health, whereas Figure 2 shows the pathway through which short-chain fatty acids illicit neuropsychiatric functions. Butyrate, a major metabolic by-product of fiber fermentation in the gastrointestinal tract, exhibits antidepressant properties and enhances overall mental health (Evans et al., 2013; Ríos-Covián et al., 2016). Similarly, SCFAs produced by gut microbiota stimulate serotonin synthesis in enterochromaffin cells (Legan et al., 2022; Reigstad et al., 2015). The gut microbiome also produces a broad spectrum of neuroactive compounds, including neurotransmitters such as serotonin, dopamine, norepinephrine, and γ-aminobutyric acid (GABA) and their precursors, which can affect mood and neuropsychiatric functions (Miri et al., 2023). For instance, their absence and reduced levels can lead to several mental health disorders, such as Parkinson’s disease, depression, anxiety, Alzheimer’s disease, and poor memory and cognition (Bäuerl et al., 2018; Eltokhi et al., 2020; Moraga-Amaro et al., 2014; Pawluski et al., 2019). Increasing evidence has highlighted the potential use of probiotics and/or prebiotics to exert antidepressant effects, improve cognition and mood, or promote the growth of inversely depression-linked gut microbes (Narli & Ozcan, 2022; Paesani et al., 2020; Swann et al., 2020; H.

Zheng et al., 2021). In this review, we discuss the prebiotic potential of fibers as a growth-promoting strategy targeting the gut microbiome, psychobiotics, and depression-inversely linked gut microbes.

2. Dietary fibers: diversity and digestion in the human tract

Box 1: Definitions

- The cereals and grains association defined Dietary fiber as the "edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine" (DeVries et al., 2001). Dietary fiber includes polysaccharides, oligosaccharides and associated plant substances. Dietary fibers have beneficial physiological effects, including laxation, blood cholesterol attenuation, and blood glucose attenuation (DeVries et al., 2001).
- The term prebiotics was first defined in 1995 by Gibson and Roberfroid as "nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of beneficial bacteria in the colon, thus improving host health" (Gibson & Roberfroid, 1995). The idea of prebiotics has evolved since this initial definition and was modified by the International Scientific Association of Probiotics and Prebiotics (ISAPP) as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Farias et al., 2019).
- The International Scientific Association of Probiotics and Prebiotics (ISAPP) defines probiotics as live microorganisms that confer health benefits to the host when administered in adequate amounts (Hill et al., 2014).
- Psychobiotics are living organisms and groups of probiotics that confer mental health benefits upon ingestion in adequate and sufficient and adequate proportions to the host (Dinan et al., 2013). They differ from traditional probiotics in their ability to produce or induce the produce neurotransmitters, short-chain fatty acids, enteroendocrine hormones, and anti-inflammatory cytokines (Sharma et al., 2021). Despite their small populations, these bacteria exhibit anxiolytic and antidepressant properties (Evrensel et al., 2019).
- Short-chain fatty acids (SCFAs) are the main byproducts of gut bacterial fermentation of dietary fibers in the gastrointestinal tract. SCFAs and lactic acid have been reported to be significant products of the fermentation of undigested carbohydrates by the gut microbiota (Ríos-Covián et al., 2016), which may be beneficial in the modulation of enteroendocrine serotonin secretion (Evans et al., 2013).

2.1. Overview of dietary fibers

Dietary fibers are structurally complex carbohydrates that are undigested by the host and fermented in the colonic environment (Benítez-Páez et al., 2016). These fibers include non-starch polysaccharides, such as fructooligosaccharides (FOS), galactooligosaccharides (GOS), celluloses, and hemicelluloses (such as arabinoxylans and arabinogalactans, pectins, and fructans)

(Caprita et al., 2010). Beyond fermentability, many dietary fibers possess several health-promoting properties, including satiety promotion and glycemic index reduction (Øverby et al., 2013).

Dietary fibers reach the colon as undigested polysaccharides and serve as primary growth substrates for gut microbiota. These undigested fibers are primarily used as fermentation substrates and are broken down into monomeric sugar units through the secretion of hydrolytic enzymes (Rowland et al., 2018). Dietary fibers are classified according to their I) solubility, II) molar mass, III) monomeric composition, and IV) branching patterns. However, classification by solubility is among the most commonly used classification methods: water-soluble fibers (e.g., water-extractable arabinoxylans (AX), arabinoxylan oligosaccharides (AXOS), galactooligosaccharides (GOS), and fructooligosaccharides (FOS)) and water-insoluble fibers (e.g., water-unextractable AX, resistant starch (RS), cellulose, and lignin) (Abdi & Joye, 2021). These unabsorbed or undigested dietary carbohydrates are fermented in the large intestine by gut microbes, producing SCFAs as one of their end products (Ríos-Covián et al., 2016).

2.2. Cereal Arabinoxylan: A major dietary fiber with potential prebiotic properties?

Arabinoxylans (AXs) are the main kind of hemicelluloses in many cereal grains. They primarily originate from the bran and starchy endosperm and cannot be degraded by mammalian enzymes. Most major cereal grains, including rye, wheat, barley, oats, sorghum, corn, and malt, have been found to contain arabinoxylan (Table 1) (Fadel et al., 2018; Izydorczyk, 2021). AXs are classified according to their solubility in water, i.e., water-extractable AX and water-unextractable AX, and they are formed when a substitution occurs between xylose chains bonded by β 1-4 bond and arabinose units bonded by 1α-2 and 1α-3 bonds on the xylose chain (Paesani et al., 2019). Arabinoxylans can be found in various foods, including maize, rye, barley, oats, sorghum, wheat, rice, and other non-cereals, such as bananas (Chen et al., 2019). Regarding the structure of AXs, the primary sugar substituent on the linear β-(1,4) $_{\text{D}}$-xylopyranose (Xylp) linked xylan backbone in most cereal arabinoxylans is L-arabinofuranose (Araf). They substitute for Xylp residues at O-2 and/or O-3 through α-1, two and α-1, three glycosidic linkages, resulting in the conformation of four unique structural components within the polymer: mono-substituted Xylp at O-2 or O-3, di-substituted Xylp at O-2 and O-3, and unsubstituted Xylp (Zhang et al., 2015). However, the structure of arabinoxylan may vary depending on the origin of the raw materials and extraction method, which confers distinct structural characteristics such as sugar composition, molecular weight, and branching patterns (Chen et al., 2019; Zhang et al., 2015). For example, the structural and functional properties of water-extracted wheat arabinoxylan (WEAX) from three different varieties were compared (Sun et al., 2019). The results of this study demonstrated that each variety of WEAX has a differential impact on gut microbes. Australian white wheat, with a greater viscosity and less branched patterns, improved the growth of *Bifidobacteria* and *Lactobacilli* and led to increased concentrations of propionic acid compared to the arabinoxylan from Jinqiang wheat and American red hard spring wheat (Sun et al., 2019). In another study, AXs generated from the green leaves of *Litsea glutinosa* had a unique branching pattern (Das et al., 2013), whereas galactose or glucuronic acid were found in AXs made from banana peel hemicellulose and corn hull (Chen et al., 2019). Similarly, the extraction process influences the molecular structure of AXs. Chemical, enzymatic, or physical methods can be used to extract AXs. Multiple studies have reported that alkaline treatment changes the functional group of AXs, reduces ferulic acid concentration, and maintains the natural molecular weight of AXs (Chen et al., 2019).

Arabinoxylans are important dietary fibers that have recently received considerable attention because of their emergent and potential prebiotic ability. Arabinoxylans are non-starch polysaccharide dietary fibers and are a significant component of dietary fiber in most cereal crops (Izydorczyk, 2021). Compared to other well-known plant dietary fibers, such as fructooligosaccharides, galactans, and -glucans, the prebiotic activity of AXs has not been studied extensively (Gu et al., 2021). In a multi-omics study involving the effects of arabinoxylan oligosaccharides (AXOS) on the human gut microbiota, researchers reported that the consumption of AXOS enhanced the abundance of beneficial bacterial taxonomic groups such as *Actinobacteria, Bifidobacteriaceae,* and *Bifidobacterium* (Benítez-Páez et al., 2019). In addition to this established bifidogenic function, the study also showed that AXOS improved the ability of the gut microbiota to secrete amino acids, such as phenylalanine and tryptophan, precursors for serotonin and dopamine, among others.

Furthermore, AXOS intake has been associated with significant GABA biosynthesis (Benítez-Páez et al., 2019). In some studies, GABA has been linked to antidepressant effects (de Noronha et al., 2017). Another study reported the modulation of the gut microbiota in obese and overweight individuals with long-chain arabinoxylan isolated from corn bran (Nguyen et al., 2020). This study reported that the administration of arabinoxylan altered the community structure and caused modifications in the gut microbiota composition that appeared after one week of treatment but did not persist at W6. At the genus level, the authors observed an increase in the population of *Bifidobacterium* and *Prevotella.* They also found that treatment with arabinoxylan increased SCFA production (specifically propionate) compared to a control group treated with microcrystalline cellulose (Nguyen et al., 2020). Butyrate-producing bacteria, such as *Eubacterium* and *Roseburia*, have been linked to AX fermentation (Rivière et al., 2016). Butyrate is an essential metabolite of the human colon, because it is the primary source of energy for colonocytes (Mendez-Encinas et al., 2018; Rivière et al., 2016).

Table 1. Percentage composition of arabinoxylan in various cereals.

Type of cereal	Type of tissue	% of AX	Reference
Wheat	Bran	13.2–22.1	(Ibba et al., 2021)
	Endosperm	1.35–2.75	(Ibba et al., 2021)
Barley	Whole grain (hulled)	3.98-5.44	(Izydorczyk & Dexter, 2008)
	Whole grain (hulless)	3.50-6.05	(Izydorczyk & Dexter, 2008)
	Flour	7.1-8.0	(Han, 2000)
Oat	Bran	5.2	(Westerlund et al., 1993)
	Endosperm	1.2	(Westerlund et al., 1993)
	Whole grain	2.0-2.4	(Izydorczyk, 2021)
Sorghum	Bran	3.0-4.1	(Izydorczyk, 2021)
Malt	Flour	3.1-4.0	(Han, 2000)
Rye	Whole grain	8.0–12.1	(Boskov Hansen et al., 2002)
	Flour	3.2-3.64	(Cyran, 2015)
	Bran	12.6	(Vinkx & Delcour, 1996)
	Endosperm	3.56-4.25	(Vinkx & Delcour, 1996)
Corn	Bran	26.0	(Kundu et al., 2018)
	cob	26.24	(Kundu et al., 2018)

2.2.2. Digestion in the gut environment

It is no longer news that undigested carbohydrates in the GI tract serve as substrates and carbon sources for most gut microbes. Carbohydrates heavily influence the microbiome, as they can modulate their composition (Wardman et al., 2022) while adding other health benefits (Flint et al., 2012). Competition for substrates and nutrients is unavoidable in the gut microbiome because of its vast microbial community. Consequently, a microorganism's capacity to break down carbohydrates gives it an advantage over its competitors (Martens et al., 2014). Furthermore, Kemezik et al. (2021) suggested that the competitive advantage exhibited by specific microorganisms could be attributed to their ability to produce carbohydrate esterases. These enzymes are responsible for removing ester linkages from carbohydrates to promote the activity of glycosyl hydrolases in complex carbohydrates (Armendáriz-Ruiz et al., 2018).

Humans cannot independently break down these dietary carbohydrates, because most enzymes that break down carbohydrates are not encoded in the human genome. Therefore, we relied on intestinal bacteria (Flint et al., 2012; Kaoutari et al., 2013). Gut microorganisms can break down dietary carbohydrates for their benefits. However, humans also gain from this process in the form of SCFAs, a byproduct of carbohydrate fermentation by resident gut bacteria (Wardman et al., 2022). Therefore, the relationship between gut microbes and humans is mutual, because humans are responsible for ingesting these carbohydrates in the first place.

Polysaccharide utilization loci (PUL) are genomic loci found in bacterial species that encode carbohydrate-active enzymes (CAZymes) that are responsible for the digestion and transportation of complex carbohydrates (Flowers et al., 2017; Wardman et al., 2022). Carbohydrate-active enzymes (CAZymes) degrade carbohydrates produced by resident gut microbes and can function on various substrates (Cantarel et al., 2009; Wardman et al., 2022). CAZymes in the microbiota play a significant role in the specialized degradation of simple to complex carbohydrates, both endogenous and exogenous (Kaoutari et al., 2013). Glycoside hydrolases are the most abundant and well-studied class of CAZymes and are involved in carbohydrate degradation (Lombard et al., 2014). *Bacteroides* species, specifically *Bacteroides thetaiotaomicron* and *Bacteroides ovatus,* are some of the largest producers of this enzyme in the gut (Kaoutari et al., 2013). Onyango et al. (2021) reported that approximately 313 CAZyme families involved in carbohydrate metabolism are encoded by *B. thetaiotaomicron*.

Furthermore, Research has shown that complex carbohydrates, such as wheat bran, porcine gastric mucin, and starch, can be mainly broken down by *Bifidobacterium* species and some members *of the Firmicutes* phylum (Leitch et al., 2007; Ze et al., 2012). Kaoutari and coauthors reported that members of the *Bacteroidetes* phylum are the primary degraders of complex polysaccharides, because these groups of bacteria have a vast repertoire of CAZymes that are either absent or minimal in other phyla of gut bacteria (Kaoutari et al., 2013). Novel insights into the variety and functions of these CAZymes in the human gastrointestinal tract will deepen our understanding of the connection between humans and their gut microbiomes. Furthermore, it will provide researchers with new knowledge regarding the wide range of applications of these enzymes.

3. Gut Microbiome: Implication in host Physiology

3.1. Beyond fiber digestion: Implication in health and diseases

Dietary fibers play an essential role in modulating and maintaining a healthy gut by increasing and strengthening the population of beneficial microorganisms. It is already known that certain organisms prefer a particular kind of substrate (Louis, 2017), and changes in the number of different bacteria strains may be caused by dietary fiber consumption (So et al., 2018). The definition of a healthy gut microbiome in adults is unclear, as it may vary from person to person. Nonetheless, significant improvements have been made in determining a healthy phylogenetic core, leading to a consensus that *Firmicutes, Bacteroidetes, Actinobacteria,* and *Verrucomicrobia* dominate the gut of healthy adults (Gevers et al., 2012). After uptake and use by host cells, dietary fibers are fermented by the gut microbiota to produce SCFAs, which improve the intestinal environment directly or indirectly by lowering pH, crossing the epithelial barrier, and metabolizing butyrate primarily by epithelial cells, propionate by liver cells, and acetate by muscles (Kasubuchi et al., 2015; Koh et al., 2016). SCFAs play a critical role in gut homeostasis with implications for host health and metabolism (Rivière et al., 2016), particularly butyrate, which has been reported to exhibit antidepressant properties (Hao et al., 2019). In addition to the metabolic impacts (by the production of SCFAs), animal studies have indicated that a healthy microbiome plays a role in brain growth and function by boosting tryptophan and energy synthesis (Gareau et al., 2011). Johnstone et al. (2021) reported that the consumption of galactooligosaccharides (GOS) for four weeks improved the overall gut microbiota composition and emotional well-being of young females. In humans, those with inflammatory bowel disease, type 2 diabetes, and atherosclerosis have been demonstrated to have a different microbial composition than those without these illnesses (Swann et al., 2020). In addition, infusing individuals with metabolic syndrome with a healthy microbiome enhances insulin sensitivity (Vrieze et al., 2012).

A growing body of research suggests that various psychiatric and neurological illnesses are associated with gut dysbiosis. Microbial dysbiosis is defined as a change in the composition of the human gut microbiota from a generally diversified and commensal microbial community to a more maladaptive and pathogenic profile (Keightley et al., 2015; Patterson et al., 2014). Furthermore, Jones et al. (2014) characterized dysbiosis as an increase in the population of adherent and/or invasive *Escherichia coli* as well as a decrease in *Bacteroidetes* and *Firmicutes* phyla, including the clinically relevant *Faecalibacterium prausnitzii*. Increased intestinal permeability can generate an inadequate immune response when natural gut microbiota shifts to a more pathogenic or harmful composition. Micro-damage occurs in the intestinal epithelial wall due to a shift in microbiota composition and increased endotoxins. Hence, intestinal epithelial permeability increases (Evrensel & Ceylan, 2019). Intestinal dysbiosis impairs the gut-brain connection and promotes bacterial translocation by increasing gut permeability. In patients with MDD, bacterial translocation results in elevated blood levels of bacteria-led lipopolysaccharide (LPS, endotoxin) metabolite (Maes et al., 2012). LPS maintains depression-induced neuroinflammatory changes (Walker et al., 2014). Dysbiosis has also been reported in patients with schizophrenia, anxiety disorders, depression, and other mental illnesses (Dinan et al., 2014).

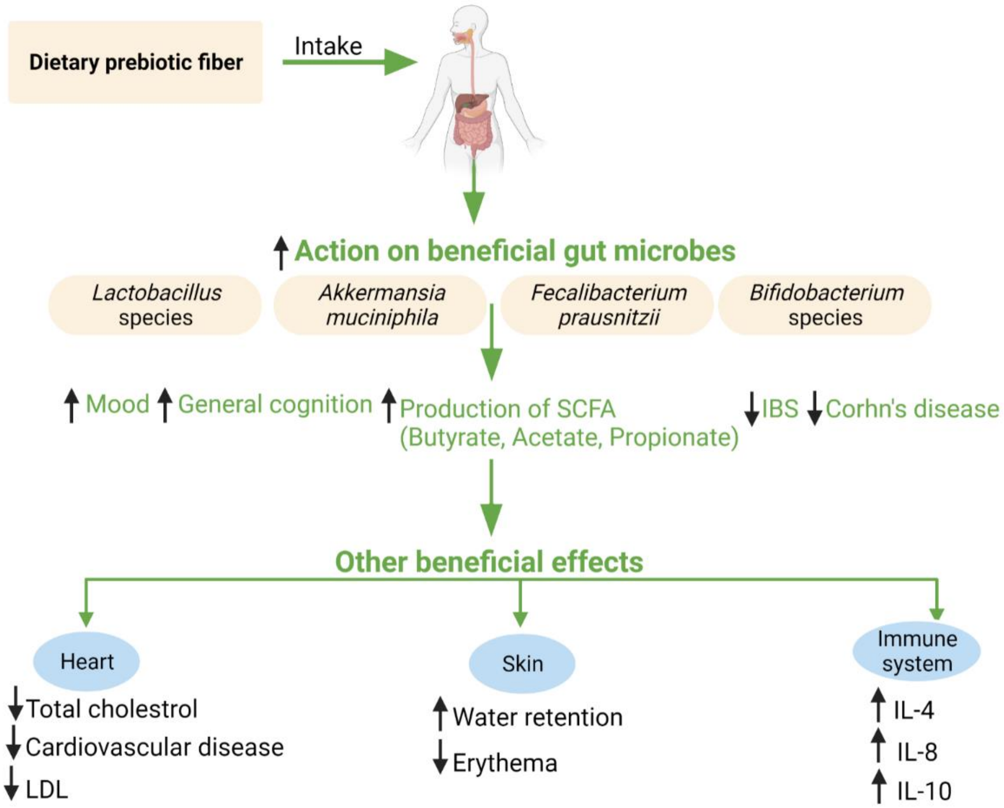

Figure 1. Effect of dietary prebiotic fiber on human health. Prebiotics positively affect the gastrointestinal tract and other body parts such as the brain, skin, and immune system. LDL, low-density lipoprotein; IBS, irritable bowel syndrome; IL-4: interleukin 4; IL-8: interleukin 8; IL-10. Symbols-: increase; ¯: decrease.

3.2. The Microbiota-Gut-Brain Axis

The primary role of gut microbiota in the development of mental illnesses is unclear and is still being studied. The bidirectional link between the brain and gastrointestinal tract through the gut-brain axis is crucial for the relationship between gut microbiota and mental disorders. Researchers have reported a significant relationship between the gut microbiota and the brain through the gut-brain axis and its role in mental health disorders (Cryan & Dinan, 2012; Szyszkowicz et al., 2017). The role of the gut-brain axis in the pathogenesis of mood disorders can be attributed to the production of bacterial metabolites acting as neurotransmitters (i.e., dopamine and GABA) and the production of SCFAs, such as butyrate, acetate, and propionate, which are known to exhibit antidepressant effects (Foster & McVey Neufeld, 2013). Cryan et al., (2019) confirmed a bidirectional relationship between gut microbiota composition and anxiety and depression. In addition to the gut-to-brain relationship, other aspects of the gut-brain axis have been reported. Recently, it was shown that fecal microbiota transplantation from chronic unpredictable stress mouse donors affected anxiety-like and depression-like behaviors in recipient mice (N. Li et al., 2019). Similarly, Zheng et al. reported that FMT of germ-free mice that received microflora from individuals with Major Depressive Disorder (MDD) led to increased depression-like behaviors compared with mice that received FMT from healthy individuals (P. Zheng et al., 2016).

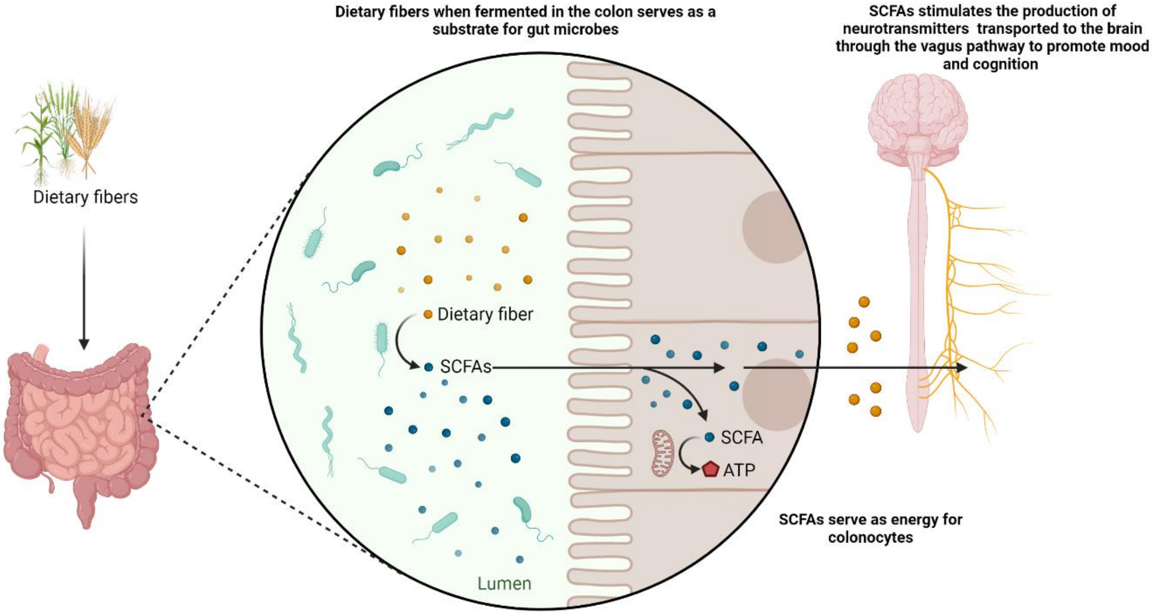

Figure 2. SCFAs produced from dietary fiber fermentation can improve mood and emotional well-being.

3.3. Microbes inversely linked to mental health diseases

Some bacterial species in the gut microbiota, such as Lactobacillus, F. prausnitzii, and Akkermansia muciniphila, have been negatively correlated with depression. In the past, researchers reported an increase in the availability of *A. muciniphila* and *F. prausnitzii* could regulate metabolic functions and improve resistance to the development of mental illness, type 2 diabetes, atherosclerosis, and obesity (de Goffau et al., 2013; Z. Gao et al., 2009; J. Li et al., 2016). *F. prausnitzii*, also regarded as the next-generation probiotic, is a species of *Faecalibacterium*, which comprises approximately 5% of the total fecal microbiota in healthy individuals (Hold et al., 2003; Martín et al., 2017). Multiple studies have reported a positive effect of prebiotics on the abundance of *F. prausnitzii*. According to Hustoft et al. (2017), patients with diarrhea or mixed irritated bowel syndrome experienced increased availability of *F. prausnitzii* after treatment with fructooligosaccharides compared to the control (maltodextrin). In their study, Dewulf and colleagues (Dewulf et al., 2013) showed that the abundance of *F. prausnitzii* increased after treatment with inulin-type fructans in obese females compared with maltodextrin. In addition, dietary inulin-type fructans also enhance the availability of *Bifidobacterium* species in obese women (Dewulf et al., 2013). A similar result was observed in another study, where Hooda and coauthors found that *F. prausnitzii* increased in healthy males who consumed either polydextrose or soluble corn fiber supplementation (Hooda et al., 2012). Ramirez-Farias et al. (2008) reported that inulin-oligofructose enhanced the abundance of *F. prausnitzii* in healthy individuals.

A. muciniphila accounts for approximately 0.5-5% of the total bacterial population in human intestinal microbiota, making it one of the most abundant bacterial species in the human gut (Collado et al. 2007). It was first identified in (2004) by Muriel Derrien et his colleagues. *A. muciniphila* has been reported to be more present in healthy guts than in the guts of patients with several illnesses, such as bowel diseases, diabetes, obesity, and ulcerative colitis (Karlsson et al., 2012; Png et al., 2010; Rajilić–Stojanović et al., 2011; Sanz Herranz, n.d.; Tilg & Moschen, 2014). Prebiotic fructooligosaccharides (FOS) have improved the abundance and growth of *A. muciniphila* in animal models. Everard and colleagues in 2011 and 2013 from their study reported that FOS administration could enhance the availability of *A. muciniphila* (Everard et al., 2011, 2013). After five weeks of oral treatment with FOS (FOS,0.3 g/d with a high-fat diet for eight weeks) in ob/ob mice. The abundance of *A. muciniphila* increased by over 80 folds (Everard et al., 2011). A similar result was reported when the administration of FOS restored the abundance of *A. muciniphila* in diet-induced obese (DIO) mice, which was altered by feeding the mice a high-fat diet for eight weeks. Upon administering FOS supplements for eight weeks, the concentration of

A. muciniphila was restored in mice, similar to that in mice fed a standard diet (Everard et al., 2013). In another study, mannan-oligosaccharide (MOS), a type of oligosaccharide, increased the intestinal microbial diversity in mice (Wang et al., 2018). The authors observed an increased relative abundance of *Firmicutes* in mice fed a high-fat diet compared to mice fed a regular diet, and a decline in *Bacteroidetes*, *Actinobacteria*, and *Verrucomicrobia*. Administration of MOS stimulated the abundance of *Bacteroidetes*, *Actinobacteria*, and *A. muciniphila,* a phylum of *Verrucomicrobia* (Wang et al., 2018).

4. Psychobiotic Potential of dietary fibers

Bacterial fermentation of nondigestible carbohydrates in the colon produces SCFAs, mainly butyrate, propionate, and acetate (H. C. Harris et al., 2021). Butyrate and other SCFAs are the major metabolic products produced after dietary fiber fermentation by *F. prausnitzii* (Louis & Flint, 2009; Roychowdhury et al., 2018). Butyrate is particularly interesting because it has been reported to improve intestinal barrier function and reduce inflammation (Peng et al., 2009). Furthermore, butyrate is the primary source of energy for colonocytes (Knudsen et al., 2003), and has been associated with antidepressant properties (Hao et al., 2019). Over the past decade, research has focused on the psychobiotic ability of dietary fibers *in vitro*, *ex vivo,* and *in vivo*. Several studies have shown that dietary prebiotic fibers are essential in modulating gut microbiota composition by enhancing the abundance of beneficial microorganisms that have been reported to be negatively correlated with anxiety and depressive-like disorders and improving the production of SCFAs (Table 2).

Table 2. Summary of some dietary fiber intervention studies

Dietary Fibers	**Method**	**Effects on gut microbiota**	**References**
Inulin	*in vivo*	↑ *Bifidobacterium*	(Petry et al., 2012)
Soluble corn fiber, polydextrose	*in vivo*	↑ *Akkermansia, Faecalibacterium*, & *Lactobacillus*	(Hooda et al., 2012)
FOS + GOS	*in vivo*	↑ *Bifidobacterium* & *Lactobacillus*	(Burokas et al., 2017)
DOP	*in vitro*	↑ *Faecalibacterium, Lachnospiraceae*, and *Ruminococcaceae*	(Y. Fu et al., 2019)
Acorn prebiotics	*ex vivo*	↑ *Lactobacillus*	(Ahmadi et al., 2019)
Alpha-GOS	*in vitro*	↑ *Bifidobacterium*	(Fehlbaum et al., 2018a)
Beta-GOS	*in vitro*	↑ *Lactobacillus*	(Fehlbaum et al., 2018a)
Inulin/Oligofructose	*in vivo*	↑ *F. prausnitzii* and *Bifidobacterium*	(Dewulf et al., 2013)
Lupin flour, tapioca fibers, tiger nut flour, gold kiwifruit powder	*ex vivo*	↑ *Lactobacillus, Collinsella and Erysipelotrichaceae*	(Mottawea et al., 2020)
Wheat arabinoxylan	*in vivo*	↑ [acetic and butyric acids] ↑*Bifidobacterium* & *Lactobacillus*	(Paesani et al., 2020)
Gynostemma pentaphyllum polysaccharide	*in vivo*	↑ overall gut diversity	(S. Li, Wang, et al., 2022)
Chicory pulp and oligofructose Citrus pectin and lime peel	*in vitro*	↑ *Bifidobacterium* and *Lactobacillus*	(Uerlings et al., 2019)
Rice bran arabinoxylan	*in vitro*	↑ *Bifidobacterium* ↓ *Sutterella* and *Clostridium*	(Gu et al., 2021)
GOS	*in vitro*	↑ *Bifidobacterium*	(Duque et al., 2021)
Australian white wheat	*in vitro*	↑ *Bifidobacterium* and *Lactobacillus*	(Sun et al., 2019)

4.1. Evidence from in vivo studies

Studies involving in vivo models have revealed valuable information regarding the impact of fibers on mood, depressive-like behaviors, and depression-inversely linked microorganisms. An in vivo study by Burokas et al. reported that the administration of fructooligosaccharides (FOS), galactooligosaccharides (GOS), and a combination of both for three weeks was able to modify brain chemistry and behavior with respect to depression and anxiety in mice (Burokas et al., 2017). In the same study, they also found that an increase in SCFAs quantities may be responsible for the favorable behavioral and neurochemical improvements exhibited by the mice. Furthermore, it is worth mentioning that combining FOS and GOS yielded a better overall effect than administering each prebiotic fiber independently (Burokas et al., 2017). This could be because combining them increases the diversity and abundance of beneficial microbes. In addition, the combined administration of FOS and GOS was instrumental in preventing a decline in the abundance of *Bifidobacterium* and *Lactobacillus* (Burokas et al., 2017), which have been previously reported to be low in individuals with major depressive disorders (Kelly et al., 2016). In a randomized, double-blind, placebo-controlled crossover study, researchers tested the impact of soluble corn fiber (SCF) and polydextrose (PDX) on the gut microbiota composition of healthy adults. Their study reported a beneficial shift in the gut microbiome composition of individuals who consumed SCF and PDX. At the genus level, *Akkermansia*, *Faecalibacterium*, and *Lactobacillus* were predominant in all treatments (Hooda et al., 2012). The intake of PDX and SCF increased the abundance of *Faecalibacterium*, whereas that of SCF increased the availability of *Lactobacillus*. In addition, the abundance of *Akkermansia* increased after the intake of PDX compared with the other treatments (Hooda et al., 2012). Paesani et al. evaluated the prebiotic effects of water-soluble extractable arabinoxylans (WE-AX) in soft and hard wheat using C57BL6 mice. They reported that WE-AX increased the abundance of beneficial bacterial species (*Bifidobacterium and Lactobacillus*) and decreased the abundance of *Clostridium* in mice. The growth of these microbes is significantly higher in mice that consume WE-AX from hard wheat (Paesani et al., 2020). In addition, SCFA concentrations, mainly of acetic and butyric acids, were higher in mice fed the WE-AX-supplemented diet. Mice fed WE-AX from soft wheat exhibited the highest SCFA levels. The authors suggested that this might result from the high final concentration of bifidobacteria in the cecal content of mice that consumed WE-AX from soft wheat (Paesani et al., 2020). In another study, the authors investigated the effects of galactooligosaccharides (GOS) on women's gut bacterial composition, mood, and emotional well-being. Their research demonstrated that the consumption of GOS by participants promoted the abundance of beneficial gut bacteria (*Bifidobacterium*) compared to that in the control group (Johnstone et al., 2021). *Bifidobacterium* is associated with improved mood and mental well-being (Messaoudi et al., 2011; Pinto-Sanchez et al., 2017; Tian et al., 2022). Furthermore, their study demonstrated that an increase in *Bifidobacterium* abundance was accompanied by anxiolytic effects, with participants in the highly anxious prebiotic group reporting much lower levels of trait anxiety (Johnstone et al., 2021). More recently, Li et al. reported that the administration of *Gynostemma pentaphyllum* polysaccharide for three weeks enhanced the amount of acetate, butyrate, and propionate in the cecum contents of normal mice (S. Li, Wang, et al., 2022). According to their findings, the consumption of this polysaccharide was also linked to increased diversity and richness of the gut microbiota (S. Li, Wang, et al., 2022).

4.2. Evidence from in vitro studies

Clinical studies on the prebiotic effects of fibers are generally considered questionable owing to ethical concerns. Nevertheless, these constraints have been significantly reduced using an in vitro approach for this type of study. According to Payne et al. (2012), *in vitro* studies are important because they allow independent research in a highly regulated environment without host interference. Yousi and coauthors, while investigating the effects of *Dendrobium officinale* Polysaccharides (DOP) on human intestinal microbiota, revealed that DOP showed a potential prebiotic impact by increasing the availability of beneficial bacteria such as *Bacteroides*, *Prevotella*, *Faecalibacterium*, *Lachnospiraceae*, and *Ruminococcaceae* while decreasing the amount of the pathogenic microorganisms (Y. Fu et al., 2019). They also observed a significant increase in the abundance of SCFAs in the DOP group compared to the control group. The differences in SCFA production were consistent with the pH shifts in both groups, with the pH of the DOP group showing a significant decrease as fermentation time increased (Y. Fu et al., 2019). The increased level of SCFA production in this study is intriguing because of the benefits of SCFAs on human health. In their research on the effects of prebiotic fibers on microbiome composition. In another study, Fehlbaum et al. (2018) reported an increase in the abundance of *Bifidobacterium* and *Lactobacillus* after fermentation for 24 h. Among all fibers used in this study, alpha-GOS yielded a higher amount of *Bifidobacterium* than the other fibers. At the same time, beta-GOS after fermentation increased *Lactobacillus* more than the other fibers used (Fehlbaum et al., 2018a). Furthermore, an increase in the *Bifidobacterium* population was observed for all fibers. A similar result was observed for *Lactobacillus*, except for inulin and FOS, which did not affect the *Lactobacillus* population. The authors reported a dose-dependent increase in the amount of SCFAs (acetate, propionate, butyrate, isovalerate, and isobutyrate) produced in all fibers (Fehlbaum et al., 2018a). Uerlings et al. fermented four types of fructan-based ingredients and nine types of pectin-based ingredients using an *in vitro* model of the piglet gastrointestinal tract. This study showed positive prebiotic abilities with respect to the fermentation attributes of all the ingredients (Uerlings et al., 2019). For the fructan-based ingredients, chicory pulp and oligofructose yielded the highest amount of *Lactobacillus* and *Bifidobacterium* species, respectively, while for the pectin-based ingredients, citrus pectin and lime peel resulted in the highest amount of *Lactobacillu*s and *Bifidobacterium* respectively after fermentation for 12 hours (Uerlings et al., 2019). Although the prebiotic potential of arabinoxylan has not been fully explored, there have been specific investigations regarding its prebiotic and bifidogenic characteristics. In their *in vitro* fermentation study, Gu et al. (2021) stated that AX from rice bran produced more SCFAs, specifically acetic acid and propionic acid, than FOS after 8 h of fermentation. In the same study, they reported that at the phylum level, AX fermentation resulted in an abundance of *Actinobacteria* when compared to other treatments, while at the genus level, AX treatment increased the availability of *Bifidobacterium*, *Blautia*, *and Collinsella*, and decreased *Sutterella* and *Clostridium* XIVb after fermentation for 24 h (Gu et al., 2021). In another study, the authors reported that galactooligosaccharides (GOS) increased the abundance of *Bifidobacterium* in autistic children (Duque et al., 2021). Previous Research has demonstrated that people with autism spectrum disorder have lower levels of beneficial bacteria, including *Bifidobacterium* (Liu et al., 2019). *Bifidobacterium* is one of the most recognized beneficial gut microbes, including some species that produce GABA, a non-proteinogenic amino acid found in microorganisms, plants, and vertebrates, with numerous physiological properties, including anti-hypertensive and antidepressant effects (Ngo & Vo, 2019).

4.3. Evidence from ex-vivo studies

More recently, in a study involving an *ex-vivo* continuous fermentation model, Mottawea and coauthors tested the psychobiotic potential of commercial formulas consisting of lupin flour, tapioca fibers, tiger-nut flour, gold kiwifruit powder, and probiotic powder of *Bacillus coagulans* (Mottawea et al., 2020). After fermentation, acetate, butyrate, and propionate were the most abundant SCFAs. In addition, they reported a significant increase in butyrate levels after treatment with the test formulation (Mottawea et al., 2020). Ahmadi et al., examined the prebiotic potential of acorn and sago prebiotics in healthy and diseased fecal microbiome *ex vivo* culture systems after 9 h of incubation and compared their effects with those of one of the most widely studied prebiotics (inulin) (Ahmadi et al., 2019). In the healthy fecal microbiome, SCFAs, such as lactate, acetate, propionate, and butyrate, are produced during the fermentation of prebiotics. Sago prebiotics increase acetate production, whereas acorns increase lactate production. In contrast, acetate production decreased in the diseased fecal microbiome, whereas prebiotics did not affect lactate production (Ahmadi et al., 2019). Prebiotics had no significant effect on butyrate production compared with the control in healthy and diseased fecal microbiome cultures. Worthy of note is that propionate production in healthy and diseased fecal microbiome cultures was increased by acorns prebiotics. Inulin increases propionate production in a healthy fecal microbiome, whereas sago probiotics increase propionate production in diseased fecal microbiome cultures (Ahmadi et al., 2019). In diseased cultures, improved propionate production by acorn prebiotics correlated with *Bacteroidetes* and *Bacteroides*. In contrast, a significant increase in propionate production by inulin and acorn prebiotics was associated with the abundance of *Lactobacillus* in healthy fecal microbiome cultures (Ahmadi et al., 2019). Their study observed that the selected prebiotics had different effects on healthy and diseased fecal microbiome cultures. This indicates that the health of the host determines the potency of prebiotics.

5. Other metabolic functionalities of dietary fibers

In addition to its prebiotic and psychobiotic effects, dietary fiber can help regulate metabolic diseases, such as oxidative stress and glucose homeostasis (Cani et al., 2009). It has also been reported to boost the production of gut hormones by improving butyrate and propionate production by the gut bacteria. Free fatty acid receptor 2, free fatty acid receptor three, and G protein-coupled receptors are activated (Lin et al., 2012). These hormones play roles in appetite control, glucose metabolism, and insulin resistance (Aliasgharzadeh et al., 2015). Furthermore, in a control clinical trial involving fifty-five women, Aliasgharzadeh et al. concluded that the prebiotic used in their study (resistant dextrin) could modulate inflammation and improve insulin resistance in women with type 2 diabetes (Aliasgharzadeh et al., 2015). In addition, butyrate also helps maintain gut barrier function and has been reported to exhibit immunomodulatory and anti-inflammatory properties (Rivière et al., 2016). Indeed, phenolic acids from AX, such as ferulic acid (FA), have been shown to have antioxidant properties. AXs have attracted attention in the pharmaceutical industry owing to their intriguing functional and biological features. In addition to their antioxidant ability, phenolic acids have anticancer, anti-inflammatory, and antidiabetic effects and reduce some chronic and cardiovascular diseases (Ayala-Soto et al., 2014; Rivière et al., 2016). According to findings from the literature, the European Food Safety Authority (EFSA) in 2010 stated that increasing dietary fiber intake is associated with a lower risk of impaired glucose control (EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA), 2010). This has been supported by some recent studies. For example, Reynolds et al. stated that changing from low to moderate or high intake showed significant improvements in glycemic control (Reynolds et al., 2020). Thus, in persons with diabetes, increasing daily fiber intake by 15 or 35 g might be a reasonable strategy to minimize mortality risk (Reynolds et al., 2020). Moreso, another research has suggested that the consumption of whole-grain oats, barley, and β-glucans may improve glucose control by effectively managing postprandial glycemia (Tosh & Bordenave, 2020). In addition, Basu, and coauthors demonstrated that blueberry and soluble fiber consumption could enhance glycemic control and reduce inflammation (Basu et al., 2021).

6. Conclusion

It has been established that gut health can be improved, and gut microbiota composition can be modulated through dietary interventions, primarily by consuming prebiotic fibers. Common carbohydrates, polysaccharides, vegetables, and fruits possess prebiotic properties and are easily accessible. As many standard pharmaceutical products, such as antidepressants, have side effects on the gut microbiome, dietary intervention via prebiotics is most likely a promising option for the treatment, management, and prevention of neuropsychiatric diseases such as depression and anxiety disorders. In addition, dietary fiber can be produced on a larger scale with a reduced cost of production. The promising effects of dietary fiber and its psychobiotic potential cannot be overemphasized, as they can shift an individual's gut microbiome from unhealthy to healthy.

Therefore, its consumption is likely a feasible and cost-effective approach for managing and treating mental health disorders. However, further research is needed to determine how dietary fibers modulate the gut microbiota and exert their psychobiotic effects. It is also essential to determine an appropriate dosage for therapeutic purposes. Hence, more human/*in vivo* studies and trials are required to establish and validate the psychobiotic potential of dietary fibers to facilitate their adoption as a means to manage some mental health disorders. Current studies point to the effects of some dietary fibers on the abundance of *A. muciniphila* and *F. prausnitzii,* among other gut microbes. However, further studies are needed to support these claims, as they have the potential to be next-generation probiotics.

Acknowledgments

This study was supported by a strategic grant from the University of Ottawa. ENN was supported by a Nutrition and Mental Health Master's Scholarship, University of Ottawa.

CHAPTER 2: HYPOTHESIS, RESEARCH QUESTIONS, SPECIFIC AIMS, AND OBJECTIVE

Gut bacterial species have preferential dietary fiber substrates, primarily depending on the nature of the enzymes they can produce and the nature of the sugar monomers they metabolize, which affects the composition of their secondary metabolites (Louis, 2017; Pastell et al., 2009). Previous studies have shown the potential prebiotic ability of modified dietary fibers and carbohydrates on gut bacteria (Demuth et al., 2021; F. Huang et al., 2019), where the chemical structure was altered. To the best of our knowledge, the effects of enzymatic modification of wheat arabinoxylan on the composition, bioactivity, and metabolism of the gut microbiome and gut microbes have not been fully explored. In this study, we hypothesized that remodelling the structure of wheat arabinoxylan would promote the growth of selected depression-inversely linked microbes (*Lacticaseibacillus rhamnosus* GG and *Faecalibacterium prausnitzii*) and lead to differential microbial shift and metabolism by the gut microbiota of healthy adults.

2.1. Research Questions

This thesis will address the following research questions:

1. What is the impact of the molar mass and structure of wheat arabinoxylan fiber on the growth and metabolism of gut microbes inversely-correlated to depression?
2. What is the impact of the structure of wheat arabinoxylan fiber on microbial population shifts and metabolite production in healthy adults' gut microbiomes?

2.2. Research Objective

The objective of this research was to understand the relationship between the structural modification of wheat arabinoxylan on gut microbes, and the gut microbiome of healthy adults.

2.3 Research Aims

The specific aims of this research are:

1. To produce structurally different wheat arabinoxylan fibers through enzymatic digestion (chapter 3).
2. To determine the effects of these bioengineered wheat arabinoxylan on the growth rate and metabolism of depression-inversely linked microbes (*Lacticaseibacillus rhamnosus* GG and *Faecalibacterium prausnitzii*) (chapter 3).
3. To evaluate the impact of bioengineered wheat arabinoxylan on the composition and metabolism of the gut microbiota using an *in vitro* human colon fermentation model (chapter 4).

CHAPTER 3: BIOENGINEERED WHEAT ARABINOXYLAN – FOSTERING NEXT-GENERATION PREBIOTICS TARGETING DEPRESSION-INVERSELY LINKED GUT MICROBES

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Abstract

Dietary prebiotic fibers play an important role in modulating the gut microbiota via enhancing the abundance of beneficial microorganisms and their bioactive metabolites. However, dietary fibers are a structurally heterogeneous class of polysaccharides, varying by molar mass, branching patterns, and monosaccharidic composition, which could influence their utilization by various gut microorganisms. The present study aimed to investigate the effect of molar mass and chemical structure of wheat arabinoxylan fiber (AX) on the growth and metabolism of two key gut resident bacteria (*Faecalibacterium prausnitzii* and *Lacticaseibacillus rhamnosus* LGG) that were previously reported negatively correlated with anxiety and depressive-like disorders. For this purpose, low, medium, and high molar mass AX (LAX, MAX, and HAX, respectively), were modified with specific α-arabinofuranosidases to leave only singly substituted, only doubly substituted, or not substituted xylose units. Almost all modified AX had a better prebiotic score than the unmodified AX across all molar masses. Modified LAX showed a better prebiotic effect than HAX and MAX. Also, LAX with only doubly substituted xylose units exhibited the highest prebiotic potential and SCFA production on both microorganisms. Furthermore, AX, either only singly or doubly substituted, had a more consistent effect on the growth of *L. rhamnosus,* while AX, with all arabinose residues removed, showed a greater effect on *F. prausnitzii*. Findings from this study suggest that bioengineered AX could be considered a promising strategy for fostering next-generation prebiotics targeting depression-inversely linked gut microbes.

Keywords: depression-inversely linked microbes, enzymatic bioengineering, wheat arabinoxylan, prebiotic effect, *Faecalibacterium prausnitzii*, *Lacticaseibacillus rhamnosus* LGG

1. Introduction

Some microbial species populating the human gut are known to produce important bioactive compounds through the fermentation of dietary fibers, majorly short-chain fatty acids (SCFA), and neurotransmitters such as gamma-aminobutyric acid (GABA). For example, two *Lactobacillus* strains, *L. acidophilus* LP16-2 and *L. plantarum* LP9, were reported to have high GABA production abilities under conditions that imitate the physiological and microbiological conditions of the human colon (Mousavi, Mottawea, Hassan, et al., 2022). These microbial metabolites could positively affect the host's health due to their antidepressant, antihypertensive, and psychobiotic capabilities (de Noronha et al., 2017; Evrensel & Ceylan, 2019; Hao et al., 2019; Ngo & Vo, 2019). In turn, *Lactobacillaceae* (Dong et al., 2022), *Faecalibacterium prausnitzii* (Jiang et al., 2015; Leylabadlo et al., 2020), *Bacteroides* (Carlson et al., 2018), *Bifidobacterium breve* (Tian et al., 2019, 2022), and *Akkermansia muciniphila* (Cheng et al., 2022) have shown to be negatively correlated with depressive-like and anxiety-like disorders.

Additionally, dietary fibers are a class of structurally complex, indigestible carbohydrates. The different species composing the gut microbial population exhibit distinct abilities to utilize dietary fibers as substrates, according to the nature and structure of the fibers and according to the carbohydrases these species can express (Cultrone et al., 2015; Larraufie et al., 2015; Ranaivo et al., 2022; Tuncil et al., 2017). Consequently, alterations in the structure of dietary fibers have been shown to modify their utilization by gut microbiota and their potential prebiotic activity (Demuth et al., 2021; F. Huang et al., 2019). Therefore, one could wonder whether specific structural features of dietary fibers could favor psychobiotic effects through the growth of gut microbial species correlated with decreased depressive-like and anxiety-like disorders and through the production of neuroactive metabolites such as SCFA and GABA.

In this context, arabinoxylans are a prominent constituent of cereal hemicelluloses and a major contributor to dietary fibers intake worldwide (Mendis et al., 2016), and therefore a relevant target to evaluate the potential of common dietary fibers as potential substrates with psychobiotic effects. Cereal arabinoxylans are essentially constituted of a linear backbone of (1,4)-linked β-D-xylopyranosyl units with side groups of L-arabinofuranosyl units, the xylopyranosyl units bearing either two, one, or no L-arabinofuranosyl units (BeMiller, 2019). The selection of specific fractions of arabinoxylans has already been shown to modulate the fermentation profile of the gut microbiota (Rose et al., 2010a; Rumpagaporn et al., 2015); however, to the best of our knowledge, the effect of arabinoxylan structure on their metabolism by psychobiotic bacteria is yet to be explored. Therefore, the purpose of the present study was to investigate the effect of molar mass and branching pattern of wheat arabinoxylans on the growth and metabolism of selected depression-inversely linked bacterial strains encountered in the human gut. For this purpose, low, medium, and high molar mass wheat arabinoxylans (LAX, MAX, and HAX, respectively) were modified with specific α-L-arabinofuranosidases to remove either: (1) only L-arabinofuranosyl units on singly substituted β-D-xylopyranosyl units, (2) only L-arabinofuranosyl units on doubly substituted β-D-xylopyranosyl units, or (3) all L-arabinofuranosyl units from β-D-xylopyranosyl units, as shown in Figure 3.

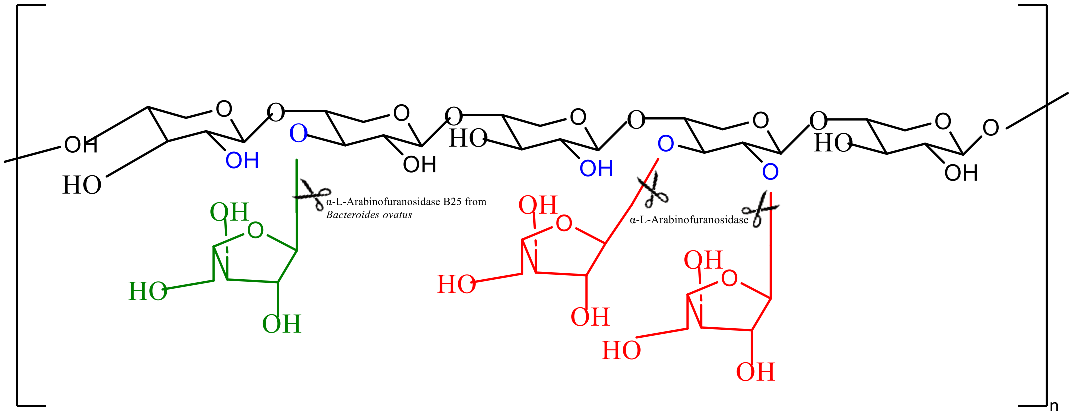

Figure 3. Chemical structure of the arabinoxylan polymer with the potential catalytic site of the enzyme α-L-arabinofuranosidase B25 from *Bacteroides ovatus* (specifically hydrolyzing α-L-arabinofuranose units from singly substituted xylose residues, in green) and α-L-arabinofuranosidase from *Bifidobacterium adolescentis* (specifically hydrolyzing α-L-arabinofuranose units from doubly substituted xylose residues, in red). Both enzymes combined cleave all α-L-arabinofuranose units from the xylan backbone.

2. Materials and methods

2.1 Arabinoxylan fibers, enzymes, and standards

Wheat arabinoxylan with high (P-WAXYH, Mw ≈ 370,000 Da, arabinose:xylose = 38:62)), medium (P-WAXYM, Mw ≈ 323,000 Da, arabinose:xylose = 38:62), and low (P-WAXYL, Mw ≈ 56,700 Da, arabinose:xylose = 38:62) molar masses (HAX, MAX, and LAX, respectively) were purchased from Megazyme International (Ireland). α-L-Arabinofuranosidase B25 from *Bacteroides ovatus* (E-ABFBO25) and α-L-Arabinofuranosidase from *Bifidobacterium adolescentis* (E-AFAM2) were also purchased from Megazyme International (Ireland). 2-Ethylbutyric acid and volatile free acid mix (respective internal and external standards for GC analysis short-chain fatty acid) were purchased from MilliporeSigma (Oakville, ON, Canada).

2.2 Culture media, bacterial strains, and culture conditions

De Man, Rogosa and Sharpe broth (MRS) and Fastidious Anaerobe Broth (FAB) media were obtained from Criterion (Santa Maria, CA, USA). Two bacterial strains, *Lacticaseibacillus rhamnosus s* GG 53103 and *Faecalibacterium prausnitzii* ATCC 27768, were purchased from American Type Culture Collection. All strains were cultured in their recommended media, MRS

L. rhamnosus GG and FAB for *F. prausnitzii*. Strains were kept frozen at -80°C until they were used. To achieve a robust and evenly growing culture, bacteria strains were cultivated in their respective media at 37 °C at least three times before each experiment. For the prebiotic score, the *Escherichia coli* was provided by the Hammami laboratory while the Luria-Bertani broth was prepared as described by Sezonov and colleagues (Sezonov et al., 2007).

2.3 Arabinoxylan fiber dissolution and enzyme digestion

0.3g of wheat AX (LAX, MAX, or HAX) was dissolved according to the supplier's recommendations: 2.4 ml of 95% ethanol was added, followed by 27 ml of sterilized water, and then stirred using a magnetic stirrer at 100°C until the fiber completely dissolved. The solution was allowed to cool, and the volume was adjusted to 30 ml. Then, 200 µL of either enzyme or 100 µL of each enzyme were added to the solutions, which were incubated at 40°C for 24 h. Enzymatic reactions were terminated by heating the solutions at 95°C for 5 minutes. The solutions were subsequently centrifuged at 6428 g for 10 minutes. The resulting supernatants were transferred to test tubes and stored for the prebiotic assays. AX modified with α-L-Arabinofuranosidase B25 from *Bacteroides ovatus* was coded B25-AX, AX modified with α-L-arabinofuranosidase from *Bifidobacterium adolescentis* was coded α-L-AX, and AX modified with both enzymes were coded B25- α-L-AX, with AX being either LAX, MAX or HAX.

2.4 Microbial growth assays

The strains were grown in a suitable medium in a 96-well microplate (Randor, PA, USA). Media with or without inoculum microorganisms were used as respective positive and negative controls. The other wells were filled with 100 µL of AX sample, 50 µL of bacteria culture, and 100 µL of suitable media. The microplates were then incubated at 37°C for 48 hours. Samples were collected at different time intervals (0, 8, 16, and 24 h for LGG; 0, 14, 24, and 40h for *F. prausnitzii*) for colony counting using the drop plate technique. After serial dilution, 20 µL drops of each sample were plated in triplicate on the appropriate agar plate medium and incubated anaerobically at 37°C for 48 h, and the results were expressed as log colony forming units (CFU) per mL (log CFU/mL).

2.6 Prebiotic score of AX

A quantitative prebiotic score was calculated to assess the extent to which each AX supported the selective growth of *F. prausnitzii* or *L. rhamnosus* GG against an enteric strain (*E. coli*). The prebiotic assay was performed as described by (Huebner et al., 2007) with slight modifications. Briefly, unmodified and modified LAX, MAX, and HAX, and glucose were added to either FAB and inoculated with *F. prausnitzii*, to MRS and inoculated with *L. rhamnosus* GG, or to Luria-Bertani broth and inoculated with *E. coli*. The mixtures were homogenized vigorously and incubated at 37 °C for 48h. Afterward, bacterial enumeration was done on either MRS, FAB, or MacConkey agar, according to the bacterial strain, and the results were expressed as log CFU/mL. The prebiotic activity score was then determined using the following formula (Huebner et al., 2007; Zhang et al., 2018):

$$\begin{aligned} Prebiotic\ score &= \left[\frac{Count_{test\ strain\ on\ AX\ at\ 48h} - Count_{test\ strain\ on\ AX\ at\ 0h}}{Count_{test\ strain\ on\ glucose\ at\ 48h} - Count_{test\ strain\ on\ glucose\ at\ 0h}}\right] \\ &- \left[\frac{Count_{E.\ coli\ on\ AX\ at\ 48h} - Count_{E.\ coli\ on\ AX\ at\ 0h}}{Count_{E.\ coli\ on\ glucose\ at\ 48h} - Count_{E.\ coli\ on\ glucose\ at\ 0h}}\right] \end{aligned}$$

where $Count_{(X)}$ is the result of bacterial enumeration (in log CFU/mL) for a specific strain (*F. prausnitzii*, *L. rhamnosus* GG, or *E. coli*) in a specific medium (AX or glucose) and at a specific time (0 or 48 h). A positive score reflects the prebiotic activity of the test substrate, and higher scores among substrates reflect the greater prebiotic activity of these substrates.

2.7 Short-chain fatty acids quantification

The concentrations of short-chain fatty acids (SCFAs), such as butyric, acetic, and propionic acids, were assessed using gas chromatography with a Flame Ionization Detector (GC-FID) (Mottawea et al., 2020). Standards (MilliporeSigma, Oakville, ON, Canada) were used for peak identification and quantification, and data expressed in mM. All the samples were analysed twice (two technical measures).

2.8 Statistical analysis

GraphPad Prism v8.3 was used for statistical analyses. To evaluate the statistically significant difference ($P < 0.05$) among experimental variables, a two-way analysis of variance (ANOVA) was used with treatment and time as the main factors, followed by Tukey's multiple comparisons. The data were expressed as duplicate experiments' mean (standard error mean) for the analysis of short-chain fatty acids while data from bacterial population count were expressed as triplicate experiments' mean ± standard deviation (SD).

3. Results

3.1. Impact of AX on the growth of *Faecalibacterium prausnitzii*

F. prausnitzii grew comparably in all solutions of unmodified and modified AX and across all molar masses, reaching maximum population density between 14 and 24 h (Figure 4), with B25-MAX showing the highest population density with 9.96 ± 0.07 log CFU/mL at 14h (Figure 4C). Comparatively, growth with the control medium was significantly slower (population density significantly lower at 14 and 24h, $p < 0.05$), and maximum population density was reached after 40 h of incubation (9.47 ± 0.16 log CFU/mL). A significant population density decrease was observed at 40 h with all AX-containing media, except for B25+α-L-AX, for which a non-significant decrease was observed, and that exhibited population density similar to the control (Figure 4).

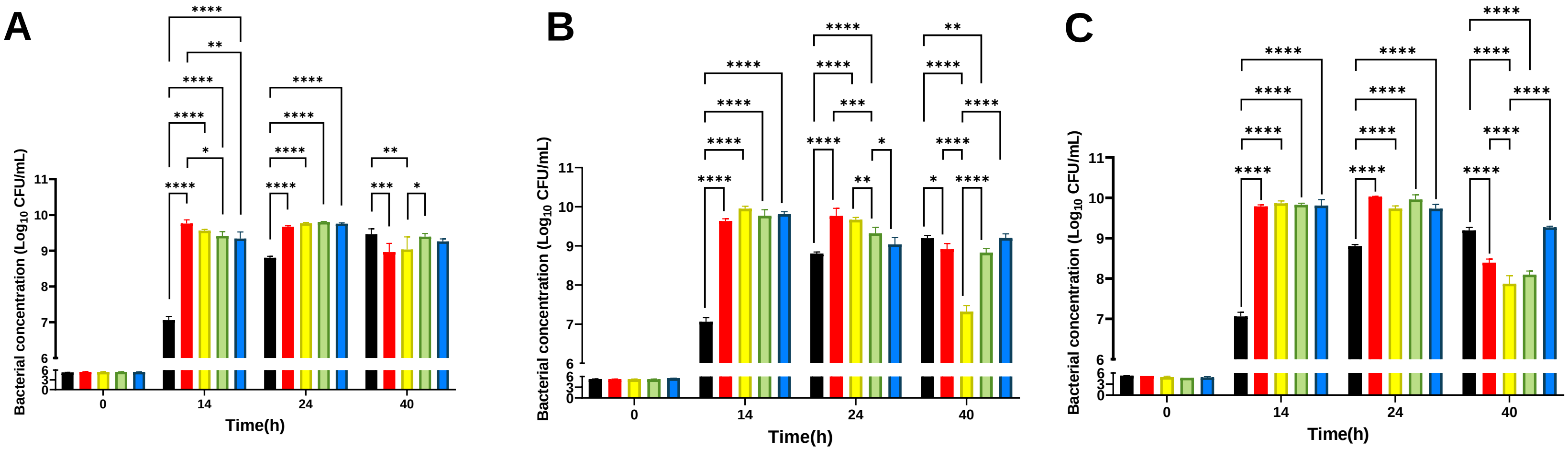

Figure 4. _Bacterial population density (log CFU/mL) of Faecalibacterium prausnitzii in the presence of LAX (A), MAX (B), and HAX (C) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$). Control (black), unmodified AX (red); B25-AX (yellow); α-L-AX (green); and B25-α-L-AX (blue)._ Data are the mean of triplicates; vertical error bars represent the standard error of the mean.

3.2. Impact of AX on the growth of *Lacticaseibacillus rhamnosus* GG

L. rhamnosus grew comparably in all solutions of unmodified and modified AX and across all molar masses, reaching maximum population density between 16 and 24 h (Figure 5), with B25-LAX showing the highest population density with 10.05 ± 0.04 log CFU/mL at 24h (Figure 5A). Comparatively, growth with the control medium was significantly slower (population density significantly lower at 8, 16, and 24 h, $p < 0.05$), and maximum population density was reached after 24 h of incubation (9.21 ± 0.04 log CFU/mL significantly lower than all AX-media). Finally, all α-L-AX induced a significantly higher population density than unmodified and modified LAX and MAX (Figures 5A and 5B).

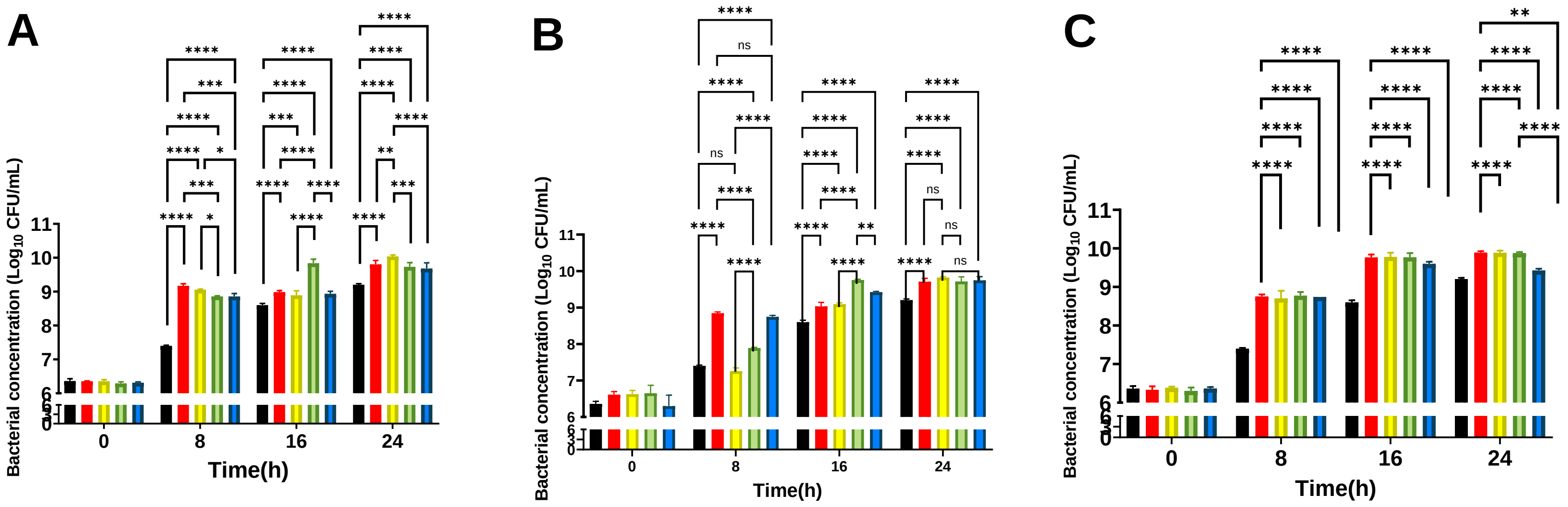

Figure 5. *Bacterial population density (log CFU/mL) of Lacticaseibacillus rhamnosus GG in the presence of LAX (A), MAX (B), and HAX (C) (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001). Control (black), unmodified AX (red); B25-AX (yellow); α-L-AX (green); and B25-α-L-AX (blue).* Data are the mean of triplicates; vertical error bars represent the standard error of the mean.

3.3. Impact of AX on the production of short-chain fatty acids by *Faecalibacterium prausnitzii*

For most SCFA produced by *F. prausnitzii*, concentration slightly increased from 14 to 24 h although it was not significantly different (p >0.05), it tended to be higher than the control, with acetate being the most abundant metabolite, ranging between 2 and 5 mM (Figure 6A). Acetate concentrations at 14 h were not significantly different across all AX, whereas acetate concentrations from unmodified LAX, B25-LAX, B25-HAX, and α-L+B25 LAX were slightly higher than from the control. At 24 h, the acetate concentration from most AX were significantly different from the control and 1.48 to 2.03 times greater than the control (Figure 6A). Butyrate concentrations were about ten times as low as acetate concentrations overall. Nevertheless, butyrate concentrations from all AX were higher than from the control at 14h (Figure 6B), with the highest production from B25-HAX. However, no significant difference was observed across test samples in butyrate concentration after 24 h (Figure 6B). Finally, isovalerate concentration was low across all samples and throughout the test, and it increased non-significantly from 14 to 24 h (Figure 6C).

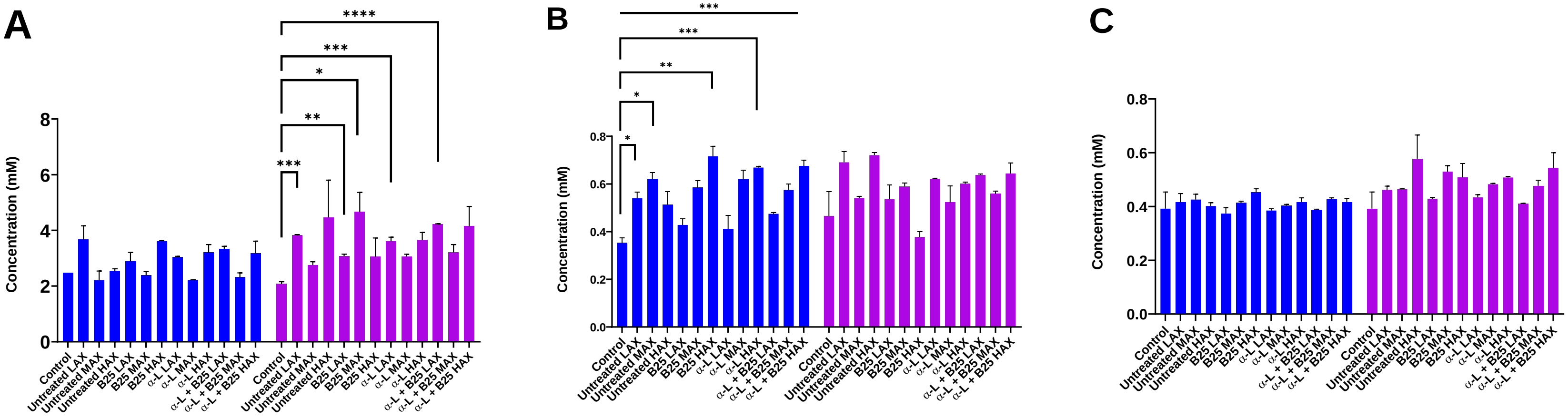

Figure 6. *Concentrations of acetate (A), butyrate (B), and isovalerate (C) produced by Faecalibacterium prausnitzii at 14h (blue) and 24h (magenta) of fermentation.* Data are the mean of duplicates; vertical error bars represent the standard error of the mean. (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

3.4. Impact of AX fibers on the production of short-chain fatty acids by *Lacticaseibacillus rhamnosus* GG

As with *F. prausnitzii*, acetate was the most abundant metabolite produced by *L. rhamnosus* GG, ranging from 30 to 70 mM. Most AXs induced a significant increase in acetate production over the control from 8 to 16 h, with α-L-LAX and α-L+B25-LAX having the greatest effect (Figure 7A). Furthermore, the amount of acetate produced by *L. rhamnosus* GG was significantly higher with LAX treatments as compared to MAX and HAX treatments (Figure 7A). Butyrate and isovalerate were sparsely produced, ranging from 0.3 to 1.2 mM and from 0.4 to 0.8 mM, respectively (Figures 7B and 7C). No significant differences in butyrate and isovalerate concentrations were found between treatments, this can be attributed to the low concentrations observed.

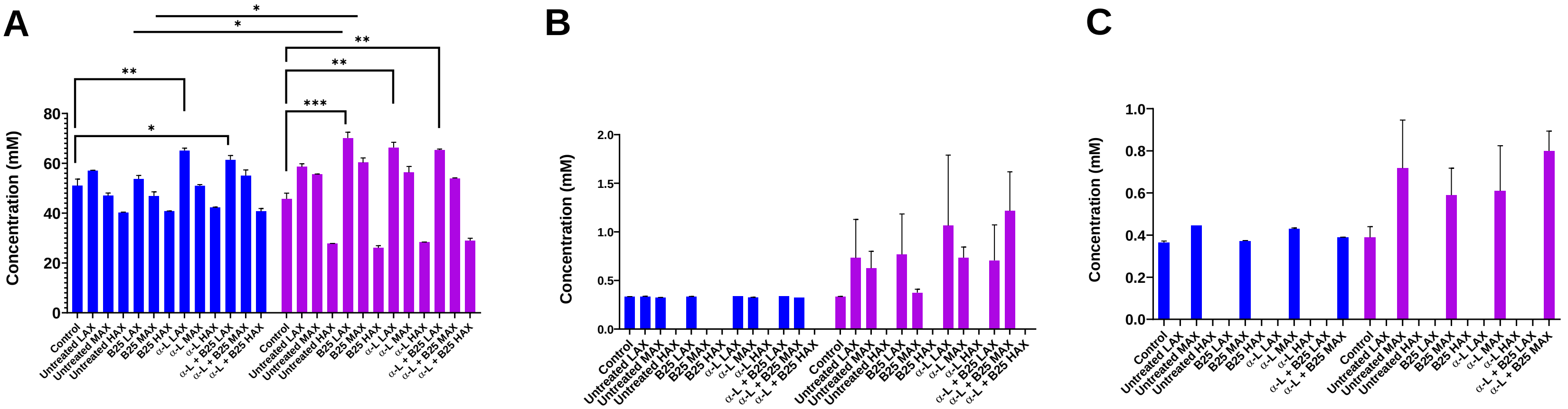

Figure 7. *Concentrations of acetate (A), butyrate (B), and isovalerate (C) produced by Lacticaseibacillus rhamnosus GG at 14h (blue) and 24h (magenta) of fermentation.*

Data are the mean of duplicates; vertical error bars represent the standard error of the mean. (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

3.5. Prebiotic scores of AX with *Faecalibacterium prausnitzii* and *Lacticaseibacillus rhamnosus* GG

All unmodified and modified AXs showed positive activity scores for *F. prausnitzii* and *L. rhamnosus* GG (Table 3). The overall highest score was observed for B25-LAX used with *L. rhamnosus* (0.56 ± 0.02). Similarly, B25- LAX had the highest prebiotic score of all AX types when used with *F. prausnitzii* (0.47 ± 0.04). The lowest overall score was for α-L-HAX used with *L. rhamnosus* (0.16 ± 0.02). In addition, ANVOA showed that the molar mass of AX significantly influenced prebiotic scores, with average scores being significantly different between LAX, MAX, and HAX treatments for the two bacterial strains ($p < 0.05$).

Table 3. Prebiotic scores of unmodified and modified AX with *F. prausnitzii and L rhamnosus* GG. *Values are expressed as the mean ± standard deviation of duplicates. Results of ANOVA between AX types are indicated by superscript uppercase letters within each column. ANOVA results between F. prausnitzii and L. rhamnosus GG are indicated by superscript lowercase letters within each row.* Values sharing a letter are not significantly different ($p < 0.05$).

Type of AX	***Faecalibacterium prausnitzii***	***Lacticaseibacillus rhamnosus s* GG**
LAX	0.25 ± 0.05^{BCDb}	0.41 ± 0.02^{Ba}
B25-LAX	0.47 ± 0.04^{Ab}	0.56 ± 0.02^{Aa}
α-L-LAX	0.31 ± 0.04^{BCDb}	0.52 ± 0.02^{Aa}
B25+α-L-LAX	0.38 ± 0.06^{ABa}	0.46 ± 0.06^{Ba}
MAX	0.24 ± 0.03^{CDb}	0.33 ± 0.02^{Ca}
B25-MAX	0.25 ± 0.05^{CDb}	0.35 ± 0.02^{Ca}
α-L-MAX	0.36 ± 0.04^{ABCa}	0.33 ± 0.03^{Ca}
B25+α-L-MAX	0.24 ± 0.03^{CDb}	0.33 ± 0.02^{Ca}
HAX	0.24 ± 0.07^{CDa}	0.18 ± 0.02^{Da}
B25-HAX	0.23 ± 0.06^{CDa}	0.20 ± 0.03^{Da}
α-L-HAX	0.23 ± 0.03^{Da}	0.16 ± 0.02^{Da}
B25+α-L-HAX	0.30 ± 0.03^{BCDa}	0.19 ± 0.02^{Db}

4. Discussion

Among the AX samples, LAX showed the highest prebiotic potential on *L. rhamnosus* and *F. prausnitzii,* while B25-treated LAX exhibited the best individual prebiotic effect on the two bacterial strains. Observations based on our findings demonstrated that molecular weight and prebiotic impact are often inversely correlated because the lower the molecular weight of AX, the greater its prebiotic effect. This data was anticipated, given that earlier studies suggested that reduced molecular weight polysaccharides exhibit improved bioactivity and encourage the proliferation of gut microbiota (J. Gao et al., 2017; Wei et al., 2022; Zhao et al., 2012). Furthermore, structural differences, degree of polymerization, or substitution patterns can influence how samples promote bacterial growth (Paesani et al., 2019). As observed from our results after the viable cell count, the AX treated with the combination of both enzymes, which resulted in zero arabinose substitution units, had a consistent impact on the growth of *F. prausnitzii* across all levels of molecular weight. Conversely, in most cases, AX treated with only α-L- arabinofuranosidase enzyme exhibited better growth on *L. rhamnosus* . Similarly, Paesani and colleagues found that the molecular size and crosslinking patterns of water-extractable arabinoxylan impacted the growth selectivity of several bacterial strains (Paesani et al., 2020). Also, gut bacterial species have preferential dietary fiber substrates primarily depending on the enzymes they can produce and the sugar monomers they metabolize, which affects the composition of their secondary metabolites (Louis, 2017). In addition, we observed a slight decrease in the impact of bacterial growth after 40h for *F. prausnitzii*. Although the circumstances leading to this are unclear, we hypothesize that the AX was rapidly consumed by the bacterial strain, leaving a limited amount of substrate available toward the end of the experiment. Our assertion is consistent with our finding, which showed that *F. prausnitzii* reached maximum growth at 14 h for all AX-containing samples. Therefore, in order to sustain higher growth for a more extended period, it is only logical to increase the amount and concentration of the substrate (AX). Likewise, Huang and coauthors reported that bacterial proliferation increased as the concentration of polysaccharides increased from 0.5% to 2% (F. Huang et al., 2019). The prebiotic activity score for our study was similar to that reported in several studies for *Lactobacillus* (Lee et al., 2021; Mondal et al., 2022; Paesani et al., 2019, 2020; Vaz Rezende et al., 2022; Zhang et al., 2018) and other gut microbes such as *Bifidobacterium* (Lee et al., 2021; Mondal et al., 2022; Paesani et al., 2019, 2020; Vaz Rezende et al., 2022; Zhang et al., 2018), *Bacteroides* (Paesani et al., 2019, 2020). More specifically, after incubation with pearl millet oligosaccharides (PMOs), Mondal and colleagues observed a prebiotic activity score for *L. rhamnosus* that was comparable to what was seen in our investigation. (Mondal et al., 2022).

Short-chain fatty acids are one of gut microbes' main by-products of carbohydrate fermentation. SCFAs reduce the risk of infectious gastrointestinal disorders by preventing the growth of pathogenic microorganisms, which in turn promotes the absorption and bioavailability of some nutrients (Mondal et al., 2022; Ríos-Covián et al., 2016). Of the three short-chain fatty acids produced, acetate was the most abundant in our study. This aligns with a recent study where the researchers reported acetate to be the most abundant SCFA produced by gut microbes after fermentation (Demuth et al., 2021; Mondal et al., 2022). Acetate has additional benefits that include reducing cognitive decline in mice (H. Zheng et al., 2021). Among all, *L. rhamnosus* inoculated with α-L-treated LAX produced the highest amount of acetate at the end of 14 h, while B25-treated LAX induced the highest amount of acetate at 24 h. Comparatively, we observed a significant difference between the control and five other test samples (untreated LAX, B25 LAX, B25 MAX, α-L LAX, α-L+B25 LAX) at 24 h. Among these five samples, four of them are LAX fibers. Previous research studies have reported that low molecular weight polysaccharides show better bioactivity and promote the growth of gut bacteria (J. Gao et al., 2017; Wei et al., 2022; Zhao et al., 2012). HAX fibers produced the lowest amount of acetate for *L. rhamnosus.* Butyrate and isovalerate were produced in limited amounts. We observed an increase in the production of these SCFAs. However, the increase was not statistically significant. Moreover, *L. rhamnosus* is not a butyrate-producing bacteria, but the traces of butyrate produced during this study is intriguing and might suggest that the suitable substrate can influence the production of butyrate by the bacterial strain. This finding is consistent with a recent study by Mondal and coauthors, who reported that different *Lactobacillus* species produced low amounts of butyric acid when cultured with pearl millet oligosaccharides (PMOs). Likewise, their study showed a slight increase in butyric acid produced from 24 h to 48 h (Mondal et al., 2022). The butyrate produced by *F. prausnitzii* significantly differed between the control and some test samples at 14 h. HAX fibers treated with an α-L-Arabinofuranosidase B25 enzyme produced the most butyrate at 14 h. Butyrate is regarded as the most important SCFA because of its antidepressant properties (Hao et al., 2019) and its impact on other psychiatric and neurological disorders, such as anxiety, depression, autism, Parkinson, and Alzheimer's (Silva et al., 2020). Besides its antidepressant and psychiatric functions, it is the primary source of energy for the colonocytes (Mendez-Encinas et al., 2018; Rivière et al., 2016) and is important in the management of gastrointestinal disorders, hemoglobinopathies, and colorectal cancer (J. Huang et al., 2018). Our result shows a promising sign that the wheat AX fibers induced the production of this important metabolite. Similarly, isovalerate (isovaleric acid) is a product of the microbial fermentation of leucine in the colon and serves as an energy source for the colon. When it enters the bloodstream, it helps to control the metabolism of cholesterol, glucose, and fatty acids (Blakeney et al., 2019; Niccolai et al., 2019). In this study, bioengineered AX fibers increased isovalerate production by both *F. prausnitzii* and *L. rhamnosus* , but the differences were non-significant.

5. Conclusion

L. rhamnosus and F. prausnitzii were reported to be inversely correlated with depressive disorders, with *L. rhamnosus* being widely recognized as a probiotic to date. Low molecular weight wheat arabinoxylan fibers (LAX) showed a better prebiotic effect than MAX and HAX on these key microbes. In most cases, regardless of the treatments, LAX had a greater prebiotic effect than MAX and HAX (LAX > MAX > HAX). Also, LAX fiber treated with α-L-arabinofuranosidase B25 enzyme exhibited the highest prebiotic potential on both bacterial strains. Almost all treated AX had a better prebiotic score than the untreated AX across all levels. These findings suggest that novel food prototypes, including altering the chemical structure of AX, may be an important method to improve the availability and growth of depression-inversely linked microbes in the gut microbiome. Furthermore, wheat arabinoxylan fibers treated with either α-L-arabinofuranosidase B25 enzyme or α-L-Arabinofuranosidase enzyme had a more consistent effect on the growth of *L. rhamnosus,* while those treated with the enzyme combination showed a better effect on *F. prausnitzii*. This result also supports and aligns with studies that certain bacterial species may have a preferred chemical composition or structure for their substrate for optimum growth and metabolism. The observations in the present study imply that bioengineered wheat arabinoxylan can modulate the gut microbiome through the production of SCFAs, and foster the proliferation of beneficial gut microbes, which in turn promotes the host's health. The use of bioengineered wheat arabinoxylan could be considered a promising strategy for fostering next-generation prebiotics targeting depression-inversely linked gut microbes.

Authors' contributions

NB and RH conceived and designed the study. ENN, HH, WM, and AG acquired the data. ENN, HH, WM, AG, and RH analyzed and interpreted the data. ENN drafted the manuscript. WM, NB, and RH revised the article. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

Author NB is a member of the Scientific Advisory Board of Agrifiber Solutions LLC (Mundelein, IL).

Acknowledgments

This study was supported by a strategic grant from the University of Ottawa to NB and RH. ENN was supported by the Nutrition and Mental Health Master's Scholarship, University of Ottawa.

CHAPTER 4: ON THE PREBIOTIC CAPACITY OF NOVEL BIOENGINEERED WHEAT ARABINOXYLANS IN A BATCH CULTURE MODEL OF THE HUMAN GUT MICROBIOME

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Abstract

Arabinoxylan (AX) is an essential component of dietary fiber with potential prebiotic properties. However, owing to its complex structure, fermentation of AX by gut microbes is structure-dependent. In this study, we evaluated the effect of bioengineered wheat arabinoxylan on the metabolism and composition of the gut microbiota using an *in vitro* fermentation model. We compared the effect of bioengineered arabinoxylan with that of untreated arabinoxylan and a control. Structurally modified AX did not significantly alter gut microbiome composition within 48 h of treatment. However, it enhanced the abundance of health-promoting bacterial taxa such as *Bacteroides, Bifidobacterium, Anaerofustis, and Eubacterium.* Furthermore, the bioengineered AX significantly increased the level of acetate produced over 24 h. The amount of microbiota-generated butyrate was significantly increased 24 h after adding α-L-arabinofuranosidase-treated AX. AX treated with the α-L-arabinofuranosidase B25 enzyme induced higher production of total short-chain fatty acids by the microbiota from four donors. The results of this study provide evidence that enzymatic-structural modification of AX has the potential to modulate gut microbiome composition and metabolic activities.

Keywords: Gut microbiome, enzymatic bioengineering, wheat arabinoxylan, prebiotics, prebiotic effect, short-chain fatty acids, *in vitro* fermentation.

Introduction

The human gut is home to a highly diverse ensemble of microorganisms, which comprises approximately 10^{14} microbial cells from more than 1000 species and contains nearly 100 times as many genes as the human genome (Demuth et al., 2021; Qin et al., 2010). It has enormous metabolic capability (Putignani et al., 2014); hence, it plays a significant role in an individual's health and overall well-being. Microbial dysbiosis refers to a change in the composition of the human gut microbiota from a generally diversified and commensal microbial community to a more maladaptive and pathogenic profile (Keightley et al., 2015; Patterson et al., 2014). For example, using some common antidepressants and poor dietary feeding patterns can negatively alter the gut microbiome composition and reduce the production of beneficial metabolites by gut microbes (Ait Chait et al., 2020; Beam et al., 2021). Furthermore, Jones and coauthors characterized dysbiosis as an increase in the population of adherent and/or invasive *Escherichia coli* as well as a decrease in *Bacteroidetes* and *Firmicutes* phyla, including the clinically meaningful *Faecalibacterium prausnitzii* (Jones et al., 2014).

Dietary fibers are one of the core components of food that strongly influence the composition and activity of the gut microbiome (Romero Marcia et al., 2021). Hence, ingesting dietary fiber is key to a healthy diet and diverse gut microbiome (Demuth et al., 2021). In addition, dietary intervention studies have recently explored diet as an avenue to modulate the composition and function of gut microbes in the gastrointestinal tract (D. Li et al., 2019; Mottawea et al., 2020; Rinninella et al., 2019). However, it is essential to note that not all dietary fibers are prebiotics. To be considered a prebiotic, dietary fiber must illicit certain health benefits to the host and support the growth of beneficial gut microbes (Gibson et al., 2017). In addition to modulating the GI tract and enhancing the availability of beneficial gut microbes, prebiotic dietary fibers are important for improving the metabolic profile of the gut microbiome. Numerous studies have revealed that short-chain fatty acids are produced by the fermentation of prebiotic dietary fibers by human colonic microbes (Demuth et al., 2021; Fehlbaum et al., 2018a; Marín-Manzano et al., 2020; Zhu et al., 2022). These SCFAs regulate metabolic syndrome, serve as important energy sources for colonic epithelial cells, and confer health benefits to the host. Acetate, butyrate, and propionate are the most dominant SCFAs produced by the gut microbiota during the fermentation of prebiotic fibers (Ding et al., 2019; X. Fu et al., 2018).

Arabinoxylan is one of the most common dietary fibers available in various cereals, including wheat, corn, and millet sorghum (Hughes et al., 2007). Wheat remains one of the primary sources of daily dietary fiber consumption in western diets (Stevenson et al., 2012), considering that wheat is widely accessible at a moderate cost (Deroover et al., 2020). Furthermore, arabinoxylans have garnered considerable attention owing to their potential prebiotic properties, which can be affected by their complex structural features (Sun et al., 2019). Rose and colleagues have also identified wheat arabinoxylan as a candidate prebiotic (Rose et al., 2010b). Several studies have revealed that slight structural differences in prebiotic dietary fibers can affect gut microbiota (Deehan et al., 2020; Tuncil et al., 2020), and these structural variations may target certain microbial species (Cantu-Jungles & Hamaker, 2020; Louis, 2017), thus affecting the production of SCFAs. Although arabinoxylan has some favorable health-promoting properties, it is still uncertain how its enzymatic structural modification influences the human gut microbiota and SCFA production. Previous studies have demonstrated the influence of prebiotics and arabinoxylan on the human gut microbiota (Demuth et al., 2021; Fehlbaum et al., 2018b; Gu et al., 2020; Holgado et al., 2022). However, the effect of enzymatic structural modification of arabinoxylan on the gut microbiota composition and potentially beneficial microorganisms found within is yet to be fully explored.

In the present study, we investigated the impact of enzymatic-structural modification of wheat arabinoxylan on the composition and metabolism of the human gut microbiome using batch culture and an *in vitro* fermentation model. First, α-L-arabinofuranosidase B25 and α-L-arabinofuranosidase enzymes were used for the structural modification of the AX fiber. Individual treatment of AX with α-L-arabinofuranosidase B25 enzyme produces only L-arabinofuranosyl units on doubly substituted β-D-xylopyranosyl units, whereas treatment with α-L-arabinofuranosidase enzyme produces only L-arabinofuranosyl units on singly substituted β-D-xylopyranosyl units. Both untreated and treated AX were subjected to *in vitro* fecal fermentation for 48 h, and the microbiota composition was assessed using 16s-RNA sequencing, while metabolic activity was evaluated by quantifying SCFAs produced using gas chromatography.

2. Materials and methods

2.1 Arabinoxylan fibers, enzymes, and standards

Wheat arabinoxylan with a high (cat no. P-WAXYH, viscosity:56cSt; hereafter termed HAX), medium (P-WAXYM, viscosity:31cSt; hereafter termed MAX), and low (P-WAXYL, viscosity:13cSt; hereafter termed LAX) molecular weights, along with α-L-arabinofuranosidase B25 enzyme (product code: E-ABFBO25) from *Bacteroides ovatus* and α-L-arabinofuranosidase enzyme (product code: E-AFAM2) from *Bifidobacterium adolescentis* were purchased from Megazyme International (Ireland). The internal standard 2-Ethylbutyric acid and the external standard Volatile Free Acid Mix used for short-chain fatty acid analysis were purchased from MilliporeSigma (Oakville, ON, Canada).

2.2 Arabinoxylan fiber dissolution and enzyme digestion

0.3 g of wheat AX fiber (LAX, MAX, or HAX) was weighed into a sterilized conical flask. To ensure complete fiber dissolution, 2.4 ml of 95% ethanol was added, followed by 27 ml of sterilized water, and the fiber was stirred using a magnetic stirrer at 100°C until complete dissolution. The solution was allowed to cool and the volume was adjusted to 30 ml.

Subsequently, 200 μL of α-L-arabinofuranosidase B25 was added to obtain fibers with doubly substituted xylose units, and 200 μL of α-L-arabinofuranosidase was added to obtain fibers with singly substituted xylose units. The samples were incubated at 40°C for 24 h, followed by termination of the enzymatic reaction by heating the samples at 95°C for 5 min and subsequent centrifugation at 7,400 rpm for 10 min. The resulting supernatants were transferred to test tubes and stored for in vitro fecal fermentation experiments.

2.3. Fecal sample collection and cell immobilization in gel beads

Fecal samples were obtained from four healthy adult donors (2 males and 2 females) without any known medical conditions, who had not been exposed to antibiotic treatment, antidepressant treatment, or probiotic or prebiotic supplements for at least three months prior to sample collection. The collection of fecal samples was approved by The University of Ottawa Research Ethics Board and Integrity (Ethics file number: H-02-18-347; Approval date:05/03/2018). The feces were processed into slurries by dilution in reduced peptone water (20%, w/v), homogenized, and further immobilized in 1–2 mm gel beads consisting of gellan gum (2.5%, w/v), xanthan (0.25%, w/v), and sodium citrate (0.2%, w/v) under anaerobic conditions, as described previously (Le Blay et al., 2012). The immobilized microbial population from each donor was used to inoculate one bioreactor that was run in continuous fermentation mode for 12 days to develop a stable, highly diverse microbiome community.

2.4. Experimental setup and fermentation procedure

2.4.1. Culture medium

MacFarlane broth was used as the nutrient medium in this experiment. Macfarlane broth is a complex nutritional medium that closely replicates the nutrients found in the large intestine of healthy adults (Macfarlane et al., 1998).

2.4.2. Ex-vivo development of fecal microbiome community

Continuous fermentation was carried out for 12 days using an *ex vivo* model mimicking the human proximal colon (N*u*GUT Research Platform, University of Ottawa), as previously described (Mottawea et al., 2020; Mousavi, Mottawea, Audet, et al., 2022). The experimental setup consisted of four bioreactors. Each reactor was set up to mimic the microbiological and physiological conditions of the adult proximal colon (pH 5.7, stirring at 120 rpm, 37°C, and a mean retention time of 8 h). Anaerobiosis was ensured through continuous headspace flushing of N_2 and CO_2 at a ratio of 0.9:0.1, while the addition of NaOH (2.5M) maintained a constant pH of 5.7. The fermentation process was initiated by inoculating 60 mL of immobilized gel beads into each of the four bioreactors containing 140 mL of newly prepared sterile MacFarlane culture medium, as previously described (Mousavi, Mottawea, Audet, et al., 2022). The colonic model was run in batch culture fermentation mode for 48 h to enhance bead colonization. After 48 h, the colonic model was switched to continuous mode for the remaining 12 days for microbiota stabilization. Subsequently, the developed microbial community was employed for *in vitro* testing of the impact of the treated and untreated AX fibers.

2.4.3. In-vitro fecal fermentation of treated and untreated AX

The microbiota was derived from four independent bioreactors containing microbiota from four different individuals. In vitro fecal fermentation was performed using MacFarlane medium (Macfarlane et al., 1998). MacFarlane medium without fibers was prepared as described by (Demuth et al., 2021) with slight modifications. The mixture of AX and the medium solution was added to 24 well plates and inoculated with the cultivated microbiota at an inoculation rate of 1% (v/v) and a final fermentation volume of 2 ml. The plates were incubated anaerobically at 37°C for 48 h. The samples (2 ml) were collected at 0, 6, 12, 24, and 48 h. The collected samples were separated by centrifugation at 14,000 g for 5 min at 4 °C. The pellet was used for metagenomic DNA extraction and the supernatant was used to determine the presence of short-chain fatty acids (SCFAs). The fermentation experiment was conducted in duplicate for each treatment per donor and per time point (figure 8)

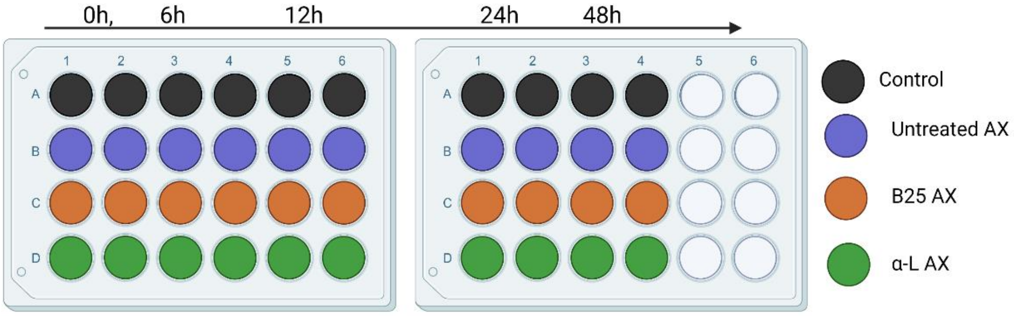


Figure 8. Experimental setup of in vitro fecal fermentation for 48h.

2.5. Microbial Community analyses

2.5.1. Genomic DNA extraction

Following the manufacturer's instructions, genomic DNA was extracted from the pellet of fecal slurry and fermentation samples using the Fast DNA Spin Kit (MP Biomedicals, Solon, OH, USA), and mechanical lysis was performed using a Bead Mill-24 homogenizer (Fisher Scientific, Ottawa, ON, Canada) as previously described (Mottawea et al., 2020). The quantity of extracted DNA was measured using a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA) and stored at −20°C until further analysis.

2.5.2. 16s rRNA Metagenomic Sequencing

Microbial community composition and diversity of the fecal slurry and fermentation samples were evaluated using 16S rRNA gene-based MiSeq sequencing (Illumina, CA, USA). The V3-V4 regions of the 16S rRNA gene were amplified using dual-barcoded primers, and an amplicon library for sequencing was constructed using the Illumina standard protocol. The amplicon libraries were pooled in equimolar amounts and paired-end sequenced using the Illumina MiSeq platform (N*u*GUT Research Platform, University of Ottawa) using the MiSeq Reagent Kit v3 (600-cycle) (Illumina; San Diego, CA, USA) according to the standard protocol.

2.5.3. Metagenomic Sequencing Data Analysis

The generated sequences were processed using the QIIME 2.2020.8 pipeline (Caporaso et al., 2010). Initially, Sequences were quality-filtered and denoised using the DADA2 pipeline (Callahan et al., 2016) and clustered into observed features based on 97%similarity using the

Greengenes database (v13.8). The observed features were rarefied into an equal number of 20,000 reads per sample using QIIME. Shannon entropy was calculated to compare alpha diversity. Beta diversity among samples was determined using the Bray-Curtis distance and visualized using Principal Coordinate Analysis (PCoA). Permutational multivariate analysis of variance (PERMANOVA) and 999 permutations (Bolyen et al., 2019) were employed to assess the contribution of different factors to gut microbiota diversity. To identify significantly different taxa, a linear mixed model analysis of the relative abundance of different taxon levels was performed (Segata et al., 2011). The treatment type served as the sample class and the time points served as the subclass for labeling the samples. Significant differential taxa were those with log10 LDA ≥ 2 and $P < 0.05$. The Kruskal-Wallis test followed by the two-stage Benjamini, Krieger, and Yekutieli false discovery rate (FDR) procedure was used for statistical analysis when needed.

2.6 Quantification of Short Chain Fatty Acids (SCFAs)

The concentrations of short-chain fatty acids (SCFAs), including butyric, acetic, and isovaleric acids, were assessed by gas chromatography with a Flame Ionization Detector (GC-FID) (Mottawea et al., 2020). All samples were analyzed twice (two technical measures). External standards (MilliporeSigma, Oakville, ON, Canada) were used for peak identification and quantification, and the data are expressed as the concentration of SCFAs in mM.

2.7. Statistical analysis

Data from Gas Chromatography analyses were analyzed using GraphPad Prism v8.3. (GraphPad Software, USA) to assess the significance of the results among treatments. Statistical comparisons were conducted among different treatments simultaneously using repeated measures of two-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test ($p < 0.05$).

3. Results

3.1. Gut Microbiota Diversity

The alpha diversity of the microbiota from *in vitro* fecal fermentation between 0 and 48 h using microplates was evaluated using Shannon entropy (figure 9). No significant differences were found between the control and AX-containing samples ($p > 0.05$). Nevertheless, the decrease in gut microbiota diversity following plate inoculation tended to be reversed faster in the untreated and AX groups than in the control group. The beta diversity across treatments, time, donor, and concentration was evaluated using principal coordinate analysis (PCoA) based on Bray-Curtis distances. The results revealed that the microbial communities of the four donors differed from one another. The microbial communities were similar at 0 h, and showed a slight shift after 0 h. PCoA plots are shown in figure 10.

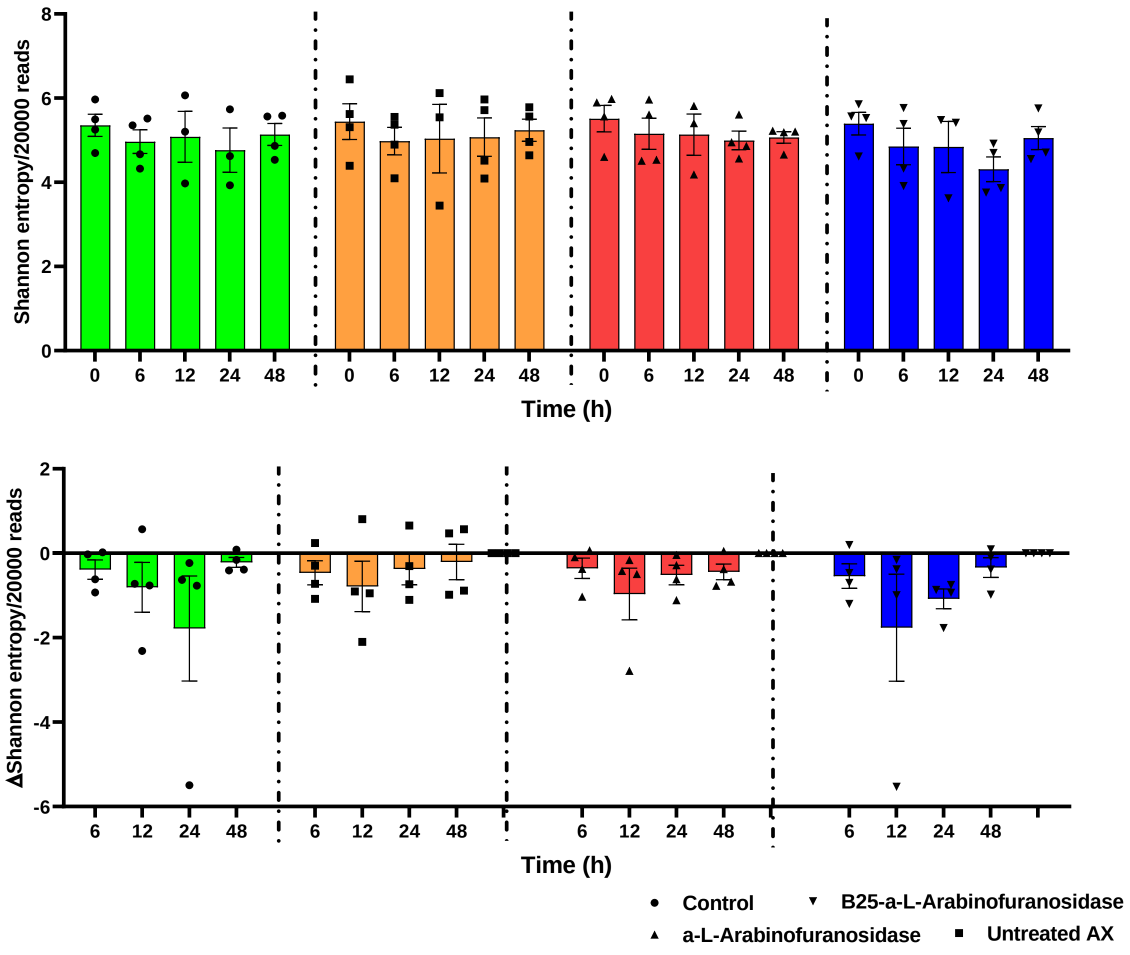


Figure 9. Shannon entropy of the identified microbiota from microplates with untreated control, α-L-arabinofuranosidase B25 enzyme-treated AX, α-L-arabinofuranosidase enzyme-treated AX, and untreated AX at different time intervals. The resulting data were analyzed using the Kruskal-Wallis test and Two-stage Benjamini, Krieger, and Yekutieli FDR procedures ($p > 0.05$). The results were calculated using rarefied 20,000 reads per sample. The middle lines represent mean values.

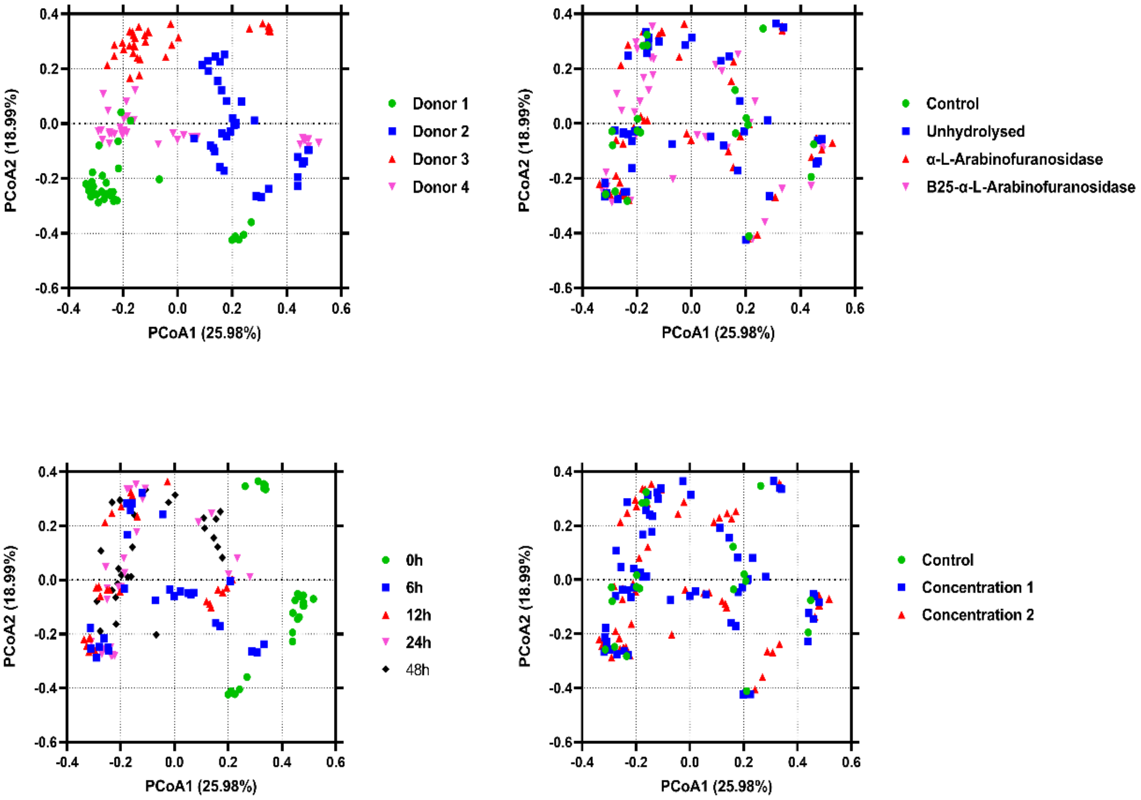


Figure 10. Plots of Principal Coordinate Analysis (PCoA) among the identified microbiota in different samples showed clustering based on the type of treatment, donor, and time. The samples are colored as indicated in the legends.

3.2. Effects of wheat arabinoxylan on the gut microbiota composition

The linear mixed model analysis results identified differential microbial taxa among the tested groups at various phylogenetic levels by adjusting for donor and time as covariates (Figures 4-6).9 taxonomy features showed significant differences, specifically the *Bacteroidetes* phylum (figure 11). Overall, 22 bacterial taxa belonging to Proteobacteria, Bacteroidetes, and Actinobacteria were significantly different at the phylum level (figure 12). At the genus level, the α-L fibers increased the abundance of *Bacteroides* and *Bifidobacterium*. On the other hand, the B25-treated fiber improved the abundance of *Anaerofustis* and *Eubacterium* compared with the control and untreated fiber groups. At the family level, *Enterobacteriaceae*, *Bifidobacteriaceae*, *Bacteroidaceae*, and *Eubacteriaceae* were the most differential taxa.

Proteobacteria, *Bacteroidetes,* and *Actinobacteria* were more abundant at the phylum level in the treated- and untreated-AX groups than in control. Figure 6 shows the effects of each AX fiber group on the microbiota composition shift and the significant bacterial taxa for each donor.

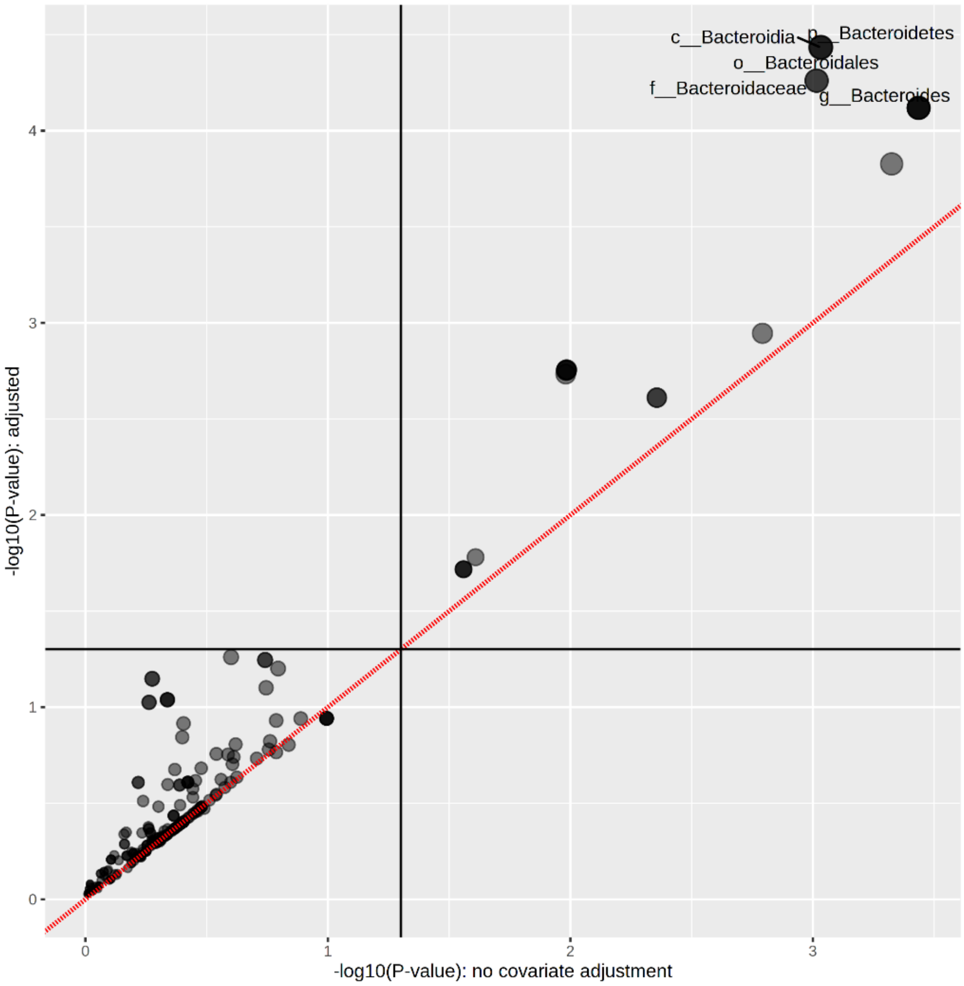


Figure 11. Graph showing significant differential taxa identified by linear mix model analysis considering donor and time as covariates.

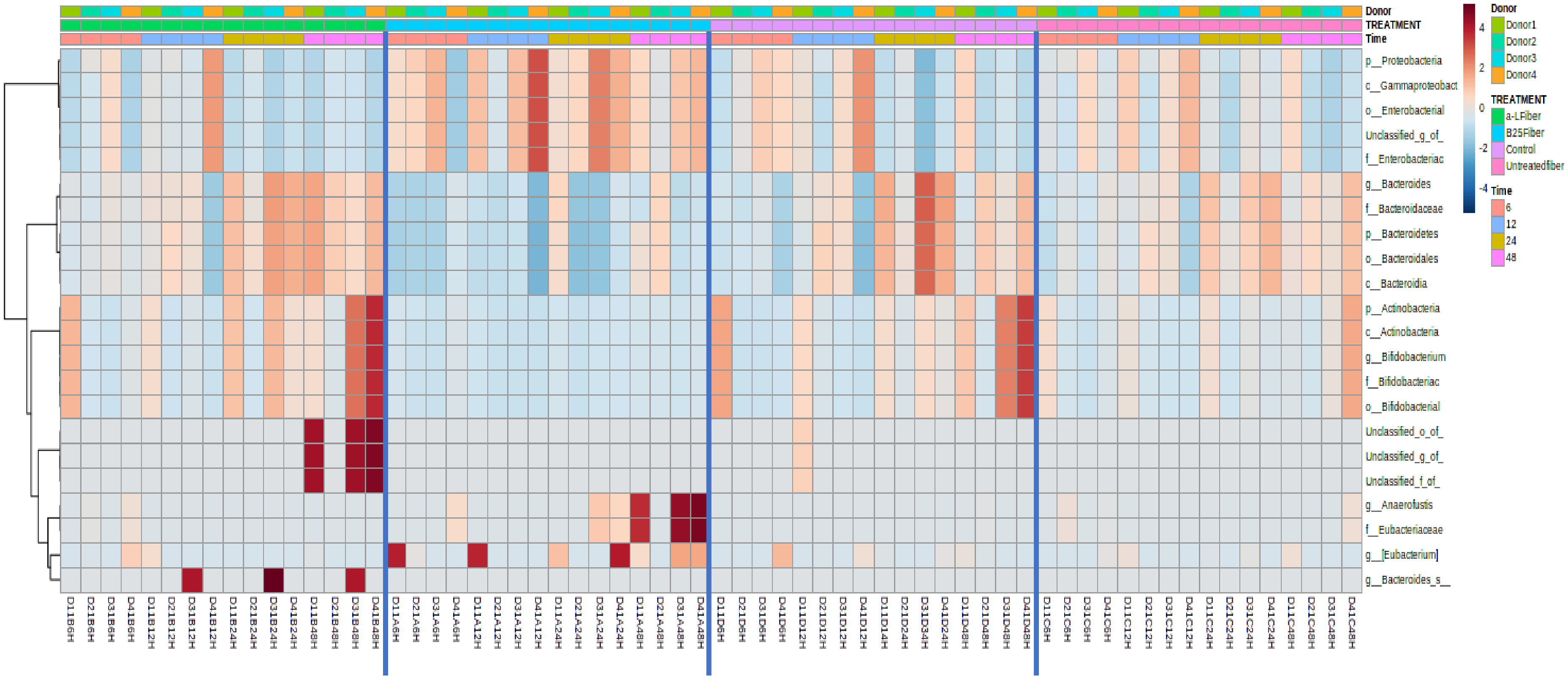
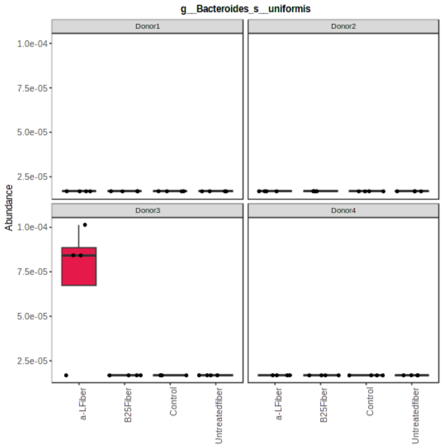
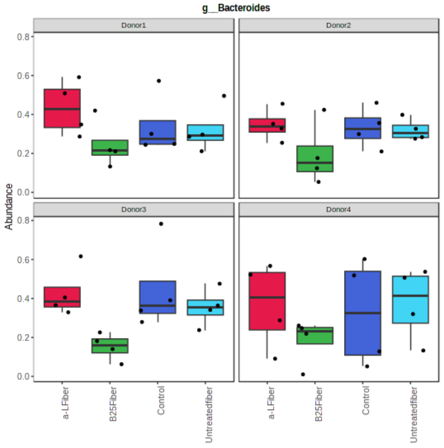
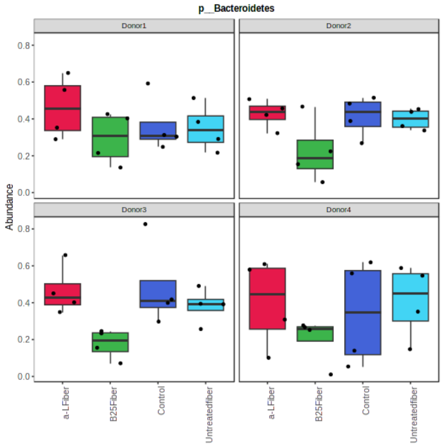
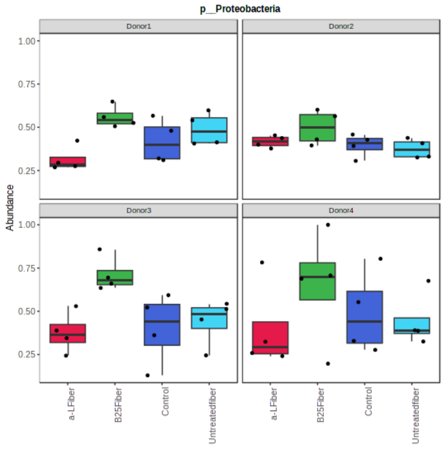


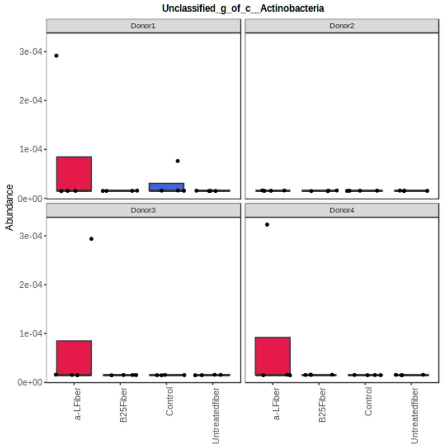
Figure 12. Heatmap showing the significant differential taxa identified by linear mix model analysis, considering donor and time as covariates. The relative abundances of each taxon were summed, normalized, and autoscaled, and the autoscaled data were used to draw the heatmap and the scale shown. The samples were arranged by treatment, time, and donor.

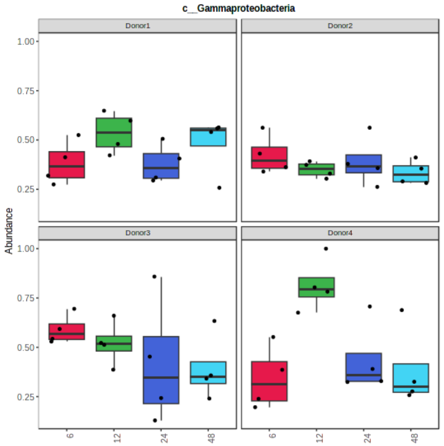

g__Bacteroides_s__uniformis

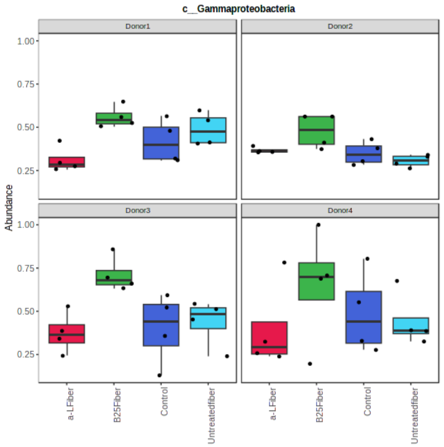

g__Bacteroides

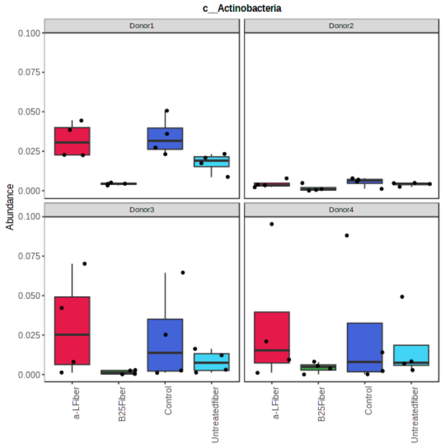

p__Bacteroidetes


p__Proteobacteria


Unclassified_g_of_c__Actinobacteria


c__Gammaproteobacteria


c__Gammaproteobacteria


c__Actinobacteria

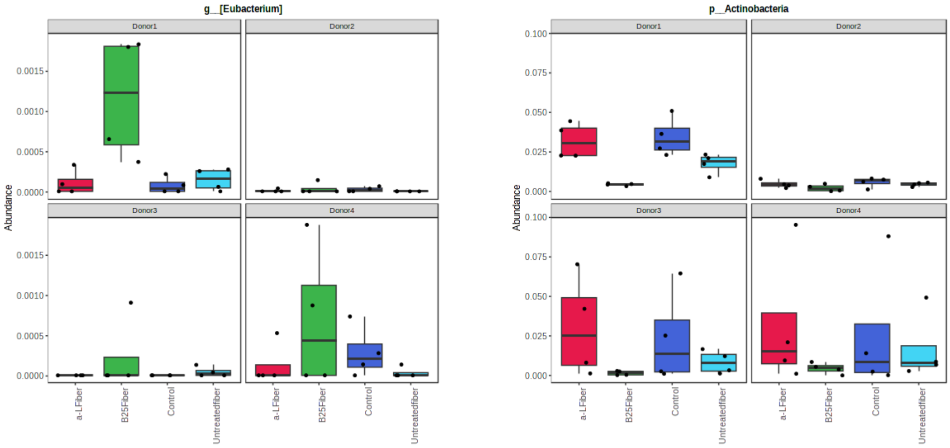


Figure 13. Histograms showing significant differential taxa identified by linear mixed model analysis. Each column and panel represents a treatment group and a donor, respectively.

3.3 Effects of wheat arabinoxylan on gut microbiota metabolism

The effect of wheat arabinoxylan (treated and untreated) on SCFA generation was quantified using GC. In all treatment groups, acetate, butyrate, and propionate were the three major SCFAs produced by gut microbes (Figure 14). Bioengineered AX significantly increased the production of these metabolites ($P < 0.05$). For example, AX treated with the α-L-arabinofuranosidase B25 enzyme increased acetate production and significantly differed from the control and untreated AX at 24 and 48 h. In comparison, treatment with α-L-arabinofuranosidase enzyme increased butyrate production, which was statistically significant at 24 h. For propionate, no significant differences were observed ($p > 0.05$) (Figure 14 C). AX treated individually with α-L-arabinofuranosidase B25 and α-L-arabinofuranosidase enzymes produced the highest number of total short-chain fatty acids from all four donors and were significantly different from the untreated AX and control (figure 15). The total SCFAs produced by both treated AXs were significantly greater than those of the untreated AX and control groups ($p < 0.05$).

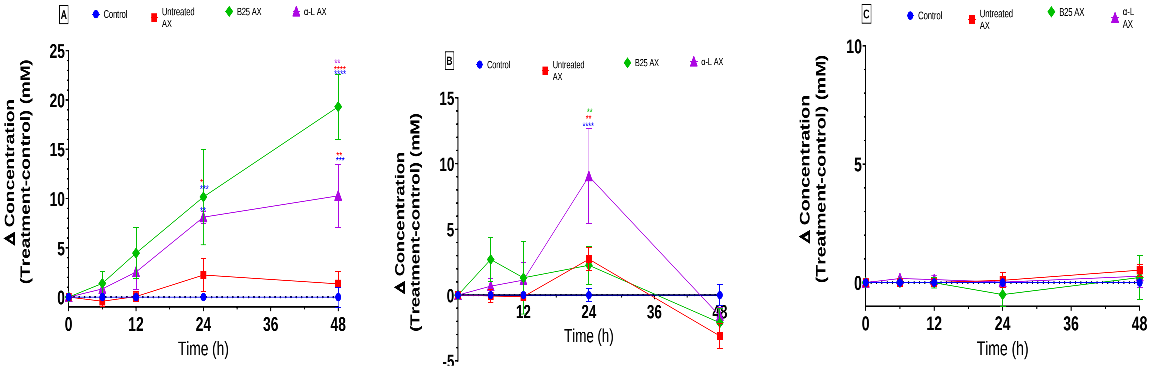


Figure 14. Concentration fold increase in short-chain fatty acids (SCFAs) over the control for all donors. (Δ concentration = test treatment −control) measured by GC over 48 h. Acetate, butyrate, and propionate (A-C) Control (circle, blue); B25 (diamond, green); untreated AX (square, red); and aL (triangle, purple). Statistical comparisons were conducted using repeated measures 2-way ANOVA test with Tukey's multiple comparison test (P < 0.05). To determine significant differences, the samples were compared among themselves. (* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001).

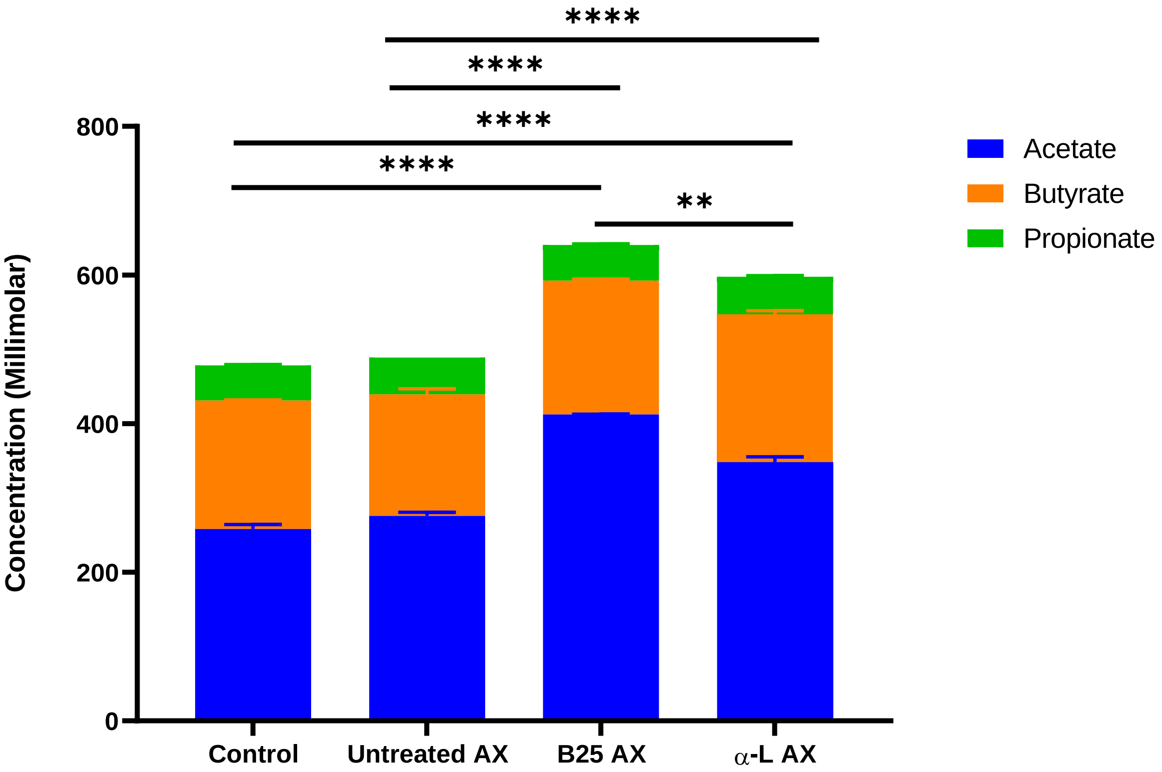


Figure 15. Concentration of total SCFAs from four healthy donors for each treatment.

4. Discussion

Numerous *in vitro, ex vivo* and *in vivo* studies have investigated the modulatory abilities of wheat arabinoxylan and other prebiotic fibers on the gut microbiota. However, only few studies have focused on the impact of structural modification and composition of wheat can have on the gut microbiota (Demuth et al., 2021; Gong et al., 2019). The dietary fiber structure is essential in determining its impact on gut microbiota composition. Studies have already demonstrated that different microbial species have varying preferences for dietary fibers as substrates, primarily depending on the fiber’s nature and structure (Louis, 2017; Tuncil et al., 2017). Hence, we hypothesized that modifying the structure of wheat arabinoxylan would cause a differential effect on the gut microbiota composition by prompting the abundance of health-associated bacterial taxa and increasing SCFA production. Demuth and coauthors investigated the effect of milling and extrusion of arabinoxylan on the gut microbiome (Demuth et al., 2021), while Gong and colleagues studied the impact of whole and refined wheat on the gut microbiota as well (Gong et al., 2019). Our study aimed to understand better the structure-function relationship between wheat arabinoxylan and the human gut microbiota. Here, we employed enzymatic digestion to modify the structure of wheat arabinoxylan to produce two distinct groups of arabinoxylan. AX treatment with α-L-Arabinofuranosidase B25 enzymes was used to produce only L-arabinofuranosyl units on doubly substituted β-D-xylopyranosyl, while treatment with α-L-Arabinofuranosidase enzyme was used to produce AX only L-arabinofuranosyl units on singly substituted β-D-xylopyranosyl units. After that, the treated and untreated AX were subjected to *in vitro* fermentation with human colon microbiota from four healthy adults.

Actinobacteria, *Bacteroidetes,* and *Proteobacteria* were the predominant bacterial groups at the phylum level, consistent with previous studies (Mousavi, Mottawea, Audet, et al., 2022; Wu et al., 2021). The AX fiber treated with the α-L-arabinofuranosidase B25 enzyme enhanced the abundance of the genus *Anaerofustis*. *Anaerofustis* is considered a beneficial bacterial taxon owing to its association with butyrate production (X. Li et al., 2016). Butyrate is considered the most crucial SCFA because of its antidepressant and immunological properties (Smith et al., 2013). Additionally, the enrichment of *Anaerofustis* with other dietary carbohydrates has been reported in several studies. For example, an animal study reported that the abundance *of Anaerofustis* increased in the presence of xylitol (Xiang et al., 2021). A similar result was observed in another study, which revealed that dietary supplementation with shredded, steam-exploded pine particles (SSPPs) resulted in an increased abundance of *Anaerofustis* (Goel et al., 2022). The SSPPs used in the study were rich in carbohydrates, similar to the wheat arabinoxylan used in our study. The population of *Anaerofustis* was reduced in patients with gastrointestinal diseases, such as inflammatory bowel disease, compared to their healthy counterparts (Masoodi et al., 2019). Additionally, a recent study revealed that *Anaerofustis* was a bacterial taxon significantly negatively associated with anxiety (S. Li, Lin, et al., 2022). The treated AX fibers were also enriched *with Eubacterium*. *Eubacterium* is a known dietary fiber fermenter and its presence in the gut is strongly correlated with increased dietary fiber consumption (David et al., 2014; Duncan et al., 2002). Furthermore, *Eubacterium* is a significant butyrate producer (Xiao et al., 2022), which is important for gut health, especially for controlling inflammation, managing immune reactions, and preserving gut barrier integrity (Mukherjee et al., 2020).

Furthermore, treating AX with α-L-arabinofuranosidase induced a bifidogenic effect by increasing the predominance of the *Bifidobacterium* genus. Likewise, Demuth et al. reported that wheat bran arabinoxylan, structurally modified through milling and extrusion, rapidly improved the growth of *Bifidobacteria* compared to native AX after *in vitro* colonic fermentation (Demuth et al., 2021). Other studies have reported that wheat AX supports the growth of this bacterial group (Hughes et al., 2007; Paesani et al., 2019). *Bifidobacterium* is an important microorganism, and its presence has been linked to numerous therapeutic benefits and improved overall gastrointestinal health (Hidalgo-Cantabrana et al., 2017). For example, *Bifidobacterium* has been reported to be instrumental in lowering depressive-like symptoms and management of gut dysbiosis (Pinto-Sanchez et al., 2017; Tian et al., 2019, 2022), preserving intestinal barrier function and protecting against pathogens (Rivière et al., 2016), and exhibits anticancer properties by preventing the growth of colon cancer cells (Bahmani et al., 2019). Another *in vivo* study showed that the ingestion of wheat arabinoxylan oligosaccharides (AXOS) significantly increased the abundance of *Bifidobacterium* in mice (Neyrinck et al., 2012). They also postulated that the prebiotic and bifidogenic properties of AXOS were inversely related to obesity, inflammatory disorders, and gut barrier integrity (Neyrinck et al., 2012). Another study showed that wheat arabinoxylan, when compared to inulin, promoted the growth of *Bifidobacterium* along with other beneficial microbes (Paesani et al., 2019). In addition, an *in vitro* study revealed that whole wheat and refined wheat after 24 h of fermentation increased the amount of *Bifidobacterium* (Gong et al., 2019). Interestingly, we observed a significant enrichment of *Bacteroides uniformis* with the addition of AX treated with the α-L-arabinofuranosidase enzyme. Some studies have reported that certain dietary fibers promote the abundance of *B. uniformis*. Cantu-Jungles et al. demonstrated that the abundance of *B. uniformis* increased after the fecal fermentation of glucans from *Cookeina speciosa* (Cantu-Jungles et al., 2018). Similarly, insoluble β-glucan fibers have been reported to enhance the population of *B. uniformis* to over five times the initial population and increase butyrate production (Cantu-Jungles et al., 2021). Another study showed that pectin utilization by *B. uniformis* led to an increase in butyrate levels and the generation of gamma-aminobutyric acid (GABA) (Benítez-Páez et al., 2017). These results concur with our observations, where the AX fiber treated with α-L-arabinofuranosidase enzyme supported the abundance of B. uniforms and increased butyrate production. Therefore, this might explain the high butyrate concentration in the α-L-arabinofuranosidase enzyme-treated AX fiber. Additionally, the results of our study support the fibrolytic capabilities of *B. uniforms*. Finally, it is important to mention that the enzymes used in this study were from two bacterial species. α-L-arabinofuranosidase B25 enzyme from *Bacteroides ovatus* and α-L-arabinofuranosidase enzyme from *Bifidobacterium adolescentis*. Consequently, we observed the abundance of these two microbial taxa after fermentation. For example, AX treated with α-L-arabinofuranosidase enzyme significantly enhanced the abundance of *Bifidobacterium* and *Bacteroides*. However, this is not surprising as a recent study revealed that the mice treated with *Bifidobacterium animalis* subsp. *lactis* XLTG11 resulted in a greater abundance of *Bifidobacterium* and *Bacteroides* than the control group (Xu et al., 2022).

The production of short-chain fatty acids by the gut microbiota is essential due to their valuable effects. In addition to being the primary carbon source for gut microbes, they also maintain intestinal pH to support the growth of beneficial bacterial taxa (Putri et al., 2022). The impact of bioengineered AX on the metabolic activity of the gut microbiome was evident. All AX samples led to the production of major SCFAs, which agrees with previous studies involving AX (Demuth et al., 2021; Gu et al., 2021; Paesani et al., 2020). Of the three SCFAs, acetate and butyrate were the most abundant, whereas propionate was produced in a lower quantity than acetate and butyrate. This finding is consistent with previous studies (Gu et al., 2021; Mottawea et al., 2020; Mousavi, Mottawea, Audet, et al., 2022; Tuncil et al., 2020). Nevertheless, the amount of SCFAs produced differed among the AX treatments. AX treated with the α-L-arabinofuranosidase B25 enzyme produced the highest amount of total SCFAs, mainly acetate. In contrast, AX treated with α-L-arabinofuranosidase enzyme exhibited a significant butyrogenic effect by inducing the highest butyrate production at 24 h. The increased butyrate production by this group of treated AX could be related to the abundance of *Eubacterium* because the abundance of *Eubacterium* was induced by AX treated with the α-L-arabinofuranosidase enzyme. Butyrate is considered an essential SCFA for human health owing to its strong antidepressant potential (Hao et al., 2019). It is also the preferred source of energy for intestinal epithelial cells (Rivière et al., 2016; Smith et al., 2013). The absence of butyrate has been linked to inflammatory bowel disease and colorectal cancer (Cresci et al., 2017; Mottawea et al., 2016). Mottawea et al. revealed that their synbiotic test formulation containing mainly polysaccharides, including tapioca fiber, lupin flour, and tiger-nut flour, substantially improved the amount of butyrate produced after 12 h of fermentation (Mottawea et al., 2020). Acetate is the most abundant SCFA that is produced during fermentation. This finding agrees with the results of several studies (Demuth et al., 2021; Fehlbaum et al., 2018a; Gong et al., 2019; Mottawea et al., 2020; Wu et al., 2021). Our results showed that treated AXs produced more acetate than the control and untreated AX. Specifically, AX treated with the α-L-arabinofuranosidase B25 enzyme induced the highest concentration of acetate production throughout the experiment. Interestingly, the acetate level increased exponentially towards the end of the experiment at 48 h. Acetate is an essential metabolite linked to various beneficial functions, such as modulating the release of colonic serotonin (Bhattarai et al., 2017) and, more recently, reducing cognitive decline (H. Zheng et al., 2021). Previous studies have reported an association between the presence of *bifidobacteria* and acetate production (D'hoe et al., 2018; Gong et al., 2019). This may explain why we observed significantly high acetate levels between 24 and 48 h. The bifidogenic properties of arabinoxylan are not surprising, as previously demonstrated (Paesani et al., 2019).

AX treated individually with α-L-arabinofuranosidase B25 and α-L-arabinofuranosidase produced the highest amount of total short-chain fatty acids. The untreated- and treated-AX produced comparatively higher SCFAs than the control. More recently, Mio et al. reported similar results in their animal study. Their research showed that the fermentation of arabinoxylan and β-glucan from barley resulted in increased production of total SCFAs compared to the control (Mio et al., 2022). Likewise, another recent study also revealed that agarooligosaccharide produced by enzymatic hydrolysis generated more SCFAs after 48 h fermentation, when compared to the control and agarooligosaccharides from acid hydrolysis (Putri et al., 2022)

Indeed, the results of our research support the structure-function relationship between AX and the gut microbiota, as different AX structures induce differences in the production of SCFAs. Similarly, Demuth et al. showed that extruded and milled wheat bran arabinoxylan had differential effects on SCFA production after fermentation (Demuth et al., 2021). Furthermore, this research showed that a slight structurally modification in the structure of AX was key to increasing the abundance of beneficial gut microbes. Thus, this could be a potential strategy to target specific microbes in the gut. Finally, different bacterial groups selectively fermented treated and untreated AX. This finding may be attributed to the structure of the AX substrate, because gut microbes have preferential substrates depending on factors such as the type of enzymes they produce and the sugar monomers they metabolize (Hughes et al., 2008; Louis, 2017).

5. Limitations

This *in vitro* study eliminated ethical concerns affecting prebiotic research in vivo/human studies. Nevertheless, this study also has limitations. The initial fecal microbiota was pre-cultured at pH 5.7. Maintaining the same pH in this batch fermentation model is complex and may affect the microbial diversity and activity. Second, the short fermentation time of 48 h fails to provide a view of long-term interactions between the arabinoxylan substrate and gut microbes. Thus, it may be difficult to project long-term effects on the gut microbiota. Notwithstanding, our test samples exhibited a more positive effect on gut microbiota composition and metabolism and could potentially alleviate dysbiosis, which is worth noting.

6. Conclusion

Previous studies have focused on the prebiotic potential and effects of AX from different cereals on the gut microbiome for the treatment and management of obesity and other diseases. However, this study is among the first to investigate the structure-function relationship and impact of bioengineered AX on the human gut microbiome. The effect of bioengineered AX on the gut microbiota compared to untreated AX and the control can be considered a prebiotic. In our study, bioengineered AX enhanced the abundance of *Roseburia inulinivorans and Bifidobacterium*, while reducing the availability of *Ruminococcaceae*. Similarly, both treated and untreated AX produced more metabolites than the control. These changes in the metabolism and composition of the gut microbiome indicate potential benefits to gut health. However, further comprehensive and long-term *in vivo* studies are recommended to strengthen the link between the structural modification of prebiotic fibers and overall gut health. Nevertheless, our research provides a scientific foundation for future studies involving the structural modification of dietary fibers. In addition, our research provided more evidence that the effect of dietary fibers on gut microbiome composition and metabolism can be structurally dependent by demonstrating how enzymatically bioengineered AX induces changes in the microbiota composition and metabolic profile. Finally, our research confirmed the prebiotic and bifidogenic properties of structurally modified AX and supported the structure-function relationship between AX and the gut microbes. The results of this study also indicate that bioengineered AX selectively stimulates the growth of beneficial microorganisms, which concurs with the definition of a prebiotic.

Acknowledgments

This study was supported by a strategic grant from the University of Ottawa. ENN was supported by the Nutrition and Mental Health Master's Scholarship, University of Ottawa.

CHAPTER 5: GENERAL DISCUSSION AND CONCLUSION

This study investigated the prebiotic effect of bioengineered wheat arabinoxylan (AX) on resident gut microbes, which were inversely correlated with depression. In the second phase of this research, we focused on the impact of bioengineered wheat AX on the composition and metabolism of the human gut microbiome in a batch culture fermentation model. The results from these studies authenticated the prebiotic properties of structurally modified wheat AX. Furthermore, our research demonstrated relationship between the structure of AX, human gut microbiota, and depression inversely linked gut microbes.

In Chapter 3, we produced 12 structurally distinct wheat AX fibers (LAX, MAX, and HAX) and investigated their impact on the growth rate and metabolism of selected depression inversely-linked microbes (*Faecalibacterium prausnitzii* and *Lacticaseibacillus rhamnosus*). Previous studies have focused on the prebiotic effects of dietary fibers and other polysaccharides on common beneficial gut bacterial species such as *Limosilactobacillus reuteri* and *Bifidobacterium breve* (Paesani et al., 2019), *Lactobacillus* (Hernandez-Hernandez et al., 2012), *Bacteroides* (Paesani et al., 2020)*, and Bifidobacterium* (S. Harris et al., 2019). All the studies mentioned above used traditional polysaccharides without altering their chemical composition. In this study, we evaluated the effect of enzymatically modified wheat AX on the growth rate and metabolism of *F. prausnitzii* and *L. rhamnosus* GG. Here, we observed that the structural modification of wheat AX positively affected the growth rate and metabolism of the selected bacterial strains more than that of the control. This observation was consistent for the low-, medium-, and high-molar mass wheat AX fibers.

Furthermore, structural differences, degrees of polymerization, and substitution patterns can influence how samples promote bacterial growth (Paesani et al., 2019). In agreement with this, our results revealed that the two bacterial strains had different preferences for bioengineered AX based on their xylose and arabinose substitution patterns. For example, regardless of the molecular weight, AX treated with both enzymes, which produced AX with no arabinose substitution units, had a positive influence on the growth of *F. prausnitzii*. In contrast, the proliferation of *L. rhamnosus* was influenced mainly by AX treated with only the α-L-arabinofuranosidase enzyme, which produced AX with a single substituted xylose unit. In addition, all AX-containing samples achieved maximum growth rates at earlier time intervals ahead of the control. For the AX-containing samples, optimum growth was reached after 24 h for *F. prausnitzii* compared to the control, where optimum growth was achieved after 40 h. A similar result was observed for *L. rhamnosus*, where most of the AX-containing samples peaked after 16 h, whereas similar growth was reached after 24 h for the control. Concerning size, we observed that the lower-molecular-weight AX (LAX) induced a faster growth rate than the medium- and high-molecular-weight AXs. This result was expected, and is consistent with previous studies where the authors reported that lower molecular weight polysaccharides exhibit improved bioactivity and encourage the proliferation of gut commensal bacteria (J. Gao et al., 2017; Wei et al., 2022; Zhao et al., 2012).

We also evaluated the concentration of short-chain fatty acids (SCFAs) produced by microbes at different time intervals after fermentation. Acetate, butyrate, and isovalerate were the most abundant SCFAs produced by both bacterial strains at the end of the experiment. However, acetate was the most abundant. Our findings are consistent with those of previous studies, where acetate was produced as the predominant metabolite (Demuth et al., 2021; Mondal et al., 2022). Moreso, butyrate have been reported as one of the main SCFAs produced, but gut microbes also produce acetate (Demuth et al., 2021; Gu et al., 2021; Paesani et al., 2020). These SCFAs have beneficial effects on both the host and the microbes. Butyrate has been reported to possess antidepressant characteristics (Hao et al., 2019) and to positively impact neuropsychiatric disorders (Silva et al., 2020). Beyond its beneficial effects on the host, it serves as the primary source of energy for colonocytes (Mendez-Encinas et al., 2018; Rivière et al., 2016). Acetate also has additional benefits, such as reducing cognitive decline in mice (H. Zheng et al., 2021). Isovalerate helps to control the metabolism of cholesterol, glucose, and fatty acids (Blakeney et al., 2019; Niccolai et al., 2019). Overall, bioengineered wheat AX significantly improved the metabolites produced by these microbes. However, the differences were visible in some cases, but were not statistically significant, especially for isovalerate. The observations and results from this section guided us in selecting LAX for the second part of this project.

In Chapter 4, we streamlined and focused on LAX, because our previous results (Chapter 3) demonstrated a more significant prebiotic potential than MAX and HAX. This chapter aims to investigate and better understand the structure-function relationship between wheat AX and its effect on the composition and metabolism of the human gut microbiota. To achieve this, we used two enzymes to modify the chemical structure of wheat AX. α-L-Arabinofuranosidase B25 enzyme was used to produce AX with double substituted xylose units, while α-L-Arabinofuranosidase enzyme was used to produce AX with a single substituted xylose unit. Subsequently, the original and modified wheat AX were fermented with human colon microbiota from four healthy adults in an *in vitro* batch culture fermentation model. Alpha and beta diversity results were not significantly different from those of the control group. However, the gut microbial diversity increased slightly after 48 h for all samples.

Actinobacteria, *Bacteroidetes*, and *Proteobacteria* were the most common bacterial phyla in our study. This observation is consistent with the results of previous studies (Mousavi, Mottawea, Audet, et al., 2022; Wu et al., 2021). Moreover, our study demonstrated the prebiotic potential of bioengineered wheat AX, as it caused a shift in the gut microbial composition and enhanced the abundance of some beneficial gut microbes. Interestingly, the abundance of *Anaerofustis,* an important gut-resident microbe with a high butyrate-producing ability (X. Li et al., 2016), was enhanced by the AX treated with α-L-arabinofuranosidase B25 enzyme. A similar result was observed in another study, which revealed that dietary supplementation with shredded, steam-exploded pine particles (SSPPs) resulted in an increased abundance of *Anaerofustis* (Goel et al., 2022). Also, the same group of treated AX fiber enriched the population of *Eubacterium*. *Eubacterium* is a known dietary fiber fermenter, and its presence in the gut has been strongly correlated with the increased consumption of dietary fiber (David et al., 2014; Duncan et al., 2002) and butyrate production (Xiao et al., 2022). Another important observation from this study was the abundance of *Bifidobacterium* after 24 h of fermentation. This observed bifidogenic effect was induced by AX treatment with α-L-arabinofuranosidase enzyme. *Bifidobacterium* is an important gut microbe and is associated with several health benefits, such as preserving the intestinal barrier function and protection against pathogens (Rivière et al., 2016), anticancer properties (Bahmani et al., 2019), as well as antidepressant potential and management of gut dysbiosis (Pinto-Sanchez et al., 2017; Tian et al., 2019, 2022). Previous studies have also shown that prebiotic fibers enhance the population of this bacterial taxon (Demuth et al., 2021; Gong et al., 2019; Neyrinck et al., 2012; Paesani et al., 2019).

SCFAs are essential metabolites produced by gut microbes through fiber fermentation, with acetate, butyrate, and propionate being the most common metabolites in the GI tract (Luo et al., 2021). In our study, the SCFAs mentioned above were the most abundant, with the highest concentrations for acetate and butyrate. A similar observation has been reported in previous studies (Gu et al., 2021; Mottawea et al., 2020; Mousavi, Mottawea, Audet, et al., 2022; Tuncil et al., 2020). The treated AXs produced acetate and butyrate that were significantly different from the untreated AX and control at almost all time intervals. Interestingly, each modified AX had a different effect on SCFA production. For instance, AX treated with α-L-arabinofuranosidase B25 induced the highest acetate concentration, whereas AX treated with α-L-arabinofuranosidase produced the most butyrate. This finding implies that the production of a specific metabolite may be related to the substrate structure. In addition, we can link high concentrations of acetate and butyrate to the presence of *Bifidobacterium* and *Anaerofustis,* which are closely associated with the production of both metabolites (D'hoe et al., 2018; Gong et al., 2019; X. Li et al., 2016).

Despite the important findings of this study, it has some limitations. The 48h fermentation period will not provide ample time for the interaction between the gut microbes and AX fibers. Additionally, the batch culture model adopted in this study made it impossible to maintain the initial pH of the microbial community at 5.7. Hence, determining the long-term impact of bioengineered wheat AX may be challenging. Nevertheless, our results showed that the short-term use of wheat AX potentially modulates the gut microbiome structure and metabolism. Indeed, the results from our research support the structure-function relationships between AX and gut resident microbial communities, leading to differential modulation of the gut microbiome metabolism and composition.

For future studies, the next direction to consider in this area of research is as follows:

i. Repeat this study in a continuous *ex vivo* model of the human colon using bioreactors to properly mimic the gut physiological and microbiological conditions.
ii. Compare different dosages of AX intake and determine the most effective dose to modulate and improve the abundance of beneficial microbes in the gut.
iii. Study the effectiveness of bioengineered wheat AX on patients with depressive- and anxiety-like disorders.
iv. Replicate this study with more donors and conduct *in vivo* trials using mice models.
v. Conduct further clinical trials on both healthy and depressed individuals to evaluate the impact of our modified wheat AX on gut microbiota composition, structure, and metabolism.

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