Assessment of diet in relation to public speaking anxiety

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Abstract

Public speaking is an important skill that can positively influence an individuals' everyday life, career, relationships and much more; yet, it has been consistently ranked as one of the top fears in the United States over the past five decades. Recent studies into the role of the microbiota-gut-brain axis has discovered a direct link between gut microbiota and hypothalamic-pituitary-adrenal (HPA) reactivity, suggesting new ways of thinking regarding the prevention and treatment of anxiety such as public speaking anxiety. Among the factors that influence the composition of the gut microbiota, diet is considered the single most critical one.

Against this backdrop, the proposed study aims to compare the anxiety level in public speaking and the dietary choices of adults aged between 18 and 55. It assesses the hypotheses: (a) individuals on a plant-based diet experience lower levels of anxiety associated with public speaking than those on a Western diet; (b) a higher intake of prebiotic and probiotic foods is associated with a lower level of public speaking anxiety among individuals on a Western diet. Implications of the findings for diet in relation to public speaking anxiety will be discussed.

Keywords: Public speaking anxiety, gut-brain axis, diet, diet-microbiota-gut-brain axis, plant-based diet, prebiotics and probiotics

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Chapter I.

Introduction

Whether voluntarily or as a requirement, the majority of people have given some form of speech or presentation at least once during their lifetime. Most commonly these situations are for a school project, a wedding toast, or maybe a professional presentation. Public speaking is an important skill that can positively influence an individual's everyday life, career, relationships and much more. Despite, or maybe as a result of the importance of this skill, public speaking has been consistently ranked as one of the top fears by many, regardless of either gender or age, in the United States over the past five decades (Bruskin, 1973; Dwyer & Davidson, 2012; Ingraham, 2014). For many people, giving a speech in front of a group or audience is not an easy task to manage, let alone something enjoyable. A more recent 2021 survey by Chapman University suggested that the situation has not changed much and that one third of Americans indicated that they fear public speaking (Amirazizi, 2022). Stage fright is still quite real.

Millions of people invest themselves financially and spiritually with the goal of building their skill and confidence in public speaking. Whether through training videos, workshops, books, or classes, people always look for ways to improve. Unfortunately, despite their best efforts, the results are often disappointing. Many individuals find themselves continuing to experience nervousness and anxiety when speaking in front of audiences and falling short of being able to deliver their speeches as hoped.

One cannot help but ask, is there a secret antidote to public speaking anxiety that works and is at the same time cost-effective? The key to answering this question lies in

the understanding of what might be the cause of the anxiety and how the brain and body connect. To find that answer requires that we gain an understanding of the physiology of stress-related anxiety.

The Biology of Anxiety

In a situation of threat or stress, the brain activates the hypothalamic-pituitary-adrenal (HPA) axis, resulting in an immediate release of cortisol to prime the body for the "fight or flight" response (Sandhu et al., 2017). This is the response that prepared our hunter-gatherer ancestors to escape from danger some six million years ago, and it is the same response that our brain has when facing public speaking (Adolphs, 2013; Davis, 1992).

Being viewed as a variety of fears, anxiety is a more tonic state compared to other types of fear such as a panic attack (Adolphs, 2013). Nevertheless, it shares the common theme of biological, emotional, and cognitive responses that are associated with the cohesive concept of fear. In DMS-5, symptoms categorized as generalized anxiety include heart pounding, dry mouth, upset stomach, increased respiration, and difficulty in concentrating (Goldstein & DeVries, 2017).

Although the exact root cause of anxiety is currently unknown, research supports that stimuli plays an important role in the etiology of anxiety states. In his review, Adolphs (2013) suggested that fear can be caused by particular sets of threat-related stimuli. He stated that, unique to humans, being publicly evaluated, the basic scenario of public speaking, is a category of social stimuli that can induce fear and anxiety. Adolphs took his statement even further and suggested that fear in humans can be triggered not

only by occurrent stimuli but also merely by thinking about such stimuli, i.e., people can have a fear reaction to and anxiety about the possibility of public speaking.

The expression and development of fear or anxiety is largely regulated by brain structures such as the amygdala and the hypothalamic-pituitary-adrenal (HPA) axis (Adolphs, 2013; Cryan et al., 2019; Davis, 1992). HPA axis is a major neuroendocrine system in the human body that directly orchestrates responses to fear (Adolphs 2013). Freezing, increased heart rate, sweaty palms, and shortness of breath are common behavioral responses and autonomic changes when one is in a state of fear. This emotional state also influences all aspects of cognition from attention and memory to judgement and decision-making.

It is important to note that the HPA axis does not act alone. Cryan et al. (2019) suggest that the HPA axis interacts with other pathways of communication between the brain and the body, such as the autonomic nervous system (ANS), the enteric nervous system (ENS), the central nervous system (CNS), and the immune system.

Cryan and colleagues emphasized that the HPA axis interacts with the vagus nerve which is the fastest and most direct route connecting the gut and the brain. The vagus nerve is a key component of the autonomic nervous system and plays a critical role in communicating changes from the GI tract to the CNS and regulating behavior (Bravo et al., 2011). What is of particular interest is that the vagus nerve contains 80% afferent fibers which transmit vital information from the gut to the brain, and 20% efferent fibers which sends the signals from brain down to gut (Cryan et al., 2019). In other words, communication lines between the gut and the brain are disproportionally allocated toward “bottom-up” signaling.

In recent studies, vagus nerve stimulation (VNS) therapy has been demonstrated as an effective treatment option and a successful approach in treating stress-related psychiatric disorders such as depression (Elger et al., 2000; George et al., 2000; Rush, Marangell, et al., 2005; Rush, Sackeim, et al., 2005; Sackeim et al., 2001). VNS refers to techniques that simulate the vagus nerve via manual or electrical stimulation (Howland, 2014). Evidence from these studies suggest that, by increasing the expression of neurotransmitters and activating their receptors, VNS can influence or alter the brain activities dysregulated in mood disorders (Howland, 2014; Rush, Marangell, et al., 2005; Rush, Sackeim, et al., 2005).

The functional role of brain structures, including those involved in development and the expression of fear, depends on neurotransmitters and their receptors. Several key neurochemicals (e.g., serotonin, GABA, catecholamines, among others) are involved in mood, behavior, and cognition. Dysfunction of neurotransmitters and their receptors can lead to physical and psychological disorders (Zarrindast & Khakpai, 2015).

Serotonin (5-HT) has long been believed to play a role in facilitating anxiety but inhibiting panic (Deakin & Graeff, 1991). The theory proposed by Deakin and Graeff suggests that, when threats are detected, the components of the defense reaction are retrained by 5-HT release. The theory is supported by clinical studies. Empirical evidence from these studies show treatments with the antidepressant selective serotonin reuptake inhibitors (SSRIs) are effective at treating mood and anxiety disorders (Bell et al., 2002; Bigos et al., 2008; Deo & Redpath, 2022; Hood et al., 2010; Martin et al., 2020). It is worth noting that over 95% of 5-HT in the body is produced and synthesized in the GI tract (Cryan et al., 2019).

GABA, the major inhibitory neurotransmitter of the nervous system, is known to be synthesized from glutamate and mediates synaptic inhibition through different receptors (i.e., $GABA_A$, $GABA_{B,}$ $GABA_{C)}$ (Cromer et al., 2002). Increasing evidence suggests that the GABAergic system, which connects the prefrontal cortex (PFC) and other brain structures, is not only involved in cognitive and behavioral processes, but also in the neurobiology of anxiety (Hasler et al., 2010). Hasler and colleagues (2010) suggest that a decrease in GABA concentration in PFC is related to a down regulation of glutamate decarboxylase associated with acute stress. $GABA_A$ receptors in particular play an important role in modulating and controlling anxiety, fear, and stress depression. Activation of $GABA_A$ receptors can reduce emotional responses (Solati et al., 2013). Interestingly, evidence from studies have shown bacterial species such as *Escherichia* and *Lactobacillus* have the capacity to synthesize GABA (Cryan et al., 2019).

Catecholamines play diverse roles in physiology, ranging from fight-or-flight responses to social behavior (Starkman et al., 1990; Terbeck et al., 2016). There is evidence suggesting that both norepinephrine and dopamine are involved in anxiety and are important in modulating mood disorders (Paine et al., 2015; Zarrindast & Khakpai, 2015; Zweifel et al., 2011). These studies have shown that, in response to aversive stimuli, dopamine and norepinephrine neurons are either inhibited or excited, implicating their activation is critical for aversive conditioning and emotional processing. A variety of bacteria such as *Escherichia* are known to produce catecholamines. *Bacillus* is known to produce dopamine and norepinephrine (Cryan et al., 2019).

All of these non-neuronal as well as neuronal pathways contribute to controlling the stress response and regulating many body processes. A growing body of research has

shown that these systems are directly affected by gut microbiota and that, in turn, each exhibits alterations in stress-response and overall behavior (Cryan & O'Mahony, 2011; Hyland & Stanton, 2016).

Anxiety and Microbiota

The human microbiota consist of 100 trillion microbial cells in each host body, primarily in the gut (Ursell et al., 2012). The microbial community is complex, diverse, and composed mainly of bacteria, archaea, viruses, fungi and protozoa (Cryan et al., 2019; Sommer & Bäckhed, 2013; Ursell et al., 2012). The microbial colonization begins during the birth of the host and the composition of microbiota changes throughout the host's development (Sommer & Bäckhed, 2013). In general, the microbial composition of an individual depends on their age, lifestyle, medication, and diet (Cryan et al., 2019; Kostic et al., 2014; Lee & Kim, 2021; Liang et al., 2018).

The genes that these microbial cells harbor are the human microbiome (Ursell et al., 2012). The gut microbiome begins developing at birth and is usually complete within 3 years; however, it can be modified when the composition of microbiota changes (Wilson et al., 2020). More than ninety-nine percent of the genes in the human body are microbial and those microbiomes help produce the biochemicals, such as serotonin, on which the brain depends (Cryan et al., 2019; Kellman, 2017). The important role that the microbiome plays in the production of brain chemicals indicates why it is crucial to understand the functional potential of the microbiome and its influence on the host and the gut-brain axis in particular.

Even though gut microbiota is well-known for its importance in processes such as digestion, metabolism, and immune responses (Sommer & Bäckhed, 2013), in recent

years, a growing body of evidence suggests that it also plays a critical role in brain function and mental health.

Sudo et al. (2004) used the GF mouse model to demonstrate that microbiota can affect the HPA axis. According to their investigation, the level of HPA response to stress by GF mice was reduced via reconstitution with *Bifidobacterium infantis*, a representative of probiotic bacteria. Cyran et al. (2019) reviewed the research using animal models and supported the idea that "the amygdala appears to be a brain region that is central to the influence of the microbiota on social behavior" (p. 67). A few further studies in humans via fMRI convinced Cryan and colleagues that there is a link between microbiota and the stress response, and that this relation is bidirectional, i.e., stress can alter the gut microbiota and in turn the gut microbiota can modulate stress-induced anxiety and anxiety-like behaviors. One of these studies, for example, demonstrated that the consumption of a fermented milk drink with probiotics was able to reduce neural responses to threat stimuli in healthy women (Tillisch et al., 2013).

Further, other studies suggest that the gut microbiota plays a key role in the etiology of anxiety disorders such as post-traumatic stress disorder (PTSD) and panic attacks (Leclercq et al., 2016; Liang et al., 2018; Turna et al., 2016). Those studies have shown that mood disorders are likely related to the abnormalities of gut microbiota, and that regulating the microbiota can produce therapeutic effects. In other words, changes in the composition of the gut microbiota influences the expression of corresponding genes (i.e., microbiome). In turn, these can result in changes to behavior, cognition, and mood (Cryan et al., 2019; Ursell et al., 2012).

Once it was understood that gut microbiota can communicate with the brain and regulate behaviors, the microbiota-gut-brain axis, through which the microbiota communicates with the brain, emerged as an exciting concept related to health and disease.

The gut-brain axis is a bidirectional communication system between the gut and the brain with the involvement of the CNS, atomic, and ENS (Cryan et al., 2019; Turna et al., 2016). As described above, recent research has suggested that the gut microbiota is a mediator of this axis. Like the gut-brain axis being bidirectional, the microbiota-gut-brain axis is a bidirectional communication network between the brain and microbial community (Cryan et al., 2019; Lee & Kim, 2021). Investigations have reported bi-directional signaling mechanisms between each of the individual components of the axis (i.e., microbiota-to-gut, gut-to-microbiota, gut-to-brain, brain-to-gut, microbiota-to-brain, and brain-to-microbiota). The bi-directional communication within the axis enables both top-down modulation of gastrointestinal function by the brain and emotions, as well as bottom-up regulation of the brain function and emotions by microbiota and the gut (Mayer, 2011).

There are many communication pathways within the microbiota-gut-brain axis. The main three pathways are: the nerve pathway, the neuroendocrine pathway, and the immune pathway (Cryan et al., 2019; Liang et al., 2018).

The nerve pathway, with neurotransmitters as a key element, is the fastest way through which the microbiota can affect the brain and behavior (Liang et al., 2018). Research has shown that the microbiota synthesize and respond to neurochemicals such as 5-HT and GABA (Liang et al., 2018).

The gut microbiota can also influence the brain and behavior through the endocrine pathway. Even though enteroendocrine cells (EECs) only represent 1% of gut epithelial cells in the GI tract (Mayer, 2011), the gut is the largest endocrine organ in the human body and gut microbiota play an important role in the activity of these EECs (Liang et al., 2018).

The gut is known as the biggest immune organ of the human body with 70%-80% of a person's immune cells contained within the gut-associated lymphoid tissue (Liang et al., 2018; Mayer, 2000). These immune cells are in constant communication with the trillions of microbiota (Mayer, 2011). As such, by regulating the function of innate and adaptive immunity, the gut microbiota influences neuroimmune and inflammation to change the brain and its behavior (Liang et al., 2018).

It is important to note that, in addition to neurotransmitters and the nervous systems, there are other pathways such as bacterial metabolites. For instance, short-chain fatty acids (SCFAs), the most well-known gut microbial-derived metabolites, have been reported to be associated with vagus nerve activation and have effects on the brain and behavior. This indicates that SCFAs are potentially another key player in the microbiota-gut-brain axis communication (Cryan et al., 2019).

Through these major pathways of communication between the gut-microbiota and the brain, the microbiota-gut-brain axis impacts not only energy use but also mood and behavior. A great deal of recent research has been actively focusing on the relation between the mechanism and various mood disorders such as anxiety, PTSD, depression, and schizophrenia (Jiang et al., 2015; Leclercq et al., 2016; Mikocka-Walus et al., 2016;

Rao et al., 2009). This suggests that regulating the microbiota can bring therapeutic effects to these mood disorders.

Microbiota and Diet

Many factors can influence the composition of the gut microbiota and therefore modulate the brain and its function. Diet, however, is considered the single most critical factor because the microbiota depend on food residues for survival and metabolism (Ceppa et al., 2019; Cryan et al., 2019; Wilson et al., 2020).

A link has been found between the diversity of diet and microbiota, i.e., the more diverse the diet, the more diverse the microbiota (Claesson et al., 2012; Cryan et al., 2019). Besides diversity of diet, dietary patterns (e.g., plant-based and Western diets)al so has been found to have long-term and acute effects on gut microbiota (Wu et al, 2011).

In other words, food shapes gut microbiota and has a profound impact on the microbiome, and therefore drives changes in the health and overall well-being of the host. Studies have reported an inverse relation between diet quality and mental health across countries, ages, and genders (Hyland & Stanton, 2016, Sandhu et al., 2017; Wilson et al., 2020). A plant-based diet has demonstrated reductions in stress responsiveness and anxiety (Ein et al., 2019; Wilson et al., 2020). On the other hand, Western diets rich in sugar and fat, have generally been considered a key risk factor for mental disorders such as depression and anxiety (Bukhari et al., 2018; Hicks et al., 2016; Hyland & Stanton, 2016).

Plant-based Diet

Plant-based diets have been gaining popularity globally in recent years. The most recent version of the Dietary Guideline for Americans highly recommends adults aged 19 through 59 years old follow plant-based diets and consume nutrient-dense foods and beverages (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2020).

In general, a plant-based diet refers to a diet that is based on foods derived from plants with no or low intake of animal products (Hargreaves et al., 2023). Diets such as Vegetarian diets, Semi-vegetarian diets (e.g., pescetarian diet), and Mediterranean diets are plant-based. Vegetables, fruits, whole grains, seafood, eggs, fat-free and low-fat dairy products are the main components of these diets. Lean meats and poultry, when prepared with little to no added sugars, saturated fat, and sodium are also included amongst nutrient-dense foods (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2020).

Plant-based diets are fiber-rich and abundant in Polyphenols. High fiber intake promotes the growth of bacteria that ferments fiber into metabolites as short-chain fatty acids (SCFAs) which is associated with improved immunity and regulation of critical functions of the intestine (Tomova et al., 2019).

Studies have found that plant-based diets create distinctive and identifiable gut microbiota characteristics, e.g., lower *E.coli* counts, increased level and prevalence of *C. albicans,* and greater molar ratio of acetate (Mitsou et al., 2017). All of these characteristics are microbial indicators of balanced inflammation and a healthy metabolism. In other words, plant-based diets impact the gut microbiota composition and

the creation of microbial metabolites. They promote more diverse and stable microbial systems which in turn affect health of the host (Tomova et al., 2019). These diets have demonstrated the ability to not only reduce the risk of westernized diseases, including cancers of the colon, breast, cardiovascular system, and diabetes (O'Keefe, 2019), but also help improve mental health (Daneshzad et al., 2020; Jacka et al., 2011; Sánchez-Villegas et al., 2009).

Western Diet

A typical Western diet is characterized by being high in fat and refined sugar, low in fiber, and composed largely of processed food (So et al., 2023), with a lower intake of fruits, vegetables, whole grains, and seafood. This diet has been linked to various health conditions, both physical and psychological. It is not only associated with obesity, diabetes, and cardiovascular diseases, but also mood disorders (e.g., depression and anxiety) and brain dysfunction (e.g., HPA dysregulation and hippocampal dysfunction) (Lopez-Taboada et al., 2020).

A high-fat and high-sugar Western diet can dramatically alter the composition of the gut microbiota and its microbiome. In an experiment conducted on humanized mice, Turnbaugh et al. (2009) demonstrated that, compared to mice fed a low-fat and plant polysaccharide-rich (LF/PP) diet, mice fed a Western diet had a significant decrease in the types of *Bacteroidetes* and a significant increase in adiposity and Erysipelotrichi, a bacterial family belonging to the Firmicutes. A significant increased ratio in the *Firmicutes* to *Bacteroidetes* usually is considered as a predictive factor for obesity (Kostic et al., 2014).

Turnbaugh and colleagues further suggested that, by switching from a LF/PP diet to a Western diet, gene expression in the microbiome changed in the mice and that 69 genes were significantly upregulated on the Western diet (Turnbaugh et al., 2009). The shift in the microbial community structure, they suggested, took place in less than a single day, supporting the evidence that dietary change impacts gut microbiota and that microbiota responds to dietary change in a rapid manner.

Similar findings were noted in human studies. According to David et al. (2014)) and Toribio-Mateas et al (2021), individuals who switched from an animal-based diet to a plant-based diet or vice versa experienced rapid and significant changes in their microbial community structure. They observed a significant change in diet-associated microbial activity and gene expression (e.g., increased expression of ß-lactamase genes associated with the animal-based diet) only one day after the start of the diet (David et al., 2014; Toribio-Mateas et al., 2021).

Probiotics and Prebiotics

Probiotics are live microorganisms that provide health benefits to the host via their effects on the gut microbiome when they are administrated in adequate amounts (National Institutes of Health, 2022b). The intake of probiotics can modify the colon's fermentation capacity, which may have health-related effects. Enhancing immune system and reducing the risk of colon cancer are among these effects.

Some commonly known probiotics include *Lactobacillus, Bififobacterium, Saccharomyces, Streptococcus, Enterococcus, Escherichia, and Bacillus*. They are almost exclusively used in fermented dairy products such as yogurt or freeze-dried cultures (Roberfroid, 2000).

The definition of prebiotics, however, is not that straightforward. It was defined originally by Gibson and Roberfroid (1995) as non-digestible food ingredients that benefit the host via stimulating the growth or activity of one or a limited number of bacteria in the colon. This definition was later expanded by the International Scientific Association of Probiotics and Prebiotics (ISAPP) in 2008 to address the benefits that prebiotics exert on host health beyond the colon (Roberfroid et al., 2010). As such, a prebiotic is re-defined as a non-digestible compound that modulates composition and/or activities of the gut microbiota and confer beneficial physiological effects on the host (Bindels et al., 2015).

Revision of the definition shifted the focus of the concept towards ecological and functional characteristics of the microbiota that are more relevant for host physiology (e.g., ecosystem diversity), instead of subjectively selective targets. With the new definition, Bindels et al. (2015) proposed more compounds such as galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS), whole grains and non-carbohydrate compounds (e.g., polyphenols) be added on the list of the prebiotics.

It is important to note that, besides the chemical structure, the dosage of prebiotics is also an important factor that influences the microbiota composition. Studies have demonstrated that the larger the prebiotic dosage, the greater the diversity of bacteria affected (Valcheva & Dieleman, 2016).

Dietary Interventions in Treatment of Anxiety and Related Disorders

In recent years, with the recognition of the microbiota-gut-brain mechanism, an increasing volume of research has been conducted in the pursuit of developing new

therapeutic solutions for mood disorders. A great deal of success has been achieved via modifying diet and composition of gut microbiota.

Generalized Anxiety Disorder (GAD). Amongst the popular diet interventions aiming to reduce anxiety, the impact of probiotics and prebiotics is widely studied. In a previously mentioned preclinical study with mice, Bravo and colleagues suggested that *L rhamnosus* (JB-1), a type of probiotic bacteria, has a direct effect on the receptors of the main inhibitory neurotransmitter of CNS, i.e., GABA (Bravo et al., 2011). The study showed *L rhamnosus* (JB-1) reduced stress-induced corticosterone and anxiety-related behavior in mice.

In a clinical trial, Rao et al. (2009) conducted an experiment with thirty-five chronic fatigue syndrome (CFS) patients and found that administration of the probiotics, *Lactobacillus* and *Bifidobacteria*, significantly reduced the anxiety symptoms in patients who were in the experimental group. The results of the experiment further supported the presence of the microbiota-gut-brain axis and that targeting the gut microbiota could lead to a decrease in depression or anxiety. In addition to beneficial bacteria such as probiotics used by Rao et al. dietary intervention can also improve the composition and the function of the microbiota.

Multiple other randomized controlled studies have also demonstrated the use of probiotics increased a buffer against stress-related detrimental effects on cognition and alleviated anxiety (Aizawa et al., 2016; Hyland & Stanton, 2016; Messaoudi et al., 2011; Papalini et al., 2019; Pirbaglou et al., 2015; Rao et al., 2009; Tillisch et al., 2013).

Improvements in specific anxiety measures have been shown in a number of prebiotic intervention studies as well. For example, experimental research has suggested

administration of bananas, a representative of prebiotics, was effective in influencing the brain and reducing overall anxiety (Putra et al., 2018; Setyarini et al., 2020). Bananas contain a great amount of Vitamin B_6, which helps maintain the body's immune system, as well as fructo-oligosaccharide and inulin (Kumar et al., 2012; Singh, 2016). Fructo-oligosaccharide is an oligosaccharide and can significantly decrease inflammatory cytokine, while inulin is the most well-known prebiotic and has been demonstrated to be able to reduce anxiety (Cryan et al., 2019; Sandhu et al., 2017).

Major Depression Disorder (MDD). Major Depression Disorder is the most common disorder in the US. According to the 2015-2020 National Survey on Drug Use and Health, in 2020, nearly one in ten Americans reported having depression in the past year (Goodwin et al., 2022).

Depression and anxiety are often closely related and are studied together in many of the microbiota-gut-brain axis research, including measures. Although the beneficial effects of the prebiotics need further investigation through clinical studies, recent studies have found improvement in depression scores resulting from the use of probiotics such as an *L. casei* strain (Benton et al., 2007), a triple-strain probiotic containing *L acidophilus, L. casei, and B. bifidum* (Akkasheh et al., 2016), and *L.rhamnosus* HN001 (Slykerman et al., 2017). Administration of these probiotics have been demonstrated to either reduce stress-induced corticosterone responses or prevent hyperactivation of the HPA axis in response to stress.

Other Mental Disorders Related to Anxiety. Despite strong preclinical and clinical evidence on the effectiveness of the gut microbiota in modulating stress response, direct investigation of diet and the microbiota in treatment of Post-traumatic Stress Disorder

(PTSD), panic disorder, social phobia, and other anxiety related disorders have been lacking.

The Present Study and Hypotheses

The fear of public speaking is the most common social fear among the general population (Amirazizi, 2022). Potential humiliation and negative evaluation induces anxiety and distress in individuals. At its worst, those with debilitating anxiety in public speaking can be diagnosed as social phobic (Blöte et al., 2009). For those with less severe symptoms, who might not meet the diagnostic requirements, the anxiety is still real and should be dealt with. Otherwise, the effect can become debilitating and the impact on an individuals' physical and mental well-being can be severe.

There have been numerous research studies done in various domains, all in the pursuit of finding an antidote to public speaking anxiety. Google search can return 57 million results in less than a second when you type "best books on how to reduce public speaking anxiety" in the search bar. The top recommended techniques include: practicing (i.e., memorization), visualization, and managing breathing. The actual effectiveness, benefits, and more importantly the sustainability of the results from these books and techniques are still unknown. Furthermore, most of those techniques focus on the brain only, and fail to recognize the connection between the brain and the gut, as well as the key role that our gut plays in influencing our brain.

As discussed in previous sections, more and more studies demonstrate the ability of the microbiota to affect the amygdala and the HPA axis. A growing body of research on the gut-brain axis has provided strong evidence suggesting that administration of

microbiota-targeted diets has a positive effect on mitigating the risk of and the treatment of mood disorders such as anxiety (Foster & McVey Neufeld, 2013).

Even though significant progress has been made in recognizing the importance of the diet-microbiota-gut-brain connection and the effect of food consumption in treating general anxiety, whether those findings hold true for specific anxieties needs further examination. Against this backdrop, the proposed study will utilize a correlational study to assess the relation of diet to anxiety associated with public speaking in adults.

The present study has two hypotheses. The primary hypothesis is that individuals who are on a plant-based diet experience lower levels of public speaking anxiety compared to those on a Western diet, i.e., a high-fat and high-sugar diet. In addition to the main hypothesis, the study also hypothesizes that a higher intake of prebiotic and probiotic foods is associated with a reduction in public speaking anxiety among individuals on a Western diet.

To the date, no other studies have been identified that specifically evaluates food intake in relation to anxiety in the setting of public speaking. If the findings support the hypotheses, this study will contribute to the body of knowledge in understanding of the diet-microbiota-gut-brain connection, and in finding cost-effective, easily-implemented, and non-medical treatments for public speaking anxiety amongst adults.

Chapter II.

Method

This study was approved by the Harvard University - Area Committee on the Use of Human Subjects on December 14, 2022 and took place from December 18th, 2022 to January 15th, 2023.

Participants

The recruitment followed the following inclusion and exclusion criteria.

Due to the hypotheses of the study focusing on public speaking anxiety, volunteers who had performed public speaking were sought out. The age range consideration is based on the aimed study population (i.e., adults) and the American Medical Associations' age designations (National Institutes of Health, 2022a). People aged 18 to 55 are in their prime working lives and private lives, which increases the occurrence of performing public speaking.

Inclusion criteria:

- Has had experience with public speaking
- Ages between 18 and 55. No restrictions on sex, race, ethnicity, or social economic status.

Exclusion criteria:

- Has never had experience in public speaking
- Ages below 18 years old or above 55 years old

Prospective volunteers were sought via social media, crowdsources, and emails. To be more specific, the platforms included Facebook, Twitter, Reddit, Amazon Mechanical Turk (Mturk), and www.socialpsychology.org/addstudy. On social media, the recruitment flyer was posted on the Principal Investigator's personal page only.

Two hundred and two individuals participated in the survey online. Fifty-seven of them were excluded due to the following reasons: 12.4% failed the screening questions; 9.4% did not consent to enroll in the study; 3.4% did not complete the survey; 3.0% provided false responses and/or inaccurate data (e.g., inconsistent responses on more than one item). The final study sample consisted of 145 subjects. A detailed description of the inclusion and exclusion criteria applied is presented in figure 1.

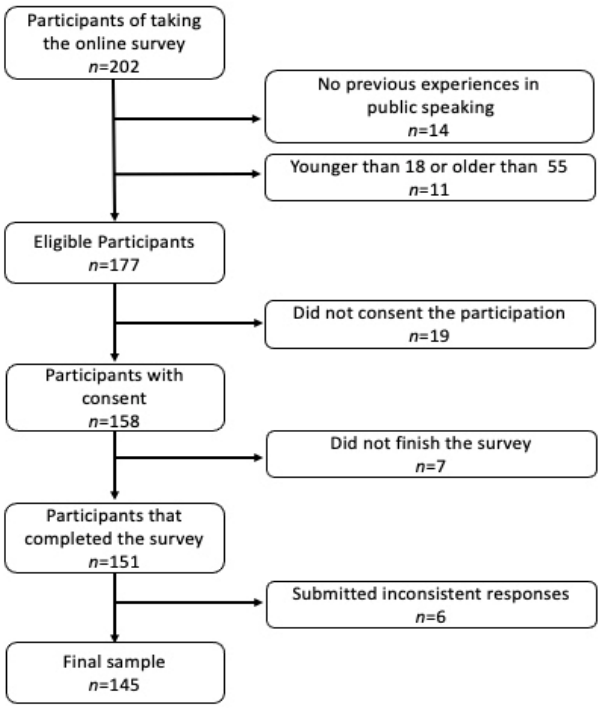

Figure 1. Flow Chart of the Study Sample

Materials and Measures

Materials

Survey webpage. The study was conducted and the data was collected online through Qualtrics.

Measures

Public Speaking Anxiety Scale (PSAS) survey (See Appendix 1). The PSAS is a 17-item self-report measure with five items that are reverse coded. It is widely used to assess and track public speaking anxiety through multiple properties (e.g., physiological, behavioral, and cognitive).

Among those 17 items, 8 items are cognitive measures of speech anxiety, 4 items are behavioral measures, and 5 items are physiological measures. Responses are measured in a Likert-format with score ranking from the minimum (1) “not at all” to the maximum (5) “extremely”, yielding overall scores between 17 - 85 with higher total scores reflecting higher levels of anxiety. Barthnomay and Houlihan (2016) reported extensive evidence for the internal consistency, concurrent validity, and convergent and discriminant validity of the test. For example, they documented a significant relation (r = .835 - .845, $p < .001$) between the PSAS and Personal Report of Confidence as a Speaker (PRCS) as well as Survey of Speech of Anxiety (SSA) for a sample size of 375, suggesting good concurrent validity. In addition, they reported a moderate to high correlations (r = .350 - .511, $p < .001$) between PSAS and Social Interaction Anxiety Scale (SIAS) as well as the Intolerance of Uncertainty Scale (IUS), indicating a good convergent validity. Furthermore, they showed that the PSAS had weaker correlations (r

= .136 - .180, $p < .011$) with measures of depression and dissociation, i.e., Depression Questionnaire (DQ) and Wessex Dissociation Scale (WDS), indicating discriminant validity. In the proposed study, the total PSAS scores will be considered the major measure.

There were four additional questions attached to the PSAS. These questions were created by the research team to gather information from participants about the frequency of performing public speaking, food and beverages regularly consumed before speaking events, as well as the timing and results of their most recent public speaking events.

Dietary Recall Questionnaire (See Appendix 2). A 7-day self-administrated food frequency questionnaire developed by the Harvard Medical School was used to access the participants' usual dietary intake over the past 7 days. The questionnaire has been used for both research and clinical purposes. It includes total 21 food and drink items which the participants were asked to provide the info on how often they consumed. Consumption frequencies include: never in the past 7 days, within the past 4 to 7 days, within the past 2 to 3 days, yesterday 1 to 2 times, and yesterday 3 or more times.

Background Information Questionnaire (See Appendix 3). Participants were asked to complete a self-reported survey that was designed with 14 questions regarding demographics (i.e., age group, gender, race, height/weight), dietary classification and restrictions, mental illness related to anxiety disorders, lifestyle, physical activities, sleeping habit, supplement intakes, and antibiotics intakes.

Study Design

The focus of the present study is on the correlations between diet and anxiety levels in public speaking. The dependent variable is the level of public speaking anxiety.

The independent variables are individual food items and dietary patterns (i.e., plant-based vs Western diets).

This study was conducted online. The participants took the PSAS survey, dietary recall questionnaire, and background information questionnaire. The results of PSAS survey were used to assess the individuals' public speaking anxiety level. The dietary assessment was performed based on the responses recorded via the dietary recall questionnaire and background information questionnaire.

Procedures

The study and procedure were fully explained to the participants in writing. Participants completed following tasks online via *Qualtrics* in the order presented. There were no requirements for the participants to change their diet during the study.

1. Screening questions. The screening questions were presented to potential volunteers. These volunteers were asked to answer the following questions.
    - Have you ever done public speaking before (e.g., giving a speech to a group)? (Yes, I have/No, never)
    - Are you between the ages of 18 and 55? (Yes/No)

Eligibility was verified based on their response. If they passed, they became eligible to participate in the study; if they failed, they were disqualified automatically from the study.

2. Informed consent. Once eligibility had been verified, participants were provided the informed consent in electronic format. The consent form outlined the nature and procedures of the study, clearly indicated that participation was voluntary and

that they may discontinue their participation at any time. The online consent form was set up in a way that the potential participants must click on the "I Agree" button indicating that they have read the consent and agree to participate. Once the button was selected, the potential participants were directed to the research survey questionnaires.

3. Public Speaking Anxiety Scale (PSAS). Participants were asked to complete PSAS and four additional questions.
4. Dietary Recall Questionnaire. Participants were asked to complete the questionnaire.
5. Background Information Questionnaire. Participants were asked to complete a survey that was designed with questions regarding demographics, health status, lifestyle, and public speaking experiences.

Data Collection

All data was collected electronically via *Qualtrics*. Data collection began on December 18, 2022 and ended on January 15, 2023.

Data Analysis

The study yielded two types of data: parametric (e.g., scores on PSAS) and nonparametric (e.g., dietary patterns and demographic characteristics including gender and the age of participants from the General Questionnaire). The data was analyzed using both descriptive and inferential statistics.

Association of food items and metadata (age, gender, BMI, weight, exercise frequency, and etc), as well as association of food items with the magnitude of change in

anxiety level, were analyzed using Spearman's correlation for each group separately. Spearman's was selected over Pearson's correlation due to the ordinal nature of some variables. Anxiety levels were compared using the one-way ANOVA for numerical values.

Descriptive statistics included the use of, for example, histograms and Normal Q-Q plot distributions to portray both the demographic characteristics of the participants as well as the means and standard deviations of the outcome measures. Further, Spearman's correlation coefficients were presented to indicate the relations between and among given variables.

With respect to inferential statistics, the data analytics strategy was as follows. To determine whether or not there is a relation between: (a) diet and public speaking anxiety level, the correlation was computed between the Dietary Recall Questionnaire responses and the first self-reported PSAS scores; (b) prebiotics and probiotics and public speaking anxiety levels, the correlation was determined between the Dietary Recall Questionnaire responses on the prebiotics and prebiotics food items and their PSAS scores.

Data was analyzed using IBM SPSS (Statistics for iOS, Version 28.0). The variables were coded based on the types of measurement scales they associated with. For example, the dietary patten groups were given dummy codes 0 (Plant-based diet group) and 1(Western diet group) for use as independent variables in statistical analysis.

Public Speaking Anxiety (PSA)

The PSA scores for the participants ranged from 23 to 78 with a mean (*M*) of 46.9 and standard deviation (*SD)* of 12.1. In addition, when asked how often they speak publicly, of 145 participants, 4.8% spoke in public daily (n = 7, M = 49.4, SD= 10.7),

11.0% regularly (*n* = 16, *M* = 42.1, *SD* = 11.8), 34.5% occasionally (*n* = 50, *M* = 45.0, *SD* = 9.9), 29.0% rarely (*n* = 42, M = 45.9, *SD* = 13.2), and 20.7% extremely rarely (*n* = 30, *M* = 53.5, *SD* = 12.4) (Figure 2).

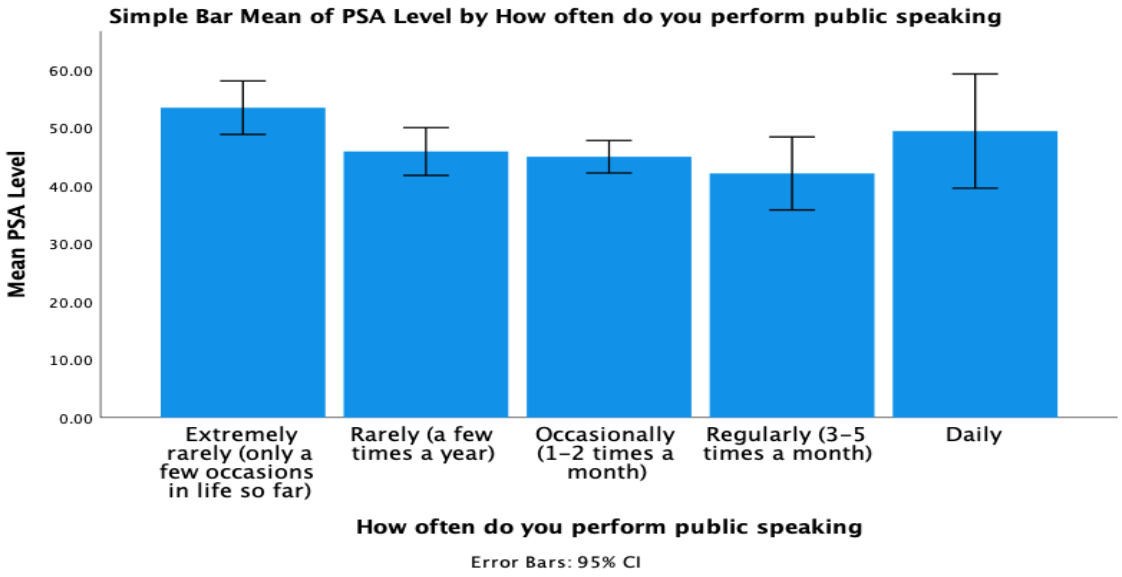

Figure 2. PSA Level by Frequency of Performing Public Speaking

Dietary Assessment

Dietary intake was assessed using 7-day dietary recall responses. The participants were asked to report the frequency of 21 types of food and beverages (Table 1) consumed over last 7 consecutive days. These food items were the core foods and beverages that make up the dietary patterns for the American population (Krebs-Smith et al., 2018; U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2020).

Table 1. Food Items Evaluated in the Present Study

Food Items	Dietary Recall Items Included
Water	Water
Tea or coffee	Tea or coffee no sugar and no sugar replacement
Soft drinks	Corn syrup, maple syrup, cane sugar, tea or coffee with sugar
Diet soft drinks	Stevia, Equal, Splenda, tea or coffee with sugar substitute
Alcohol	Alcohol
Fruit juice	Orange, apple, cranberry, prune, etc
Probiotic	Yogurt or other foods containing active bacterial cultures, Kefir, sauerkraut
Dairy	Milk, cream, ice cream, cheese, cream cheese
Vegetables	Salad, tomatoes, onions, greens, carrots, peppers, green beans
Beans	Tofu, soy, soy burgers, lentils, Mexican beans, lima beans
Whole grains	Wheat, oats, brown rice, rye, quinoa, wheat bread, wheat pasta
Refined grains	White rice, bread, pizza, potatoes, yam, cereals, pancakes
Eggs	Eggs
Processed meat	Other red meat and other white meat such as lunch meat, ham, salami, bologna, sausage, kielbasa, hotdog, bacon
Red meat	Beef, hamburger, pork, lamb
White meat	Chicken, turkey
Seafood	Shellfish – shrimp, lobster, scallops Fish – fish nuggets, breaded fish, fish cakes, salmon, tuna
Sweets	Pies, jam, chocolate, cake, cookies

The frequency of consumption of the food items was then quantified in terms of the number of times of consumed within the past 7 days. Participants were assigned a score for each food item based on their self-reported consumption frequencies, i.e., never

in the past 7 days (zero consumption) = 1 point, within the past 4 to 7 days = 2 points, within the past 2 to 3 days = 3 points, yesterday 1 to 2 times = 4 points, and yesterday 3 or more times = 5 points.

As shown in Table 2, on average, water, vegetables, fruits, starch, whole grains, dairy, sweets, and eggs are among the foods that were consumed by the participants most often with the average frequency score of 3.00 and above. Probiotics were the food type that was consumed least among the participants with the average frequency score of 2.00.

Table 2. Frequency of Food Intake by Participants

	M	*SD*
Sample size = 145		
Tea or coffee no sugar and no sugar replacement	2.58	1.36
Soft drinks, tea or coffee with sugar	2.87	1.29
Diet soft drinks, tea or coffee with sugar substitute	2.39	1.25
Fruit juice	2.52	1.24
Water*	**4.48**	.94
Alcohol	2.06	1.26
Yogurt or other foods containing active bacterial cultures	2.28	1.24
Dairy*	**3.28**	1.11
Probiotic	2.00	1.24
Fruits (no juice) *	**3.34**	1.02
Vegetables*	**3.70**	1.03
Beans	2.91	1.18
Whole grains*	**3.30**	1.05
Starch*	**3.32**	1.01
Eggs*	**3.12**	1.14
Processed meat	2.57	1.20
Red meat	2.63	1.22
White meat	2.86	1.05
Shellfish	2.03	1.81
Fish	2.16	1.15
Sweets*	**3.17**	1.02

M = mean; SD = standard deviation
**Food items with M ≥3.00*

The dietary pattern on an individual level was determined mainly based on the self-reported diet classifications, with necessary adjustments according to the dietary recall questionnaire responses. Of 145 respondents, 44.8% (*n* = 65) were on a plant-based diet while 55.2% (*n* = 80) were on a Western diet.

When asked if there were any particular foods and/or drinks they would usually have before the speaking events, 33.8% of participants reported 'yes' (*n* = 49, *M* = 47.6, *SD* = 12.3) and 66.2% reported 'no' (*n* = 96, *M* = 46.8, *SD* = 12.2).

Among those who responded with 'yes', 87.8% of them listed some type of drinks (*n* = 43, *M* = 46.3, *SD* =11.9) with water being the most popular one (*n* = 25, *M* = 45.2, *SD* = 12.8). 6.1% of participants listed they would regularly eat food (*n* = 3, *M* = 60.0, *SD*= 15.5), with fruits being the most popular item, and the remaining 6.1% listed a combination of drinks and food (e.g., water and bananas) (*n* = 3, *M* = 53.0, *SD* = 5.6).

Demographics, Health Status, and Lifestyle

The details about the demographics, health status, and lifestyles of the participants of the study were presented in Table 3.

Demographics. The majority of the participants were male (*n* = 86, 59.3%), aged 26 to 35 years old (*n* = 64, 44.1%), and Caucasian (*n* = 121, 83.4%).

Body Mass Index(BMI) in kg/m^2. BMI ranged widely from 7.2 to 51.3 with a mean of 24.4 (*SD* = 6.0). Almost half of them (*n* = 71, 49.0%) were with a healthy weight, while

42% were either overweight (*n* = 48, 33.1%) or had obesity (*n* = 13, 9.0%). 44.6% of the participants (*n* = 29) in the Plant-based group reported they were either overweight or obese, compared to 40.0% of those (*n* = 32) among the Western group.

Anxiety related mental disorders. When asked about mental health history, less than one third of the participants (*n* = 38, 26.2%) reported they had been diagnosed with a mental illness related to anxiety. Among these 38 participants, the majority (*n* = 21) reported they were on Western diets, while the remaining said they were on plant-based diets (*n* = 17).

Frequency of exercise. With regard to exercise, the majority of the participants (*n* = 116, 80.0%) reported that they exercise fairly regularly or daily. When examining the two diet groups, more than 85% of those who were on plant-based diets reported they would exercise at least once a week (*n* = 56, 86.2%), compared to 75.1% of those (*n* = 60) who were on Western diets.

Hours of sleep. When it comes to sleep, 71.1% reported, on average, that they get 6-8 hours of sleep (*n* = 103) each day. Slightly fewer participants in the plant-based group (*n* = 25, 38.5%) reported sleeping 7 hours or more per day on average, in comparison to those in the Western group (*n* = 34, 42.6%).

Frequency of dining-out. When asked how often they would dine out, including dining at a restaurant, getting take out, or having a meal delivered, 62.1% (*n* = 90) said they chose to dine out at least once a week. The percentage is much higher, closer to 70% in the Western group (*n* = 55, 68.9%), compared to those in the plant-based group (*n* = 35, 53.9%).

Daily multivitamin intake. Less than half of participants (*n* = 66, 45.5%) reported they would take daily vitamins. Slightly more people in the plant-based group (*n* = 35, 53.8%) reported that they would take vitamins on a daily basis compared to those in the Western group (*n* =31, 38.8%).

Antibiotics intake in past 12 months. When asked when was the last time that they took antibiotics, 64.8% (*n* = 94) reported they did so in the past year, while only 35.2% (*n* = 51) said they did not take any antibiotics in the past year. Among those who reported that they did not take any antibiotics in the past 12 months, 66.7% were in the Western group (*n* = 34) while 33.3% were in the plant-based group (*n* = 17).

Probiotics intake. More than half of the participants (*n* = 95, 65.5%) said they never have or have rarely taken probiotics. Close to 20% (*n*= 28, 19.3%) reported they take probiotics occasionally. Only 15.1% (*n* = 22) said they would take probiotics on a regular or daily basis. Among the people who were on plant-based diets, 12.3% (*n* = 8) took probiotics on a regular or daily basis, compared to 17.6% (*n* = 14) among the Western group.

Table 3. Characteristics of Participants

Characteristics	Total		Plant-based		Western	
	n	Percentage	*n*	Percentage	*n*	Percentage
Sample size	145	100%	65	44.8%	80	55.2%
Gender						
Male	86	59.3%	41	63.1%	45	56.3%
Female	59	40.7%	24	36.9%	35	43.8%
Age group						
18-25 years old	23	15.9%	9	13.8%	14	17.5%
26-35 years old	64	44.1%	27	41.5%	37	46.3%
36-45 years old	39	26.9%	22	33.8%	17	21.3%
46-55 years old	19	13.1%	7	10.8%	12	15.0%
Ethnicity						
Caucasian	121	83.4%	59	90.8%	62	77.5%
Asian or	18	12.4%	5	7.7%	13	16.3%
Pacific						
Islander						
Hispanic	5	3.4%	1	1.5%	4	5.0%
Others	1	0.7%	-	-	1	1.3%
BMI (in kg/m^2)						
Underweight	13	9.0%	5	7.7%	8	10.0%
Healthy	71	49.0%	31	47.7%	40	50.0%
Weight						
Overweight	48	33.0%	23	35.4%	25	31.3%
Obesity	13	9.0%	6	9.2%	7	8.7%
Frequency of Performing Public speaking						
Extremely rarely	30	20.7%	8	12.3%	22	27.5%
Rarely	42	29.0%	19	29.2%	23	28.7%
Occasionally	50	34.5%	27	41.5%	23	28.7%
Regularly	16	11.0%	9	13.8%	7	8.8%
Daily	7	4.8%	2	3.1%	5	6.3%
Anxiety Related Mental Disorders						
No	100	69.0%	45	69.2%	55	68.8%
Yes	38	26.2%	17	26.2%	21	26.3%
Prefer not to say	7	4.8%	3	4.6%	4	5.0%
Frequency of Exercise						
Never	5	3.4%	1	1.5%	4	5.0%
Rarely	24	16.6%	8	12.3%	16	20.0%
Occasionally	45	31.0%	24	36.9%	21	26.3%

Characteristics	Total		Plant-based		Western	
	n	Percentage	*n*	Percentage	*n*	Percentage
Regularly	48	33.1%	20	30.8%	28	35.0%
Daily	23	15.9%	12	18.5%	11	13.8%
Hours of Sleep						
Less than 5 hours	5	3.4%	5	7.7%	-	-
5-6 hours	30	20.7%	17	26.2%	13	16.3%
6-7 hours	51	35.2%	18	27.7%	33	41.3%
7-8 hours	52	35.9%	21	32.3%	31	38.8%
8 or more hours	7	4.8%	4	6.2%	3	3.8%
Frequency of Dining-out						
Never	5	3.4%	1	1.5%	4	5.0%
Rarely	50	34.5%	29	44.6%	21	26.3%
Occasionally	65	44.8%	30	46.2%	35	43.8%
Regularly	21	14.5%	4	6.2%	17	21.3%
Daily	4	2.8%	1	1.5%	3	3.8%
Daily Multivitamin Intake						
No	79	54.5%	30	46.2%	49	61.3%
Yes	66	45.5%	35	53.8%	31	38.8%
Antibiotics Intake in past 12 months						
Last week	13	8.9%	8	12.3%	5	6.3%
Last month	29	20.0%	17	26.2%	12	15.0%
Last 6 month	32	22.1%	13	20.0%	19	23.8%
Last year	20	13.8%	10	15.4%	10	12.5%
Never in the past year	51	35.2%	17	26.2%	34	42.5%
Probiotics intake						
Never	51	35.2%	21	32.3%	30	37.5%
Rarely	44	30.3%	19	29.2%	25	31.3%
Occasionally	28	19.3%	17	26.2%	11	13.8%
Regularly	15	10.3%	6	9.2%	9	11.3%
Daily	7	4.8%	2	3.1%	5	6.3%

Descriptive Statistics

The Kolmogorov-Smirnov and Shapiro-Wilk test was used to confirm whether the public speaking anxiety (PSA) scores in the sample were normally distributed. The result in Table 4 indicated that the anxiety scores, $D(145) = .066$, $p = .200$, did not deviate significantly from normality. The corresponding Q-Q plot (Figure 3) echoed this view because the data points fall very close to the "ideal" diagonal line.

Table 4. Kolmogorov-Smirnov and Shapiro-Wilk Test of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	Df	Sig.
PSA Level	.066	145	.200*	.986	145	.158

**This is a lower bound of the true significance*
[a] Lilliefors Significance Correction

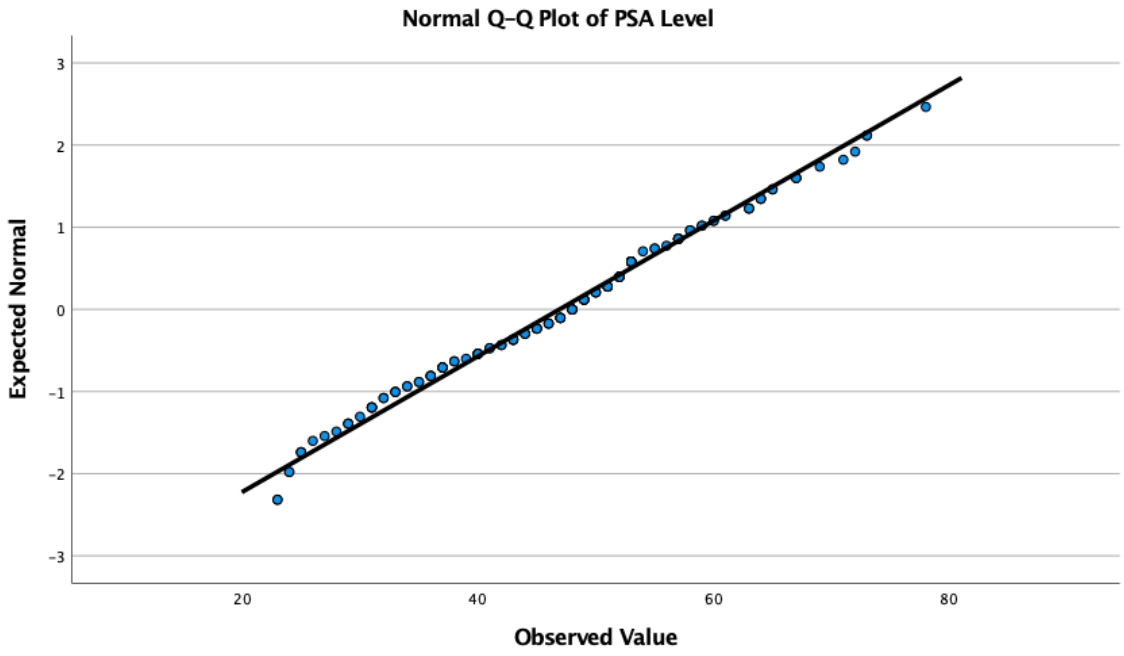

Figure 3. Normal Q-Q Plot of Public Speaking Anxiety Level

When analyzing based on the dietary patterns, on average, people on plant-based diets have lower PSA scores than those on Western diets (Figure 4). The PSA scores for the plant-based group ranged from 23 to 73 (n = 65, M = 45.7, SD = 11.8), comparing to the range of 23 to 78 for the Western group (n = 80, M = 47.9, SD =12.3).

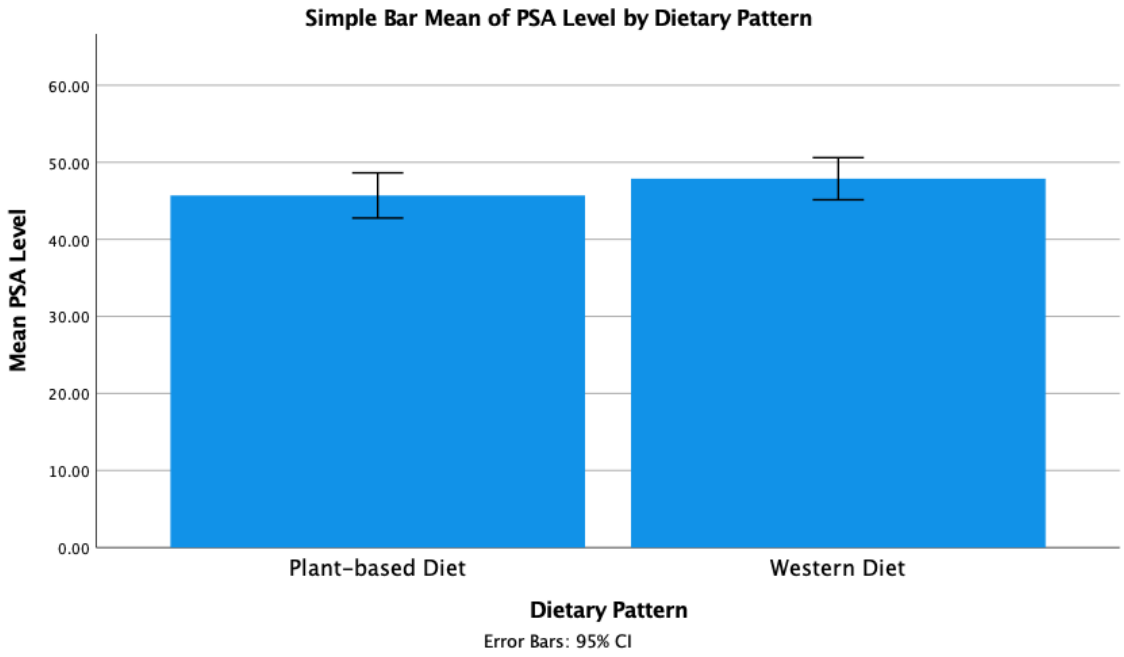

Figure 4. Comparison of Group Means by Dietary Pattern Classification

The more detailed descriptive statistics for mean scores by demographics and lifestyle variables were presented in Table 5. It appears that the average of PSA scores were higher across the groups in each category among the female participants, participants who have obesity, and participants who were diagnosed with anxiety disorders. In addition, the average of PSA scores were lower among those participants who exercised more often.

Table 5. Descriptive Statistics by Demographics and Lifestyle Variables

	Total (n = 145)		Plant-based (n = 65)		Western (n = 80)	
	M	*SD*	*M*	*SD*	*M*	*SD*
Gender						
Male	45.94	10.99	44.65	9.81	47.11	11.95
Female	48.32	13.55	47.50	14.74	48.89	12.86
Age group						
18-25 years old	44.04	12.35	42.78	9.42	44.86	14.21
26-35 years old	48.31	12.80	48.70	12.97	48.03	12.85
36-45 years old	46.23	10.62	44.82	11.14	48.06	9.94
46-55 years old	47.05	12.48	40.71	11.30	50.75	12.02
Ethnicity						
Caucasian	46.21	11.90	45.86	11.96	46.55	11.93
Asian or Pacific Islander	49.67	13.73	43.00	12.39	52.23	13.80
Hispanic	53.60	11.01	-	-	54.50	12.50
Others	48.00	12.11	-	-	-	-
BMI (in kg/m^2)						
Underweight	48.39	8.60	49.00	10.00	48.00	8.33
Healthy Weight	45.25	12.12	42.03	11.26	47.75	12.30
Overweight	47.85	11.51	47.78	12.31	47.92	10.99
Obesity	51.00	16.41	54.00	9.47	48.43	21.14
Frequency of Performing Public speaking						
Extremely rarely	53.46	12.38	46.25	8.73	56.09	12.61
Rarely	45.90	13.24	45.74	14.92	46.04	12.02
Occasionally	45.00	9.86	46.07	10.35	43.74	9.33
Regularly	42.13	11.83	41.33	11.66	43.14	12.90
Daily	49.43	10.67	58.00	8.49	47.89	12.31
Anxiety Related Mental Disorders						
No	45.20	12.98	43.00	12.00	47.00	13.57
Yes	50.71	9.30	51.47	9.41	50.10	9.40
Prefer not to say	50.71	6.95	53.67	8.08	48.50	6.14
Frequency of Exercise						
Never	51.20	19.99	-	-	55.75	19.87
Rarely	50.71	12.61	52.63	10.81	49.75	13.65
Occasionally	48.18	11.41	46.75	11.06	49.81	11.85
Regularly	44.17	12.27	43.20	12.71	44.96	12.14
Daily	45.26	9.80	44.25	11.86	46.36	7.34

	Total (n = 145)		Plant-based (n = 65)		Western (n = 80)	
	M	*SD*	*M*	*SD*	*M*	*SD*
Hours of Sleep						
Less than 5 hours	42.60	13.45	42.60	13.47	-	-
5-6 hours	51.77	10.28	47.53	9.43	57.31	8.84
6-7 hours	49.61	11.38	49.50	10.84	49.67	11.84
7-8 hours	42.04	12.15	41.10	13.84	42.68	11.06
8 or more hours	45.71	12.19	49.00	6.83	41.33	18.03
Frequency of Dining-out						
Never	45.20	17.51	-	-	48.75	18.03
Rarely	48.96	12.11	47.00	9.22	51.67	15.09
Occasionally	44.52	11.52	44.00	13.43	44.97	9.78
Regularly	49.52	11.97	48.25	14.66	49.82	11.76
Daily	48.50	13.82	-	-	43.33	11.24
Daily Multivitamin Intake						
No	45.86	13.68	46.86	10.53	49.65	8.98
Yes	48.17	9.86	44.37	13.26	46.78	13.99
Antibiotics Intake in past 12 months						
Last week	40.15	9.06	39.13	10.78	41.80	6.14
Last month	46.48	9.62	47.06	8.78	45.67	11.96
Last 6 month	46.66	13.81	44.62	13.81	48.05	14.01
Last year	48.05	11.98	48.80	12.87	47.30	11.67
Never in the past year	48.59	12.75	46.47	12.87	49.65	12.74
Probiotics Intake						
Never	48.94	14.14	47.10	14.30	50.23	14.13
Rarely	43.36	11.28	44.32	10.95	42.64	11.69
Occasionally	44.43	10.10	43.24	10.81	46.27	9.06
Regularly	53.40	8.17	49.00	7.32	56.33	7.68
Daily	50.43	7.07	55.50	12.02	48.40	4.56

M = mean; SD = standard deviation

Limitations and Potential Biases in Method

There are several limitations in method and potential biases introduced by procedures should be noted.

First, general measurement concerns raised from this study should be addressed. Because the questionnaires used in the present study were based on self-reported measures, validity is one of the most articulated problems. There might be cases in which the participants misestimated or misreported their food consumption and/or dietary classification intentionally (e.g., individuals who feel embarrassed by lack of compliance) or unintentionally. It is likely that results from the surveys should be reasonably valid; however, they may show less consistency than with other techniques.

Secondly, the 7-day recall questionnaire that was used in the study focused on the frequency of consumption of the food groups. There was no portion size indicated for each item included, therefore, the diet assessment did not measure the individual's macronutrients or the energy intake of the participants. We also acknowledged that the creation of the dietary indices (i.e., PDI and WeDI), based on the frequency scores, may have potential to oversimplify the complexity of dietary intake. A combination of dietary recall questionnaires and food frequency questionnaires, with more detailed questions about the individuals' food consumption in both quantity and frequency, may be able to produce a more in-depth analysis in the area of dietary assessment.

Additionally, online recruitment might have led to potential biases in our participant sample. The participants in the present study had an over-representation of those who were younger and of Caucasian males. As such, generalization of the results can be limited.

Last but not least, even though using an online survey makes collecting data easier and faster, the format does not provide opportunities for the participants or the

research team to interact and clarify questions, which might increase errors and reduce the accuracy of the responses.

Chapter III.

Results

A summary of the key results is presented in Table 6. The data are adjusted results after controlling for age group, gender, ethnicity, frequency of performing public speaking, and anxiety related mental disorders.

Correlation coefficient analysis revealed two food items, i.e., water (r = -.203, 95% BCa CI [-.338, -.075], p = .016), and vegetables (r = -.236, 95% BCa CI [-.397, -.067], p = .005) had an inverse association with PSA levels, respectively. There were no significant relations found with the remaining food items in Table 1.

To examine the combined effect of these food items on the level of public speaking anxiety, we looked into different combinations of the items and identified two versions of dietary indices (Table 7). It appears that higher Plant-based Dietary Index (PDI) was associated with lower levels of public speaking anxiety (r = -.201, 95% BCa CI [-.380, -.024], p = .017), while higher Western Dietary Index (WeDI) was associated with higher public speaking anxiety (r = .217, 95% BCa CI [.060, .364], p = .010) (Table 6).

PDI is comprised mainly vegetables, beans, fruits, water, seafood, and whole grains while maintaining a low intake of processed meat, meats, sugary drinks, and sweets. WeDI focuses on the consumption of high fat and high sugar foods such as processed meat, meats, starches, dairy, and sweets, while the intake of fruits, vegetables, and whole grains are low. Food items included in each index were given either positive or reverse scores. For creating the PDI, the plant-based and seafood food groups were given

positive scores, and the high fat and high sugar food groups were given reverse scores. For creating the WeDI, positive scores were given to the high fat and high sugar food items, and reversed scores were given to the plant-based food items.

In addition to the food items and dietary patterns mentioned above, we also observed that the PSA level was significantly related to the frequency of how often one performs public speaking (r = -.218, 95% BCa CI [-.378, -.045], p = .009), diagnosis of anxiety related mental disorders (r = .236, 95% BCa CI [.086, .389], p = .006), frequency of exercise (r = -.223, 95% BCa CI [-.382, -.053], p = .008), and hours of sleep (r = -.216, 95% BCa CI [-.368, -.060], p = .010) (Table 6).

To test the secondary hypothesis, we specifically looked at the data collected from the participants that were on Western diets, and examined the relation between their PSA level and their total consumption of prebiotics (i.e., vegetables, fruits, beans, and whole grains) and that of probiotics (i.e., yogurt and probiotics other than yogurt) within the group (n = 80). There was no significant relation observed with the intake of prebiotics with adjusted values for age group, gender, and ethnicity, r = -.062, 95% BCa CI [-.304, .171], p = .592. Probiotics was not found significantly related to public speaking anxiety level either, r = 0.093, 95% BCa CI [-.172, .371], p = .420.

Table 6. Association between PSA Level and Variables

	Correlation Coefficient (*r*)	Sig. (2-tailed) (*p*)	*SE*	BCa 95% Confidence Interval	
				Lower	Upper
Tea or coffee no sugar and no sugar replacement [a]	-.004	.965	.084	-.167	.148
Soft drinks, tea or coffee with sugar [a]	.056	.509	.088	-.129	.234
Diet soft drinks, tea or coffee with sugar substitute [a]	-.027	.747	.087	-.185	.171
Fruit juice [a]	-.046	.589	.088	-.218	.113
Water [a]	**-.203**	.016	.070	-.338	-.075
Alcohol [a]	.127	.131	.080	-.037	.277
Yogurt or other foods containing active bacterial cultures [a]	.091	.287	.085	-.085	.257
Dairy [a]	.022	.794	.083	-.142	.183
Probiotic [a]	.113	.182	.080	-.047	.265
Fruits (no juice) [a]	-.069	.417	.084	-.086	.214
Vegetables [a]	**-.236**	.005	.080	-.397	-.067
Beans [a]	-.001	.989	.084	-.163	.172
Whole grains [a]	-.102	.233	.084	-.264	-.080
Starch [a]	.024	.783	.089	-.142	.180
Eggs [a]	.035	.683	.085	-.133	.191
Processed meat [a]	-.036	.670	.086	-.196	.128
Red meat [a]	.156	.066	.089	-.021	.314

	Correlation Coefficient (*r*)	Sig. (2-tailed) (*p*)	*SE*	BCa 95% Confidence Interval	
				Lower	Upper
White meat [a]	.094	.271	.087	-.078	.256
Shellfish [a]	.101	.233	.087	-.096	.292
Fish [a]	-.049	.564	.082	-.195	.107
Sweets [a]	.116	.172	.089	-.056	.280
PDI [a]	**-.201**	.017	.090	-.380	-.024
WeDI [a]	**.217**	.010	.080	.060	.364
Gender[b]	.186	.066	.086	-.020	.362
Ethnicity[b]	.146	.082	.074	.016	.299
Age[b]	.027	.746	.082	-.141	.205
Frequency of Performing Public speaking [c]	**-.218**	.009	.000	-.378	-.045
Anxiety Related Mental Disorders[c]	**.236**	.006	.086	.086	.389
BMI (in kg/m^2) [d]	.113	.182	.089	-.060	.279
Frequency of Exercise [d]	**-.223**	.008	.085	-.382	-.053
Hours of Sleep [d]	**-.216**	.010	.080	-.368	-.060
Frequency of Dining-out [d]	-.041	.633	.088	-.215	.129
Daily Multivitamin Intake [d]	-.078	.359	.084	-.003	.084
Antibiotics Intake in past 12 months [d]	.154	.068	.077	-.002	.298
Probiotics Intake [d]	.004	.963	.083	-.159	.185

n=145; PSA Level = 1.000
[a]Correlation coefficient adjusted for age group, gender, ethnicity, frequency of performing public speaking and anxiety related mental disorders
[b]Correlation coefficient adjusted for frequency of performing public speaking and anxiety related mental disorders
[c]Correlation coefficient adjusted for age group, gender, and ethnicity
[d]Correlation coefficient adjusted for age group, gender, ethnicity, anxiety related mental disorders

Table 7. Plant-based Diet Index (PDI) and Western Diet Index (WeDI)

	Description	PDI	WeDI
Water	Water	Positive scores	Reverse scores
Soft drinks	Corn syrup, maple syrup, cane sugar, tea or coffee with sugar	Reverse scores	Positive scores
Dairy	Milk, cream, ice cream, cheese, cream cheese	Not included	Positive scores
Vegetables	Salad, tomatoes, onions, greens, carrots, peppers, green beans	Positive scores	Reverse scores
Beans	Tofu, soy, soy burgers, lentils, Mexican beans, lima beans	Positive scores	Reverse scores
Whole grains	Wheat, oats, brown rice, rye, quinoa, wheat bread, wheat pasta	Positive scores	Reverse scores
Refined grains	White rice, bread, pizza, potatoes, yam, cereals, pancakes	Not included	Positive scores
Processed meat	Other red meat and other white meat such as lunch meat, ham, salami, bologna, sausage, kielbasa, hotdog, bacon	Reverse scores	Positive scores
Red meat	Beef, hamburger, pork, lamb	Reverse scores	Positive scores
White meat	Chicken, turkey	Reverse scores	Positive scores
Seafood	Shellfish – shrimp, lobster, scallops	Positive scores	Not included

	Description	PDI	WeDI
	Fish – fish nuggets, breaded fish, fish cakes, salmon, tuna		
Sweets	Pies, jam, chocolate, cake, cookies	Reverse scores	Positive scores

Covariates

The study identified four covariates from the collected data, i.e., frequency of performing public speaking, anxiety related mental disorders, frequency of exercise, and hours of sleep. Age group, gender, ethnicity, BMI in kg/m^2, frequency of dining-out, intake of daily multivitamins, antibiotics, and probiotics were all non-significant.

Regression

One-way ANOVA analysis was used for summarizing the relation between the predictor variables and public speaking anxiety. Multicollinearity was detected between the potential confounding variables, i.e., anxiety related mental disorders and sleep (p = .015), frequency of performing public speaking anxiety and exercise (p = .007), exercise and sleep (p = .038). Even though the levels of the collinearity were low ($r < 0.8$), frequency of exercise and hours of sleep were removed from the adjusted model to correct for multicollinearity. The final results adjusted for anxiety related mental disorders and frequency of performing public speaking are presented in Table 8.

Water and vegetables were predictors that have significant impact on our ability to predict the public speaking anxiety level, $t(143) = -2.00$, $p = .047$, and $t(143) = -2.56$, $p = .011$.

Plant-based dietary pattern (i.e., PDI), which emphasizes the consumption of fruits, vegetables, water, beans, seafood, and whole grains with low intake of processed meat, meats, sweets, and sugary drinks, was a predictor of the PSA level, $t(143) = -2.16$, $p = .040$. Western dietary pattern (i.e., WeDI), which emphasizes the consumption of processed meat, meats, sweets, sugary drinks, and starch, with less fruits, vegetables, water, and whole grains, appeared to be a predictor of the PSA level as well, $t(143) = 2.06$, $p = .042$.

Table 8. Linear Model of Food Intake Predicting PSA Level Adjusted for Frequency of Performing Public Speaking and Anxiety Related Mental Disorders

	R Square	*F-test*	*P*	*B*	*SE*	*t*	*P*
Water	.10	5.40	.002	**-2.10** (-4.17, -.02)	1.05	-2.00	.047
Vegetables	.12	6.32	<.001	**-2.40** (-4.25, -.55)	.94	-2.56	.011
PDI	.11	5.50	.001	**-.44** (-.86, -.02)	.21	-2.16	.040
WeDI	.10	5.48	.001	**.39** (.02, .76)	.19	2.06	.042

95% Bias Corrected and Accelerated Confidence Intervals Reported in Parentheses
Confidence Intervals and Standard Errors based on 1000 Bootstrap Samples

Chapter IV.

Discussion

There have been peer reviews regarding diet and mental disorders including general anxiety. However, to our knowledge, this is the first study trying to link diet with public speaking anxiety; therefore, it is of great interest and importance.

General Discussion

The observed relations between diet and public speaking anxiety support the hypothesis that individuals who are on a plant-based diet experience lower levels of public speaking anxiety. Our results are consistent with prior studies (Cryan et al., 2019; Sandhu et al., 2017), and could be potentially explained by the diet-microbiota-gut-brain axis-based mechanism.

As mentioned in Chapter I, the gut-brain axis is a bidirectional communications network that allows the brain and the gut to communicate through gut microbes. The HPA axis is part of this network and becomes activated under stress when preparing the body for the “fight or flight” response (Sandhu et al., 2017). Dysregulation of the HPA axis appears to be associated with stress-related disorders (Leclercq et al., 2016). Gut microbiota has been demonstrated effective in normalizing the HPA axis response to stress (Sudo et al., 2004), and modulating stress-induced anxiety (Tillisch et al., 2013). Diverse gut microbiota is necessary for the continuous preservation of a healthy microglia and proper brain function (Cryan et al., 2019).

Diet is considered to be one of the most critical factors in modulating gut microbiota composition along with the brain and its behavior (Cryan et al., 2019). The positive impacts of the plant-based diet are associated with its ability to change the composition of the gut microbiota, increase the abundance of *Bacteroides*, and reduce *Firmicutes,* when compared with high-fat and high sugar Western diets (Cryan et al., 2019; Tomova et al., 2019).

Fruits and vegetables. On an individual food item level, evidence from longitudinal studies suggested that consumption of fruits and vegetables not only influences physical health in the long-run, but also plays a role in improving mental health and well-being in the short-run (Mujcic & Oswald, 2019; Ocean et al., 2019). According to these studies, mental well-being is correlated to increases in the quantity and frequency of fruit~~s~~ and vegetable~~s~~ intake in a dose-response fashion. It is important to note that an upward trend until the age of 64 in the consumption of fruits and vegetables was documented in the studies.

In the current study, the data did not indicate an association between fruit~~s~~ intake and public speaking anxiety, however, it did indicate that greater consumption of vegetables is associated with the lower levels of public speaking anxiety. The possible explanations for the different results from consumption of fruits might be due to the selection and sample size of the study population. In comparison to the current study of 145 individuals aged 18 to 55 years old, the study Ocean and colleagues conducted involved 90,448 individuals aged between 15 to 66 years old and above. Mujcic and Oswald also had a large sample size of 7,108 with the same age range in their study as Ocean's.

Water. As a food and as an essential nutrient, water was found to be positively associated with lower levels of public speaking anxiety in the present study. This result is consistent with findings from a study conducted among a large sample of adults (n = 3327) in Iran. The researchers investigated the relation between drinking plain water and the risk of depression and anxiety, and found that participants who consumed greater amounts of plain water had lower anxiety and depression scores (Haghighatdoost et al., 2018).

Whole grains. In the current study, an inverse association between whole grains and public speaking anxiety was found in the crude model, however, after adjusting potential confounders, this inverse link became insignificant.

Whole grains have been associated with a number of health benefits such as a lower risk of type 2 diabetes, colorectal cancer, and cardiovascular diseases (Hu et al., 2020; Tieri et al., 2020). The link between whole grains and positive health outcomes is not surprising given the fact that whole grains are high in dietary fiber and contain vitamins, minerals, and phytochemicals with antioxidant properties (Tieri et al., 2020).

Yet, there is no empirical consensus on whether whole grains has an effect on mood disorders in the existing literatures. Ross and colleagues (2023) reviewed 23 literatures researching the relation between whole-grain intake and cognition, mood, and anxiety, concluding that the effect of whole grains on mood and anxiety is mixed and varied by study design.

The inconsistent results before and after controlling the covariates in this study demonstrated that the association between whole-grain intake and anxiety (e.g., public speaking anxiety) is inconclusive and could be confounded by other factors such as mental health status.

Dietary pattern. In addition to the individual food items mentioned above, overall dietary intake and dietary patterns were also assessed in the current study, given the complex interactions among nutrients in our daily diets. The results suggest that a plant-based dietary pattern (PDI) characterized by high consumption of vegetables, beans, fruits, whole grains, seafood including fish and shellfish, and water, with a low intake of processed meat, red meat, white meat, sugary drinks, and sweets, has a positive correlation with lower public speaking anxiety.

On the other hand, a Western diet (WeDI) characterized by high consumption of meat (i.e., processed meat, red meat, white meat), dairy, starch, and sugary products (i.e., sweets and sugary drinks), with a low intake of fruits, vegetables, and whole grains, was found to be associated with higher levels of public speaking anxiety.

It is worth noting that the findings about correlation between the WeDI and PSA level is different compared to the examination on an individual food item level. No association was observed between PSA level and the consumption of food items that are high fat and high sugar (e.g., processed meats, red meat, sugary drinks, and sweets). The difference indicates the importance of diet composition. As prior research noted, other than the content of individual nutrients, nutrient composition could be more important (Botchlett & Wu, 2018).

Prebiotics and probiotics. In addition to examining the group of participants as a whole, the present study also zoomed in on individuals who were on Western diets and investigated the association between public speaking anxiety and the intake of prebiotics and probiotics within the subgroup. On the total intake levels, i.e., intake of all prebiotic food items (i.e., vegetables, fruits, beans, and whole grains) and intake of all probiotic

food items (i.e., yogurt and probiotics other than yogurt), there was no statistical significance found in either of these categories, as would be expected. This result contradicted some prior reviews that suggested a high-prebiotics diet or a high-probiotics diet had positive effects on the reduction of mood disturbance and anxiety (Freijy et al., 2023; Liu et al., 2019). The different findings might be a result of the sample size and data insufficiency (e.g., lack of information on dose of prebiotics and probiotics intake) of the current study.

Potential Alternative Explanations for Observed Relations

Although an association between public speaking anxiety and these food items/dietary patterns was detected, the relation is fairly weak. The correlation coefficient values of the relations discussed above were all relatively small (i.e., $0.02 < R^2 < 0.13$, Table 8) based on benchmarks in Cohen (1988). This suggests that proportion of variance explained by the model was limited. There are a few possible explanations for the weak association.

First of all, in the domain of microbiota-gut-brain mechanism, the composition of the gut microbiota is impacted by multiple factors. Even though diet is one of the critical factors modulating gut microbiota composition, it is not the only factor. The microbial composition of an individual also depends on genetic predisposition, age, overall lifestyle, diseases, medication, use of antibiotics, and etc (Cryan et al., 2019; Kostic et al., 2014; Lee & Kim, 2021; Liang et al., 2018). For example, the current study noted that, percentage-wise, the participants on Plant-based diets exercised more, dined out less, and took daily multivitamins more often (Table 5).

Secondly, besides diet and gut-brain axis, public speaking anxiety levels can also be influenced by other factors such as pre-existing mental health conditions associated with anxiety (Tran & Chambless, 1995), frequency of performing public speaking (Byers & Weber, 1995; Smith & Frymier, 2006), and/or lifestyles (Chellappa & Aeschbach, 2022; Kandola & Stubbs, 2020). As noted in the present study, higher public speaking anxiety was associated with anxiety related mental disorders, while healthy lifestyle (e.g., more exercises or more sleep) and higher frequency of performing public speaking both had a positive effect in reducing public speaking anxiety.

Lastly, the possibility exists that the small size of the effect results from the combination of all the factors mentioned above.

Research Limitations

There are a number of important limitations of this study that deserve mention.

The main limitation is the observational study design. The study was carried out via a survey without interventions. With the study not being a controlled experiment and no randomization applied, the responses collected from the survey might be influenced by unmeasured confounding biases and the conclusions drawn from these responses might not be generalized to other populations. In addition, because data collected via the survey were at a single point in time, it is difficult to measure changes in the population.

Additionally, the sample size was fairly small at 145 participants. Generalization of the results can be limited. A larger sample with more diversity in the participant's age and ethnicity, as well as occupation, education, socioeconomic status, and medical history may be able to provide more clarity to the findings.

Further, due to the limitations of the study scope, the researcher was unable to either collect stool samples or to perform shotgun metagenomic sequencing which would provide higher resolution and sensitivity in microbiome analysis. As we seek to better understand how microbiome influences the host and the gut-brain axis, recent studies have capitalized on new technologies and provided not only more reliable estimates of the composition and diversity of microbiome, but also valuable insights into human genetics and the functional potential of the microbiome. For example, a more recent study combining metagenomic sequencing and functional genomic analysis has shed light on the differences in the composition of pre-industrial versus industrial gut microbiomes, and discovered previously undescribed gut microorganisms from ancient microbiomes (Wibowo et al., 2021). Research like this showcases the immense potential of metagenomic sequencing and other advanced methodologies in the field to investigate alternative therapies for human diseases. The possibility of utilizing them in the area of public speaking anxiety research should be explored in the future.

Last but not the least, we acknowledged that there are limitations and potential biases introduced by the research method and procedures used, and addressed their potential impact in Chapter II.

Future Directions

To our knowledge, the present study is one of the first to present data suggesting an association between diet and public speaking anxiety. Results from the study confirmed that a plant-based diet, specifically vegetables and water, was associated with lower levels of public speaking anxiety. Knowing that certain foods influence public

speaking anxiety levels may have the potential of affecting an individual's food choices and dietary behaviors. This study highlights the need for further investigation in this area.

Even though the findings on the association between dietary patterns and public speaking anxiety were consistent with prior reviews, we failed to detect a statistical significance specifically concerning the association between public speaking anxiety and the intake of prebiotics and probiotics among participants who were on a Western diet. That being the case, there would be additional value to investigate this area further via controlled studies in the future.

In conclusion, this correlation study revealed an association between diet and public speaking anxiety level. As this study is observational, further examination of the same research questions in well-designed cross-sectional studies should be pursued to strengthen the evidence and findings.

Additionally, due to the limitation of the research design of the current study, it is not possible to make any conclusions about the causal nature of the relation between diet and public speaking anxiety. Further research is needed to determine the direction of such a relation. Future studies could focus on research questions such as: what is the effect of diet on public speaking anxiety? We recommend that researchers investigate the topic with a larger and more diverse population through randomized controlled dietary intervention trials targeting the role of dietary components and gut microbiota in the onset of public speaking anxiety levels in adults.

A thorough understanding the effect of diet on public speaking anxiety is integral to understanding the role of diet in the microbiota-gut-brain axis, and to facilitate the

development of a next generation of specific, efficacious, and cost-effective non-medical solutions for treating public speaking anxiety.

Appendix 1.

Public Speaking Anxiety Scale

1= not at all, 2= slightly, 3=moderately, 4= very, 5= extremely

6. Giving a speech is terrifying.
7. I am afraid that I will be at a loss for words while speaking.
8. I am nervous that I will embarrass myself in front of the audience.
9. If I make a mistake in my speech, I am unable to re-focus.
10. I am worried that my audience will think I am a bad speaker.
11. I am focused on what I am saying during my speech.
12. I am confident when I give a speech.
13. I feel satisfied after giving a speech.
14. My hand shakes when I give a speech.
15. I feel sick before speaking in front of a group.
16. I feel tense before giving a speech.
17. I fidget before speaking.
18. My heart pounds when I give a speech.
19. I sweat during my speech.
20. My voice trembles when I give a speech.
21. I feel relaxed while giving a speech.
22. I do not have problems making eye contact with my audience.

Additional Questions to Public Speaking Anxiety Scale

1. How often do you perform public speaking (e.g., giving a speech or presentation to a group of people)?
    - Extremely rarely (only a few occasions in life so far)
    - Rarely (a few times a year)
    - Occasionally (1-2 times a month)
    - Regularly (3-5 times a month)
    - Daily

2. Are there any particular types of food and/or drinks (e.g., apples, bananas, water, coffee, etc.) you usually have before your speaking events?
    - None
    - Yes (please specify)

3. When was the last time you gave a speech or performed any other type of public speaking?

    ____ day(s) ago

4. (cont. Q3) How did it go?
    - Better than usual
    - Worse than usual
    - No significant difference than usual

Appendix 2.

Dietary Recall Questionnaire

Dietary Recall	**Did you eat or drink the following products in the last 7 days?**	**If yes, how recently?** *please choose only <u>one</u> response per category, from this column
Example: **Vegetables** (salad, tomatoes, onions, greens, carrots, peppers, green beans, etc.)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times
Tea or coffee <u>no</u> sugar and <u>no</u> sugar replacement	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times
Soft drinks, tea or coffee <u>with</u> sugar (corn syrup, maple syrup, cane sugar, etc.)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times

Dietary Recall	**Did you eat or drink the following products in the last 7 days?**	**If yes, how recently?** *please choose only <u>one</u> response per category, from this column
Diet soft drinks, tea or coffee <u>with</u> sugar substitute (Stevia, Equal, Splenda, etc.)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times
Fruit juice (orange, apple, cranberry, prune, etc.)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times
Water	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times
Alcohol (beer, brandy, spirits, hard liquor, wine, aperitif, etc.)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times

Dietary Recall	**Did you eat or drink the following products in the last 7 days?**	**If yes, how recently?** *please choose only <u>one</u> response per category, from this column
Yogurt or other foods containing active bacterial cultures (kefir, sauerkraut, etc.)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times
Dairy (milk, cream, ice cream, cheese, cream cheese)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times
Probiotic (other than yogurt)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times
Fruits (no juice) (Apples, raisins, bananas, oranges, strawberries, blueberries, etc. (frozen or fresh)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times

Dietary Recall	**Did you eat or drink the following products in the last 7 days?**	**If yes, how recently?** *please choose only <u>one</u> response per category, from this column
Vegetables (salad, tomatoes, onions, greens, carrots, peppers, green beans, etc.)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times
Beans (tofu, soy, soy burgers, lentils, Mexican beans, lima beans, etc.)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times
Whole grains (wheat, oats, brown rice, rye, quinoa, wheat bread, wheat pasta, etc.)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times
Starch (white rice, bread, pizza, potatoes, yam, cereals, pancakes, etc.)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times

Dietary Recall	**Did you eat or drink the following products in the last 7 days?**	**If yes, how recently?** *please choose only <u>one</u> response per category, from this column
Eggs	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times
Processed meat (other red meat and other white meat such as lunch meat, ham, salami, bologna, sausage, kielbasa, hotdog, bacon, etc.)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times
Red meat (beef, hamburger, pork, lamb)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times
White meat (chicken, turkey, etc.)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times

Dietary Recall	**Did you eat or drink the following products in the last 7 days?**	**If yes, how recently?** *please choose only <u>one</u> response per category, from this column
Shellfish (shrimp, lobster scallops, etc.)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times
Fish (fish nuggets, breaded fish, fish cakes, salmon, tuna, etc.)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times
Sweets (pies, jam, chocolate, cake, cookies, etc.)	○ No, I did <u>not</u> consume these products in the last 7 days	○ Within the past **4 to 7 days** ○ Within the past **2 to 3 days** ○ **Yesterday**, **1 to 2** times ○ **Yesterday**, **3 or more** times

Appendix 3.

Background Information Questionnaire

1. Gender: How do you identify?

    - Male
    - Female
    - Non-binary/third gender
    - Prefer not to say

2. What is your age group?

    - 18-25 years old
    - 26-35 years old
    - 36-45 years old
    - 46-55 years old

3. What race or ethnicity best describes you?

    - Caucasian
    - Asian or Pacific Islander
    - African American
    - Hispanic
    - Other (please specify)

4. Please tell us about your height.

   ___ Feet___Inches

5. Please tell us about your weight.

   ___lbs

6. Approximately how many hours of sleep do you get on an average night?

   - Less than 5 hours
   - 5-6 hours
   - 6-7 hours
   - 7-8 hours
   - 8 or more hours

7. Are you taking a daily multivitamin?

   - Yes
   - No

8. When is the last time you took any antibiotics?

   - Last week
   - Last month
   - Last 6 months

- Last year
- I have not taken antibiotics in the past year
- Other (please specify)

9. Have you ever been diagnosed with mental illness associated with anxiety?

- No
- Yes
- Prefer not to say

10. How would you classify your diet?

- I eat anything with no exclusions (omnivore)
- I eat anything except red meat
- Mediterranean
- Vegetarian
- Vegan

11. Do you follow any other special diet restrictions?

- No
- Yes (please specify)

12. How often do you exercise?

- Never
- Rarely (a few times/month)
- Occasionally (1-2 times/week)
- Regularly (3-5 times/week)
- Daily

13. How often do you dine out (including dine at a restaurant, get take out or have a meal delivered)?

- Never
- Rarely (a few times/month)
- Occasionally (1-2 times/week)
- Regularly (3-5 times/week)
- Daily

14. How often do you take a probiotic?

- Never
- Rarely (a few times/month)
- Occasionally (1-2 times/week)
- Regularly (3-5 times/week)
- Daily

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