EXAMINING THE EFFECTS OF CONSUMING MUFFINS CONTAINING BREWER'S SPENT GRAINS ON FIBER INTAKE, HEALTHY EATING INDEX SCORES, AND INFLAMMATORY BIOMARKERS IN HEALTHY ADULTS

A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER'S IN SCIENCE

IN THE GRADUATE SCHOOL OF THE

TEXAS WOMEN'S UNIVERSITY

DEPARTMENT OF NUTRITION AND FOOD SCIENCES

COLLEGE OF HEALTH SCIENCES

BY MARLEY GRAMS, B.S

DENTON, TEXAS

DECEMBER 2021

DEDICATION

To my parents, David and Kerri, my sister Madi, for their endless love, patience, and support. To my grandparents, Leona and George, whom I would not have made it through my higher education without their monetary support and constant confidence in my dreams. To my aunt, Audrey, who I know is looking down on me cheering me on.

ACKNOWLEDGEMENTS

There are several individuals I would like to acknowledge for their contributions during my graduate education at Texas Woman's University. First, I would like to thank my advisor, Dr. Cynthia Warren for her guidance and support throughout not only my graduate degree and thesis project but also my dietetic internship and new careers as a Registered Dietitian. Dr. Warren's guided my application for grants and her expertise in grant writing helped me to obtain the money I needed to complete my assays. Thank you to Dr. Shanil Juma for teaching me the proper techniques to complete my assays and for allowing me to use his lab bench to complete my study. Thank you to Dr. Carolyn Moore for having confidence in me and my research study. Dr. Moore awarded me the necessary funds to complete my blood sample analysis through the Moore-Khourie award. I want to thank Dr. Derek Miketinas and Dr. Wanyi Wang for providing me much needed support and guidance regarding my statistical analysis. Thank you to Dr. Mindy Patterson for your support on study design, methodology, and thesis writing. Her support in helping to create my thesis project and in connecting me with Dr. Shannon Combest to complete nutrient intake analyses and assay is much appreciated. Thank you to Dr. Shannon Combest for allowing me to complete a secondary analysis on her dissertation project and allowing me to learn alongside her in the lab. Finally, I want to acknowledge and thank my family who has loved, encouraged, and cared for me during my graduate education.

ABSTRACT

MARLEY EVAN GRAMS

EXAMINING THE EFFECTS OF CONSUMING MUFFINS CONTAINING BREWER'S SPENT GRAINS ON FIBER INTAKE, HEALTHY EATING INDEX SCORES, AND INFLAMMATORY BIOMARKERS IN HEALTHY ADULTS

DECEMBER 2021

Brewer's spent grain (BSG) is the barley byproduct that is created during the beer brewing process. This nutrient-dense waste product is not only rich in dietary fiber, but also protein. This study evaluated the impact of consuming BSG on dietary fiber intake, Healthy Eating Index (HEI) scores as well as the pro-inflammatory cytokines IL-6, IL-1beta, and anti-inflammatory cytokine IL-10 in healthy adults (n = 37; 26.0 ± 4 y; BMI 22.9 ± 3 kg/m^2) using secondary data from a single-blinded, randomized, controlled study. Diet records were collected for two weekdays and one weekend day at three timepoints from participants who either consumed muffins with 0 g BSG (control; n = 18) or 10.4 g BSG (treatment; n = 19) daily for eight weeks. These diet records were evaluated to determine dietary fiber intake as well as HEI scores. The inflammatory cytokines were analyzed using fasting serum samples collected at baseline and final time points of the study. While the mean dietary fiber intake was increased within the BSG group (5g/day, 26%, p = .003), no significant effects were found on HEI scores, IL-6, IL-10, and IL-1beta. Though significance was not achieved, within-group IL-10 and IL-1 beta increased in concentration were approaching significance (p = .060 and p = .066) in the treatment group. There were also moderate to strong, positive, and significant

correlations observed between the change within the interleukins as well as between moderate correlations between fiber and HEI scores. In conclusion, BSG does not significantly affect inflammatory biomarkers, fiber intake, or contribute to improvements in overall healthy diets in a healthy population in an 8-week timeframe while consuming this amount.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Globally, nearly one-third of all foods produced are lost due to waste, spoilage, or as byproducts., and in the United States, millions of tons of food are lost annually.[1-4] The wasted foods utilize the same amount of water, land, and fertilizer as foods that are consumed. Food waste contributes to 21% of the solid waste disposed of through landfills and incinerators.[5] Utilization of foods that would typically be lost as waste, especially those that may provide health benefits, can not only increase the nutrition content of foods and access to more nutritious foods.

WHOLE GRAINS AND HEART-HEALTHY DIETS

Whole grains are consumed worldwide as a major source of energy and nutrition. This important food staple provides 56% of energy and 50% of protein recommendations worldwide.[6] Throughout the world, grains such as wheat, corn, rye, rice, oats, sorghum, quinoa, and barley are widely consumed. According to the US Food and Drug Administration (FDA), whole grains are the intact fruit of the grains. The individual components of this grain are the endosperm, germ, and bran.[7] The endosperm is the starchy interior of the grain, which is the largest of the three components. The endosperm provides nutrients and energy to the germ, or the embryo, of the grain, so that the single grain may grow into a new plant. The bran or outer casing of the grain is composed of cellulose and hemicellulose, which serve to give the grain structure and protection. While the bran is an insoluble fiber, the endosperm and the germ are made up of fermentable

oligosaccharides, resistant starch, soluble fibers, vitamins, minerals, fatty acids, antioxidants, lignans, polyphenols, and phytochemicals.[8] With this extensive list of bioactive compounds, the exact nature of physiological health benefits provided from whole grains is unknown as it is hard to pinpoint exactly which interaction is providing these benefits. However, it is likely that these compounds act synergistically to provide not only physical benefits, such as increased transit time and the bulking action of insoluble fibers but also antioxidative effects from polyphenols as well as anti-inflammatory and anti-carcinogenic effects from an array of micronutrients housed within the grain.[9]

DIETARY FIBER INTAKE AND DISEASE (INFLAMMATION)

Many epidemiological studies have demonstrated that whole grain and fiber consumption is inversely related to many chronic diseases such as obesity, type 2 diabetes, cancer, and cardiovascular disease.[10] Moreover dietary fiber intake is associated with reducing systemic inflammation.[10] Therefore, comparing pro-inflammatory versus anti-inflammatory cytokines is a possibly important relationship to examine when investigating the effects of whole grain consumption on health. Inflammatory cytokines such as IL-6, IL-1beta, and IL-10 are closely related, with IL-6 and IL-1beta acting as a pro-inflammatory cytokine and IL-10 acting as an anti-inflammatory cytokine. [11,12]

On average, adults in the US do not consume the recommended servings of whole grains or fiber per day.[13] For adults 19-30 years of age, the dietary reference intake (DRI) is 28 g/day and 34 g/day, for men and women respectively. The adequate intake level for adults is 14 g/day per 1000 calories. The mean fiber intake for all adults between 19-30

years old was 16.1 g/day in 2015, which was a significant increase from 2001-2010 data.[13] While it is important to note that this value was significantly increased from 2001-2010, mean fiber intakes remain far below the DRI recommendations. The 2020-2025 Dietary Guidelines for Americans (DGA) focus heavily on fiber as a nutrient of concern.[14] Consuming whole grains provides higher dietary intakes of fiber. While the edible portion of most vegetables provides < 6 g/100 g total fiber, whole grain wheat contains 9-17 g/100 g total fiber.[9] To explore a relationship between diet and overall health, the United States Department of Agriculture (USDA) created the Healthy Eating Index score (HEI) to better analyze the diet quality of Americans based on the DGA. Dietary fiber intake has been found to improve overall health. Therefore, it is important to look at the role that daily dietary fiber intake has on the improvement of an individual's overall health.

BARLEY AND ITS NUTRITIONAL VALUE

Barley is a commonly used grain in the beer brewing process, but in many countries, it is a staple grain used similarly to rice in cuisine. Barley contains greater concentrations of fiber (17 g/100 g) than more commonly consumed whole grains like wheat and rice.[15] Barley consumption is associated with increased health benefits, including reduced risk factors of cardiovascular disease and diabetes through lowering cholesterol and reducing glycemic response. For this reason, barley, or more specifically BSG, may provide opportunities to develop food products with increased nutritional content from a source that is readily available and is produced at a high yield for a low cost.

BREWER'S SPENT GRAIN

In this study, we proposed that the use of brewer's spent grain (BSG) as a reusable resource that has the potential to add nutritional value to food products.[16,17] BSG is the barley mash byproduct of the beer brewing process. Once the wort or liquid portion of the mash is siphoned off to continue in the brewing process, the leftover spent grains are typically discarded. During the brewing process, the fermentable and non-fermentable sugars are released from the grain's starchy endosperm and the proteins are broken down into peptides and amino acids.[16,17] This results in the formation of BSG, which is made up of fiber (cellulose and hemicellulose), protein, and lignin. In addition to fiber and protein, a compound known as arabinoxylans is released from the hemicellulose of the grain.[16,17] Arabinoxylans are found in the cell walls of most cereal grains and are the main component of soluble and insoluble fibers. Arabinoxylans have been shown to have antioxidant activity due to the abundance of phenolic acids found within their chemical structure.[18] The high fiber and protein content of BSG holds a promising opportunity to reduce food waste and add nutritional value to grain-based foods that might otherwise be considered less healthy.

CURRENT USES OF BSG

Currently, BSG is primarily being utilized as feed for dairy cattle. BSG has also been successfully used as feed for poultry, pigs, and fish.[16,17,19,20] BSG used for dairy cattle feed has been found to provide all essential amino acids when combined with the urea that the cattle need for increased milk production. While there have been several small ventures of food companies adding BSG to protein bars to increase fiber content,

there has not yet been extensive research into the benefit of using BSG as a nutritive addition to foods.[16,17,19,20] The utilization of BSG as a functional food will not only create a reusable resource for human consumption as it relates to increasing the nutritional content of foods, but also provide invaluable health benefits to a large population.[16,17,19,20]

PURPOSE OF THE STUDY

The overall purpose of this secondary study is to determine the effect of BSG consumption on increased fiber intake, diet quality, and its impact on anti- and pro-inflammatory cytokines, IL-6, IL-1beta, and IL-10 in human intervention.

HYPOTHESIS AND AIMS

We conducted a secondary analysis to determine whether regular consumption of BSG would change participants' dietary fiber intake, as well as HEI scores, and fasting blood inflammatory biomarkers. This secondary study analyzed data collected from a previous study on BSG, including 3-day diet records and fasting blood samples from 37 healthy men and women participants who consumed muffins with BSG or a control muffin for 8 weeks.[21] Diet records were collected at baseline, 4 weeks, and 7 weeks, while blood samples were collected at baseline and 8 weeks.

Aim 1: To examine changes in dietary fiber (mean g/d) intake and HEI scores (overall mean score) at baseline, midpoint, and 8 weeks following the daily consumption of control or treatment groups. The first sub aim compares the mean daily fiber intake between and within the control and the treatment groups at baseline, midpoint, and after 8 weeks. The second sub aim compares mean overall HEI scores within and between the control and the treatment groups at baseline, midpoint, and after 8 weeks.

***H_{01}*:** No between or within-group difference in fiber intake will be observed following the daily consumption of the control or treatment groups in healthyadults for 8 weeks.

H_{02}: No between or within-group difference in HEI score will be observed from baseline to the end of the intervention and between-group difference at week 8 inthe control) and treatment groups.

Aim 2: To compare within-group change in the control and treatment groups on fasting IL-6, IL-10, IL-1 beta biomarkers.

H_{03}: No within-group differences in IL-6, IL-1 beta, and IL-10 concentrations will be observed between the control and treatment groups.

Exploratory Aim: To evaluate if mean overall HEI scores are influenced by total dietary fiber intake using change scores (final-baseline) of all participants.

CHAPTER II

LITERATURE REVIEW

GLOBAL FOOD WASTE

Approximately one-third of all food produced is lost or wasted, globally. In the US, 60 million tons of food are lost annually.[1-4] Wasted food utilizes the same valuable resources (water, land, fertilizer) as food that is consumed. Food waste also reaches landfills and incinerators that contributes to 21% of discarded municipal solid waste.[5] Reducing this food loss by utilizing food waste for a different purpose, especially in foods that may influence human health, can increase access to more nutritious foods.

BREWER'S SPENT GRAIN

Brewer's spent grain consists of the leftover grains that are present after the liquid portion, or the wort, is separated from the barley mash. As seen in Figure 1, the wort continues through the beer brewing process, while the BSG is left behind as a byproduct. These grains represent around 85% of the total waste byproduct from the brewing process.[16,17] During the malting process of beer making, fermentable and non-fermentable sugars are released from the grain's starchy endosperm and the proteins are broken down into peptides and amino acids. This results in the formation of BSG, which is made up of fiber (cellulose and hemicellulose), protein, and lignin.[16] Additionally, 1,3- and 1,4-b-glucans and arabinoxylans, substances that would otherwise be inaccessible, are released from the grain. [16,17] These substances, especially arabinoxylans, have been found to improve dietary health when they are released from the hemicellulose of a grain.[16] The

high fiber and protein content of BSG has created interest as a reusable food substance that can add value and improve the nutritional quality of food products.

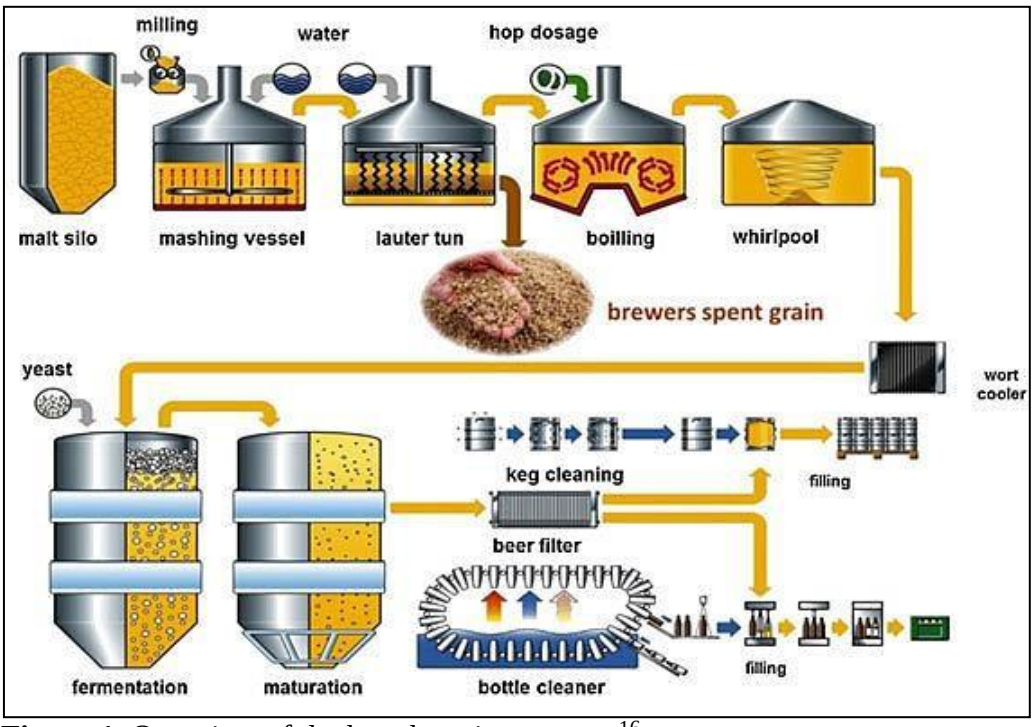

Figure 1. Overview of the beer brewing process[16]

DIETARY FIBER AND DISEASES PREVENTION

Dietary fiber and human health have a close relationship. Increased fiber intake has been found to improve satiety and reduce intake of energy-dense foods.[22] Among the many health benefits of regular and adequate consumption of dietary fiber are their ability to lower blood cholesterol, regulate blood glucose levels, and protect against developing certain cancer types.[17,23,24] Cancer development often coincides with chronic inflammation, but increased intake of dietary fibers has been found to reduce pro-inflammatory cytokines such as IL-6 and increase anti-inflammatory cytokines such as IL-10, and IL-1 beta.[23] These dietary fibers interact with immune cells to promoteimmune

response and the production of local cytokines. Thus, the immune response of the body that reduces the production of IL-6 and IL-1beta, and increase production of IL-10, may decrease the development of some gastrointestinal (GI) cancers[25,26]

MICRONUTRIENT COMPOSITION

BSG also has an interesting protein profile as it contains a relatively high amount of essential amino acids, with about 30% of the total protein content of the grain being essential amino acids. The levels of lysine in BSG, when compared to other cereal grains, are quite high at 14.3% of the total available amino acids.[16,17] Lysine is often a limiting amino acid in cereal foods. To counteract insufficient protein in a food, protein hydrolysates, a complex combination of oligopeptide, peptide, and free amino acids, are utilized to add nutritional value in the form of proteins to a food.[27] These hydrolysates are more bioavailable and are absorbed more easily in the gastrointestinal tract.[27] While most protein hydrolysates that are added to foods are milk-based, hydrolysates isolated from plant-based crops such as soy, rapeseed, wheat, and barley have been found to elicit antioxidant properties and antihypertensive effects.[16,17] Since most beer is made using barely, there is potential for BSG protein hydrolysates to act as a functional ingredient for the management of hypertension. In many studies, increased intake of dietary fiber has been found to significantly decrease both systolic and diastolic blood pressure, especially when increased fiber intakes are coordinated with weight loss[28,29]

Arabinoxylans are a non-cellulose polysaccharide found in the outer layers of cereal grains such as wheat and barley and are considered dietary fibers.[23] Arabinoxylans can have ferulic acids ester-linked to them. In oxidative conditions, these ferulic acid

residues function as radical scavengers.[23] As such, these compounds have potential health benefits as an antioxidant. Consumption of arabinoxylans has been shown to have many health benefits including immunomodulatory activity, cholesterol-lowering activity, and help modulate postprandial glycemic response.[23]

CURRENT USES IN INDUSTRY

Currently, BSG is primarily being incorporated into animal feeds. BSG used for dairy cattle feed has been found to provide all essential amino acids when combined with urea and can increase milk production in dairy cattle without affecting the animal's fertility.[17] Urea is often added to feed formulated for ruminants, as it provides a nonprotein nitrogen source for the animal. This allows their gut microflora to restructure the proteins they consume into usable forms within their body.[30] BSG has also been successfully used as feed for poultry, pigs, and fish. However, the transportation costs of BSG are high due to the water content, which can constitute 70-80%, of the weight of the grain.[16,17] This increased water content makes BSG expensive to transport as most transported items are priced by weight.[19] It costs approximately $16 to transport one ton of wet BSG a distance of five miles.[19] For this reason, the grain byproduct is only being transported to local farmers to decrease the cost of eliminating the BSG.[19] Additionally, its high water content can harbor microbial growth if it is not stored at refrigerated temperatures.[19] Although transport and storage can be costly, the use of BSG as a nutritious additive to food has become increasingly popular.

Manufacturers have used BSG in the manufacturing of bread, muffins, snacks, tortillas, cereals, and cakes. BSG flour has been successfully incorporated into these

baked goods by replacing 5-30% of the total flour and still maintaining consumer acceptance of the food.[16] The addition of BSG to these grain-based foods significantly increased their nutritional value by increasing their protein, dietary fiber, and mineral content, which varies with the variety, harvest time, and quality of barley used in the beer brewing process.[17]

DIETARY FIBER AND OVERALL HEALTH

Three-day diet records can provide accurate reporting of the participant's diets because they are completed most often in real-time as the study participant is preparing and eating food or a meal.[31] One downside to this method of reporting is people tend to make changes to their diet to make it seem as though they are "healthier" than they are.[27] This can lead to either over or under-reporting. If the 3-day diet records are completed in real-time, they are more accurate than that of 24-hour recalls and food frequency questionnaires.[31]

The HEI is a measurement tool used to assess diet quality and how well dietary patterns align with the recommendations made by the DGA.[32] The most current version of the HEI is HEI-2015, which conforms with the key dietary recommendations of the 2015-2020 DGA.[32] HEI scores range from 0 to 100. The highest HEI score that can be achieved is 100, indicating that a particular set of foods consumed aligns with the DGA.[32] Currently, the average total HEI-2015 score in the US is 59 out of 100, indicating that the overall diet of most Americans does not meet key dietary recommendations.[32] Having a higher HEI score is correlated with a reduced risk of developing chronic diseases such as heart disease, cancer, and type 2 diabetes.[32] HEI scores are computed based on the intake

of adequate components and moderate components that are consumed in the diet. For the adequate components, such as fruits, vegetables, protein, and dairy, a higher score reflects a higher intake of those foods. For the moderate components, such as refined grains, sodium, and added sugars, a higher score reflects lower consumption of those foods.[33] While HEI scores measure diet quality independently of total energy intake, changes in energy consumption can impact the HEI scores.[34] Fiber is a major contributor to changes in energy consumption as it promotes satiety and can delay gastric emptying.[35] Increased satiety can promote decreased energy intake and has been associated with the loss of body weight.[35] Delayed gastric emptying will extend the feeling of fullness and can also contribute to lower energy intake as well as the delayed absorption of glucose and other nutrients.[35]

DIETARY FIBER AND INFLAMMATION

Dietary fiber intake not only improves the rate of digestion but also alters the inflammatory responses of the body. Previous studies have found that systematic inflammation is increased with the consumption of refined grains but decreased with the consumption of whole grains, fruits, and vegetables.[36] Consistent consumption of refined grains has been found to increase the incidence of acute hyperglycemia, and increase the circulating levels of free radicals and pro-inflammatory cytokines throughout the body.[36] In an observational study by Ma et al., increased levels of IL-6 were found to be associated with increased risk for chronic diseases such as diabetes mellitus, cardiovascular disease, metabolic syndrome, and various forms of cancer.[37] High fiber

diets were found to reduce systematic inflammation and therefore reduce the risk of developing chronic diseases associated with increased inflammation.[37]

Inflammatory cytokines such as IL-6, IL-1b, and IL-10 are closely related. IL-6 promotes increased inflammation within the body while IL-10 reduces inflammation within the body.[20] IL-10 is produced by immuno-active cells such as monocytes and lymphocytes and is regarded as one of the most important anti-inflammatory cytokines.[38] Secretion of IL-10 is delayed and always follows a pro-inflammatory event. This anti-inflammatory cytokine functions systematically as an auto- or a paracrine hormone.[38] Although IL-10 functions in many ways to reduce the production of a wide variety of pro-inflammatory cytokines, it most commonly and effectively down-regulates pro-inflammatory cytokines such as IL-1, IL-6, and TNF-alpha.[38] Similarly, IL-6 circulates systematically to have both an anti- and a pro-inflammatory effect on the body. During exercise, IL-6 acts as a myokine and is released from the muscles to promote down-regulation of inflammatory agents such as TNF-alpha and IL-1. This activation of IL-6 as an anti-inflammatory agent is typically followed by a later influx of the anti-inflammatory cytokine IL-10.[39] IL-6 promotes inflammation by activating lymphocytes and leukocytes, differentiating B cells, and inducing an acute phase protein response from the liver.[38,39] IL-6 strongly affects inflammation in the body as it can circulate to activation sites far from the site of origin. Consumption of whole grains, such as barley, may consequentially improve systematic inflammation by reducing circulating IL-6 levels and increasing levels of IL-10 throughout the body. [40]

Il-1beta is a pro-inflammatory cytokine whose mechanism remains unclear. Though there have been many proposed mechanisms, the true mechanism remains to be elucidated.[26] The proposed mechanisms suggest that the production of Il-1beta is determined by a stimulus, and they go even further to illustrate that the intensity of the stimulus plays a role in the production and action of Il-1beta.[26] While this interleukin is pro-inflammatory and has been studied as such to work against inflammatory diseases such as irritable bowel disease (IBD), periodontitis, and cardiovascular diseases, it has also been found to promote tumor growth and immunosuppression.[41] Because of the theory that the intensity of the stimuli impacts the amount of IL-1beta that is produced, it makes sense that over-production can have equally negative effects as underproduction.[42] In essence, too much of a good thing can cause major problems throughout the body. Because of this, the concentration on IL-1beta in our blood serum samples must befurther evaluated to determine if its presence is positive or negative in nature.[42]

CONCLUSIONS AND SIGNIFICANCE

In conclusion, it has been found that increased fiber intake can improve an individual's diet quality, reduce inflammation, and even has the potential to prevent the development of many chronic diseases. Not only does fiber, in general, provide countless health benefits, but also barley beta-glucans has been shown in numerous studies to provide additional benefit as the grain is higher in protein. BSG has the potential to become an invaluable resource to add nutritive value to a wide variety of foods due to its barley content. In this study, we looked at the potential benefits of BSG on fiber intake, HEI scores, and inflammatory biomarkers.

CHAPTER III

METHODOLOGY

All methods and protocols for the parent study and secondary analysis were approved by the Texas Woman's University (TWU) Institutional Review Board (IRB). A copy of the IRB approval letter for the secondary study can be found in Appendix C. Participants for the parent study were recruited from the TWU-Houston campus and the surrounding area. All participants provided written informed consent before participating in the study. For the parent study, a priori power analysis was used to determine that a minimum sample size of 34 participants (17 control and 17 treatment) was necessary to test for mean differences.

METHODS TO ADDRESS AIM 1

In the parent study, the principal investigator (PI) educated participants on how to fill out each diet record as well as how to follow a low fiber (<19 g/day) diet throughout the study. Demographic information and diet records were de-identified by the parent study PI and sent to the secondary study PI via excel spreadsheets and Google Drive files. The 3-day food records were analyzed using the Nutrition Data System for Research (NDSR; University of Minnesota, Minneapolis, MN, USA, 2020) available through the Department of Nutrition and Food Sciences, TWU-Denton. The analysis used to estimate each participant's 3-day average fiber intake was collected at baseline, 4-weeks, and 7-weeks. HEI scores computed based on the HEI-2015 criteria.[33] HEI composite scores are calculated using the data collected in NDSR that is then transferred to the publicly

available statistical analysis system (SAS) 9.4 macros. This statistical algorithm also scores to be generated using the 13-component scoring system detailed below in Figure 2.[33]

HEI-2015[1] Components and Scoring Standards

Component	Maximum points	Standard for maximum score	Standard for minimum score of zero
Adequacy:			
Total Fruits[2]	5	≥0.8 cup equivalent per 1,000 kcal	No Fruit
Whole Fruits[3]	5	≥0.4 cup equivalent per 1,000 kcal	No Whole Fruit
Total Vegetables[4]	5	≥1.1 cup equivalent per 1,000 kcal	No Vegetables
Greens and Beans[4]	5	≥0.2 cup equivalent per 1,000 kcal	No Dark-Green Vegetables or Legumes
Whole Grains	10	≥1.5 ounce equivalent per 1,000 kcal	No Whole Grains
Dairy[5]	10	≥1.3 cup equivalent per 1,000 kcal	No Dairy
Total Protein Foods[4]	5	≥2.5 ounce equivalent per 1,000 kcal	No Protein Foods
Seafood and Plant Proteins[4,6]	5	≥0.8 ounce equivalent per 1,000 kcal	No Seafood or Plant Proteins
Fatty Acids[7]	10	(PUFAs + MUFAs) / SFAs ≥2.5	(PUFAs + MUFAs)/SFAs ≤1.2
Moderation:			
Refined Grains	10	≤1.8 ounce equivalent per 1,000 kcal	≥4.3 ounce equivalent per 1,000 kcal
Sodium	10	≤1.1 grams per 1,000 kcal	≥2.0 grams per 1,000 kcal
Added Sugars	10	≤6.5% of energy	≥26% of energy
Saturated Fats	10	≤8% of energy	≥16% of energy

[1] Intakes between the minimum and maximum standards are scored proportionately.
[2] Includes 100% fruit juice.
[3] Includes all forms except juice.
[4] Includes legumes (beans and peas).
[5] Includes all milk products, such as fluid milk, yogurt, and cheese, and fortified soy beverages.
[6] Includes seafood; nuts, seeds, soy products (other than beverages), and legumes (beans and peas).
[7] Ratio of poly- and mono-unsaturated fatty acids (PUFAs and MUFAs) to saturated fatty acids (SFAs).

Figure 2. HEI-2015 Components and Scoring Standards[33]

METHODS TO ADDRESS AIM 2

Participant blood samples were collected in the fasting state at baseline and 8 weeks. At each collection 2 ethylenediaminetetraacetic acid (EDTA) vacutainers, with 14ml of blood each, were collected. The EDTA vacutainers, were centrifuged at 4000 rpm for 15 minutes, the plasma was transferred to pre-labeled (participant ID, date, time of collection) 1.5 mL microcentrifuge tubes and stored in white cardboard boxes at -80°C. Plasma samples were transported by the secondary study PI from Houston to Denton in

an insulated container with dry ice. IL- 6, IL-1beta, and IL-10 concentrations were analyzed using 25 µL aliquots of plasma from each sample using MILLIPLEX magnetic bead-based assay kits in duplicate (Millipore Sigma, MA, USA) according to the manufacturer's protocol. All biomarker concentrations were determined using the MAGPIX microplate reader (Millipore Sigma, MA, USA).

STATISTICAL ANALYSIS

Descriptive statistics of continuous variables were displayed as means and standard deviations. Categorical variables were displayed as percentages and frequencies. Between-group differences obtained from independent sample *t*-test. Repeated measures analysis of variance (ANOVA) was used to assess the impact of the intervention on fiber intake, HEI scores, IL-6, IL-1 beta, and IL-10. A repeated measures analysis of covariance (ANCOVA) was used to assess the impact of fiber intake and intervention assignment on HEI scores. Pearson's correlation was used to identify relationships between the change scores for HEI scores and IL-6 and IL-10 values. Linear modeling predicted IL-6, IL-1 beta, and Il-10 values as a function of HEI scores while controlling for any necessary covariates such as age, body mass index (BMI), and gender. Statistical analysis for this study was completed using IBM SPSS Statistics 25 for Macintosh and SAS/STAT® software 9.4. Statistical significance was considered as a p-value < 0.05.

CHAPTER IV

RESULTS

AIM 1 RESULTS

Participants

Diet records and plasma samples of 37 (76% female) participants were included in the data analysis. Baseline characteristics of participants who completed the study did not differ between the control (n = 18) or the treatment (n = 19) groups (see Table 1).

Table 1. Baseline characteristics of participants who completed the study

Characteristic	Control (n = 18)	BSG (n = 19)	*P*-value*
Female, n (%)	14 (77.8)	14 (73.7)	
Age, years	26.0 ± 4.0	26.8 ± 3.9	0.545
Weight (kg)	63.6 ± 12.8	62.1 ± 11.1	0.706
BMI (kg/m^2)	22.9 ± 2.9	22.8 ± 3.0	0.923

BSG: brewers' spent grain, BMI: body mass indexContinuous variables presented as mean ± SD
Total number of participants who completed the study (n = 37)
*Between-group differences obtained from independent sample *t*-test. Significance set a $p \leq 0.05$.

The majority of the study participants were female (77.8% control, 73.7% treatment), with a mean age of 26 ± 4 years, and mean BMI of 23 ± 3 kg/m^2. No significant difference between the control and treatment participants was found for age, weight, and BMI. Subject characteristics can be found in Table 1.

Intervention on Dietary Fiber Intake

Using NDSR software, 3-day dietary data were analyzed to determine the intakeof dietary fiber by participants across three time points. The means and standard deviations of fiber consumed at each time point for control and treatment groups are displayed in

Table 2. At baseline, mean fiber intake was similar between the two groups, control (19.64 ± 7.55), and treatment groups (15.19 ± 6.60). Themean fiber intake increased at the midpoint for both the control group (17.46 ± 4.69) andBSG group (13.28 ± 4.66) Both groups maintained a relatively low fiber intake throughout the study, indicating that the participants likely followed the recommended low fiber diet. It is interesting that the BSG group has lower fiber intakes than the controlgroup thorough out the study, it is possible that there were between baseline fiber consumption of the two groups; however, there were no significant findings to indicate this would impact the results.

Table 2. Means and standard deviations of dietary fiber (g) intake by time and group

			Baseline		Midpoint		Final	
		N	Mean(g)	Standard Deviation	Mean (g)	Standard Deviation	Mean(g)	Standard Deviation
DietaryFiber	BSG	18	15.19	6.60	13.28	4.66	13.87	5.15
	Control	19	19.64	7.55	17.46	4.69	17.84	5.98
	Total		17.48	7.36	15.43	5.07	15.90	5.87

Data presented as mean and SD except for qualitative values.
Between-group differences obtained from repeated measures ANOVA. Significance set a $p \leq 0.05$.

The average amount of fiber consumed across each time point for control and BSG groups is displayed in Figure 3. Dietary fiber intakes decreased from baseline to midpoint, and then remained stable through the final time points of the study for both groups. This suggests that the participants followed the suggested low fiber diet. Over time, the group consuming BSG muffins had an increased fiber intake daily; however, no significance was established between baseline and final time points on total intakes of fiber and the increased fiber intakes of the BSG group.

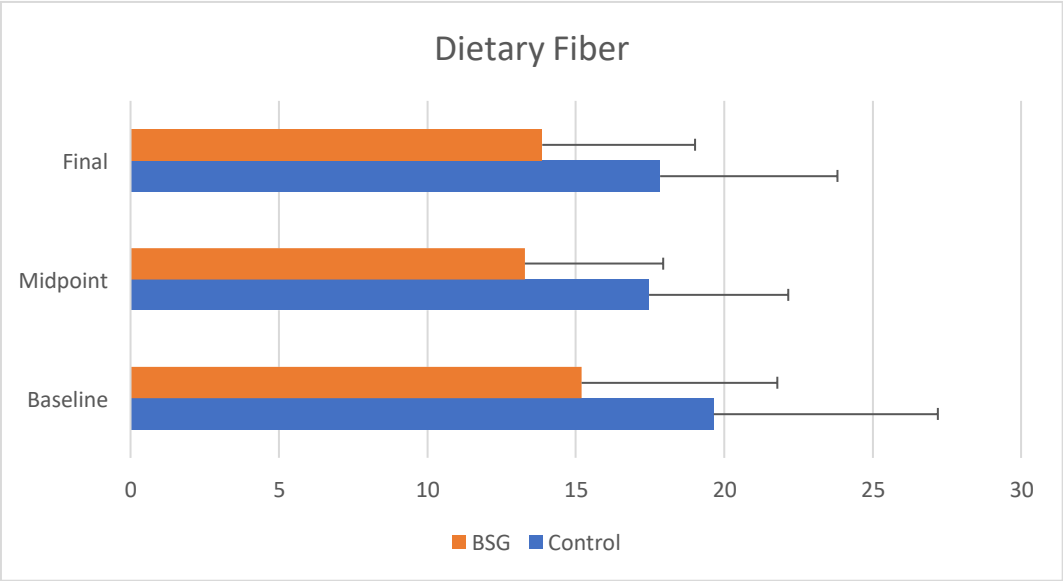

Figure 3. Change in dietary fiber intake (g) over time

A repeated measures ANOVA test was conducted to determine if fiber intake differed based on the treatment group (see Table 3). Results indicated that consuming BSG onfiber intake was not statistically significant, $p = 0.663$.

Table 3. Means and standard deviations of HEI scores by time and group

			Baseline		Midpoint		Final	
		N	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
HEI	BSG	18	55.38	14.28	51.26	8.85	51.00	11.77
	Control	19	52.24	12.22	52.15	11.80	49.78	12.90
	Total		53.77	13.18	51.72	10.33	50.38	12.21

Data presented as mean ± SD except for qualitative values.

Figure 4 shows the changes in values of HEI scores and fiber from baseline, midpoint, and final between the two groups. The intervention did not significantly improve the HEI scores versus the control groups.

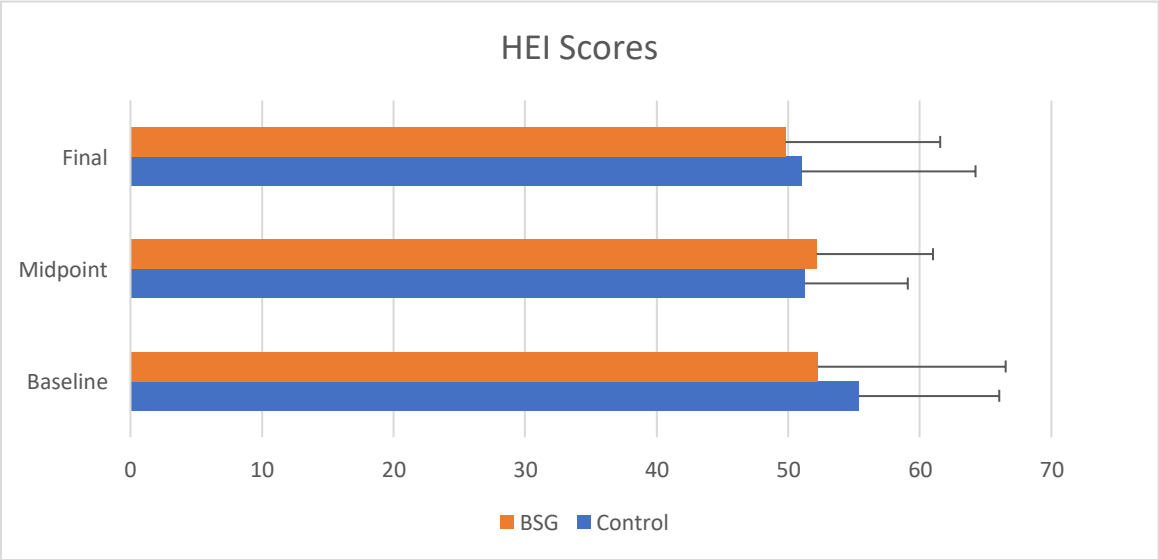

Figure 4. Change in HEI scores over time

Intervention on Healthy Eating Index Scores Analysis

A repeated measures ANOVA test was conducted to determine if HEI scores differed based on the intervention group. Results indicated that the effect of the BSG treatment in both groups on HEI score did not change significantly throughout the intervention. The same was true of the control group. Table 3 shows the change in mean and standard deviation values of HEI scores from baseline to final. At baseline, mean fiber intake was similar between the two groups, control (52.24 ± 12.22) and BSG groups (55.38 ± 14.28). The mean HEI score remained the same at the midpoint for the control group (52.15 ± 11.80), but interestingly it decreased in the BSG group (51.26 ± 8.85). At the final time point, both control and BSG groups experienced a decrease in mean HEI score (control: 49.78 ± 12.90, BSG: 51.00 ± 11.77). This unexpected, though slight, decrease may indicate that the participants did not maintain a consistent diet throughout the study.

The intervention did not significantly improve the HEI scores versus the control groups (p = 0.626) indicating the possibility that participants were already eating an overall healthy diet based on the DGA.

However, as seen in Table 4, changes in dietary fiber and HEI scores (final - baseline) show a moderate positive correlation and are statistically significant (r = .424, p = 0.009). While the within-group differences displayed no significance for change offiber or HEI scores over time, the overall effect of increasing dietary fiber in the dietcorrelates to increased HEI scores, and therefore improves overall diet per the DGA.

Table 4. Pairwise comparison between change scores of HEI and dietary fiber data

		Change IL-10	Change IL-1 beta	Change IL-6	Change HEI	Change Fiber
Change HEI	Pearson Correlation	-.376	-.084	.154	1	.424**
	Sig. (2-tailed)	.034	..619	..364		.009

**Correlation is significant at the 0.05 level (2-tailed).

AIM 2 RESULTS

In the parent study, a total of 37 participants provided consent and were randomized for the intervention on the first visit. A total of 37 participants were included in the secondary study analysis. Participant characteristics can be found in Table 1.

Intervention on Inflammatory Cytokines

The means and standard deviations of Interleukin concentrations at each time point for Control and BSG are displayed in Table 5. At baseline, mean IL-6, IL-10, and IL-1beta concentrations were not statistically different between the two groups, IL-10 (control; 6.58 ± 5.26) and IL-10 (BSG; 4.78 ± 5.04), IL-1beta (control; 1.11 ± 0.75) and IL-1beta (BSG; 0.78 ± .49), IL-6 (control; 5.27 ± 6.19) and IL-6 (BSG; 4.50 ± 4.75). The

mean concentrations increased at final for the BSG groups and decrease slightly for the control groups. It is possible that the BSG did not have significant acute effects on these biomarkers, or that the control group was not following their intended low fiber diet.

Table 5. Means and standard deviations of inflammatory biomarkers by time and group

			Baseline		Final	
		N	Mean (pg/mL)	Standard Deviation	Mean (pg/mL)	Standard Deviation
IL-10	BSG	18	4.78	5.04	6.26	5.88
	Control	19	6.58	5.26	6.03	4.43
	Total		5.71	5.16	6.14	5.11
IL-1 beta	BSG	18	.78	.49	1.08	.72
	Control	19	1.11	.75	1.08	.68
	Total		.95	.65	1.08	.63
IL-6	BSG	18	4.50	4.75	4.56	4.31
	Control	19	5.27	6.19	5.33	5.83
	Total		4.90	5.46	4.96	5.07

Data presented as mean ± SD except for qualitative values.

Within and between group comparisons for all blood biomarkers at baseline and final time points were completed for each group as shown in Table 6. There were no significant effects of BSG on IL-6 (p = .991), but there were significant findings for IL-10 (p = .020) and IL-1 beta (p = .015).

Table 6. Within subjects' effect on inflammatory cytokines adjusted for time point and treatment group

		Sig.
Time*test group	IL-10	.020
	IL-6	.991
	IL-1beta	.015

*Within-group differences obtained from Repeated measures ANOVA. Significance set a $p \leq 0.05$.

The effects of BSG on IL-10 and IL-1beta were significant, though their partial eta values suggest that effect size is small. There were no changes in IL-10, IL-1beta, and

IL-6 between the groups over time, though there are strong, positive, and significant correlations between the change in IL-6 and IL-1beta over time.

Pearson's Rho correlations were run on the change scores of each interleukin biomarker from baseline to final, as well as the change in HEI score and fiber over the three time points. The values calculated can be seen in Table 7.

Table 7. Pairwise comparison between change scores of inflammatory cytokine data

Correlations		chgIL10	chgIL1beta	chgIL6
chgIL10	Pearson Correlation	1		
	Sig. (2-tailed)			
	N	32		
chgIL1beta	Pearson Correlation	-.194	1	
	Sig. (2-tailed)	.287		
	N	32	37	37
chgIL6	Pearson Correlation	-.004	.614**	1
	Sig. (2-tailed)	.984	.000	
	N	32	37	37

**Correlation is significant at the 0.01 level (1-tailed)

The relationships between the pro-inflammatory cytokines IL-6 and IL-1beta were found to be statistically significant with IL-1beta and IL-6 showing strong correlations. The correlation between IL-10 with IL-1beta and IL-6 showed weak negative or no correlations. The changes in interleukin concentrations displayed a negligible correlation between the biomarkers and fiber or HEI scores. The correlations between each interleukin were found to be positively correlated, which was the original assumption based on the known pathways of each biomarker.

CHAPTER V

DISCUSSION AND CONCLUSION

DISCUSSION

BSG is the byproduct left over after the beer brewing process. These leftover grains contain protein, dietary, fiber, minerals, and antioxidants in substantial quantities. While BSG is primarily used as animal feed to date, the US food industry has recently integrated this grain into baked goods to increase the nutritional value of a baked item. Addition of BSG to a baked good increase the fiber, protein, and micronutrient content of the food item in a way that is dose dependent.[16,17,20,43]

The primary aim of this study was to evaluate changes in dietary fiber intake depending on BSG consumption as well as evaluating the effects of BSG intake on pro- and anti-inflammatory biomarkers, IL-6, IL-10, and IL-1beta. Another aim of this study was to explore the effects of dietary fiber intake on HEI scores. The results demonstrate there were no differences between groups on age, gender, BMI, and baseline intake values. Dietary fiber intake of the BSG group was 26% of dietary intake on average compared to 10% nationally. This increased fiber intake, however, did not result in any changes to inflammatory cytokines or HEI scores.

In the present study, participants were educated and encouraged to follow a low fiber diet throughout the intervention so changes in dietary fiber intake based on the intervention group might be seen. In other study findings, it is suggested that increased dietary fiber intake can contribute to improved overall health as well as disease

prevention. The most notable of those being colon cancer. [44,45,46] Currently, the DGA recommends that adults between the ages of 19 and 50 years of age consume three 1-ounce equivalent servings (oz eq/day) of whole grains and 25-34 g/day dietary fiber.[11] In recent studies it has been found that mean whole grain and fiber intakes in the US were 0.97 oz eq/day and 16.1 g/day, respectively. This indicates most US adults do not consume the recommended quantities of fiber and whole grains each day.[10,47] In the current study, daily servings of BSG muffins provided slightly less than the 7.2 g dietary fiber content from daily whole grain recommendations.[48] However, due to the lack of adherence to a low-fiber diet, the treatment group, on average, consumed about 19 g/day dietary fiber throughout the study.[21] Between the intervention groups, no significance was found in the dietary fiber intake after 8 weeks. It is possible that the selected participants followed a higher fiber diet (25-34 g/day) before the start of the study, therefore skewing the baseline numbers contributing to insignificant effect on fiber intake, inflammation, and overall healthy eating. In future studies, it may be beneficial to observe the differences in consumption of diet higher in refined grains versus whole grains before observing the effects of increasing fiber in the daily diet.

In previous studies, it has been found that increased intake of dietary fiber can show effects on the improvement of pro- and anti-inflammatory biomarkers in the blood. IL-6 promotes increased inflammation within the body while IL-10 reduces inflammation within the body.[20] Previous studies using a similar study design also had insignificant findings for their inflammatory biomarkers after 6 weeks. Thus, the present study length may be a limiting factor in our ability to determine significant effects of BSG on

inflammatory cytokines IL-1, IL10, and IL-1beta.[49,50,51] Increasing the amount of BSG consumed daily, thus increasing the amount of fiber consumed, should be considered in future human trials.

Based on the results, these interleukins reacted as they should be based on the current understanding of their mechanisms. Inflammatory cytokines IL-6 and IL-10 are demonstrated to be similar in value to previous studies (5.16 pg/mL, 5.10 pg/mL), while IL-1beta is somewhat higher in value (0.40 pg/mL).[53,54,55] The relationship between IL-6 and IL-1beta is strong, positive, and significant. (r^2 = .614). IL-10 is weakly correlate to IL-1beta and IL-6.[13,26,52] IL-6 and IL-1beta (both pro-inflammatory cytokines) reacted similarly. IL-10, to counteract the effects of these pro-inflammatory cytokines, increased in a positive manner. However, there were no differences in the changes of these biomarkers between groups.

The HEI is a measurement tool used to assess diet quality and how well dietary patterns align with the recommendations made by the DGA. In observational studies, participants were educated to increase a certain food groups and the impacts of those intakes were determined based on changes to HEI scores over time.[56,57] In these studies it was found that changes to these food groups increased HEI scores significantly over time. However, the previous studies showed significant impact in a population with an age range of 20-75 and with a BMI of >30. As the results of the current study suggest, the increased fiber content of BSG had no significant effect on the overall healthy eating patterns and HEI scores in either intervention group. The groups in this study were between considered to be healthy at baseline with BMI <30 and ages ranging from 18-60

years, though the average age was 26 years. Interestingly, the mean HEI score in both intervention groups was lower than the national average HEI score of 59.[33] This is likely due to the small population size that did not generate a true representation of the American population. Other studies completed on a young and healthy population have shown similar HEI scores, however. Woglom et al. details that the age group 18-30 years have a mean HEI scores of 47.6, 48.6, 50.3 in the age ranges of 18-21, 22-25, and 26-30, respectively.[58] Further research on BSG consumption should include higher BSG levels in a variety of grain-based foods. It is possible that the current group of subjects were already compliant about an increased fiber diet, therefore the fiber intake values would not significantly change from the first to the last time point. Future studies may benefit from selecting a broader group of participants, perhaps those who more habitually consume refined grains or consume less fiber in their diet, or those with diabetes.

CONCLUSION

In conclusion, consumption of 10.4 g of BSG in muffins over an 8-week period did not significantly improve dietary fiber intake, HEI scores, or inflammatory biomarkers. Therefore, BSG does not significantly affect inflammatory biomarkers, fiber intake, or contribute to improvements in overall healthy diets in a healthy population in an 8-week timeframe while consuming this amount. It is important to note that study design is a key limitation of this study. As this is a secondary analysis of data collected from the parent study, it is possible that the study design, intervention time, and population size were not sufficient to show an effect of BSG on fiber intake, HEI, and inflammatory cytokines. This is the first study of our knowledge to investigate the effect

of BSG on the health improvement of healthy individuals. Baseline health status, BSG dosage, study design, and sample size may be key determinants of outcome measures and future studies will be needed to evaluate this subject further.

REFERENCES

1. Alexander P, Brown C, Arneth A, Finnigan J, Rounsevell MD. Human appropriation of land for food: the role of diet. *Global Environ Change*.2016;41:88-98. doi:10.1016/j.gloenvcha.2016.09.005
2. Kester C. Analysis of U.S. food waste among food manufacturers, retailers, and wholesalers. Food Waste Reduction Alliance. 2013. Accessed May 15, 2020. https://furtherwithfood.org/wp-content/uploads/2016/12/FWRA_Food-Waste-Assessment-2013_FINAL.pdf.
3. Galanakis CM. Recovery of high added-value components from food wastes: conventional, emerging technologies and commercialized applications. *TrendsFood Sci Tech*. 2012;26(2):68-87. doi:10.1016/j.tifs.2012.03.003
4. Golan E, Buzby JC. Innovating to meet the challenge of food waste. *FoodTechnol.* 2015;69(1)
5. Papargyropoulou E, Lozano R, Steinberger JK, Wright N, Ujang ZB. The foodwaste hierarchy as a framework for the management of food surplus and food waste. *J Clean Prod*. 2014;76:106-115. doi:10.1016/j.jclepro.2014.04.020
6. Aune D, Keum NN, Giovannucci E, et al. Whole grain consumption and risk of cardiovascular disease, cancer, and all cause and cause specific mortality: systematic review and dose-response meta-analysis of prospective studies. *Br. Med. J.* 2016:i2716. doi:10.1136/bmj.i2716
7. Center for Food Safety and Applied Nutrition. Draft guidance on what FDA considers to be "whole grain" . U.S. Food and Drug Administration. AccessedJuly 22, 2021. https://www.fda.gov/food/guidanceregulation/ guidancedocumentsregulatoryinformation/ucm059088.htm
8. Jonnalagadda SS, Harnack L, Liu RH, McKeown N, Seal C, Liu S, Fahey GC. Putting the whole grain puzzle together: health benefits associated with whole grains--summary of American Society for Nutrition 2010 Satellite Symposium. *JNutr.* 2011;141(5):1011S-1022S. doi:10.3945/jn.110.132944
9. Fardet A. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? *Nutr. Res. Rev.* 2010;23(1):65-134. doi:10.1017/S0954422410000041
10. Lattimer J., Haub M. Effects of dietary fiber and its components on metabolichealth. *Nutrients*, 2010;2(12):1266-1289. doi:10.3390/nu2121266
11. Stenvinkel P, Ketteler M, Johnson RJ, et al. IL-10, IL-6, and TNF-α: central factors in the altered cytokine network of uremia—the good, the bad, and the ugly. *Kidney Int*. 2005;67(4):1216-1233. doi:10.1111/j.1523-1755.2005.00200.x
12. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochem Biophys Acta*. 2011;1813(5):878
13. McGill C, Fulgoni III, V, Devareddy L. Ten-Year trends in fiber and whole grain intakes and food sources for the United States population: national health and

nutrition examination survey 2001–2010. *Nutr*. 2015;7(2):1119-1130. doi:10.3390/nu7021119.
14. U.S. Department of Agriculture, U.S. Department of Health and Human Services. 2020-2025 dietary guidelines for Americans. 9th edition. Dietary Guidelines for Americans. Accessed July 29, 2021. https://www.dietaryguidelines.gov/sites/default/files/2021-03/Dietary_Guidelines_for_Americans-2020-2025.pdf.
15. U.S. Department of Agriculture. FoodData central: barley, hulled . FoodDataCentral 2019. Accessed July 29, 2021. https://fdc.nal.usda.gov/fdc- app.html#/food-details/170283/nutrients
16. Lynch KM, Steffen EJ, Arendt EK. Brewers spent grain: a review with anemphasis on food and health. *J I Brewing*. 2016;122(4):553-568. doi:10.1002/jib.363
17. Mussatto S, Dragone G, Roberto I. Brewers spent grain: generation, characteristics and potential applications. *J Cereal Sci*. 2006;43(1):1-14. doi:10.1016/j.jcs.2005.06.001
18. Rao RS, Muralikrishna G. Water soluble feruloyl arabinoxylans from rice andragi: changes upon malting and their consequence on antioxidant activity. *Phytochemistry*. 2006;67(1):91-99.doi:10.1016/j.phytochem.2005.09.036
19. Mussatto, SI. Brewer's spent grain: a valuable feedstock for industrial applications. *J. Sci. Food Agric*., 2014;94:1264-1275. doi:10.1002/jsfa.6486
20. Ikram S, Huang L, Zhang H, Wang J,Yin M. Composition and nutrient value proposition of brewers spent grain. *J Food Sci.* 2017;82(10):2232-2242. doi:10.1111/1750-3841.13794
21. Combest S. *Upcycling brewers' spent grain for functional food development andthe effect on markers of cardiovascular disease risk in healthy adults.* Dissertation. Texas Women's University; 2021.
22. Cioffi I, Ibrugger S, Bache J, et al. Effects on satiation, satiety and food intake of wholegrain and refined grain pasta. *Appetite*. 2016;107:152-158. doi:10.1016/j.appet.2016.08.002
23. Mendis M, Simsek S. Arabinoxylans and human health. *Food Hydrocoll*. 2014;42:239-243. doi:10.1016/j.foodhyd.2013.07.022
24. Johnstone M, Donohoe D. Pro-inflammatory cytokine, IL-1B, diminishes butyrate oxidation in colorectal cancer cells. *FASEB J*. 2015;29(S1). doi:10.1096/fasebj.29.1_supplement.394.5
25. Andersen V, Egeberg R, Tjønneland A, Vogel U. Interaction between interleukin-10 (IL-10) polymorphisms and dietary fibre in relation to risk of colorectal cancer in a Danish case-cohort study. *BMC Cancer*. 2012;12(1). doi:10.1186/1471-2407-12-183
26. Gupta P, Barthwal MK. Il-1 β genesis: the art of regulating the regulator. *Cell Mol Immunol* 2018;15(11):998-1000. doi:10.1038/s41423-018-0054-7
27. Nsari M. Protein hydrolysates and biopeptides: production, biological activities,and applications in foods and health benefits. a review. *Adv Food Nutr Res.* 2017;81:109-159. Academic Press. doi:https://doi.org/10.1016/bs.afnr.2016.10.003

28. McCarthy AL, O'Callaghan YC, Connolly A, Piggott CO, FitzGerald RJ, O'Brien NM. In vitro antioxidant and anti- inflammatory effects of brewers' spent grain protein rich isolate and its associated hydrolysates. *Food Res Int*. 2013;50:205– 212.
29. El Khoury D, Cuda C, Luhovyy BL, Anderson GH. Beta glucan: health benefitsin obesity and metabolic syndrome. *J Nutr Metab*. 2012;2012:1-28. doi:10.1155/2012/851362
30. Kertz AF. Review: urea feeding to dairy cattle: a historical perspective andreview. *Prof Anim Sci*. 2010;26(3):257-272.
31. Subar AF, Freedman LS, Tooze JA, et al. Addressing current criticism regardingthe value of self-report dietary data. *J Nutr*. 2015;145(12):2639-2645. doi:10.3945/jn.115.219634
32. Healthy eating index (HEI). USDA. Accessed April 10, 2020. https://www.fns.usda.gov/resource/healthy-eating-index-hei
33. How the HEI is icored. USDA. Accessed April 24, 2020. https://www.fns.usda.gov/how-hei-scored
34. Guenther PM, Kirkpatrick SI, Reedy J, et al. The healthy eating index-2010 is a valid and reliable measure of diet quality according to the 2010 dietary guidelinesfor Americans. *J Nutr*. 2014;144(3):399-407. doi:10.3945/jn.113.183079
35. Slavin JL. Dietary fiber and body weight. *Nutrition*. 2005;21(3):411-418. doi:10.1016/j.nut.2004.08.018
36. Giugliano D, Ceriello A, Esposito K. The effects of diet on inflammation: emphasis on the metabolic syndrome. *J Am Coll Cardiol*. 2006;48(4):677. doi: 10.1016/j.jacc.2006.03.052
37. Ma Y, Hébert JR, Li W, et al. Association between dietary fiber and markers of systemic inflammation in the women's health initiative observational study. *Nutrition*. 2008;24(10):941-949. doi:10.1016/j.nut.2008.04.005
38. Stenvinkel P, Ketteler M, Johnson RJ, et al. IL-10, IL-6, and TNF-α: central factors in the altered cytokine network of uremia—the good, the bad, and the ugly. *Kidney Int*. 2005;67(4):1216-1233. doi:10.1111/j.1523-1755.2005.00200.x
39. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochem Biophys Acta*. 2011;1813(5):878
40. Aune D, Keum NN, Giovannucci E, et al. Whole grain consumption and risk of cardiovascular disease, cancer, and all cause and cause specific mortality: systematic review and dose-response meta-analysis of prospective studies. *Br Med Jl* 2016:i2716. doi:10.1136/bmj.i2716
41. Erthal Leinig C, Pecoits-Filho R, Kunii L, et al. Low-fiber intake is associatedwith high production of intraperitoneal inflammation biomarkers. *J Ren Nutr*. 2019;29(4):322-327. doi:10.1053/j.jrn.2018.10.013
42. Claude-Taupin A, Bissa B, Jia J, Gu Y, Deretic V. Role of autophagy in il-1βexport and release from cells. *Semin Cell Dev Biol*. 2018;83:36-41. doi:10.1016/j.semcdb.2018.03.012
43. Waters DM, Jacob F, Titze J, Arendt EK, Zannini E. Fibre, protein and mineral fortification of wheat bread through milled and fermented brewer's spent grain

enrichment. *Eur Food Res Technol*. 2012;235(5):767-778. doi:10.1007/s00217-012-1805-9
44. Larsson SC, Giovannucci E, Bergkvist L, Wolk A. Whole grain consumption andrisk of Colorectal cancer: a population-based cohort of 60 000 women. *Br J Cancer*. 2005;92(9):1803-1807. doi:10.1038/sj.bjc.6602543
45. McCullough ML, Robertson AS, Chao A, et al. A prospective study of wholegrains, fruits, vegetables and colon cancer risk. *Cancer Causes Control*. 2003;14(10):959-970. doi:10.1023/b:caco.0000007983.16045.a1
46. AbuMweis SS, Jew S, Ames NP. B-Glucan from barley and its lipid-lowering capacity: a meta-analysis of randomized, controlled trials. *Eur J Clin Nutr*. 2010;64(12):1472-1480. doi:10.1038/ejcn.2010.178
47. Albertson AM, Reicks M, Joshi N, Gugger CK. Whole grain consumption trends and associations with body weight measures in the United States: results from the cross sectional national health and nutrition examination survey 2001–2012. *Nutr J*. 2015;15(1). doi:10.1186/s12937-016-0126-4
48. U.S. Department of Agriculture. Nutrient profiles for food groups and subgroups in the 2015 USDA food patterns. Accessed August 22, 2021. https://www.fns.usda.gov/usda-food-patterns
49. Cooper D, Kable M, Marco M, et al. The effects of moderate whole grain consumption on fasting glucose and lipids, gastrointestinal symptoms, andmicrobiota. *Nutr*. 2017;9(2):173. doi:10.3390/nu9020173
50. Ampatzoglou A, Atwal KK, Maidens CM, et al. (2015). Increased whole grain consumption does not affect blood biochemistry, body composition, or gut microbiology in healthy, low-habitual whole grain consumers. *J Nutr*. 145(2), 215-221. doi:10.3945/jn.114.202176
51. Ampatzoglou A, Atwal KK, Maidens CM, et al. Effects of increased wholegrain consumption on immune and inflammatory markers in healthy low habitual wholegrain consumers. *Eur J Nutr*. 2016;55(1):183-195. doi:10.1007/s00394-015-0836-y
52. Ellul P, Boyer L, Groc L, Leboyer M, Fond G. Interleukin-1 β-targeted treatment strategies in inflammatory depression: toward personalized care. *Acta Psychiatr Scand*. 2016;134(6):469-484. doi:10.1111/acps.12656.
53. Di Iorio A. Serum il-1β levels in health and disease: a population-based study. 'the inchianti study'. *Cytokine*. 2003;22(6):198-205. doi:10.1016/s1043- 4666(03)00152-2.
54. Ndishimye P, Seghrouchni F, Domokos B, et al. Evaluation of interleukin-10 levels in the plasma of patients with various stages of tuberculosis. *Med PharmRep*. 2015;88(2):164-167. doi:10.15386/cjmed-459
55. Said EA, Al-Reesi I, Al-Shizawi N, et al. Defining il-6 levels in healthyindividuals: a meta-analysis. *J Med Virol*. 2020;93(6):3915-3924. doi:10.1002/jmv.26654
56. Crowe-White KM, Ellis AC, Mehta T, Locher JL, Ard JD. Dietary qualityassessed by the HEI-2010 and biomarkers of cardiometabolic disease: an exploratory analysis. *J Am Coll Nutr*. 2019;38(7):640-647. doi:10.1080/07315724.2019.1580168

57. Drewnowski A, Rehm C. Consumption of low-calorie sweeteners among U.S. adults is associated with higher healthy eating index (HEI 2005) scores and morephysical activity. *Nutrients*. 2014;6(10):4389-4403. doi:10.3390/nu6104389
58. Woglom C, Gray V, Hill M, Wang L. Significant relationships exist between perceived and objective diet quality in young adults. *J Acad Nutr Diet.* 2020;120(1):103-110. doi:10.1016/j.jand.2019.06.002.
59. Chen G-C, Tong X, Xu J-Y, et al. Whole-grain intake and total, cardiovascular,and cancer mortality: a systematic review and meta-analysis of prospective studies. *Am J Clin Nutr*. 2016;104(1):164-172. doi:10.3945/ajcn.115.122432
60. Geng Z, Alisa G, Hu FB, Qi S. Whole grain intake and mortality from all causes, cardiovascular disease, and cancer: A meta-analysis of prospective cohort studies. *Circulation*. 2016;133(24):2370-2312. doi:10.1161/CIRCULATIONAHA.115.021101
61. Hoevenaars FP, Esser D, Schutte S, et al. Whole grain wheat consumption affects Postprandial inflammatory response in a randomized controlled trial in overweight and obese adults with mild hypercholesterolemia in the Graandioos Study. *J Nutr*. 2019;149(12):2133-2144. doi:10.1093/jn/nxz177
62. Maki K, Palacios O, Koecher K, et al. The relationship between whole grain intake and body weight: results of meta-analyses of observational studies andrandomized controlled Trials (FS18-07-19). *Curr Dev Nutr*. 2019;3(Supplement_1). doi:10.1093/cdn/nzz041.fs18-07-19
63. Malik A, Kanneganti T-D. Function and regulation of il-1α in inflammatory diseases and cancer. *Immunol Rev*. 2017;281(1):124-137. doi:10.1111/imr.12615
64. Newby PK, Maras J, Bakun P, Muller D, Ferrucci L, Tucker KL. Intake of whole grains, refined grains, and cereal fiber measured with 7-d diet records and associations with risk factors for chronic disease. *Am J Clin Nutr*. 2007;86(6):1745-1753. doi:10.1093/ajcn/86.6.1745
65. Nilsson A, Johansson-Boll E, Sandberg J, Björck I. Gut microbiota mediated benefits of barley kernel products on metabolism, gut hormones, and inflammatory markers as affected by co-ingestion of commercially available probiotics: a randomized controlled study in healthy subjects. *Clin Nutr ESPEN*.2016;15:49-56. doi:10.1016/j.clnesp.2016.06.006
66. Robertson JA, I'Anson KJ, Brocklehurst TF, Faulds CB, Waldron KW. Effect of storage conditions on the microbial ecology and biochemical stability of cell wall components in brewers' spent grain. *J Ag Food Chem*. 2010;58(12):7266-7272. doi:10.1021/jf1001099.
67. Schioldan AG, Gregersen S, Hald S, et al. Effects of a diet rich in arabinoxylanand resistant starch compared with a diet rich in refined carbohydrates on postprandial metabolism and features of the metabolic syndrome. *Eur J Nutr*. 2017;57(2):795-807. doi:10.1007/s00394-016-1369-8
68. Schioldan AG, Gregersen S, Hald S, et al. Effects of a diet rich in arabinoxylanand resistant starch compared with a diet rich in refined carbohydrates on postprandial metabolism and features of the metabolic syndrome. *Eur J Nutr*. 2017;57(2):795-807. doi:10.1007/s00394-016-1369-8

69. Shah PK, Lecis D. Inflammation in atherosclerotic cardiovascular disease. *F1000Research*. 2019;8:1402. doi:10.12688/f1000research.18901.1.
70. Tiwari U, Cummins E. Meta-analysis of the effect of β-glucan intake on blood cholesterol and glucose levels. *Nutrition*. 2011;27(10):1008-1016. doi:10.1016/j.nut.2010.11.006
71. Tong L-T, Zhong K, Liu L, et al. Effects of dietary wheat bran arabinoxylans on cholesterol metabolism of hypercholesterolemic hamsters. *Carbohydr Polym*. 2014;112:1-5. doi:10.1016/j.carbpol.2014.05.061
72. Vanegas SM, Meydani M, Barnett JB, et al. Substituting whole grains for refined grains in a 6-WK randomized trial has a modest effect on gut microbiota and immune and inflammatory markers of healthy adults. *Am J Clin Nutr*. 2017;105(3):635-650. doi:10.3945/ajcn.116.146928
73. Zhou D, Yu H, He F, et al. Nut consumption in relation to cardiovascular diseaserisk and type 2 diabetes: a systematic review and meta-analysis of prospective studies. *Am J Clin Nutr*. 2014;100(1):270. doi:10.3945/ajcn.113.079152

APPENDIX A

HEI Components and Scoring Standards

HEI-2015[1] Components and Scoring Standards

Component	Maximum points	Standard for maximum score	standard for minimum score of zero
Adequacy:			
Tolal Fruits'	5	20.8 cup equivalent per 1,000 kcal	No Fruil
Whole Fruits•	5	20.4 cup equivalent per 1,000 kcal	No Whole Fruit
Tolal Vegetables'	5	21.1 cup equivalent per 1,000 kcal	No Vegetables
Greens and Beans•	5	20.2 cup equivalent per 1,000 kcal	No Dark-Green Vegetables *ex* Legumes
Whole Grains	10	21.5 ounce equivalent per 1,000 kcal	No **Whole** Grains
Dairy'	10	?1.3cup equivalent per 1,000 kcal	No Dairy
Tolal Prolein Foods'	5	22.5 ounce equivalenl per 1,000 kcal	No Protein Foods
Seafood and Planl Proleins'·'	5	0.8ounce equivalent per 1,000 kcal	No Seafood or Planl Proleins
Fatty Acids'	10	(PUFAs + MUFAs) I SFAs 22.5	(PUFAs + MUFAs)ISFAs S1.2
Moderation:			
Refined Grains	10	S1.8 ounce equivalent per 1,000 kcal	H.3 ounce equivalent per 1,000 kcal
Sodium	10	S1.1 grams per 1,000kcal	22.0 grams per 1,000 kcal
Added Sugars	10	SG.5% of energy	26% of energy
Saturated Fats	10	S8% of energy	16% of energy

' Intakes between the minimum andmaximum standards are sccxed proportionately. 'Includes 100% fruit juice.

3 Includes all fcxms except juice.

4 Includes legumes (beans and peas).

'Includes all milk products, such as fluidmilk, yogurt, and cheese, and fortified sey beverages.

'Includes seafood: nuts, seeds, soy products (olher than beverages), andlegumes (beans andpeas). 7 Ratioof poly- and mono-unsaturatedfatty acids (PUFAs and MUFAs) to saturated fatty acids (SFAs).

APPENDIX B

IL-6, IL-1beta, and IL-10 Assay Analysis

Participant blood samples were collected in the fasting state at baseline and 8 weeks. The EDTA vacutainers were centrifuged at 4000 rom for 15 minutes, the plasma was transferred to pre-labeled (participant ID, date, time of collection) microcentrifuge tubes and stored in white cardboard boxes at -70°C. IL- 6, IL-1beta, and IL-10 concentrations were analyzed using 5 ml aliquots of plasma from each sample using MILLIPLEX® magnetic bead-based assay kits in duplicate (Millipore Sigma, MA, USA) according to the manufacturer's protocol. All biomarker concentrations were determined using the MAGPIX microplate reader (Millipore Sigma, MA, USA) to determine the effect of BSG on those biomarkers.

APPENDIX C

Institutional Review Board Approval

Texas Woman's University
Institutional Review Board (IRB)
irb@twu.edu
https://www.twu.edu/institutional-review-board-irb/

March 3, 2020

Marley Grams
Nutrition and Food Sciences

Re: Exempt - IRB-FY2020-227 Examining the Effects of Consuming Muffins Containing Brewer's Spent Grains on Fiber Intake, Healthy Eating Index Scores (HEI), and Inflammation Biomarkers in Healthy Adults

Dear Marley Grams,

The above referenced study has been reviewed by the TWU IRB - Denton operating under FWA00000178 and was determined to be exempt on March 2, 2020.

Note that any modifications to this study must be submitted for IRB review prior to their implementation, including the submission of any agency approval letters, changes in research personnel, and any changes in study procedures or instruments. Additionally, the IRB must be notified immediately of any adverse events or unanticipated problems. All modification requests, incident reports, and requests to close the file must be submitted through Cayuse.

On May 31, 2021, this approval will expire and the study must b e renewed or closed. A reminder will be sent 45 days prior to this date.

If you have any questions or need additional information, please contact the IRB analyst indicated on your application in Cayuse or refer to the IRB website at http://www.twu.edu/institutional-review-board-irb/.

Sincerely,

TWU IRB - Denton

APPENDIX D

Institutional Review Board Closure Letter

s University
eview Board (IRB)

u.edu/institutional-review-board-irb/

July 9, 2021

Marley Grams
Nutrition and Food Sciences

Re: Closure for IRB-FY2020-227 Examining the Effects of Consuming Muffins Containing Brewer's Spent Grains on Fiber Intake, Healthy Eating Index Scores (HEI), and Inflammation Biomarkers in Healthy Adults

Dear Marley Grams,

The TWU IRB - Denton has received all required documents necessary to close the protocol referenced above. As applicable, the final study report has been submitted. As of this date, the protocol file hasbeen closed.

If you have any questions or need additional information, please email your IRB analyst at irb@twu.edu or refer to the IRB website.

To download a copy of this letter, please login to Cayuse, click on "Studies" and locate the study under the "Archive" tab. After clicking on the above referenced study, the closure letter will be located under the "Letters" tab of the Closure Submission.

Sincerely,

TWU IRB - Denton