ANALYSIS OF RESISTANT STARCH CONTENT AMONG DIFFERENT POTATO VARIETIES AND THE IMPACT OF ONE VARIETY ON SATIETY

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ABSTRACT

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Resistant starch (RS) content can be impacted by cooking method and potato variety and have effects on satiety when consumed. This study analyzed RS content among three potato varieties (Red Norland, Russet, and Yukon Gold) where each were cooked using five different methods and serving temperatures (boiled used hot, baked used hot, baked then chilled one day, baked then chilled three days, and baked then chilled five days). RS content was the highest in Russet potatoes baked then chilled for five days (6.21g/100g) and lowest in Yukon Gold potatoes boiled used hot (1.84 g/100g). Cooking method showed an effect on RS content ($p < 0.001$) but RS did not differ among potato variety ($p = 0.247$). Then, Russet potatoes were utilized in a randomized crossover trial that examined their impact on subjective satiety measured by a visual analogue scale (VAS) and satiety biomarkers glucagon-like peptide-1 (GLP-1) and peptide YY (PYY). In the trial, the females consumed boiled potatoes served hot and baked then chilled potatoes consumed on separate occasions with VAS scores and GLP-1 and PYY. No differences in the area under the curve (AUC) for $AUC_{(0\text{-}120\ min)}$ for GLP-1 and PYY and overall subjective satiety were found between the boiled and chilled potatoes.

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CHAPTER I

INTRODUCTION

Potatoes are a predominant staple within the American diet. Potatoes are the second most consumed vegetable commodity in individuals two years or older.[1] In 2018, approximately 114 pounds of potatoes were consumed per person.[2] Potatoes can be processed using many different methods prior to consumption, the most common being processed potato products, such as French fries and chips.[3]

Potatoes have an average cost of $0.60 per pound and can contribute several nutrients to the diet in a cost-effective manner.[4] The nutritional profile of potatoes includes complex carbohydrates, vitamin C, potassium, vitamin B6, magnesium, fiber, and naturally low in fat content.[5] Consuming potatoes not only contributes to a nutrient-dense eating pattern, but also may modulate post-meal hunger and subsequent food intake. Compared to rice and pasta, potatoes can be more subjectively satiating, which contributes to a lower desire to eat following a meal.[6] Subjective satiety varies among different potato products, with boiled showing higher satiety post-meal when compared to French fries.[7,8] In a study comparing different carbohydrate products eaten ad libidum within a meal, 30-40% less kcals were consumed in meals with boiled and mashed potatoes than rice, pasta, fried, or baked French fries.[7]

Recommending potatoes in the diet has been a source of controversy due to reports from epidemiology studies. Potato consumption has been linked to increased risk of obesity, type 2 diabetes mellitus (T2DM), and cardiovascular disease (CVD).[9-11] These associations often

overlook the differences in nutritional and caloric value that may be altered due to various preparation methods, the addition of other ingredients prior to consumption, quantity consumed, and impact of other foods within the overall diet. The most popular potato-containing products utilize methods of preparation that involve frying or secondary addition of fat content, such as butter, sour cream, and cheese. In an eight-year longitudinal study, potato consumption was not associated with increased mortality risk until sub-group analysis of fried potato consumption frequency of two to three times per week and greater or equal to three times per week.[12] A cohort study found no significant association with specific or overall mortality when considering consumption of a variety of potato preparations, except with French fry consumption, which was associated with cancer-related mortality.[13] Although there is conflicting research on the impact of potato consumption on health, there is a consensus to show that different cooking methods alter chemical nature of potatoes that can influence health outcomes.

Starch is a complex carbohydrate found within a variety of commonly consumed plant foods, including potatoes. The composition of starch includes amylose and amylopectin, with differing ratios impacting digestibility within the human gastrointestinal (GI) system. For example, a higher amylose to amylopectin ratio reduces the digestibility, thus, reducing the available carbohydrate from the starch molecule. Classification of starches are organized based on transit time in the digestive process in the small intestine, which include rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). RDS and SDS both are completely digested in the small intestine while RS is resistant to enzymatic digestion, and instead bypasses the small intestine to the large intestine where specific types of RS can be fermented.[14]

Fermentation of RS through microbiota of the colon can encourage microbial diversity and produce by-products that may be beneficial to host health.[15] The byproducts of RS fermentation include short-chain fatty acids (SCFA), butyrate, propionate, and acetate, which are released and utilized by the host. SCFA have been recognized to influence host energy metabolism and appetite through colonic signaling of anorexic hormones glucagon like peptide-1 (GLP-1) and peptide YY (PYY).[16,17] Higher RS content in foods also displaces the available carbohydrate that can be digested which attenuates blood glucose concentrations.

Potatoes contain different types and amounts of RS, based on variety, degree of maturity, and preparation method.[18,19] The analysis of RS content in various potato variations and preparation techniques shows the highest amounts of RS are found in uncooked potatoes, followed by higher RS content in cooked then chilled potatoes, which contain retrograded starch, than boiled potatoes.[20] Current analysis of RS from potatoes predominantly uses RS-2 raw potato starch as the intervention but lacks further understanding from an intervention utilizing the whole food, which is more representative of a standard meal component. Variance in preparation methods and serving temperatures have been shown to affect RS content, with baked potatoes higher in RS than boiled potatoes and chilled potatoes higher in RS than hot potatoes.[18]

Purpose of the Study

The overall purpose of this study was to determine the amounts of RS in three commonly consumed potato varieties subjected to different cooking methods and serving temperatures. This study also incorporated a human intervention trial to examine the effects of RS from

Russet potatoes subjected to two cooking methods and serving temperatures on subjective satiety and GLP-1 and PYY post-prandial concentrations.

Research Aims and Hypotheses

This project examined two separate overall aims. Aim 1 examined the effects of different cooking methods and storage temperature on RS content across commonly consumed potato varieties of Russet, Yukon Gold, and Red Norland potatoes. The first sub-aim of this study compared the RS content in 1) boiled, 2) baked, 3) baked then chilled for one day, 4) baked then chilled for three days, and 5) baked then chilled for five days. The second sub-aim compared RS among the following potato varieties: Yukon Gold, Russet, and Red Norland. Each sample was measured in triplicate.

- H_{0a}: Yukon Gold, Russet, and Red Norland potatoes will have equal amounts of RS.
- H_{0b}: No difference in RS concentrations will be observed across cooking and storage methods.

According to the results of Aim 1, Russet potatoes were chosen to address Aim 2, the human intervention, because they had the highest RS content compared to the other varieties. Aim 2 utilized data from a published human intervention trial to examine effects of boiled, served hot versus baked, then chilled cooking methods of Russet potatoes on subjective satiety and GLP-1 and PYY post-prandial concentrations. The first sub-aim examined the relationship between RS content in boiled potatoes consumed hot and baked potatoes chilled for five days on mean scores of subjective satiety measured by a 100 mm visual analogue scale (VAS).[21] The second sub-aim compared the plasma area under the curve ($AUC_{(0\text{-}120\ min)}$) GLP-1 and $AUC_{(0\text{-}120\ min)}$ PYY following each potato intervention.

- H_{0c}: No differences in mean VAS scores between baked, then chilled and boiled potatoes will be observed.
- H_{0d}: No differences between $AUC_{(0\text{-}120\ min)}$ for GLP-1 and $AUC_{(0\text{-}120\ min)}$ for PYY values for baked, then chilled and boiled potatoes will be observed.

CHAPTER II

LITERATURE REVIEW

Global Potato Production

Potatoes originated approximately 8000 years ago in South America. Due to efforts of crop diversity, there are over 5000 potato varieties that have been identified globally. The large number of potato varieties is attributed to the increased usage and consumption of potatoes and potato products to meet consumer demand. Potatoes are considered a staple crop within global agricultural production. They were the fifth most produced crop in 2016, with the first being sugar cane, followed by maize, rice, and then wheat.[22] Over a period of almost 25 years, potato production has increased by 36% from 1994 to 2018, in the amount of 270 million tons to approximately 368 million tons. The top five producing countries of potatoes include China, India, Russia Federation, Ukraine, and the United States (U.S.).[23] Percentages of potato production include 51.2% from Asia, 28.6% from Europe, 12.7% from the Americas, 7.1% from Africa, and 0.5% from the Oceania region.

The Food and Agriculture Organization (FAO) of the United Nations declared the year 2008 as the "International Year of the Potato," with the mission of raising awareness to the importance the potato can play in the role within food security, poverty, sustainability and hunger on a global scale. Due to the adaptability of the plant, potatoes can grow in suboptimal soil and environmental conditions among those that utilize appropriate agricultural practices, such as rotation of cultivating land with dissimilar crops, adequate crop care and fertilization, control of pest and disease, and continual introduction of diverse genetic varieties.[24]

United States Potato Consumption

Five percent of global potato production is produced in the U.S. With 44 billion pounds of potatoes produced in the U.S. every year, approximately 39 billion are processed into potato products for consumption, while the remaining contributes to livestock feed and seeds.[3] Out of the potatoes processed, 55% are processed as frozen French fries, 20% as potato chips and shoestrings, 17% as dehydrated flakes, 4% as other frozen products, 2% as other items, and <1% in canned products potatoes.[3] Potatoes are the most commonly consumed vegetable in the U.S., with the highest potato consumption contribution from French fries.[25]

There are over 200 varieties of potatoes grown in the U.S., which are classified into seven categories: Russet, red, white, yellow, purple, fingerling, and petite. Each category of potato differs in physical characteristics of size, shape, and color, but also biological characteristics, such as varying water content, nutrient concentration, and starch content. These characteristics are utilized for various products within the food industry.

Nutrient Composition

Potatoes contain vitamin C, vitamin B6, folate, thiamin, potassium, and magnesium.[5] For every small potato (approximately 150 grams), there are 110 kcals, zero grams of fat, 26 grams of carbohydrates, two grams of fiber, three grams of protein, 30% of the daily value of vitamin C, 15% daily value of potassium, and 10% of the daily value of vitamin B6. Raw potatoes comprise of approximately 80% water and 20% dry matter. The dry matter is composed of 60-80% of starch, with the remaining 20-40% as non-starch polysaccharides.

Starch

Starch is a complex carbohydrate found within a variety of commonly consumed plant-based foods. Starch is comprised of amylose and amylopectin which are found present in granules. Amylose is an amorphous, linear structure comprised on α (1,4) glycosidic bonds while amylopectin is a highly branched structure with α (1,4) linkages and α (1,6) glycosidic bonds at the branch connections. Starches typically contain 20-30% amylose and 70-80% amylopectin. Amylose and amylopectin are compacted in a helical formation within a semi-crystalline structure.[26] Amylase cleaves the α (1,4) bonds of both structures, with debranching enzymes isoamylase and pullulanase cleaving the α (1,6) bonds in amylopectin.[26] The degree of crystallization is determined by the ratio of amylose to amylopectin and are divided into three types- A,B, and C. The A-type crystallinity includes densely packed helices, B-type is less densely packed, and C-type consists of both A and B-types.

Changes in starch structure occur under temperature and moisture treatment. When a starch granule is heated with an aqueous source, the bonds joining starch fractions amylose and amylopectin are weakened, allowing the water molecules to move into the starch granule and form hydrogen bonds. The result is gelatinization and render the granule more available to amylose enzymes due to the disruption of its native crystalline structure. When the cooked starch is cooled, the granule undergoes retrogradation and reverts back to a crystalline structure.

Starch digestibility is impacted by alterations in starch structure. Level of gelatinization is attributed by the specific starch and external factors. Some characteristics, such as granule size, ratio of amylose and amylopectin, and amylose and amylopectin chain length impact gelatinizing behavior. In general, higher amylose content is associated with both decreased digestibility from

the tightly packed structure and increased capacity for gelatinization under heat and moisture treatment. Higher levels of amylose require higher temperatures for gelatinization when the starch is cooked. Retrogradation of amylose back to crystalline structure can occur at 4°C within two days while re-crystallization of amylopectin may take weeks.[27]

Hans N. Englyst has been a large contributor to carbohydrate, specifically starch, research and provided the foundation of knowledge utilized by researchers to this day. [28] Englyst et al pioneered assay procedures to differentiate and classify starch according to digestibility characteristics through the GI system, starting in the mouth and ending in the ileum.[28] Classifications include RDS, SDS, and RS. RDS is defined by being completely digested within 20 minutes and SDS defined by complete digestion within 20 and 120 minutes within the small intestine. RS has a crystalline structure that is resistant to normal enzymatic digestion. Because of this resistance, it is bypassed into the large intestine where some types may be fermented by the gut flora.

With the variation of digestibility and absorption, each starch class induces different glycemic and hormonal responses. RDS induces a faster glycemic response from readily available glucose, which is typically followed by a higher insulin response. The SDS has a slower digestion rate imparting a slower rise blood glucose concentrations and insulin response than RDS. Unlike RDS and SDS, RS is unique due to its minimal impact on blood glucose but also demonstrated improvement on glycemic control.[29]

Resistant Starch

RS is a class of starch that is indigestible through normal digestion and is bypassed where some types can be fermented in the large intestine. Englyst originally classified the first three classes of RS. [28] Later, RS-4 and RS-5 classes were created.[30] There are now 5 classes of RS: RS-1 is nondigestible and entrapped matrix, RS-2 is ungelatinized starch found in raw potatoes, RS-3 is retrograded starch that occurs when cooked starch products are chilled, RS-4 is a chemically modified starch, and RS-5 is an amylose-lipid formed starch.[31]

RS-1

Type 1 RS is contained in physically inaccessible starch granules. The starch granules are surrounded by the thick cell wall and protein matrix, impacting the digestibility quality when consumed. The structural integrity of the RS-1 when heated under normal conditions remains intact, preventing an aqueous solution to be absorbed into the granule which would allow gelatinization and subsequent enzymatic hydrolysis.[31] RS-1 can be found in bread and pasta made of whole or coarsely-ground grain kernels.[28]

RS-2

Type 2 RS is found in unmatured, raw plants and plants with higher amylose content. RS-2 comprises of either type B or C crystallinity due to a higher ratio of amylose to amylopectin. The crystalline structure confers resistance to enzymatic hydrolysis but is vulnerable when cooked with an aqueous solution or through maturation in foods. Examples of foods with RS-2 are raw potatoes, green bananas, and high-amylose maize starch. High-amylose maize as a functional ingredient, however, has been formulated so that heat and water do not influence RS concentrations.

RS-3

Type 3 RS is formed through a process called retrogradation. When starch is cooked or heated, the granules swell from an influx of water, causing the molecule to gelatinize which improves enzymatic digestion. As the gelatinized starch is cooled at typical refrigeration temperatures, the amylose and amylopectin reforms the crystalline structure. Examples of foods containing RS-3 can be found in cooked and chilled potatoes legumes, breads, and pasta.

RS-4

Type 4 RS is created due to the chemical modification of bond formation through cross-linking or addition of chemical derivatives.

RS-5

Type 5 RS is formed when amylose and lipids create a complex structure. RS-5 is the most recently classified RS. The binding of the helical structure of amylose with various lipids exhibits resistance against enzymatic hydrolysis.[32,33] Experimental formation of various lipid structures with raw or cooked starches may alter the RS content of different foods.[34]

Resistant Starch and Physiological Response

Due to the resistance of enzymatic digestion, RS is bypassed from the small intestine to the large intestine. RS is considered a type of dietary fiber with RS-2, RS-3, and RS-4 shown to be fermentable by the gut microbiome.[35-37] Byproducts of RS fermentation include the production of SCFA, predominately acetate, propionate, and butyrate, as well as gases, organic acids, and alcohols.[38,39] Certain types of RS may exert prebiotic properties that increase the viability and abundance of specific microflora species that produce different concentrations of acetate, propionate, and butyrate. SCFA have different roles in host metabolism due to varying

routes of transport in the body. Acetate is transported to the peripheral tissues for energy metabolism. Propionate is transported through the portal vein to the liver for gluconeogenesis. Butyrate acts as the preferred energy source for colonocytes. SCFA have beneficial properties to the host, such as providing a source of energy, maintaining gut integrity, and stimulating production of hormones. SCFA bind to L-cell receptors on the colonocytes to stimulate the production of anorexic hormones of GLP-1 and PYY.[40] SCFA can also cross the blood-brain barrier and influence appetite regulation in the hypothalamus. [41,42]

There are multiple health benefits associated with RS intake. Foods with high levels of RS have a slower digestibility rate, which slows the rise in the post-prandial levels of glucose and insulin. RS intake can be beneficial for prevention and treatment of metabolic disorders, such as obesity, metabolic syndrome, abnormal glycemic control, and insulin resistance. [39] RS intake also increases levels of GLP-1 and PYY, which increases signals for appetite regulation and delayed gastric emptying which are beneficial for controlling blood sugar and reducing subsequent food intake. [43,44]

Manipulation of RS

RS content differs across potato varieties due to several factors. Environmental conditions, level of maturation, and length of storage time can alter starch content. [45,46] Potatoes can also be selectively bred to disengage genes that code for starch branching enzymes (SBE) 1 and SBE 2 and thereby inhibit amylopectin formation and increase amylose content. [47,48] These factors cannot be controlled by the average consumer when purchasing potatoes from a grocery store. The most practical application to alter RS content is heat treatment and subsequent cooling to form RS-3 through retrogradation. Degree of retrogradation is dependent on storage length

and temperature. Increased storage time in typical refrigeration temperatures improve re-aggregation of crystalline structure. [49] Retrogradation of potatoes through common cooking and storage methods can be a simple method to increase RS content of the diet. Different cooking methods and serving temperatures influence RS content in potatoes. Dry heat treatment, such as baking, significantly increase RS content more than wet heat treatment, such as boiling, after being chilled in refrigerator. Cooked potatoes stored at cold temperatures increase RS content compared with potatoes kept warm. [50] Potatoes chilled for a longer duration are also less susceptible to decreased RS content when reheated by maintaining more RS than during the first heat treatment.

Resistant Starch and Appetite Regulation

The influences of RS on appetite has been demonstrated, but still require more research. When RS was supplemented in the diet for over six weeks, results demonstrated in healthy individuals a reduction of hunger, increased subjective satiety and PYY release, and lower calorie intake in subsequent meals was observed.[51] In another study, muffins with RS were found in to be more subjectively satiating for a longer duration compared to those with other fibers in healthy individuals.[52] However, not all clinical trials have shown RS promotes satiety. One study found that neither subjective satiety nor satiety-influencing hormones were impacted following the intake of 45g RS-2 daily for 12-weeks in adults with prediabetes. [53] These findings suggest disease state may influence satiety response.

Appetite regulation encompasses multiple processes regarding meal-time physiology. The system of meal-time sensations can be described using these terms: appetite, hunger, satiation, and satiety. These terms may utilize one or multiple definitions to accurately describe

each sensation. Appetite refers to the sensory aspects related to intake, selection, motivation and food preference.[54] Hunger can be physical and mental sensations for a desire to eat, manifested through feelings of weakness or an empty stomach.[54] Satiation is a physiological desire that leads to termination of eating during a meal.[54] Satiety is the sensation of fullness after termination of a meal and decline of hunger that inhibits further eating.[54,55] These terms encompass different inter-connected physiological processes of the central nervous system, gut-brain signaling, and endocrine systems.

The process of controlling appetite is multifaceted and continues to be researched for further understanding. An early mechanistic framework that is still utilized by researchers to understand appetite is "The Satiety Cascade" proposed by Blundell et al.[56] This framework shows the relationship between psychological and physiological processes of meal-time considerations of meal quality, meal quantity, nutrient status, and energy balance that affect satiation and satiety.

The GI system provides functions beyond digestion. The GI system also plays a part in appetite and satiety regulation of food intake through secretion of hormones GLP-1 and PYY. GLP-1 is classified as an incretin hormone secreted from enteroendocrine L-cells post-prandially. After food ingestion, blood glucose in the intestinal lumen signals GLP-1 release, which stimulates insulin secretion from the pancreatic beta-cells to regulate blood glucose. Additionally, GLP-1 also inhibits release of glucagon from pancreatic α-cells to reduce the rate of nutrient absorption through reducing rate of gastric emptying and food intake.[57] A proposed mechanism behind GLP-1 on reducing food intake involves action upon the vagus nerve from GLP-1 receptors within both central and peripheral nervous systems.[55,56] Increasing GLP-1 levels

can be achieved through pharmacological methods of GLP-1 receptor agonists or by increasing GLP-1 concentrations by food selection. The Food and Drug Administration (FDA) has approved GLP-1 receptor agonists to improve blood glucose concentrations in adults with T2DM but the efficacy of GLP-1 beyond blood glucose control is still lacking. Considerations for inter-person variability, as well as dosage of medication, and long-term safety are cited. [58] Studies using exogenous GLP-1 injections have shown a reduction in appetite and bodyweight in obese individuals with T2DM.[59-61] Native GLP-1 secretion has a short half-life due to the action of the dipetidylpeptidase-1 (DPP-4) enzyme and urinary excretion. Therefore, identification of an alternative mechanisms to sustain GLP-1 concentrations may be useful for appetite control.[62]

PYY is an anorexic hormone released from enteroendocrine L-cells of the GI tract in response to nutrient ingestion. Level of PYY release is dependent on caloric intake, although specific foods and nutrient profile have been shown to be influential as well. [63,64] When eating, secretion of PYY increases, which acts upon appetite regulation mechanisms in the hypothalamus and food-reward processing in the orbital frontal cortex to reduce food intake. [65] This mechanism affects healthy and overweight or obese individuals differently. Studies supplying intravenous PYY before meal intake in both normal weight and obese individuals have shown decrease overall caloric intake for both groups but endogenous PYY levels were significantly lower in obese subjects.[63,66] The question of whether onset of obesity can be attributed due to low PYY levels or if low levels are a consequence of obesity is still unclear.

Ingestion of specific foods impact GLP-1 and PYY release. Fermentable dietary fibers, such as certain types of RS, have shown to increase production of SCFA, which act upon the free

fatty acid receptor (FFAR) 2 and 3 of the L-cells in the colon to stimulate release of GLP-1 and PYY in rats and humans.[67-69]

Subjective Satiety

Measuring satiety involves an integration of both hormonal levels and subjective regulation. Satiety hormones like GLP-1 and PYY are influential in the perception of appetite and food-pleasure attitudes. Subjective satiety is measured through completion of questionnaires before or during food intervention trials. A common and validated measure used in studies is the VAS. [21] The VAS is a validated questionnaire with eight questions, each containing a 100 mm line with the maximum value of 100 mm expressing either a positive or negative rating compared to 0 mm line. These questions assess hunger, satiety, fullness, desire or subsequent food intake, and desire to eat something sweet, salty, savory, or fatty. [21]

Conclusions and Significance

Observational trials have shown that potato consumption has been associated with increased risk of chronic diseases. [12,13] These association often lack consideration of consumption of fried potato products, other confounding food consumption, and overall surplus of caloric intake. When cooked using lower calorie cooking methods, potatoes can be part of a nutritious meal. Potatoes contain vitamin B6, potassium, vitamin C, and a good source of complex carbohydrates and fiber from RS. RS can improve satiety through stimulating the secretion of GLP-1 and PYY via fermentation by the gut microbes, lower post-prandial glucose and insulin secretion, and help with weight management. RS in potatoes can be altered through different cooking methods and storage temperatures and duration.

CHAPTER III

METHODOLOGY

The following methods addressing Aim 1 and Aim 2 is described below.

Aim 1 Methods

Aim 1 examined the effects of different cooking methods and storage temperature on RS content across commonly consumed potato varieties

Sample Preparation

Potatoes were prepared to analyze the RS content following different cooking methods and serving temperatures among Red Norland, Russet, and Yukon Gold potatoes: boiled used hot, baked used hot, baked then chilled for one day, baked then chilled for three days, baked then chilled for five days. A total of 15 samples were prepared. The boiled used hot potatoes were peeled and sliced into one-inch cubes and cooked in boiling water for approximately 10 minutes until tender and prepared for freeze drying. The protocol for baked and chilled potatoes included wrapping each potato with skin in foil and baking in a convection oven at ~204°C for approximately 60 minutes, placed in the cooler at ~4°C for one, three, and five days, then peeled and cut to 1-inch cubes for freeze drying. Baked used hot potatoes were peeled and cut into one-inch cubes after cooking and prepared for freeze drying.

Prepared potatoes were subjected to lyophilization using Labconco FreeZone Benchtop Freeze Dryer for sample preparation for RS assay analysis. The Labconco user manual provided the protocol used for freeze drying the potatoes. Prepared potato samples were weighed then stored in bags made from nylon hose and placed in -80°C freezer overnight before freeze drying. Pre-frozen samples were placed in appropriate glassware two to three times the amount of

sample, then attached to a drying chamber valve of the machine to begin the lyophilization process. Samples were freeze dried at -84°C and 0.22 mbar for approximately three days to ensure adequate drying. Following lyophilization, the sample and glassware were weighed again to determine moisture loss. Percent moisture content was then calculated through pre and post-lyophilized weight. The lyophilized samples were ground up in a mortar and pestle into a fine powder and stored in plastic zipper seal bags at room temperature until RS assay analysis.

Resistant Starch Analysis

The RS content of each sample was measured in triplicate following the protocol of a commercially available RS assay kit (K-RSTAR, Megazyme, International Ireland Ltd, Co. Wicklow, Ireland). One hundred milligrams of lyophilized potato were placed in labeled test tubes along with pancreatic α-amylase to break down digestible starch and incubated in a shaking water bath at 37°C for 16 hours. Contents were subsequently treated with ethanol and centrifuged three times, decanting the supernatants every cycle for later use for calculating non-RS content. Addition of a magnetic stirrer bar and potassium hydroxide (2 M) to each test tube was placed in a water bath over a magnetic stirrer for 20 minutes then placed into water bath at 50°C for 30 minutes. Samples were then centrifuged for 10 minutes then aliquoted in triplicate into glass test tubes with addition of glucose oxidase/peroxidase (GOPOD) reagent to incubate at 50°C for 20 minutes. RS content was analyzed for absorbance at 510 nm using a spectrophotometer (Synergy HI, BioTek® Instruments, Inc., Winooski, Vermont, U.S.A.) and calculated with the Mega-Calc software tool provided by Megazyme using absorbance, sample weight, extract volume, and moisture content values to calculate grams of RS in samples. Mean and standard deviation of each triplicated potato sample were calculated for descriptive statistics.

Non-RS content, the solubilized starch of the sample, was calculated following the protocol of the same assay kit. Each of the decanted supernatant solutions was adjusted for volume to 100 mL with sodium acetate buffer. Aliquots of each solution along with dilute amyloglucosidase solution was placed in test tubes and incubated at 50°C for 20 minutes and measured for absorbance at 510 nm in a spectrophotometer. The RS and non-RS content was used to calculate the total starch content of each sample.

Aim 2 Methods

A human trial was used to address Aim 2, which examined effects of boiled, served hot versus baked, then chilled cooking methods of Russet potatoes on subjective satiety and GLP-1 and PYY post-prandial concentrations.

The cross-over, randomized controlled trial research project titled "Influence of resistant starch in baked then chilled and boiled potatoes on glycemic and satiety responses in overweight females" was conducted between December 2017 and December 2018. Aim two used the results from Aim one that included Russet potatoes 1) boiled consumed hot and 2) baked and chilled for five days. Russet potatoes using the cooking methods stated above were used because they had the most variation in amount of RS.

Potato Intervention Preparation

The study used the cooking methods described in the Aim 1 methods to prepare the potatoes. In summary, the baked then chilled protocol consisted of Russet potatoes with skin in foil and baking at ~204°C for approximately 60 minutes, then placing in the cooler at ~4°C for five days and sliced into one-inch cubes prior to consumption. Protocol for boiling consisted of

peeling and slicing potatoes into one-inch cubes and boiling in water until tender and served immediately.

Study Design

The Texas Woman's University Institutional Review Board in Houston, TX, approved the study. Study subjects were recruited from the Houston area through flyer distribution and advertisements on internet listings. Inclusion criteria consisted of 18 to 45-year-old pre-menopausal women with BMI of 28 to 40 kg/m^2. Exclusion criteria included diagnosis of diabetes mellitus, CVD, cancer, or other disorders affecting metabolism, nicotine or drug use, on-going pregnancy or lactation status, recent significant alterations in weight ($\pm \geq 5\%$ in body weight over past six months), or following specific diet plan. All subjects meeting inclusion criteria provide written informed consent prior to data collection. A total of 30 subjects met inclusion criteria, provided informed consent, and were included in the study.

Subjects were randomized to either the boiled or chilled Russet potato on visit one. On visit one, individuals arrived fasted ($\geq$ 8 hours). Fasting blood was collected first and then the subjects consumed 250 grams of potato according to randomization within 15 minutes with eight ounces of water. Post-prandial (PP) blood draws were collected by a trained phlebotomist at 15 minutes, 30 minutes, 60 minutes, and 120 minutes. Analysis of GLP-1 and PYY concentrations from blood draws were completed by the principal researchers (Mindy Patterson, Ph.D , RDN and Joy Nolte Fong, MPH, RD, LD) and according to instructions from a commercially prepared assay analysis kits (Alpco, Salem, New Hampshire, U.S.A.; see Appendix B and C). Subjective satiety was measured using a VAS at two times points: 15 minutes PP and 60 minutes PP. A one-week washout period occurred between visit one and visit two where the second potato type was

consumed. The entire protocol was repeated for visit two for the other potato intervention. The intervention trial protocol was conducted by graduate research assistants (Stephanie Kung, Nezar Nashef, and Araz Sarkissian).

The VAS is a validated questionnaire with eight questions, each containing a 100 mm line with one side expressing a positive rating and the other side a negative rating, to assess hunger, satiety, fullness, desire or subsequent food intake, and desire to eat something sweet, salty, savory, or fatty.[21] Values of five questions were reverse coded, where a lower score is indicative of a positive response. Higher scores indicate greater subjective satiety. Utilizing the VAS will examine the level of association between the satiety biomarkers GLP-1 and PYY and subjective satiety. The VAS questionnaire is located in Appendix A.

Biomarker Analysis

Blood samples were collected in BD P800 tubes and analyzed for GLP-1 and PYY measurements. Each tube was centrifuged at 4000 rpm for 15 minutes to separate red blood cells from plasma, which was aliquoted into 1.5 mL vials to be stored at 80°C. Samples were analyzed by the principle researchers using a commercial enzyme-linked immunosorbent assay kit (Alpco, Salem, New Hampshire, U.S.A.).

Statistical Analysis

Descriptive statistics of mean and standard deviation were calculated for the triplicated samples, VAS scores, and $AUC_{(0\text{-}120\ min)}$ for GLP-1 and PYY. The $AUC_{(0\text{-}120\ min)}$ for biomarkers was calculated using the trapezoidal method. A two-way ANOVA was utilized to analyze the main and interaction effects of potato type and cooking methods/serving temperature on RS content. A paired *t*-test compared $AUC_{(0\text{-}120\ min)}$ for GLP-1 and PYY from each potato intervention.

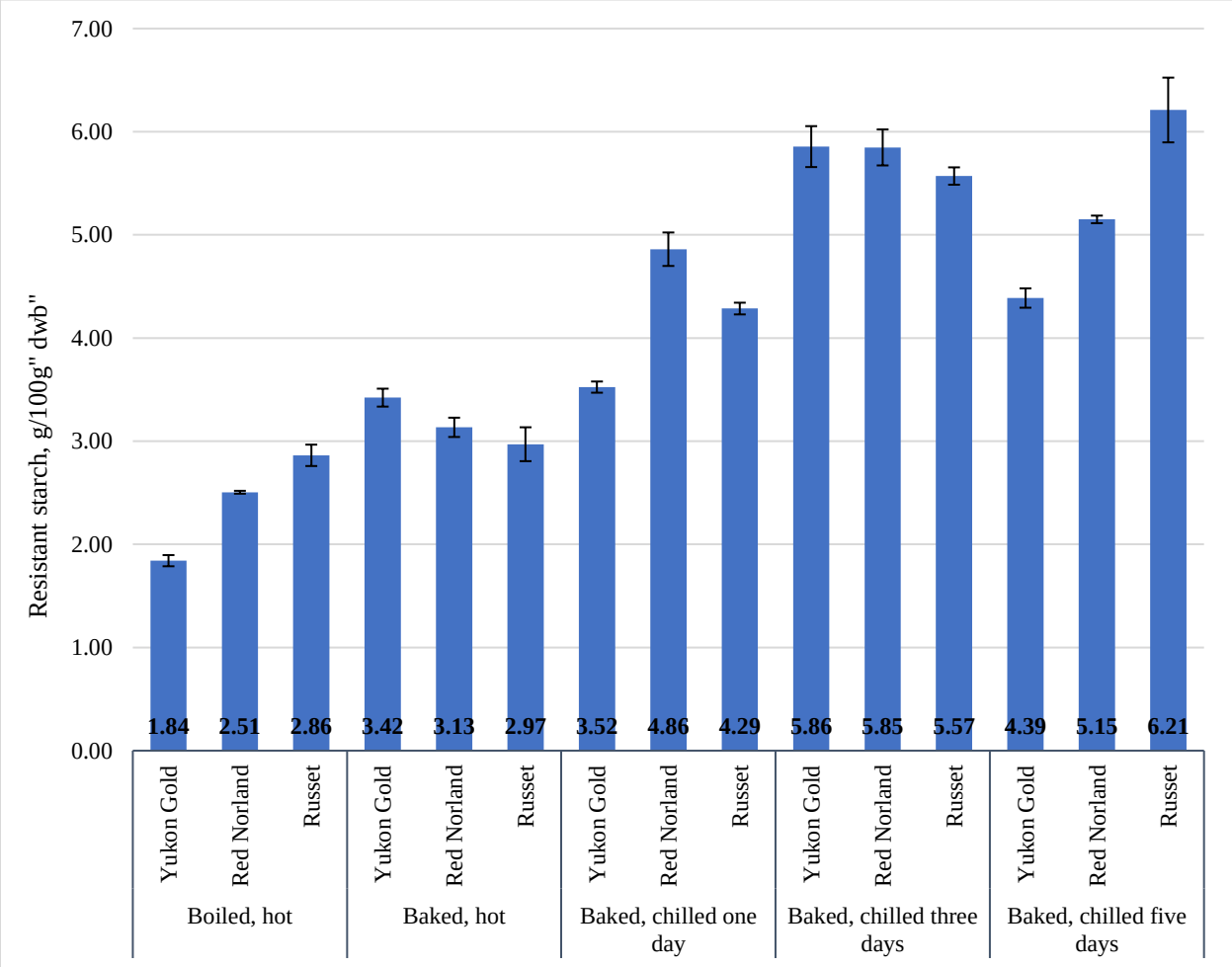
Repeated measures ANOVA determined differences in VAS scores for each potato intervention at each time point. Statistical analysis was performed using IBM SPSS version 25 software with significance set at $p < 0.05$.

CHAPTER IV

RESULTS

Aim 1 Results

The RS content across all samples are presented in Figure 1. Russet potatoes that were baked, chilled for five days contained the most RS (6.21 g/100 g). The lowest amount of RS was found in boiled, hot Yukon Gold potatoes (1.84/100 g).



†Based on dry weight

Figure 1. RS Content (Mean ± SD) of Each Potato Type and Cooking and Storage Method.

Cooking Method on RS

A significant effect of cooking method was found to influence RS content ($p < 0.001$). When looking at the mean RS values across cooking methods (see Table 1), baked and chilled for three day potatoes had the most RS overall (5.76 g /100 g), baked and chilled for five day has the second most RS (5.25 g/100 g), and boiled, hot potatoes had the least RS (2.40 g/100g). Table 2 shows pairwise comparisons between each cooking method. There was no significant difference between baked, hot potatoes and boiled, hot potatoes. Baked, chilled for one day potatoes has significantly greater RS than boiled, hot by 1.05 g ($p = 0.04$) and baked, hot potatoes by 1.82 g ($p = 0.00$). There was no significant different between baked and chilled for three day or five day potatoes ($p = 0.28$).

Table 1. Mean RS Values Across Cooking Methods

Cooking Method	Mean ± SD (g/100g) †
Boiled, hot	2.40±0.52
Baked, hot	3.17±0.23
Baked, chilled for one day	4.22±0.67
Baked, chilled for three days	5.76±0.16
Baked, chilled for five days	5.25±0.92

†Based on dry weight

Table 2. Pairwise Comparisons Between Cooking Methods

A	B	Mean difference, (A-B) †	Significance
Boiled, hot	Baked, hot	-0.77	0.12
	Baked, chilled one day	-1.82*	0.00
	Baked, chilled three days	-3.36*	0.00
	Baked, chilled five days	-2.85*	0.00
Baked, hot	Boiled, hot	0.77	0.12
	Baked, chilled one day	-1.05*	0.04
	Baked, chilled three days	-2.58*	0.00
	Baked, chilled five days	-2.07*	0.00
Baked, chilled one day	Boiled, hot	1.05*	0.04
	Baked, hot	1.82*	0.00
	Baked, chilled three days	-1.53*	0.01
	Baked, chilled five days	-1.03*	0.05
Baked, chilled three days	Boiled, hot	2.58*	0.00
	Baked, hot	3.36*	0.00
	Baked, chilled one day	1.53*	0.01
	Baked, chilled five days	0.51	0.28
Baked, chilled five days	Boiled, hot	2.07*	0.00
	Baked, hot	2.85*	0.00
	Baked, chilled one day	1.03*	0.05
	Baked, chilled three days	-0.51	0.28

†Based on dry weight

*Indicates significance at $p < 0.05$

Potato Variety on RS

Table 3 shows the mean RS values for each potato variety. Although Russet potatoes had higher mean RS content (4.38 g/100g) compared with the other varieties, there was no significant effect of potatoes variety on RS content ($p = 0.247$).

Table 3. Mean RS Values Across Potato Varieties

	Mean ± standard deviation, (g/100g) †
Yukon Gold	3.81±1.47
Red Norland	4.30±1.42
Russet	4.38±1.51

†Based on dry weight

Aim 2 Results

Subjects

Figure 2 shows enrollment, completion, and analysis of the study. A total of 35 participants provided consent and were randomized for the intervention on the first visit, with five participants discontinuing the study. A total of 30 women were included in the analysis. Subject characteristics can be found on Table 4.

Table 4. Participant Characteristics

(n = 30)	Mean ± Standard deviation
Age	29.60 ± 6.03
Weight (kg)	62.27 ± 11.82
BMI (kg/m^2)	32.83 ± 3.65

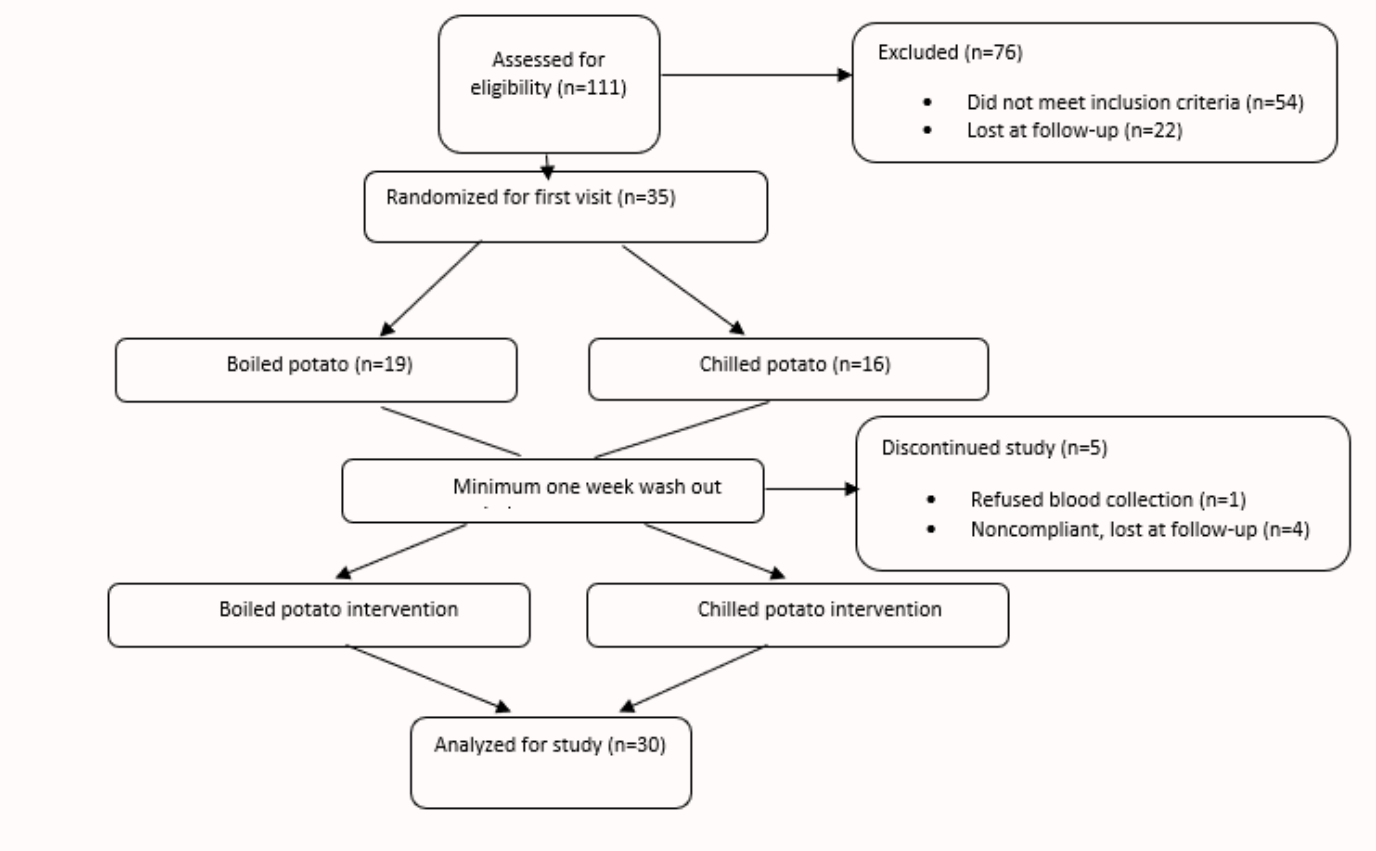
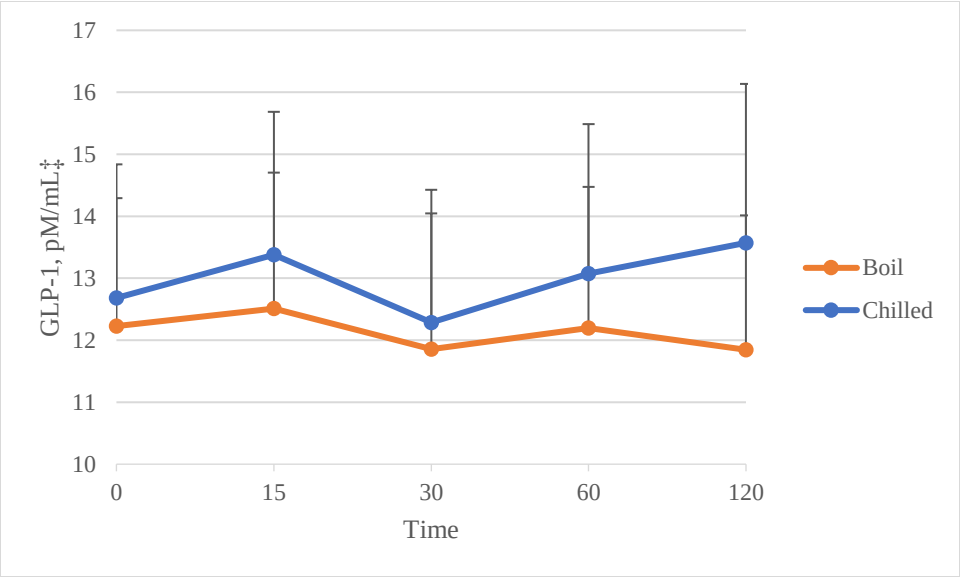


Figure 2. CONSORT Diagram

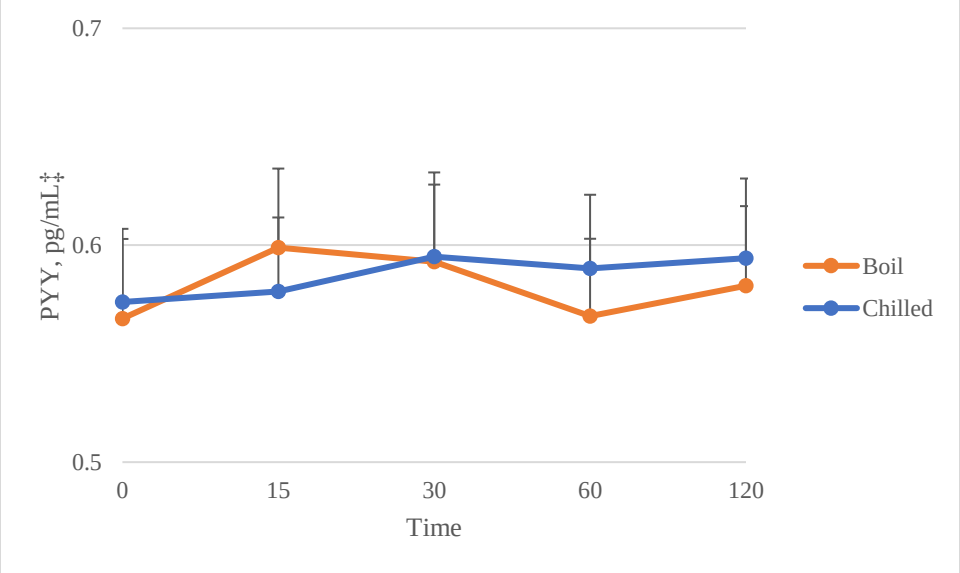
Biomarker Response

GLP-1 and PYY response from fasting (time 0) to 120 minutes post-prandial are shown in Figures 3 and 4. Mean $AUC_{(0\text{-}120\ min)}$ for GLP-1 and PYY are shown in Figures 5 and 6. There was no significant difference in $AUC_{(0\text{-}120\ min)}$ GLP after consuming boiled and chilled Russet potatoes ($p = 0.740$). There was also no significant difference between $AUC_{(0\text{-}120\ min)}$ PYY after consuming boiled and chilled Russet potatoes ($p = 0.296$).



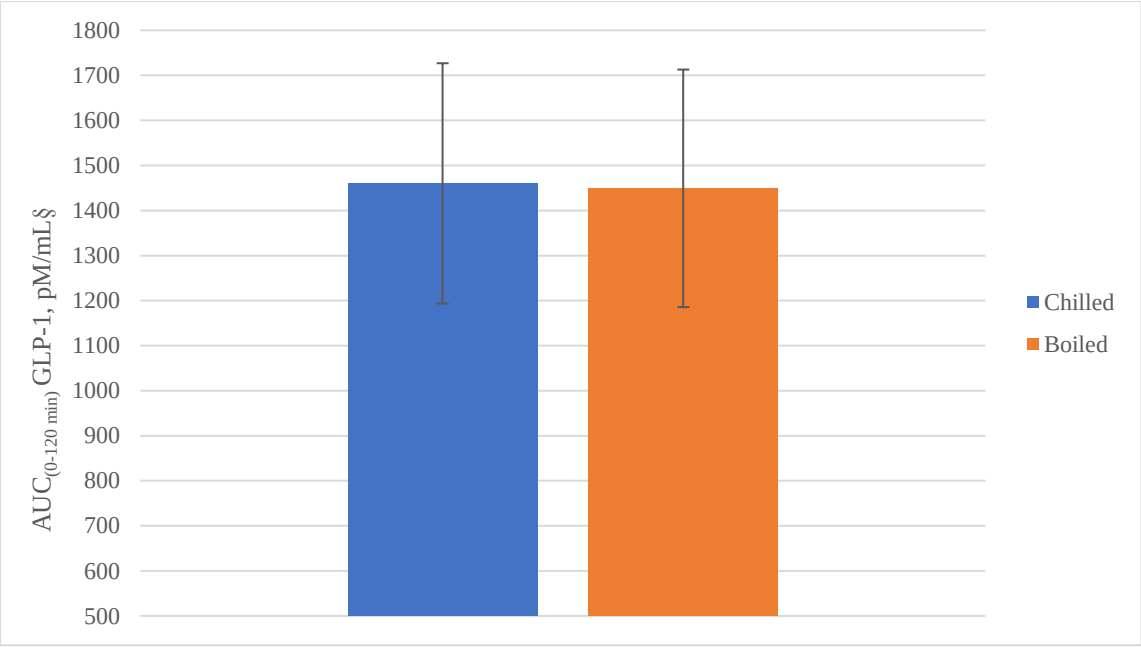
‡Standard error (SE) shown in black bars

Figure 3. GLP-1 Response from Fasting to 120 Minutes Post-Prandial of Boiled and Chilled Russet Potato Interventions



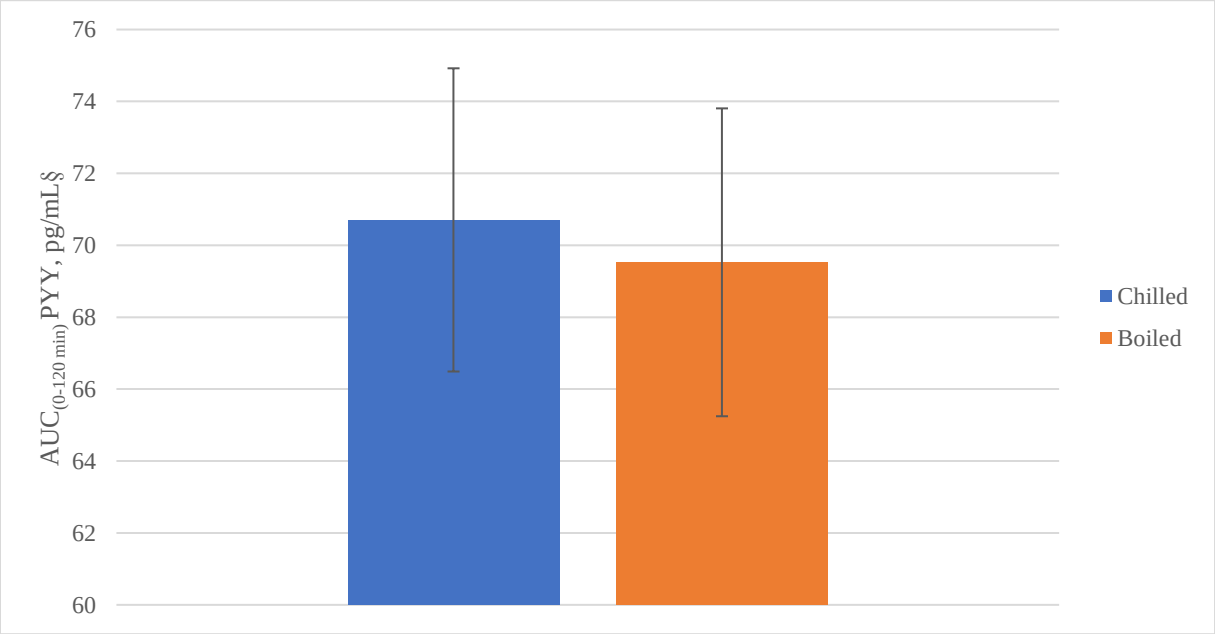
‡SE shown in black bars

Figure 4. PYY Response from Fasting to 120 Post-Prandial of Boiled and Chilled Russet Potato Interventions.



§SE bars shown

Figure 5. Mean $AUC_{(0\text{-}120\ min)}$ GLP-1 Values by Each Potato Intervention.



§SE bars shown

Figure 6. Mean ± SE $AUC_{(0\text{-}120\ Min)}$ PYY Values by Potato Intervention.

Subjective Satiety

There was no difference in mean total VAS scores at either time point between boiled or chilled Russet potatoes. The scores of VAS Question 1, “How hungry do you feel?”, and VAS Question 4, “How much do you think you can eat?”, were higher at 60 minutes compared to 15 minutes regardless of potato intervention ($p = 0.003$). When looking at each intervention, chilled potatoes had significantly higher VAS one and four scores at 60 minutes than at 15 minutes ($p = 0.026$). Based on these findings, increased hunger was observed at 60 minutes for both interventions but was significantly greater after consuming chilled potatoes. VAS question five “How pleasant would you find eating another mouthful of this food?” scores were significantly higher at 60 minutes than 15 minutes after consuming boiled potatoes ($p = 0.031$), suggesting taking another bite of boiled potatoes to be pleasant.

CHAPTER V

DISCUSSION AND CONCLUSION

This study was divided into two aims. The first aim was to examine the influence of common potato varieties and cooking methods on RS content. The second aim utilized data from the first aim for a human interventional trial that examined the impact of cooking methods on one of the varieties on satiety biomarkers and subjective satiety. Although some cooking methods and potato varieties have been previously analyzed for RS content, this study was novel in analyzing the whole cooked potato at multiple storage time points of retrograded potatoes across easily accessible potatoes.

In the present study, five different cooking methods were examined: boiled used hot, baked used warm, baked and chilled for one day, baked and chilled for three days, and baked and chilled for five days. Russet potatoes baked, chilled for five day had the greatest RS content at 6.21 g/100g. A comparable study by Raatz et al.[18] also analyzed RS content from common potato varieties and cooking methods of boiling and baking but each under three different temperatures: hot, chilled for six days, and reheated after chilled for six days. Raatz et al.[18] found approximately 4.7 g/100g in Russet potatoes that were baked and chilled for six days, more than one g less with one extra storage day than the current study. Cooking method was significantly impactful on RS content, with baking yielding higher RS content among all varieties when compared with boiled potatoes. Baking increases RS content more than boiling because of a dry heat treatment. An aqueous cooking environment, like boiling, increases the solubility of the

starch granule but dry heat cooking allows for the starch structure to maintain integrity and remain intact, which offers more resistance to enzymatic hydrolysis.[70] In addition, starch breaks down and escapes in the boiling water from potatoes prepared and cooked as cut pieces versus cooking of unpeeled, uncut potatoes. When considering retrogradation duration, RS content was found to increase across each chilled time point. Exceptions were seen in both Red Norland and Yukon Gold potatoes where RS values were higher at three days chilled than five days chilled. This may be due to characteristics of the potato variety or eventual starch degradation due to extended storage periods.

Russet potatoes contained the highest overall RS content across all cooking methods. Potato variety did not show significant effect on RS content, which was the same consensus found by Raatz et al.[18] This could be due to usage of common potato varieties grown for the average consumer. Potato varieties that have been selectively bred for higher amylose content exhibits greater RS content when cooked and subsequently cooled compared to its parent potato.[70] High amylose potatoes are specialized products not offered or advertised for consumer purchase, which reduces the practicability of the implementation of using potatoes as a source of RS outside of a research setting.

The human intervention portion of the study utilized the Russet potato under two cooking methods and serving temperatures for a cross-over intervention. Two-hundred fifty grams of boiled consumed hot Russet potatoes and baked and chilled for five days consumed cold Russet potatoes were used to analyze impacts on subjective satiety and satiety biomarkers. No differences in $AUC_{(0\text{-}120\ min)}$ for GLP-1 or PYY were shown between the two interventions. This could be due to the amount of RS provided with each intervention, along with a single

intentional exposure of each intervention to assess the response. The current literature of the effect of RS on GLP-1 and PYY in humans is mixed, despite utilizing high RS interventions. One study using 30 g high amylose RS-2 in muffins consumed daily for six weeks by healthy, overweight adults showed higher PYY response following consumption and improved fasting PYY. [71] A recent study using 45 g of high amylose RS-2 mixed in with yogurts and meals daily for 12 weeks found no effect on not only GLP-1 and PYY, but also no effect on subjective satiety in adults with prediabetes.[53] The present study utilized approximately 2.9 g and 6.2 g RS from boiled and baked then chilled for five day potatoes, respectively, which is considerably lower than other interventions and cross-sectional study design with a single exposure of each potato intervention while other trials analyzed daily RS intake over a longer period of time, which may explain the lack of significant response of these satiety biomarkers. Another consideration may be due to limited of two-hour post-prandial window in the study. Metabolism of RS to produce SCFA has shown to take five to seven hours after consumption.[72,73] Due to the post-prandial timeframe, SCFA would not be produced soon enough to promote secretion of GLP-1 and PYY after only two hours.

In addition to the satiety hormone measurements, subject satiety was measured using a VAS. A previously mentioned study of supplementing high RS-2 intake did not impact subjective satiety. Potatoes have been shown to be increase subjective satiety compared with other carbohydrate containing foods. [74] When comparing various potato products, boiled potatoes have been shown to induce higher subjective satiety compared to French fries of equal caloric value, but not with mashed potatoes.[8] The current study found that increased subjective hunger was actually higher at 60 minutes in chilled potatoes, and less palatable than boiled

potatoes. This could be due to perception of unfamiliar preparation practices, as chilled potatoes are not as commonly consumed, and differences in sensory characteristics. Influences of food odor, color, temperature, and texture have been shown to influence reward neuronal pathways, which may impact food intake and perception of food. [75] These sensory characteristics of chilled potatoes may have less impact on the reward center of the brain, increasing hunger perceptions in response.

This study has several strengths. A commonly consumed food staple was analyzed for RS content. Common preparation methods also were used to examine the influences of RS on satiety in a human trial. Most human studies with RS interventions have utilized RS delivered as supplements or as an additive to foods. In contrast, common potato varieties and cooking and serving temperatures were examined, which offers a practical application that may be used by most individuals.

There were limitations of this study. Due to the small sample size in Aim 1, an interaction effect of cooking method/serving temperature and potato variety on RS content could not be determined. Boiled potatoes were not subjected to the same chilling protocols as the baked potatoes, so retrogradation process of boiled potatoes was not studied. The post-prandial timeframe for blood collection in the human intervention trial was insufficient to allow for potential SCFA production to influence GLP-1 and PYY levels.

In conclusion, cooking methods, storage time, and storage temperature are influential in modulating RS content among commonly consumed potato varieties. Russet potatoes baked and chilled for five days had a higher concentration of RS compared with other potato varieties and storage times. When comparing impact on postprandial subjective satiety and satiety biomarkers

with boiled Russet potatoes, no differences in $AUC_{(0\text{-}120\ min)}$ for GLP or PYY were found. However, chilled Russet potatoes increased hunger and was less palatable than boiled Russet potatoes. While baked and chilled potatoes have higher RS, the impact on biomarkers could not be found within the post-prandial time and have lower palatability impart lower reward response from its sensory characteristics. Future studies analyzing other cooking methods and serving temperatures on potatoes are needed to develop further understanding on their impact on RS content. In addition, future human intervention trials should consider extended feeding trials of these potato preparations to demonstrate the impact of consistent RS from potato intake on satiety. Findings could contribute to the scientific understanding of RS and assist public health educators and Registered Dietitians in development of nutrition education of the nutritional benefits of potatoes to patients and clients.

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APPENDIX A

Visual Analogue Scale

Visual Analogue Scale Questionnaire - Potato Study

Participant ID _____ Date _____

Time _____

Instructions: Draw a line indicating how you feel at the moment regarding hunger and satiety.

How hungry do you feel?

I am not hungry at all _____ I have never been more hungry

How satisfied do you feel?

I am completely empty _____ I cannot eat another bite

How full do you feel?

Not at all full _____ Totally full

How much do you think you can eat?

Nothing at all _____ A lot

How pleasant would you find eating another mouthful of this food?

Very unpleasant _____ Very pleasant

Would you like to eat something sweet?

Yes, very much _____ No, not at all

Would you like to eat something fatty?

Yes, very much _____ No, not at all

Would you like to eat something savoury?

Yes, very much _____ No, not at all

APPENDIX B

PYY Elisa Analysis

PYY Elisa analysis began with 0.3 mL of prepared wash solution applied to each well of microtiter plate three times and inverted onto absorbent surface. 25 µL of buffer solution were placed, followed by 50 µL of sample then 25 µL of labeled antigen. Covered plate was incubated at 4ºC overnight for 16-18 hours. After incubation, plate remained at room temperature for approximately 40 minutes. Each well was washed four times with 0.3 mL of wash solution and inverted on an absorbent surface. 100 µL of SA-HRP solution was pipetted into each well. Covered plate was incubated at room temperature for 2 hours on plate shaker at 100 rpm. Each well was washed again four times and inverted, followed by addition of 100 µL of enzyme substrate solution and re-covered to incubate at room temperature in a dark place. After final incubation, addition of 100 µL of stop solution to each well discontinued color reaction. Absorbance was measured at 450 nm.

APPENDIX C

Active GLP-1 Elisa Analysis

Active GLP-1 Elisa analysis began with adding 100 μL of standard, control, and sample into designated wells. 100 μL of GLP-1 antibody mixture was pipetted into each well. Covered plate was incubated for 20-24 hours at 2-8°C. Contents were decanted and washed five times with wash buffer solution and inverted on absorbent surface. 200 μL of ELISA HRP substrate was pipetted into each well and covered with aluminum foil to incubate at room temperature for 20 minutes. 50 μL of ELISA stop solution was added and mixed to each well. Absorbance was measured at 450-620 nm with microplate reader.