Optimizing the Creation of a Protein Beer

A Major Qualifying Project Report
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With microbreweries becoming increasingly popular, the need to develop new and innovative beverages has become essential to standing out among the competition. Our project was designed to help Purgatory Brewing Company determine the feasibility of infusing protein into a craft beer, in an attempt to develop a new product. Our team explored how whey protein, leucine and high-protein ingredients influence the protein content and integrity of a beer in regards to overall drinkability when added at different times throughout the brewing process. We quantified protein concentration in our beers via a Bradford Protein Assay and were able to identify an increase in protein in a 12 oz beer. Further research into protein detection is recommended as next steps towards achieving this goal.

This MQP contains information deemed confidential to the business interest of the industrial sponsor. Please contact Stephen Kmiotek at sjkmiotek@wpi.edu for additional information.

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1 - Introduction

Behind water and tea, beer exists as the third most consumed drink in the world (Nelson, 2004). Since beer arrived in North America, brewing has grown into a multi-billion dollar industry, including companies such as Budweiser and Miller-Coors. Although industrial brewing booms, craft brewers have shown concern about the loss of traditional beer brewing, and hence the past few decades have largely been focused on bringing back the innovative side of brewing. Since the first brewing methods were developed in Babylonia around 5000 BC, additions of simple sugars and hops, as well as utilizing new techniques, revolutionized the world of craft brewing.

Throughout craft brewing history, a variety of alterations were made to improve the growth of this business. Changes to the process lead to the creation of different beers, such as ales and lagers. Furthermore, flavor additives were tested to determine how different sugars, hops, and additives improved taste profiles for customers; companies continue to test and improve their recipes. Despite rapid innovation over the past couple decades, new ideas are constantly being generated and tested. For example, adult beverages that are “clearer” are being created so that suspended particles are not in the drink to diminish the taste.

A common nonalcoholic beverage that is also gaining popularity in America today is a protein shake. The scope of infusing protein into an alcoholic drink has been tested but has mostly produced unfruitful results. With a steadily growing population that consumes both alcoholic beverages and high protein drinks individually, our group decided to take this opportunity to perform our own tests to determine if it were feasible to infuse a high amount of protein into beer. Brian Distefano and Kevin Mulvehill, our sponsors at Purgatory Beer Company, expressed interest in researching this topic, looking to see if they can add it to their arsenal of innovative drinks.

The adult beverage industry does not have published methods that have been tested, so starting from scratch, our group devised various ways to infuse beer with protein, including but not limited to protein isolates and high protein foods. Our group created a control brew to standardize the steps in the brewing process and we altered it as we saw fit throughout the brewing process. Once brewing was complete, a qualitative analysis was performed to determine taste, bitterness, and other factors that were predetermined to be important to taste. Protein content was determined by creating a Beer’s Law plot from protein standards. These were used in tandem with a Bradford Assay protocol to determine the amount of protein in our brewed beers; these could then be compared to each other, as well as commercial drinks. This project is a stepping stone to determine if a protein beer could be introduced into the market in the near future, or if additional research needs to be performed to engineer this type of beverage.

2 - Background

In recent years, the craft brewing industry has seen a boom in popularity as more local breweries are popping up around the United States and the world. Beer is produced through a fermentation process in which sugar is broken down by yeast or other microorganisms to form ethanol and byproduct carbon dioxide, as seen in Figure 1 below.

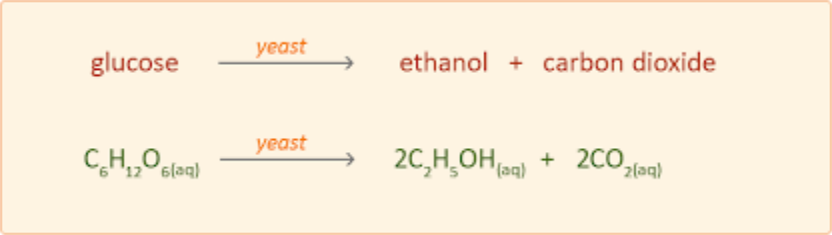


Figure 1: *General fermentation of sugar into alcohol and carbon dioxide using yeast.*

It is hypothesized that early brewing began in Babylonia around 5000 years ago. Early brewing started by fermenting fruits or wild grain, but was drastically changed when plain sugar could be utilized instead and the process became more widely investigated. Brewing remained largely unchanged until the 1100s when European nations began implementing what are called hops. As European nations imperialized overseas lands, colonies began setting up breweries and began manufacturing alcohol, mainly beer. Throughout the industrial revolution, the industry continued to prosper. Although periods such as Prohibition in the 1920s banned the manufacturing, sale, and transportation of alcohol, the craft has since been revitalized (*The oxford companion*). Since beer is one of the oldest alcoholic drinks in history, it is important to understand the process and scope of this industry that has people hooked from around the world.

2.1 Micro-brewing and Purgatory

Behind water and tea, beer exists as the third most consumed drink in the world (Nelson, 2004). Since beer arrived in North America, brewing has grown into a multi-billion dollar industry, including companies such as Budweiser and Miller-Coors. Although industrial brewing booms, craft brewers have shown concern about the loss of traditional beer brewing, and hence the past few decades have largely been focused on bringing back the innovative side of brewing. These craft brewers are typically independently owned and generally produce much smaller quantities of beer compared to the industrial breweries.

In 2016, Brian Distefano and Kevin Mulvehill started a microbrewery in Whitinsville, Massachusetts called Purgatory Beer Company. Over the past several years, they have seen immense growth and improvement in their business. Additionally, many projects have been performed to aid them in the growth of their brewing passion. As they still desire to create innovative adult beverages, we hope to help Purgatory Beer Company make a new drink for the craft brewing industry.

2.2 The Brewing Process

Beer can be broken down into two main categories: ales and lagers. Ales are fermented with top-fermenting yeast at warmer temperatures, while lagers are fermented with bottom-fermenting yeast at cooler temperatures. Although there are many variations of beer in each category, the general brewing process stays relatively consistent. The four main process ingredients are malts, water, hops, and yeast. The overall process can be lumped into 5 steps: mashing, boiling, cooling, fermenting, and packaging. A flow diagram in Figure 2 below shows the entire brewing process in more detail.

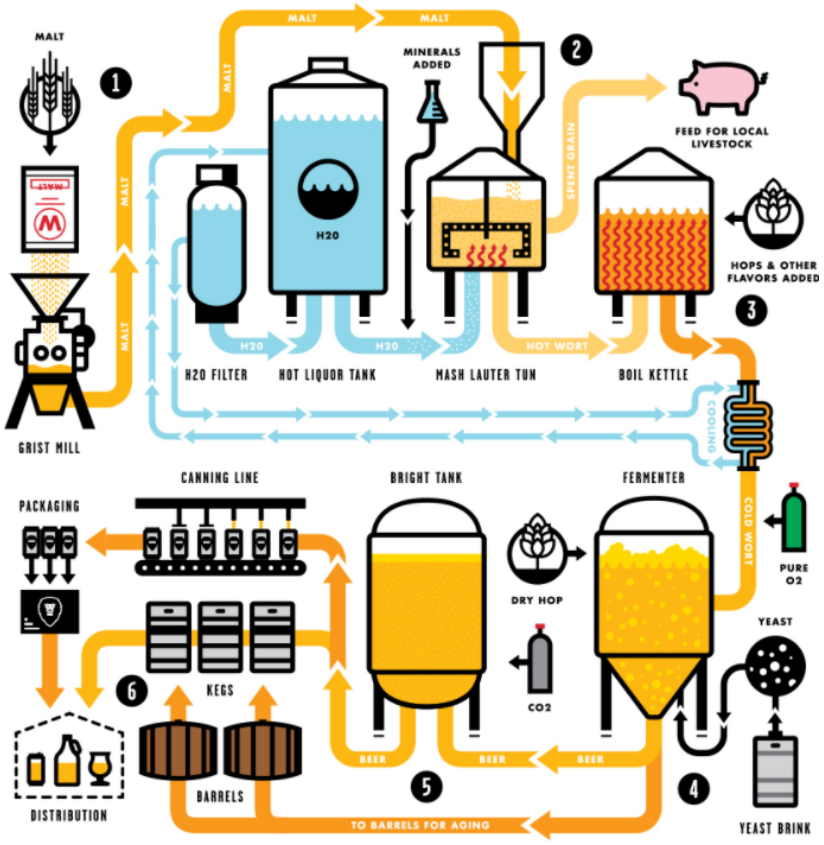


Figure 2: *Flow diagram of the Brewing Process.* (Trosset, 2018)

2.2.1 An Overview of Brewing

The brewing process starts with milling the malt, which is typically a grain. The malt is steeped in warm water, which extracts the sugars that will be used for fermentation. The creation of this sugar-liquid, called "wort," is instrumental in what type of beer will be produced. Since all malts have different enzymes, process conditions must be altered in the brewing process to ensure they are not damaged in the extraction process. Once the sugars and enzymes are separated from the malt, the wort is sent to the boil kettle. As indicated in Figure 2 above, the byproduct grain that has been steeped can be fed to livestock.

In this stage of the process, the wort is boiled, essentially pasteurizing it. At this point, hops are added to the wort, which changes the bitterness, flavoring, and aroma. Hops can be added at different times throughout the boiling process. Adding hops at the beginning mainly affects the bitterness of the beer because the longer hops are boiled, the more bitter the final taste will be. If added at the end, hops will contribute more to the flavor rather than the bitterness (Trosset, 2018). Once boiling occurs for between one to two hours, the wort is cooled and moved to the fermenter.

Fermentation is the most time-consuming part of the brewing process. Yeast is used in the fermentation process to catalyze the reaction of sugars into ethanol, as seen in Figure 1. Yeast is a living organism, and therefore it can only survive in certain conditions. The wort must be cooled to around 70 degrees Fahrenheit before addition of yeast, but depending on the type of beer, this temperature can vary slightly. The yeast is typically left in the fermentor for six to seven days, or until the yeast consumes all of the fermentable sugars in the wort. Dry hops can be added later in the fermentation step, which adds additional flavors that may have been dampened when the yeast was introduced to the wort. Once the yeast converts the sugars to ethanol and carbon dioxide, they eventually die. At this point, the beer can be filtered and moved to the bright tank, where it can be carbonated. Once carbonated, the final step is packaging in bottles or kegs.

Figure 2 shows fermentation occurring in one primary reactor; however, some breweries use a binary system, where a second fermentation tank allows the beer to ferment for an additional week or two. Overall, the fermentation process takes around two to three weeks depending on the fermentation process.

2.2.2 Stouts and Porters

Stouts and porters are subcategories of ales which tend to be very popular in Great Britain and Ireland. These beers are known for being dark in color and typically taste roasty, bitter, and have a creamy texture. Stouts and porters are commonly brewed with small amounts of hops and large amounts or dark, rich malts. Traditionally, the term stout was used to describe a porter with an alcohol content above 7%; however, today the two terms are typically used interchangeably. Some of the most common types of stouts are oatmeal stouts, milk stouts, imperial stouts, and Irish dry stouts (Encyclopædia Britannica).

Oatmeal stouts rose to popularity in the late 19th century in Scotland. These beers are brewed with an oat malt and boast a, “substantial head of thick, creamy, long-lasting foam, usually tan to light brown in color.” The oats used in the brewing process give the beer an earthy, nutty flavor which is commonly paired with mild notes of coffee and medium hop bitterness and flavor. Oatmeal stouts tend to give a smooth and velvety mouthfeel with a medium-full body (Pattinson, 2016).

Milk stouts, also known as sweet stouts, are brewed with lactose from milk. Rising to popularity in England in the early 20th century, the milk stout is dark brown or black in color with a creamy, tan head. This beer typically exhibits a roasted, malt flavor with hints of

sweetness, coffee or chocolate. Hops and bitterness are commonly present at a medium level and are accompanied by a sweet, balanced, and dry finish (Carr, 2017).

The imperial stout was created for a Russian Czar who fell in love with stouts on a trip to England. The czar requested for the creation of a stout that could withstand the trip back to Russia with him, and so brewers developed a stout with large amounts of hops and alcohol. Today, imperial stouts typically fall within the 8.0-12.0% alcohol range and appear dark brown or black with a dark mocha head. These beers are known for their intense, complex, and rich flavors of roastiness, malt tones, hoppiness & fruity esters (Carr, 2015).

The Irish dry stout was made famous by Arthur Guinness who began using high roasted malts in his beers to create coffee notes. The beer's appearance can range from almost jet black to a deep rich brown with an alcohol by volume (ABV) range of 4.0-5.0%. Guinness, as well as other Irish dry stouts, have maintained their high popularity for their smooth and creamy mouthfeel. Irish dry stouts are known for their roasty flavor profile, but may also contain hints of bittersweet chocolate and medium to high hop bitterness (Carr, 2014).

2.2.3 Anatomy of a Stout

As is common with most other types of beers, stouts are composed of the classic four ingredients: malted barley, water, hops, and yeast. The malted barley is primarily responsible for the dark brown or black color that is distinctive of a stout. Most stouts have small amounts of hop aroma and typically use hop pellets in the brewing process instead of the hop flower.

British or American pale malt makes up 60-80% of the grain bill for an oatmeal stout. Oats typically range from 3-12% of the bill, but in some cases they can comprise up to 30%. An increased amount of oats (30% or higher) typically creates a bitterness and astringency in the finished beer. Baking or toasting the oats will help bring out more flavor and increase their contribution to the finished product. It is important to note that brewing with oats will require a piece of equipment called a mash (Pattinson, 2016).

In a milk stout, about 60-80% of the grain bill will be composed of British pale malt. Common specialty malts include about 10-15% caramel/crystal malts and another 10% dark malts such as chocolate, British black malt, and roasted barley. The lactose that gives milk stouts their name can be added at various different times throughout the process. Brewers have experimented with adding lactose before the boil, after the boil, during fermentation, and just before packaging. If lactose is present during the boil, it will be more sterilized and dissolve easier; however, adding before packaging allows for the brewer to taste the final product and add the perfect amount of lactose (Carr, 2017).

The key to brewing a good imperial stout is maintaining an abundant grain to gallon ratio of about 3.5 lbs to every 1 gallon. The typical base malt for an imperial stout is a high quality 2-row base malt. Complex, dark malts such as roasted grains should make up at least 20% of the bill to bolster the flavors of chocolate, coffee, and roast. Flaked oats or specialty malts can also be added to deepen the flavor profile of the beer. The imperial stout is very accepting of experimentation since the idea is to have a big and bold flavor (Carr, 2015).

The “dry” in Irish dry stout means the beer is brewed with less unfermentable sugars. This means that the Irish dry stout favors one or two-molecule sugars which can be easily broken down and fermented into alcohol. The base malt for this type of stout should be a high quality pale ale malt. Chocolate, high roast, and black patent are commonly found in these types of beers and are often responsible for the dark, roasty qualities. Unmalted adjuncts such as corn, rice, or barely often make up 20-30% of the mash (Carr, 2014).

2.2.4 Basic Stout Recipe

The following is a basic home brewing recipe for an Irish stout. This recipe will be adjusted to use the ingredients that are available to our group and scaled to the desired batch size. This recipe will be used as our control and will be adjusted accordingly to allow for the addition of protein. If possible, the malt/grain bill will be substituted for a liquid malt extract depending on what is available at the local homebrewing store:

~ **O’Davey Stout** ~

All Grain:

- Batch size: 5 gallons
- Original Gravity: 1.045
- Final Gravity: 1.011
- IBUs: 43
- ABV: 4.4%

Malt / Grain Bill:

- 6 lb pale malt
- 2 lb flaked barley
- 1 lb roasted barley

Hops Schedule:

- 2 oz Willamette [6% AA] at 60 minutes

Yeast:

- Danstar Nottingham dry yeast

Directions:

- Mash for 60 minutes at 152°F
- Boil for 60 minutes following the hops schedule
- Ferment 10 days at 64°F
- Keg or bottle

(Carpenter, 2016)

2.3 Protein

Proteins are defined as large complex macromolecules made up of long chains of amino acids linked via peptide bonds (Haurowitz, n.d). The thousands of proteins that exist, differ in terms of the sequence of amino acids in their chains, which are dictated by the sequence of nucleotides in the genes that are contained in their genetic code (Haurowitz). These alpha-amino sequences determine each protein's unique structure and function in an organism, which is specific to each and every organism. Although there are more than 100 amino acids that occur in nature, only a set of 20 amino-acids, commonly known as the "building blocks", combine in different configurations to form proteins (*New World Encyclopedia* & *Interactive Nutrition Facts Label, 2020*). The free amino acids, di-peptides and tripeptides that constitute proteins are essential to the growth, development and overall health of all organisms. These "ingredients" are digested in an organism's gastrointestinal tract and used for the resynthesis of proteins in cells (Wu, 2016).

The name "protein," coined by Swedish chemist Jons Jacob Berzelius in 1838 who contributed to the discovery of these molecules, originated from the Greek word "proteios" meaning "primary" or "holding first place" (*New World Encyclopedia, n.d*). Time has proven this to be an appropriate name, considering that in nutrition protein has been found to be the "most fundamental component of tissues in animals and humans" (Wu, 2016). Proteins, derived from the resynthesis of amino acids, are found essentially everywhere in all living organisms - muscles, tissues, bones, skin, hair, etc (Haurowitz). They play vital roles in many bodily processes that contribute to the overall structure and function of all living cells. There are many different types of proteins that perform a vast array of functions within organisms. For example, antibody proteins respond to foregin invaders such as viruses to protect organisms; proteins known as enzymes carry out DNA replication and many other biochemical reactions; messenger proteins receive, process and transmit signals via cells; structural proteins give cells the support they need to strengthen and develop; and other proteins aid in intracellular transport, which helps move molecules such as oxygen throughout an organism (*MedlinePlus Genetics* & *New World Encyclopedia*). Many other proteins exist to achieve an assortment of other functions, however, these proteins are the most essential to an organism's survival, as they fuel the creation and maintenance of cells, powering the growth and development of an organism (*MedlinePlus Genetics*).

Although some amino acids can be produced by your body to create proteins that promote healthy development, nine amino acids (the essential amino acids) that are considered "nutritionally indispensable" for normal functioning, can't be produced by an organism (Watford & Wu, 2018). This is due to the fact that an animal's cells (including humans) are unable to synthesize their carbon skeletons. As a result, dietary protein is crucial for the survival of animals (*New World Encyclopedia*). As Nancy Waldeck (chef and dietitian at Thomas F. Chapman Family Cancer Wellness at Piedmont) states, "it is important for individuals to consume protein every day…[it] should be part of [their] daily health maintenance plan" (*Piedmont*). Animals (including humans) rely on protein rich foods to obtain the amount of

leucine, valine, isoleucine, histidine, lysine, methionine, threonine, tryptophan, and phenylalanine, needed to meet the requirements of the Recommended Dietary Allowance (RDA) (*New World Encyclopedia*).

According to Wu, in the *Journal of Food and Function* in "Dietary Protein Intake and Human Health", studies based on the meta-analysis of short-term N-balance in humans have shown that a healthy adult participating in minimal physical activity requires 0.8 g of protein per kg body weight (BW) per day to meet the requirements of the Recommended Dietary Allowance (RDA) (Wu, 2016). As for individuals participating in moderate and intense physical activity, 1.3 g and 1.6 g of protein per kg of BW per day, respectively, is needed to promote protein synthesis and stimulate growth and development in humans (Wu, 2016). It is important to note that, although this information is reliable, research on how much protein should be consumed to ensure good health remains a controversial topic (Pendick, 2019). For example, according to Nancy Rodriguez - a dietitian and professor of nutritional science at the University of Connecticut in Storrs, absorbing "twice the RDA of protein is 'a safe and good range to aim for'" (Pendick, 2019). Guoyao argues that the quantity of protein, among other things, is most essential in determining its nutritional value (Wu, 2016). This is due to the fact that dietary protein absorbed through food provides no nutritional value to an animal or human unless it is hydrolyzed by proteases and peptidases to produce constituents such as amino-acids, dipeptides, and tripeptides that are digestible in an organism's small intestine (Wu, 2016). Therefore, when too much protein (above safe upper limits) is consumed by an individual, it is not used efficiently by the body, which has the potential to impose "metabolic burden" on one's bones, kidneys, liver and heart ultimately increasing the risk of developing serious disorders over time (Delimaris, 2013). Nevertheless, both of the mentioned researchers emphasized that eating healthier foods containing high quality protein was more important than meeting daily consumption levels (Pendick, 2019 & Wu, 2016).

2.3.1 Sources of Protein

Protein is found in a variety of different food products, at an assortment of different quantities. Both plants and animals provide great sources of protein for the human body, however, in comparison, studies have shown that animal based foods provide more protein and protein that is of higher quality than plant based foods (Watford & Wu, 2018). Some examples of animal products that are rich in protein include but are not limited to meat, seafood, eggs, poultry, and dairy products such as yogurt, milk and cheese. Some plant based foods that contain considerable amounts of protein include legumes (beans, peas and lentils) and nuts/seeds (*Interactive Nutrition Facts Label, 2020)*. Other plant based products that provide protein, in lower amounts, include soy products such as tofu, and whole grains and vegetables. However, few plant based foods provide individuals with the required amounts of all the indispensable amino acids needed to meet the RDA, as a result, plant based foods are considered incomplete proteins (*Interactive Nutrition Facts Label, 2020)*.

2.3.2 Supplemental Whey Protein

While many experts suggest obtaining the bulk of one's required protein allowance from animal and plant based food products, protein supplements can be a great way to help meet or increase the amount of protein in one's diet. For athletes/bodybuilders, supplemental protein has the ability to develop lean muscle, helping to increase strength and physical function, which is essential for athletic performance (Devries & Phillips, 2015). As a dietary supplement, whey protein is commonly used by athletes and bodybuilders to support muscle hypertrophy and stimulate recovery (Devries & Phillips, 2015). Whey protein is one of the two primary proteins found in bovine milk, making up approximately 20% of milk's total protein content. Whey protein is derived from whey, which is the liquid by-product that remains after the casein (the other protein in milk) curds are formed during the production of cheese (*Nutrition Sourced the Right Way*). This liquid is dehydrated and turned into a fine powder where it is sold for mixing into beverages. Three main types of whey protein are marketed; whey protein concentrate (WPC), whey protein hydrolysate (WPH) and whey protein isolate (WPI). Unlike WPC and WPI, which differ solely based on protein content (WPC → 70 - 85% ; WPI → 90 - 95%), WPH is not dictated by its protein content, instead WPH is partially hydrolyzed, which makes it easier to digest than the other two forms (*Nutrition Sourced the Right Way*). In this report, the team focused solely on whey protein isolate.

In comparison to other protein supplements such as casein, soy, and pea protein, whey protein has shown to be superior in terms of its quality and its ability to stimulate muscle protein synthesis - the incorporation of amino acids in skeletal muscle used in muscle recovery and growth (Devries & Phillips, 2015). According to Michaela Devries, the quality of a protein is based on three attributes, its amino acid content, its digestibility, and its bioavailability (Devries & Phillips, 2015). In terms of digestibility, whey protein is more desirable in comparison to the mentioned proteins, due to the fact that it is an acid-soluble. This characteristic allows it to be rapidly digested in the gastrointestinal tract, and its high bioavailability allows it to be rapidly absorbed within the plasma and circulated throughout the body via the bloodstream (Devries & Phillips, 2015). According to Michaela Devries, these attributes play a key role in the stimulation of muscle protein synthesis (Devries & Phillips, 2015). In addition, whey protein is considered to be a complete protein, which means it possesses all of the essential amino acids in adequate amounts (*Interactive Nutrition Facts Label, 2020)*. Furthermore, whey protein is said to contain a high portion of the essential amino acid known as leucine, which aids in muscle growth, tissue repair and the production of growth hormones, is considered to be the most crucial amino acid in the stimulation of muscle protein synthesis (Devries & Phillips, 2015). In comparison to the mentioned protein supplements, which are also complete proteins, whey protein is superior in terms of its amino acid content (with high amounts of essential amino acids, in particular leucine), digestibility, and bioavailability, which contribute to muscle protein synthesis (Devries & Phillips, 2015). These superior characteristics contribute to whey being the highest-quality protein in comparison to those considered for this project. Figure 3 below provides a visual comparison of the protein quality characteristics of the mentioned protein supplements.

	Whey	Casein	Soy	Hydrolyzed collagen
Complete protein?	Yes	Yes	Yes	No
Digestibility	Fast	Slow	Fast	Fast
Amino acid content				
(g/25 g protein)				
Leucine	3.0	2.3	1.5	0.8
∑ EAA	12.4	11.0	9.0	3.8
∑ BCAA	5.6	4.9	3.4	1.4
Splanchnic AA extraction	Low	Low	High	High
PDCAAS	1.0	1.0	1.0	0.0

Figure 3: *Protein quality characteristics associated with the most commonly ingested types of protein.*

2.3.3 BCAA - Leucine

As mentioned, there are nine amino acids that are considered essential to normal function. Of these nine amino acids, three fall into a category known as “Branched-Chain Amino Acids” (BCAAs) due to their chemical structures (Saharan & Awasthi, 2019). BCAAs are vital components in the building and repairing of muscles within the body (Saharan & Awasthi, 2019). Of the three - valine, leucine and isoleucine, leucine is considered to be the most essential to improving muscle mass and aiding in muscle recovery (Devries & Phillips, 2015). Although leucine is best known for its role in strengthening the muscular system, it aids a number of different functions in the body such as the regulation of blood sugar, healing wounds, and the production of growth hormones (Saharan & Awasthi, 2019). Similar to the other essential amino acids, leucine cannot be synthesized by the human body. However, like the other essential amino acids, leucine is abundantly present in many of the high protein foods mentioned in section 2.3.1.

According to Saharan & Awasthi, leucine as a supplement is popular among athletes/bodybuilders to help increase athletic performance (2019). Lecuine’s anabolic properties and anti-catabolic properties are said to help build and strengthen muscles, and preserve existing muscle fibers respectively (Saharan & Awasthi, 2019). When taken while exercising, leucine supports muscle hypertrophy and enhances muscle endurance (Saharan & Awasthi, 2019). In a study that examined the effects of dietary leucine supplementation on exercise performance, results showed that candidates that had supplemented their workout with leucine had significantly more power and endurance performance than those that did not (Saharan & Awasthi, 2019). Its ability to stimulate the secretion of insulin allows for the cells in one's muscles to take up glucose and use it as a source of energy to function (Saharan & Awasthi, 2019). In a different study, leucine was also found to be beneficial to the body when ingested

after exercising. This is due to its ability to stimulate muscle protein synthesis through translation regulation and help skeletal muscle recovery (Saharan & Awasthi, 2019). This amino acid serves as a stimulus to the creation of proteins - the building blocks of muscles (Saharan & Awasthi, 2019).

2.3.4 Protein Solubility

To determine the feasibility of brewing a stout with whey protein, the solubility of protein must be explored. There are many different factors, both extrinsic and intrinsic that affect the solubility of protein. Some intrinsic factors that affect a protein's solubility include its amino acid sequence and composition, the structure of the protein (i.e flexibility), and whether or not the protein's surface is hydrophilic or hydrophobic. The solubility of a protein is also affected by extrinsic factors including pH of the solvent, ionic strength between the protein and the solvent, the interaction of other present molecules, and temperature.

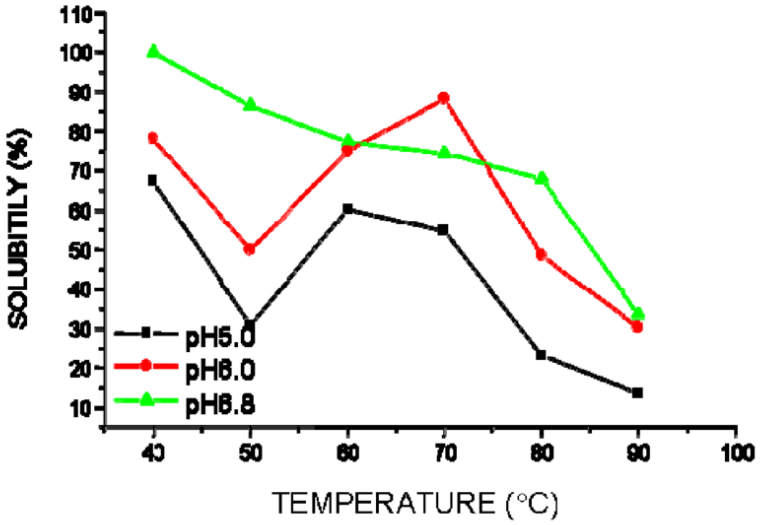


Figure 4: *Effect of pH and temperature on the solubility of whey protein in water.* (Helena Pelegrine Guimarães & Thereza Moraes Gomes Rosa, 2012)

The effects of temperature and pH on the solubility of whey protein were also researched. Figure 4 shows a plot of the % solubility of whey protein at various temperatures and pH levels. According to the figure, the whey was most soluble at the lowest temperature and a pH closest to 7. On the contrary, the trial run at the highest temperature (90ºC) and lowest pH (5.0) showed the lowest % solubility. Through the analysis of this data, it can be concluded that decreasing pH below 7.0 and increasing temperature have a negative effect on the solubility of whey protein (Helena Pelegrine Guimarães & Thereza Moraes Gomes Rosa, 2012).

The presence of alcohol also plays a major role in the solubility of whey protein powder. Since whey is a dairy-based protein powder, a study on the effect of alcohol on the solubility of lactose can provide insight on the solubility of whey. A study by Majd and Nickerson at the

University of California found that in tests of both high and low alcohol concentrations, the solubility of whey decreased as the concentration of alcohol increased. This negative effect on the solubility of lactose may prove to be an obstacle when brewing alcoholic beer with whey protein powder (Majd & Nickerson, 1976).

2.3.5 The Effect of Heat on Protein Denaturing

When brewing beer, many of the ingredients are exposed to high temperatures during the boil. If protein is to be added prior to the boil, the effects of temperature on the denaturing of protein must be considered. In a study conducted by researchers Zhu and Damodaran, whey protein isolate was heated at moderate and high temperatures to investigate heat's effect on the denaturation of whey protein. In this study, a solution of 5% WPI in water was heated to 70ºC and a solution of 9 % WPI in water was heated to 90ºC. High-performance liquid chromatography was used to analyze the protein profile of the mixtures. It was determined that heating 5% WPI to 70ºC denatures the protein in as little as 1 minute; however, the 9% sample heated to 90ºC did not experience significant changes in structure, inferring that heating whey to higher temperatures at higher concentrations helps protect against the denaturing of essential proteins (Damodaran & Zhu, 1994).

2.3.6 Solubility and Denaturation of Leucine

Similar to whey protein, leucine presents solubility challenges in regards to changes in pH. Although no data regarding the relationship between leucine and its solubility in ethanol-water solutions was identified, according to the U.S. National Library of Medicine, only 22.7 g of leucine is soluble in 1 L of water and only 0.72 g of leucine is soluble in a 1 L of a 99% ethanol solution (U.S. National Library of Medicine: *Leucine)*.

However, leucine differs significantly from whey protein in terms of heat's effect on its denaturation. As mentioned, during a brew the ingredients are exposed to high temperatures during the boil. The effects of temperature on denaturation have the ability to alter leucine's structure and negatively affect its functionality within the human body. However, research suggested that leucine will not be affected by the moderate to high temperatures it would be exposed to during the boil. This is due to studies that have proven that it doesn't sublime until 145 ºC and doesn't denature until 293 ºC, which both are well above the temperature of the boil in the brewing process.

2.4 Measuring Properties of an Adult Beverage

Producing adult beverages necessitates a way to record properties, such as alcohol content, haziness, and bitterness. Knowing these properties helps brewers label, recreate, and advertise their beverages for customers. For our project, we also need a way to measure how

much protein is dissolved in our beverage, because even if some dissolves, how much can we claim that it is truly a beer infused with protein?

2.4.1 Calculating Alcohol by Volume

Alcohol content is found by calculating the ABV. A hydrometer can be used to measure the original gravity (before fermentation) and the final gravity (after fermentation) of the beer. Once these values are found, the equation below can be used to find the alcohol percent:

$$\frac{original\ gravity}{final\ gravity} \cdot 131.25 = ABV\%$$

2.4.2 Quantifying Clarity

As a commercial product, the look of a beer also plays a role in selling the product. In general, the color of the beer is important, but in addition to this, the clarity of beer can dictate how well it sells. Also referred to as the haziness or turbidity, haze refers to the instability in beer in which insoluble material appears. This includes remaining yeast, hops, and additives that were used earlier in the process and has yet to become sediment. Clarity is one of the most desired traits by brewers, because removing the unwanted haze largely creates a better tasting beer.For our purposes, clarity was only quantified by what we could see as it was not a primary focus of this project.

2.4.3 Quantifying Protein in Beverages

UV-Vis can be used to distinguish if protein compounds are dissolved in beer through the use of a Bradford Protein Assay. A solution including Coomassie G-250, a substance that functions as a dye, can be used as an indicator for all types of proteins. Simply put, Coomassie G-250 latches onto protein in solution, and as the protein concentration increases, the solution becomes a darker blue. The UV-Vis for the Bradford Assay can be performed at 595 nm to determine the absorbance of each sample, because this analysis produces a solution with a blue hue that absorbs between 595 and 600 nm. A Beer's Law curve can then be created to define the relationship between absorbance and protein concentration. Once this has been done, the same curve can be used to interpolate or extrapolate the concentration of the created sample based on the absorbance recorded by the UV-Vis.

2.4.4 Beer's Law

Beer's Law, properly known as the Beer-Lambert law, has more uses than just how we did for this project. Beer's law is defined by the equation $A = \varepsilon lc$ where A is absorption, ε is the molar extinction coefficient, l is the length light travels and c is the concentration of a given solution. For our purposes we were only focussed on absorption and concentration as the molar extinction coefficient and length remain constant.

3 - Methodology

Our mission, with the guidance of Purgatory Brewing Company, was to determine the best method for infusing the highest amount of protein into a craft stout without compromising the taste. Our team explored three different forms of protein and two methods of infusion to understand how they affected the ability to impart protein into beer. Our team completed the following objectives to achieve the project goal:

1. Created the Bradford reagent composed of Coomassie Blue G-250 in order to identify protein concentration in beer.
2. Using predeveloped protein standards with known concentrations, a Beer's Law Plot was generated to properly calculate sample protein concentrations using a standard curve.
3. Modified different parameters/ingredients in the brewing process to isolate and eliminate problems in order to create a defined recipe for the preparation of our controlled batch.
4. Evaluated the performance of leucine, whey protein, and high-protein ingredients through UV-Vis spectroscopy to determine which ingredient produced a beer with the greatest amount of protein without compromising the taste.

In this chapter, we describe how we carried out these objectives. Figure 1 below shows the project objectives and their respective methods. The green steps represent our objectives, while the gray steps represent the methods to complete each objective and our results. We used the scientific process to complete and refine our brewing processes.

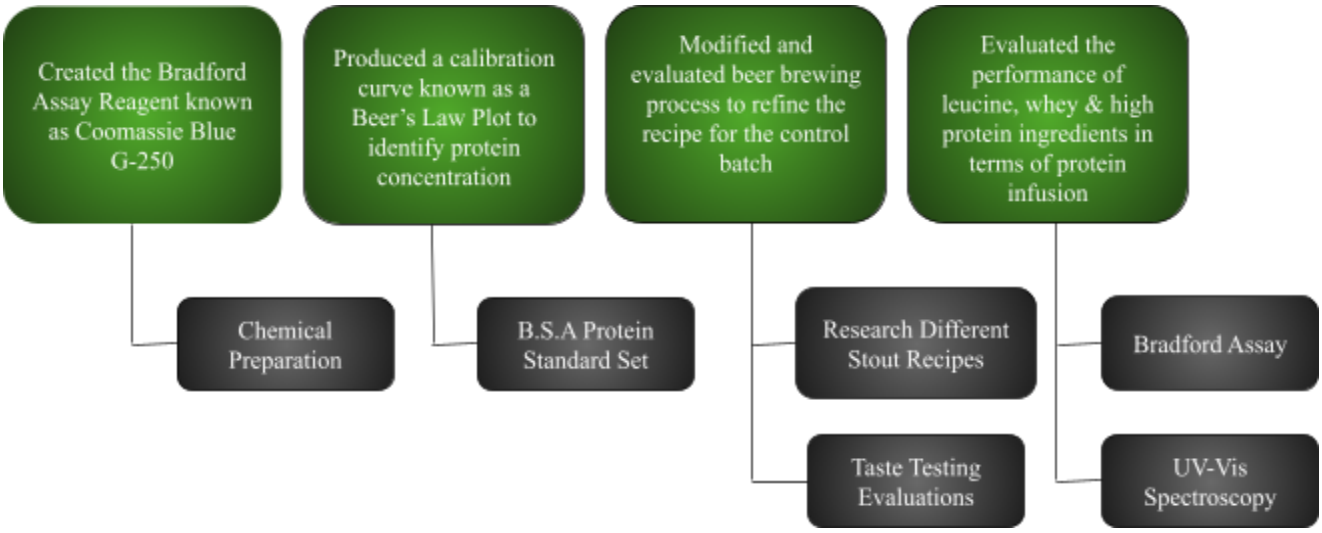


Figure 5: *Objectives and Corresponding Methods.*

3.1 - Creating Coomassie Blue G-250 Solution for Bradford Assay

Our team used the equipment and resources available in WPI's Goddard Laboratory to prepare a solution known as Coomassie Blue G-250 in order to accurately determine the concentration of protein in a solution through a process known as a Bradford Assay. Coomassie Blue G-250 is a disulfonated triphenylmethane staining reagent that interacts and binds to amino acids to form protein-dye complexes, which helps to quantify protein concentration in a solution through UV-vis spectroscopy (CITE - Thermofisher). The team began to prepare the solution by collecting the following ingredients from the WPI Stockroom: 25 mg of Coomassie Blue G-250 compound, 25 mL of methanol and 50 mL of 85 % phosphoric acid (H_3PO_4). First, the team added the 25 mg of Coomassie Blue G-250 compound that was measured out via an analytical balance to the 25 mL of methanol. This mixture was then placed on a continuous stirrer until the Coomassie Blue G-250 was completely dissolved. Once completely dissolved, the 50 mL of 85 % phosphoric acid (H_3PO_4) was added to the solution and similarly mixed via the continuous stirring apparatus. Following this step, 250 mL of DI water was added to the mixture and the solution was stirred until it was uniform. The team then filtered out the solid precipitates left in solution using a technique known as gravity filtration. The mixture was poured onto a filter medium (filter paper) and gravity was relied on to pull the liquid through; separating the solid precipitates from the filtrate. The solid precipitates that remained on the filter paper were discarded, and 175 mL of DI water was then added and mixed with the collected filtrate before the solution was sealed and stored at 4 ^{o}C in the laboratory refrigerator until use. Below is the step-by-step procedure the team followed to prepare the Coomassie Blue G-250 solution:

Step-by-Step Procedure:

1. Use an analytical balance to weigh out 25 mg of Coomassie Blue G-250 compound.
2. Measure out 25 mL of methanol and 50 mL of 85 % phosphoric acid (H_3PO_4) using a 50 mL graduated cylinder.
3. Add the 25 mL of methanol to the 25 mg of Coomassie Blue G-250.
4. Place a stirring bead in the solution, and set the beaker containing the solution on a continuous stirring apparatus.
5. Stir the solution on high until the Coomassie Blue G-250 has completely dissolved.
6. Add the 50 mL of 85 % phosphoric acid (H_3PO_4) to the mixture.
7. Stir the solution on high using the continuous stirring apparatus until the solution is uniform.
8. Add 250 mL of DI water to the mixture.
9. Stir the solution on high using the continuous stirring apparatus until the solution is uniform.
10. Retrieve a piece of filter paper and a funnel.

11. Fold the piece of filter paper to fit the funnel and wet it down with DI Water.
12. Dispose of any DI water that was collected in the beaker below during the previous step.
13. Pour the mixture, in small increments, onto the filter medium.
14. Dispose of the filter paper containing the precipitates left over in solution, and add 250 mL of DI water to the collected filtrate.
15. Stir the solution on high using the continuous stirring apparatus until the solution is uniform.
16. Seal the top of the beaker using a piece of wax paper
17. Store the sealed solution of Coomassie Blue G-250 in a 4 ^{o}C refrigerator until use.

3.2 - Generating Protein Standard Calibration Curve for Bradford Assay

3.2.1 - Determining the Proper Protein Assay Standard Kit

In order to be able to determine the unknown concentration of protein in the samples we brewed with leucine, whey protein, and high protein ingredients, a standard curve was generated. Rather than creating our own set of standardized protein samples via the use of whey protein, and running the risk of producing inaccurate protein concentrations due to complications associated with creating a uniformly solutionized product that didn't contain unfiltered homogenate, we purchased a protein assay standard kit that contained 7 prediluted concentrations. The two most common protein standards used for Bradford Protein Assays are Bovine Serum Albumin (B.S.A.) and Bovine Gamma-Globulin (B.G.G.). Based on our research, we purchased the B.S.A. Protein Standard Kit from FisherScientific™, due in part to the fact that with a Bradford Protein Assay "dye color development is significantly greater with B.S.A. than with most other proteins, including Bovine Gamma-Globulin" (*Quick Start Bradford Protein Assay - Instruction Manual*). The B.S.A. Protein Standard Kit contained the following set of 7 pre diluted concentrations of B.S.A. in 2 mL tubes: [1] 2 mg/mL; [2] 1.5 mg/mL; [3] 1 mg/mL; [4] 0.75 mg/mL; [5] 0.50 mg/mL; [6] 0.25 mg/mL & [7] 0.125 mg/mL.

3.2.2 - Preparing Standards for Bradford Assay

The team performed a 5 mL cuvette assay using the set of known protein concentrations to create a Beer's Law Plot. The team began by removing the Coomassie Blue G-250 they had previously prepared from the refrigerator and allowed it to warm to ambient temperature. While waiting for the Bradford reagent to reach room temperature, the team cleaned and sterilized 8 cuvettes with acetone and kimwipes. After an ample amount of time, the ambient temperature Coomassie Blue G-250 solution was then gently mixed on a continuous stirring apparatus. The team then carefully measured out 100 µL of the 2 mg/mL protein solution using a micropipette and dispensed it into a cleaned cuvette. A disposable plastic pipette was then used to measure out 5 mL of the Coomassie Blue G-250 solution, which was added to the previously mentioned

cuvette. The plunger of the plastic pipette was depressed repeatedly to mix the protein sample and the reagent. An additional 20 mL of DI water was measured and added to dilute the solution in the cuvette (the reason for this will be discussed later in this section). A new plastic pipette was then used to mix the protein sample and the reagent with the DI water using the aforementioned strategy. The tip of the micropipette was replaced, and this process was repeated for the remaining 6 prediluted protein concentrations.

Step-by-Step Procedure:

1. Remove Coomassie Blue G-250 solution from refrigerator and allow it to warm to ambient temperature.
2. Clean and sterilize 8 UV-Vis cuvette with acetone and kimwipes.
3. Place the Coomassie Blue G-250 solution on a continuous stirrer and gently mix for 2 minutes.
4. Use a micropipette to measure out 100 μL of the 2 mg/mL protein standard.
5. Use a disposable plastic pipette and a graduated cylinder to measure out 5 mL of Coomassie blue G-250 solution.
6. Add the 5 mL of Coomassie blue G-250 solution and the 100 μL of the 2 mg/mL protein standard to the same cuvette.
7. Repeatedly depress the plunger of the plastic pipette to mix the protein standard and the Bradford reagent.
8. Use a disposable plastic pipette and a graduated cylinder to measure out 20 mL of DI water.
9. Add the 20 mL of DI water to the cuvette containing the 5 mL of Coomassie blue G-250 and the 100 μL of the 2 mg/mL protein standard.
10. Repeatedly depress the plunger of the plastic pipette to mix the protein standard and the Bradford reagent.
11. Repeat steps 1-10 for the remaining 6 protein standards.
12. Place the 7 cuvettes containing the samples in a holding rack and allow them to incubate for 5 minutes.

3.2.2 - Measuring Standard Maximum Absorbances Using UV-Vis Spectroscopy

The cuvettes containing the samples were placed in a holding rack and allowed to incubate for 5 minutes. During this time, the UV-Vis Spectrometer was turned on and set to a wavelength of 595 nm. The team then zeroed the instrument with a blank sample, containing only 5 mL of Coomassie Blue G-250 and 20 mL of DI water. The reason why our group added an additional 20 mL of DI water to the samples was due to limitations associated with the UV-Vis Spectrometer the team used to measure absorbance. This apparatus possessed a maximum absorbance of 1.999. Therefore, to ensure the sample absorbances fell within the capabilities of the spectrometer (< 1.999) we diluted them by a factor of 5. Once the samples

had finished incubating for 5 minutes, the outer surface of each cuvette was cleaned with a kimwipe and placed in the reference holder. The absorbances of the standards were measured and recorded. This entire process was repeated 3 times, and the average absorbance for each concentration was calculated. A standard curve, also known as a Beer's Law Plot, was created by plotting the absorbances recorded at 595 nm (y-axis) against the protein concentrations in µg/mL (x-axis). The following figure displays the standard curve you would expect to see when using the 5 mL cuvette assay approach with both B.S.A. and B.G.G protein standard kits.

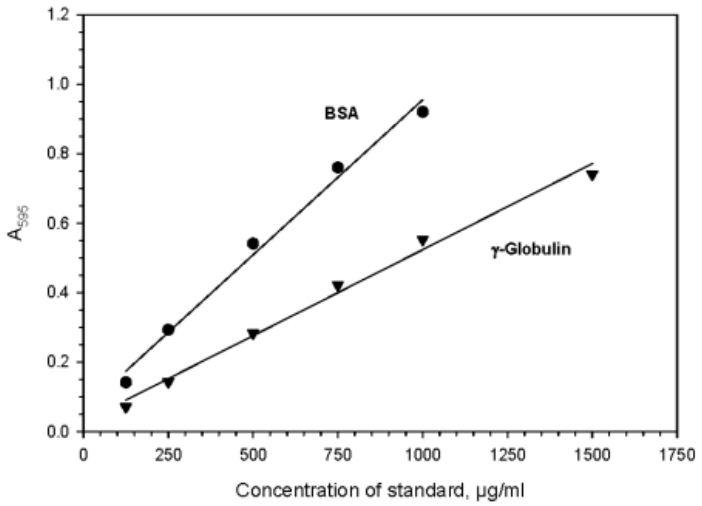


Figure 6: *Standard curve generated when running a 5 mL cuvette assay using the B.S.A. and B.G.G. standard kits at a wavelength of 595 nm.*

Step-by-Step Procedure:

1. Power on the UV-Vis Spectrometer and set the wavelength to 595 nm.
2. Prepare a "blank sample" by measuring out 5 mL of Coomassie blue G-250 solution and 20 mL od DI water using a graduated cylinder, and mix them together in the last clean cuvette.
3. Place the "blank sample" cuvette in the reference holder in the UV-Vis and run the sample. Be sure to manually set the absorbance to zero using the dial on the apparatus.
4. Before the sample is loaded into the reference holder, wipe down the outer surface of the cuvette using a kimwipe.
5. Record the measured absorbance.
6. Repeat steps 4 and 5 for the remaining 6 protein standards.
7. Recreate the 7 protein standards and measure their absorbances using the UV-Vis Spectrometer 2 more times, and calculate the average absorbance obtained from each protein standard.

8. Plot the average absorbance obtained at 595 nm for each protein standard on the y-axis versus the concentration of the protein standard in µg/mL on the x-axis to create the protein standard curve.

3.2.3 - Quantifying Protein Concentration in Unknown Samples

The standard curve our team created represents the relationship between absorbance and concentration of protein (in µg/mL). Through use of this curve, our team was able to quantitatively identify how much protein we were able to infuse into our brewed beers. By adding 5 mL of Coomassie Blue G-250 to 100 µL of our sample beer, diluting this with 20 mL of water (due to equipment limitations), and running it through the UV-Vis Spectrometer, our team was able to produce absorbance values for our beer samples at 595 nm. Using the linear equation ($y = mx + b$) generated from the trendline run through our data points, we were able to determine the concentration of protein (x), in µg/mL, in our beer samples, by plugging our measured absorbance value in for y in the trendline equation. The calculated protein concentration (x) was then multiplied by the dilution factor used (5) to determine the final concentration of the unknown samples.

3.3 - Brewing Control Batch

In this objective, we experimented with different amounts of ingredients associated with the beer brewing process to create a polished recipe that would be used to prepare our control batch. We evaluated the quality of each experimental batch through taste testing to determine which batch would be used as our control group when comparing to protein infused beers.

The beer brewing process begins with the mashing or mulling of malt or grain. In order to bypass this part of the brewing process and any complications associated with it, our team purchased *"Brewmaster Porter & Malt Extract"* to serve as our beer's source of sugar. This allowed our group to focus solely on trying to infuse protein into beer rather than perfecting the step mashing technique. Our team began by cleaning and sterilizing the home brewing kit we purchased from the Brewing Emporium in Worcester MA, and all the equipment retrieved from the Goddard Laboratory, with a cleaning reagent known as *"Easy Clean"* and iodine. After our equipment had been adequately cleaned and sanitized, we began our first brew using the "Basic Stout Recipe" defined in section 2.2.4 as a reference. To create a 1 gallon batch of beer, the team measured out 1.5 gallons of water and poured it into a 5-gallon steel kettle. This kettle of water was placed on the stove and raised to a roaring boil. While waiting for the water to come to fruition, the team measured out 1.9 pounds of malt extract using an analytical balance. Once the water reached its boiling point, the 1.9 lbs. of malt extract was added to the water and gently stirred until it had completely dissolved. This mixture, known as the wort, was then boiled at a continuous rolling boil for 1 hour. Approximately half-way through this sterilization process, the team added 0.4 oz of Fuggle Hops (received from Brian Distefano at Purgatory Brewing Co.) to the wort to give it added flavor, bitterness and an aroma. After 60 minutes had passed, the 5

gallon kettle, which now contained about 1 gallon of wort, was placed in an ice bath to cool the wort from its high temperature (~ 150 ºC) to a temperature between 70 ºC - 75 ºC. Once the wort had achieved a temperature between 70 ºC - 75 ºC, it was siphoned into a clean 250 mL graduated cylinder so that the gravity could be recorded, then siphoned into a 1.5 gallon glass beer growler (some of the wort containing leftover precipitates was not siphoned into the growler). The team then used a hydrometer to measure the specific gravity of the brew prior to adding 1 teaspoon of yeast to the beer growler, and placing an airlock containing water atop the growler to begin the fermentation process. The glass beer growler was then placed in a dark room for a total of 10 days, where it was frequently monitored. Below is the step-by-step brewing process the team followed to prepare their first brew:

Step-by-Step Procedure:

1. Measure out 1.5 gallons of tap water using the 1 gallon glass beer growler and pour it into a 5-gallon steel kettle.
2. Allow the water in the 5-gallon steel kettle to come to a boil.
3. Use an analytical balance to weigh out 1.9 pounds of *"Brewmaster Porter & Malt Extract"*.
4. Add the 1.9 pounds of malt extract to the water in the 5-gallon steel kettle once it reaches a roaring boil.
5. Gently stir the malt extract into the water until it has completely dissolved.
6. Allow this mixture to boil at a continuous rolling boil for 1 hour.
7. Use an analytical balance to weigh out 0.4 ounces of Fuggle Hops.
8. Half-way through the boiling process (30 minutes), add the 0.4 oz. of Fuggle Hops to the wort and give it a gentle stir.
9. After 1 hour, remove the 5-gallon steel kettle containing the wort from the stove and place it into an ice bath.
10. Allow the temperature of the wort to cool to between 70 ºC - 75 ºC.
11. Once the wort reaches a temperature between 70 ºC - 75 ºC use a hydrometer to measure the specific gravity.
12. Siphon the wort into a 1.5 gallon glass beer growler.
13. Use a hydrometer to measure the specific gravity of the brew.
14. Add 1 teaspoon of yeast to the wort in the 1.5-gallon beer growler.
15. Fill an airlock approximately half-way with water, and place it atop the glass beer growler.
16. Store the beer growler in a dark environment for 14 days to allow it to ferment.
17. Make sure to frequently monitor the beer growler.

3.4 Whey Protein Testing

Testing for the addition of whey protein was the first attempt at adding protein to beer. First, an initial solubility test was run to determine the amount of whey that could be added to a generic, light beer. Then, whey was added to Guinness to test the feasibility of adding whey protein to a stout post-fermentation. The taste, appearance, and protein content were all measured.

3.4.1 Initial Whey Solubility Test

To test the feasibility of brewing a stout with whey protein, the solubility of whey protein isolate must first be explored. The solubility of whey protein in beer was qualitatively examined through a preliminary test using Optimum Nutrition Gold Standard 100% Whey Protein and a home-brewed American classic ale. About 12 oz of beer was added to a glass, with ⅓ scoop (~10 g) of whey protein powder. It is worth noting that the instructions for the protein powder recommended adding 1 scoop per 6-8 oz of water; therefore, this trial was run at approximately ⅕ -⅙ the recommended amount of protein powder.

3.4.2 Testing for Protein Content

To test for the amount of whey protein that can be added to a stout post-fermentation, GNC unflavored whey protein was added to Guinness. Since whey protein does not denature in ethanol at room temperature, the amount of protein in the beer was calculated as the original protein content of Guinness plus the added protein from whey.

A Bradford Assay was used to test the original protein content in Guinness. 5 ml of our Bradford Assay solution, 20 ml of distilled water, and 100 µl of Guinness was mixed in a plastic centrifuge tube and vortexed. This solution was then poured into a test tube and the absorbance of the sample at a wavelength of 595 nm was measured using UV Vis. Our previously-created protein standard curve was then used to determine the corresponding protein concentration of the diluted sample. This process was repeated three times and the average value was determined to be the original protein content in Guinness.

The amount of whey protein to add to Guinness was determined following the results from the Initial Solubility Test (Section 3.3.1). 100 ml of Guinness was measured using a graduated cylinder and poured into a beaker. 1.7431 g of whey protein (less than 1/10th the recommended amount for 100 ml of water) was then added to the beer and stirred as best as possible. The total amount of added protein from whey was then calculated using the ratio on the container that states that 33.6 g of protein powder corresponds to 24 g of added protein.

A separate mixture of approximately the same amount of Guinness and whey protein was created without using any lab materials so that the beverage could be tasted. Each group member then rated the beverage on the following categories: taste, mouthfeel, bitterness, after taste, and appearance of the body. Mouthfeel and appearance of the body were measured qualitatively and the other categories were measured quantitatively on a scale from 1 to 10.

3.5 Leucine Testing

Testing the feasibility of adding leucine to beer was our second approach towards making a protein-rich beer. Since leucine does not denature at the boiling point of water, the addition of leucine was tested both during the boil and after fermentation.

3.5.1 Leucine Added During Boil

To test the feasibility of adding leucine to beer during the boil, Nutricost L-Leucine powder was added to a home-brewed beer. A process similar to the one outlined in Section 3.1 was used to brew a batch of beer. The differences between this brew and the initial control batch is that all of the ingredients were scaled down by half to produce half a gallon of beer. Additionally, 32 g (6 scoops) of leucine (approximately the recommended amount for half a gallon of water) was added to the boil with 30 min left (half way through the boil). A control batch was also brewed simultaneously without the leucine to be used as a baseline during testing. The beers were allowed to ferment in modified mason jars which acted as micro-reactors for two weeks.

After the fermentation process had been completed, the leucine beer and the control beer were taken to the lab for testing. Both beers were tested for protein content, taste tested, and qualitatively examined using the same methods as described for the whey beer in Section 3.3.2. The results for the leucine beer were compared to the control brew.

3.5.2 Leucine Added After Fermentation

The feasibility of adding leucine to a stout after fermentation was also tested using the leucine powder and Guinness. The results of an unaltered Guinness found in Section 3.3.2 were used as a control for the leucine testing. 100 ml of Guinness was measured using a graduated cylinder and poured into a beaker. Small amounts of leucine (approximately .2 g) were then measured using an electronic balance and stirred into the beaker in increments until there appeared to be a noticeable visible change in the beer. In total, 0.5438 g of leucine was added to the Guinness (approximately 40% of the recommended amount for 100 ml of water). The mixture was then tested for protein content, taste tested, and qualitatively examined using the same methods as described in Section 3.3.2. The results for the leucine beer were compared to regular Guinness.

3.6 Brewing With High-Protein Ingredients

To test whether brewing with high-protein ingredients is a feasible method for increasing the protein content in beer, various high-protein foods were added to small-scale brews during the boiling process. Peanuts, peanut butter, quinoa, oats, and hot cocoa powder were all tested individually.

3.6.1 Adding High-Protein Ingredients During the Boil

A process similar to the one outlined in Section 3.1 was used to brew six, half-gallon batches of beer. The differences between this brew and the initial control batch is that 1 gallon of water was used, 0.6 lb of liquid malt extract was used, and all other ingredients were scaled down by half to produce half a gallon of beer. Additionally, 1 cup of each high-protein ingredient was added to its own batch with 30 min left on the boil (halfway). A control batch was also brewed simultaneously without any high-protein ingredients to be used as a baseline during testing.

After a two-week fermentation in modified mason jars, the samples were taken to the lab for testing. Testing for each sample was conducted as outlined in Section 3.3.2 and the results were compared to the control brew.

3.6.2 Increasing the Amount of High-Protein Ingredients During the Boil

To test whether increasing the amount of high-protein ingredients has an effect on the protein content of the finished product, two additional ½ gallon batches were brewed similar to the ones outlined in Section 3.5.1. The only difference between these brews and the ones in Section 3.5.1 is that 0.75 lbs of liquid malt extract were used. Additionally, three cups of peanuts were added to the boil of one of the batches (triple the amount used in Section 3.5.1) with 30 min remaining. The other batch was used as a control and did not receive any high-protein ingredients.

After a two-week fermentation, the samples were taken to the lab for testing. Testing for each sample was conducted as outlined in Section 3.3.2 and the results were compared to the control brew.

3.6.3 Adding High-Protein Ingredients After the Boil

To test whether protein denaturing has an effect on adding high-protein ingredients during the boil, an additional ½ gallon batch was brewed similar to the ones outlined in Section 3.5.2. Instead of adding the high-protein ingredients during the boil, one cup of peanuts was added to the mason jars just before fermentation began.

After a two-week fermentation, the sample was taken to the lab for testing. Testing was conducted as outlined in Section 3.3.2, and the results were compared to the control brew used in Section 3.5.1 since no other variables were changed.

4 - Results and Discussion

4.1 Objective 1 - Create the Bradford Reagent:

An important step in this project was finding a way to quantitatively determine the amount of protein that we were able to infuse into our brew as a means to identify which approach; brewing with leucine, whey protein, or high protein ingredients, was most effective. A wide array of different methods have been developed to quantify the amount of protein in samples containing a mixture of different proteins. Through research, our team determined that a Bradford Protein Assay would best allow us to accurately measure a wide range of protein concentration in our samples. Therefore, our first objective was to create the Bradford Assay reagent known as Coomassie Blue G-250. This solution, which functions as a dye, is essential to indicating the presence and overall concentration of all types of proteins in an unknown solution. This dye is said to bind to the following amino-acids: arginine, tryptophan, tyrosine, histidine and phenylalanine. The Coomassie Blue G-250 dye exists in four different ionic forms. In its most stabilized anionic form, the light brown dye binds to soluble proteins to form blue complexes. Therefore, at a wavelength of 595 nm, a UV-Vis Spectrometer can be used to measure a sample's absorbance and ultimately identify protein concentration. The team carried out the instructions outlined in the procedure for preparing the Coomassie Blue G-250 solution described in section 3.1 in order to quantify protein. The following images provide a glimpse into the Coomassie Blue G-250 solution preparation process:

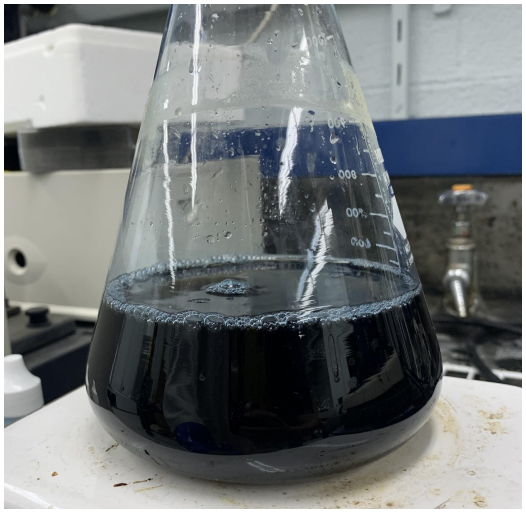


Figure 7: *Coomassie Blue G-250 solution being mixed on a continuous stirring apparatus.*

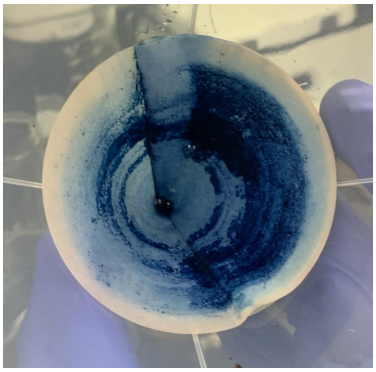


Figure 8: *A piece of Whatman filter paper containing insoluble material following the filtration of the Coomassie Blue G-250 solution.*

4.2 Objective 2 - Developing a Standard Curve

The seven samples, each containing 5 mL of Coomassie Blue G-250, 100 µL of the pre-diluted protein standards, and 20 mL of DI water, were prepared and run through the UV-Vis Spectrometer, where their maximum absorbances were measured and recorded. In an attempt to improve our precision and ensure our data was reliable, we performed a total of three trials for each protein standard sample. The following table displays the absorbances that were recorded for each protein standard during the three different trials:

Protein Standard Samples:	Absorbance:			
	1	2	3	Average
2.0 mg/mL	0.408	0.416	0.416	0.413
1.5 mg/mL	0.422	0.371	0.484	0.426
1.0 mg/mL	0.370	0.355	0.330	0.352
0.75 mg/mL	0.306	0.305	0.329	0.313
0.50 mg/mL	0.294	0.257	0.248	0.266
0.25 mg/mL	0.191	0.167	0.170	0.176
0.125 mg/mL	0.148	0.138	0.128	0.138
0 mg/mL	0.058	0.106	0.067	0.077
Protein Standard Samples were mixed with 5 mL of Coomassie Blue G-250 Solution and diluted with 20 mL of DI Water				

Table 1: *Absorbance values measured from FisherScientific protein standards used to create a Beer's Law standard curve.*

The maximum absorbance values measured by the UV-Vis Spectrometer at 595 nm for each of the protein standard samples for the three trials were plotted (y-axis) against the

concentration of the protein standard samples following dilution in µg/mL (x-axis) to generate the following Beer's Law Plots:

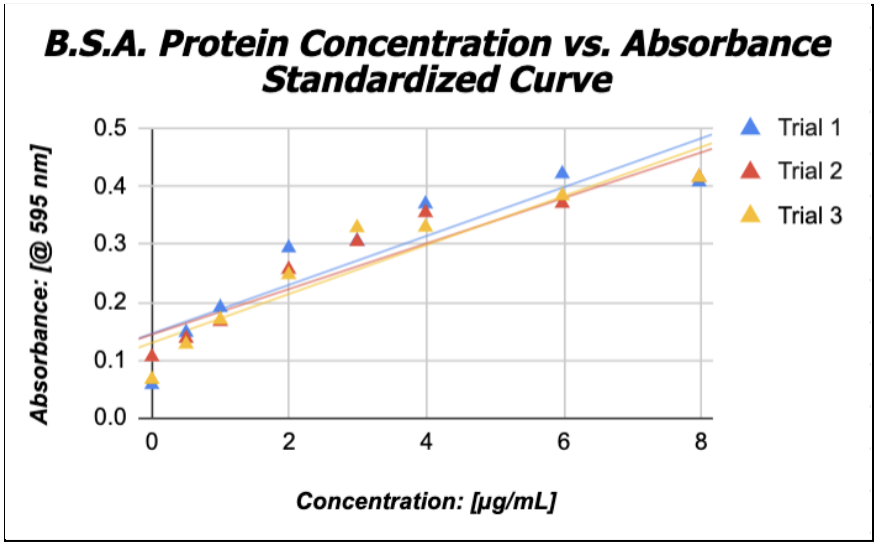


Figure 9: *B.S.A. Standard Calibration Curves for the 3 different trial runs plotting concentration of protein standard sample (µg/mL) vs. Average Maximum Absorbance (@ 595 nm).*

The measured absorbances from these trials were used to calculate the average maximum absorbance for each of the protein standard samples at 595 nm. The average maximum absorbance values were plotted (y-axis) against the concentration of the protein standard samples following dilution in µg/mL (x-axis) to produce the following calibration curve for our Bradford Assay.

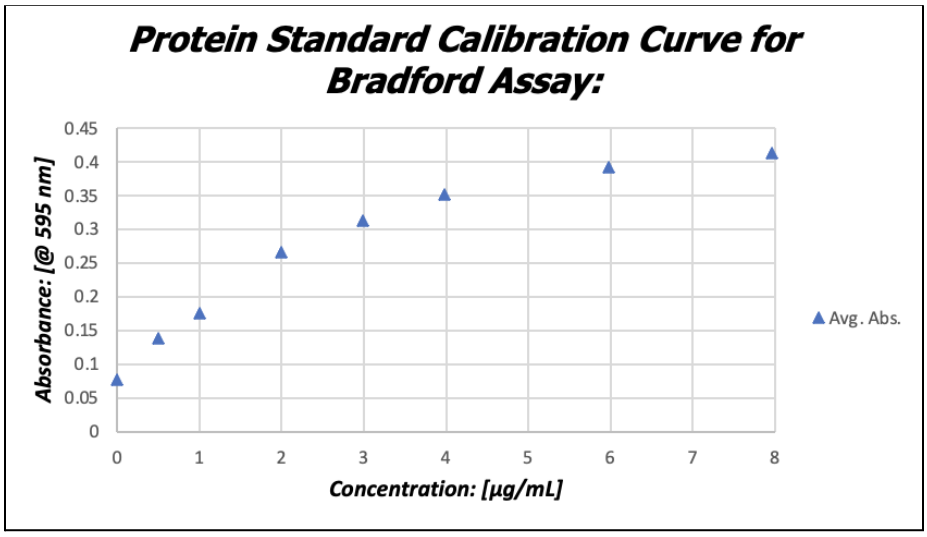


Figure 10: *"Beer's Law Protein Standard Calibration Curve" plotting concentration of protein standard sample (µg/mL) vs. Average Maximum Absorbance (@ 595 nm) for Bradford Assay.*

Beer's Law is a universally accepted relationship which aids in determining the concentration of a chemical species based on measured absorbance values. Ideally, the plot of absorbance vs. concentration is linear, with a y-intercept of zero and a slope equal to the product of the molar absorptivity and the pathlength at which the beam of radiation must travel through. However, under real conditions, positive/negative deviations from ideal linear behavior occur at high concentrations of analyte. Positive deviations occur when the measured absorbance values are more than the expected ideal absorbance, and negative deviations occur when the measured absorbance values are less than the expected ideal absorbance. In Figures 9 and 10, as a protein concentration of approximately 5 μg/mL is approached, the linearity of our Beer's Law Protein Standard Curve begins to dissipate, suggesting a negative deviation from ideal linear behavior.

Deviations from linearity in Beer's Law Plots are caused by either fundamental, chemical or instrumental limitations. The reason why our plots possess negative deviation is due to a combination of fundamental limitations and instrumental limitations associated with Beer's Law and the UV-Vis Spectrometer respectively. From a fundamental standpoint, the reason why our standard curve begins to deviate from linearity as concentration increases is due to the fact that Beer's Law is a limiting law which is essentially only valid under low concentrations of analyte. This is due to the fact that individual molecules of the analyte behave freely at low concentrations. This behavior allows for more accurate absorption of light, which in turn produces more precise radiation readings by the UV-Vis detector. Ideally, as the concentration of analyte increases, the measured absorbance should increase, as less radiation reaches the detector due to the fact that more light is being absorbed by these free molecules. However, at higher concentrations, analyte molecules tend to interact, instead of behaving freely. This causes measured absorbance to decrease, as these interactions enable more radiation in the form of light to travel through the sample and reach the detector. As a result, in some cases (such as ours), Beer's Law Plots begin to negatively deviate from the expected ideal behavior at high concentrations of analyte because these interactions change the analytes absorptivity.

In addition to the fundamental issue associated with Beer's Law, our team believes that our standard curve began to negatively deviate from Beer's Law due to an instrumental limitation known as stray radiation. Stray radiation is defined as any form of light greater than the set wavelength that enters the instrument and reaches the detector without traveling through the pathlength of the sample. The following image illustrates this phenomenon:

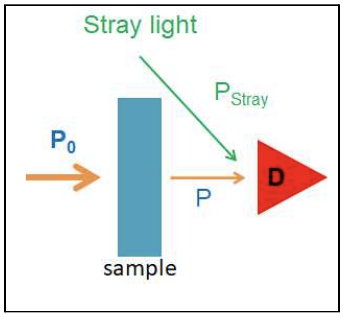


Figure 11: *Stray light enters the UV-Vis Spectrometer and strikes the detector without passing through the sample.*

According to LibreTexts, the photometric error stray light introduces is said to only negatively affect absorbance in a UV-Vis Spectrometer for solutions containing high concentrations of analyte. The following formula for calculating absorbance illustrates how stray radiation effects the linearity of Beer's Law at high concentrations:

$$A = -\log \frac{P_T + P_{stray}}{P_0 + P_{stray}}$$

Where P_T is the power from the radiation source; P_0 is the power from the radiation leaving the sample; and P_{stray} is the power from the stray radiation.

The effect of stray radiation on the linearity of a Beer's Law Plot for solutions containing a low concentration of analyte is negligible. This is due to the fact that there are less molecules to absorb radiation from the beam of light, which means the power of the radiation leaving the sample (P_0) is approximately equal to the power from the source of the radiation (P_T). Therefore, because P_T and P_0 are much greater than the P_{stray}, its small value does not affect the overall absorbance. However, for solutions containing a high concentration of analyte, P_0 is much smaller than P_T due to the fact that there are more molecules that absorb incident radiation causing less radiation to reach the detector. In other words, as the concentration increases, P_0 decreases, which causes the value of the denominator to be heavily influenced by power from the stray radiation (P_{stray}). This ultimately results in an absorbance that is smaller than expected, which leads to a negative deviation from Beer's Law, which is depicted in Figure 11 below.

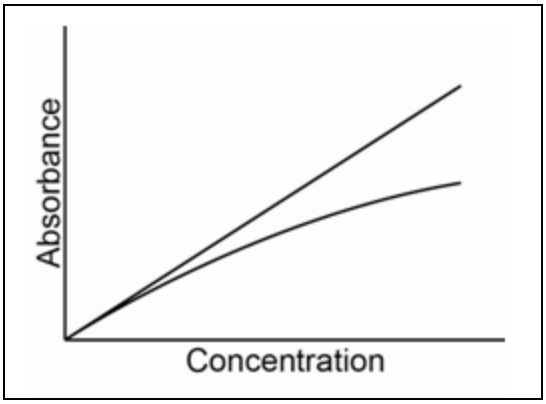


Figure 12: *The effect of concentration on the influence of stray light and its overall effect on absorbance in terms of the linearity of a Beer's Law Plot.*

Due to the fundamental limitations associated with Beer's Law, and the instrumental limitation known as stray light associated with the UV-Vis spectrophotometer, our Average Beer's Law Protein Standard Curve began to negatively deviate from the ideal Beer's Law relationship at high concentrations of protein. These limitations are rather impactful, as they required that the protein concentration of the unknown samples we tested be within a limited range (2.5 ~ 5.0 µg/mL) in order to be able to accurately determine protein concentration via our standard curve. As a result, the team linearized the standard curve through a logarithmic approximation in order to extrapolate the logarithmic trend further and create artificial data that extended beyond the limited range caused by the fundamental and instrumental limitations associated with Beer's Law and the UV-Vis Spectrometer respectively. The following figure illustrates the linearized version of our Bradford calibration curve:

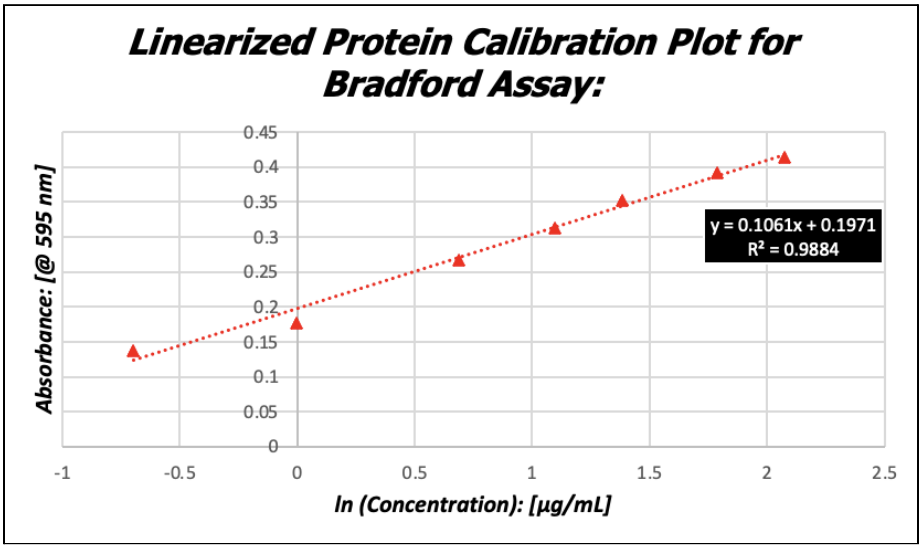


Figure 13: *Linearized version of our Average Beer's Law Protein Standard Curve that is extrapolated to accommodate a broader range of protein concentrations.*

Through use of linear regression, our team was able to model the relationship between absorbance and protein concentration by fitting a linear equation to the observed data. The following linear regression equation (Equation 1) was determined to be the "best fit" for the measured data:

$$y = 0.1061x + 0.1971 \qquad (1)$$

Where "y" is the independent variable - known absorbance value; 0.1061 is the slope of the trendline; 0.1971 is the point where the line intersects the y-axis; and x is the dependant variable - ln of the unknown protein concentration

With this linearized protein calibration curve, our team was able to more accurately identify protein concentration in unknown samples across a broader range of protein concentrations. As a result of this linear regression, which fit the experimental data well as is indicated by R^2 = 0.9884, the protein concentration of the unknown samples no longer needed to fall within the 2.5 ~ 5.0 µg/mL range (the linear range on the *Beer's Law Protein Standard Calibration Curve*) in order for the team to accurately determine how much protein it possessed. Using the absorbance value obtained from the UV-Vis Spectrometer, in place of *y* in the linear regression equation, the team was able to obtain the natural log of the protein concentration of the sample being tested. The protein concentration in the unknown sample was solved for through use of the following equation:

$$X = (251)e^{x} \qquad (2)$$

This coefficient of 251 is derived from our team diluting the 100 µL protein standard sample with 5 mL of Coomassie Blue G-250 and 20 mL of DI water in order to detect protein below the absorbance limitation of the UV-Vis Spectrometer we were using (A < 1.999). By multiplying the exponential of the natural log of the protein concentration by the inverse of the dilution factor (251), our team was able to determine the protein concentration in the 100 µL of each of the different protein sample brews we produced. The calculated value was then used to approximate how much protein this would resemble in a 12 oz. can of beer. Sample calculations depicting this process can be found in Appendix A.

4.3 Control Brew

The initial control stout was brewed to be used as a baseline for much of the testing done throughout this report. Figures 14 and 15 below compare the appearance of the initial control brew before and after the two-week fermentation process.

Figures 14 & 15: *Figure 14 on the left shows the initial control brew just before it begins the two-week fermentation process. Figure 15 on the right shows the initial control brew after it had finished its two-week fermentation.*

As can be seen in Figure Y, a light brown sediment appears to have settled to the bottom of the fermenter. This sediment is likely part of the liquid malt extract. Excess hops also appear to float at the top of the fermenter. Finally, the body of the beer appears to have gotten much darker following the fermentation process. This is likely due to the fact that less particles from the hops and malt are suspended in the body of the beer.

As discussed in Section 3.3 of the methodology, the control beer that was used in much of our testing was a home-brewed stout. Throughout the course of the testing, the recipe of the control brew was tweaked to improve the overall taste of the beer and to adjust for scaling issues with the smaller batch sizes. The first control batch of beer was brewed at a batch size of 1 gallon and was used as a control for the testing of adding leucine after fermentation (Section 3.5.2 of Methodology). The second control batch of beer was brewed at a batch size of 1⁄2 gallon and was used as the control for the initial set of high-protein ingredient testing (Section 3.6.1 of Methodology).The third control batch of beer was also brewed at a batch size of ½ gallon and was used as a control for the final two sets of high-protein ingredient testing (Sections 3.6.2 and 3.6.3 of the Methodology). Each control brew was brewed alongside different batches using the same recipe as their corresponding control.

Control Brews:			
Batch #:	**Ingredients Metrics:**	**Alcohol By Volume (ABV.): [%]**	**Protein Concentration: [g] (12 oz. Can of Beer)**
1	• Batch Size: 1.0 Gallon • Water: 1.5 Gallons • Liquid Malt: 1.9 lbs. • Hops: 0.40 oz. • Yeast: 1 teaspoon	6.96%	0.411
2	• Batch Size: 0.5 Gallons • Water: 1.0 Gallon • Liquid Malt: 0.60 lbs. • Hops: 0.20 oz. • Yeast: 1/2 teaspoon	2.36%	0.047
3	• Batch Size: 0.5 Gallons • Water: 1.0 Gallon • Liquid Malt: 0.75 lbs. • Hops: 0.20 oz. • Yeast: 1/2 teaspoon	3.01%	0.169

Table 2: *Table 2 shows the ingredient metrics, ABVs, and protein concentrations for each of the control brews used throughout testing.*

As can be seen in Table 2, the initial control brew had a batch size of 1 gallon, used 1.5 gallons of water, 1.9 lbs of malt, 0.40 oz of hops, and 1 teaspoon of yeast. The quantities of these ingredients were determined based on the basic stout recipe in Section 2.2.4 of the Background. Since our group chose to brew with liquid malt extract instead of pale malt, flaked barley, and roasted barley; the combined weight of the grain bill was used to determine the amount of liquid malt to be used in our recipe. The ABV of this control beer was 6.96% which is fairly typical of a stout and the protein concentration was 0.411 g / 12 oz beer. When tasted by the members of our group, it was apparent that this beer had a very syrupy mouthfeel which would be addressed in the second control batch.

The second control brew was scaled down to a batch size of ½ gallon and used 1 gallon of water, 0.6 lbs of malt, 0.20 oz of hops, and ½ teaspoon of yeast. After brewing our leucine brew (Section 3.5.1), our group learned that the amount of water does not scale down proportionally to the batch size. Since the surface area of the pot and boil time is the same for both batch sizes, a constant amount of water should boil off during each boil. With this information in mind, 1 gallon of water was used to produce ½ a gallon of beer. To address the syrupy mouthfeel of the first control batch, the amount of liquid malt used was also not scaled down proportionally. Since the liquid malt extract was believed to be contributing to the syrupy taste, 0.6 lbs of malt was used instead of 0.95 (½ of 1.9 lbs). The ABV of the second control beer dropped dramatically to 2.36%. Our group believed that the most likely reason for the low ABV is because of the decreased amount of malt. This logic seems to make sense because less malt means that the yeast would have less sugar to convert into alcohol during the fermentation process. The protein concentration of this beer was determined to be 0.047 g /12 oz beer. All

members of the group that tasted this beer noted that the mouthfeel was much smoother than the first control batch.

The third control brew also had a batch size of ½ gallon and used much of the same ingredients as the second control besides the malt which was increased to 0.75 lbs as seen in Table 2. Since the ABV of the second batch was so low, the malt was increased to 0.75 lbs to provide the yeast with more sugar to ferment into alcohol. 0.75 lbs was chosen because it was in between 0.6 and 0.95 which would hopefully provide the yeast with enough sugar while not giving the beer the syrup mouthfeel that the initial control batch had. Unfortunately the increase in malt did not result in a higher alcohol content since the ABV of the third control beer was only 3.01%. One possible explanation for the low alcohol content in both the second and third control batches is that our group did not accurately have a method of measuring ½ teaspoons of yeast. When making this measurement, a 1 teaspoon measuring spoon was filled by about half; however, it is possible that less than the recommended amount of yeast was added. If less yeast was added to the fermenter, less alcohol would be fermented in the two-week fermentation period. The protein concentration of this beer was also determined to be 0.169 g /12 oz beer. The third control beer also had a mouthfeel that was slightly more syrupy than the second brew but less than the initial brew. This is once again most likely due to the increase in liquid malt extract that was used in this control batch.

To quantify qualities of beer that could not be objectively measured without further instrumentation, each member of our group rated the aroma, mouthfeel, appearance, and overall taste of each of the control brews on a scale from 1 to 10. The results of these ratings can be seen in Figure 16 below.

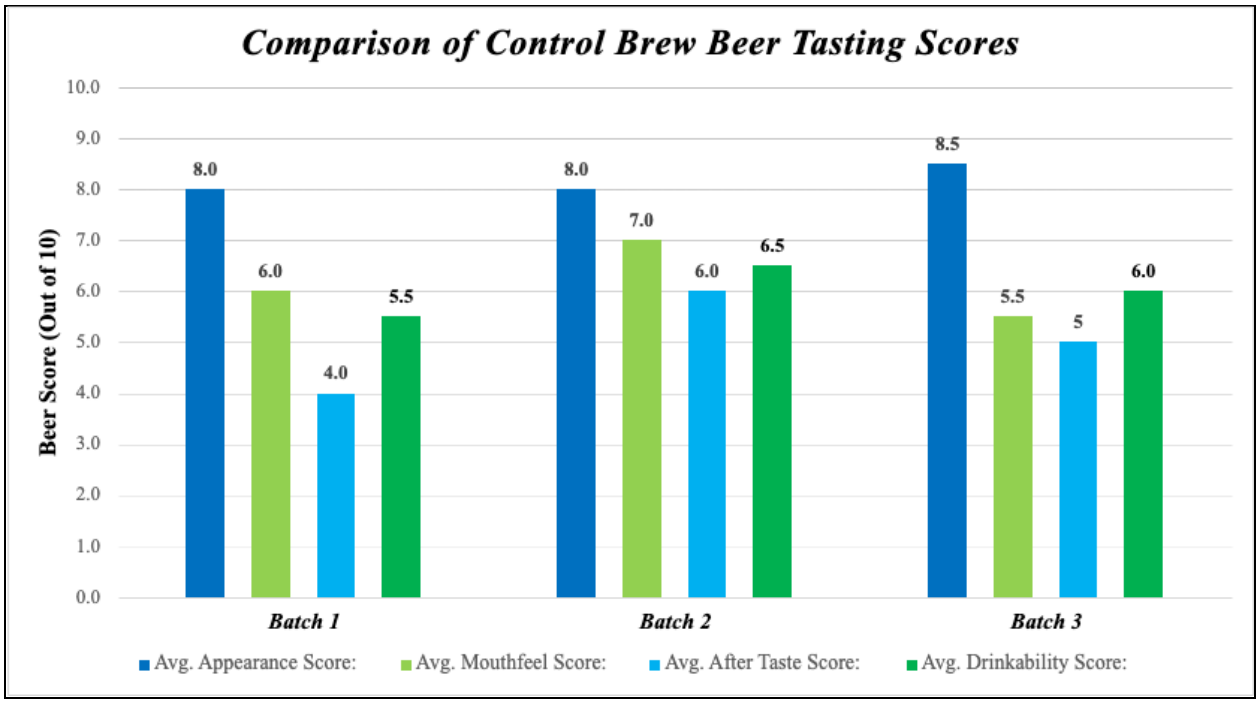


Figure 16: *Figure 16 shows the average appearance, mouthfeel, after taste, and drinkability scores for each of the three control brews used throughout the report. The beers were rated on each category on a scale from 1 to 10 by each member of the group.*

As seen in Figure 16, the three control btches were taste tested by the members of our group on a scale of 0 to 10 on appearance, mouthfeel, after taste, and drinkability. These four features were rated for every beer that was used or tested throughout the course of the experiment so that the beverages could be objectively compared to one another. When looking at Figure 16, one can see that all three control batches were rated very similarly to each other in all four categories. This makes sense since the only differences between the three controls is the amount of water and malt used.

4.4 Testing Whey

When testing the feasibility of adding whey to beer, the initial solubility was tested before comparing a mixture of whey and Guinness to a Guinness control. The average appearance, mouthfeel, after taste, and drinkability were then measured for each of the two beverages; and then their protein contents were measured.

4.4.1 Initial Whey Solubility Test

The purpose of the initial whey solubility test was to get a basic understanding of how soluble whey protein is in beer. This information was useful in helping us narrow down an

appropriate amount of whey protein powder to use in the post-fermentation test with Guinness. The results of the initial solubility test can be seen below.

Figures 17 & 18: *Figure 17, on the left, represents the American Classic Ale before whey protein was added. Figure 18, on the right shows the ale once ⅓ scoop of ON Gold Standard chocolate protein powder was added and stirred.*

Figure 17 shows the home-brewed American classic ale before the addition of chocolate protein powder, and Figure 18 shows the beer after the whey protein had been introduced and mixed into the ale. When the scoop of protein was dumped into the beer, the surface tension of the liquid prevented any powder from immediately mixing. Once mixing began, the powder immediately overtook the body of the beer. The beer turned an opaque brown color and a layer of froth began to accumulate at the top of the beverage as seen in Figure 18. Chunks were also observed in the top layer of froth, and globs of unmixed whey stuck to the sides of the glass.

From this preliminary experiment it is apparent that whey protein powder is highly insoluble in beer even when adding less than the recommended amount on the packaging to be used for water. This solubility issue would be addressed in the following experiment when testing for the feasibility of adding whey protein to a post-fermentation stout such as Guinness.

4.4.2 Testing for Protein Content

Following the initial whey solubility test, Guinness Draught Stout and unflavored GNC whey protein powder were used to test how much whey could be added to a stout post-fermentation without compromising the taste of the beer. Since the initial solubility test showed that adding about ⅛ of the recommended amount of whey was very insoluble in beer, the idea for this stage of testing was to start with an extremely small amount of whey protein and increase until we found the maximum amount of protein that could be added. The starting point

for the testing was set at approximately 1/9 of the recommended amount whey to be used in water. Since the whey was added while the beer was at room temperature, there was no denaturing of the protein. This means that the amount of protein in the beer is equal to the amount being added plus the amount in base Guinness. The starting point of this whey testing would scale up to add about 4.42 g of protein to a 12 oz beer, or 4.61 total grams when including the starting protein in Guinness. This number was chosen as the starting point because it was the smallest amount that our group felt could still add a meaningful amount of protein to the beer. The initial mixture of 100 mL of Guinness and 1.7431 g of whey protein can be seen in Figure 19 below.

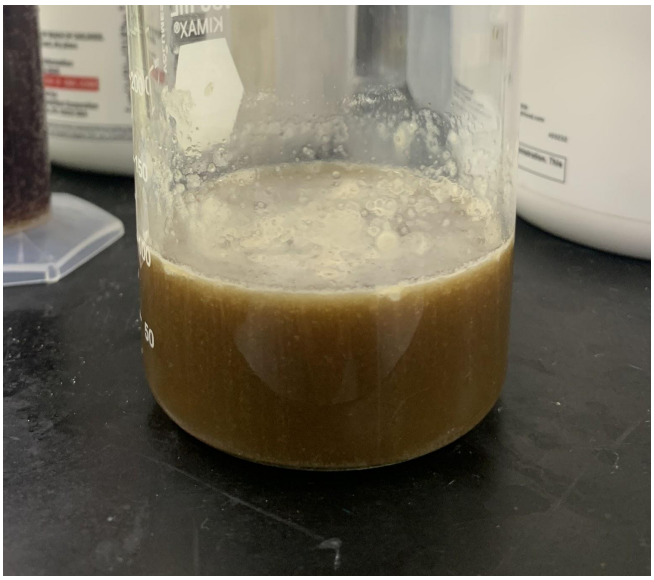


Figure 19: *Figure 19 shows the mixture of 100 ml of Guinness with 1.7431 g of unflavored GNC whey protein powder.*

As can be seen in Figure 19, the extremely small amount of whey protein used as a starting point for testing was still not able to be dissolved in the Guinness. The initial black/dark brown color of the Guinness was immediately transformed into a creamy, light brown. Globs of protein were observed floating at the top of the beer and excess powder could be seen suspended in the body of the beer. The results of the taste test comparing the whey-Guinness mixture to normal Guinness can be seen in Figure 20 below.

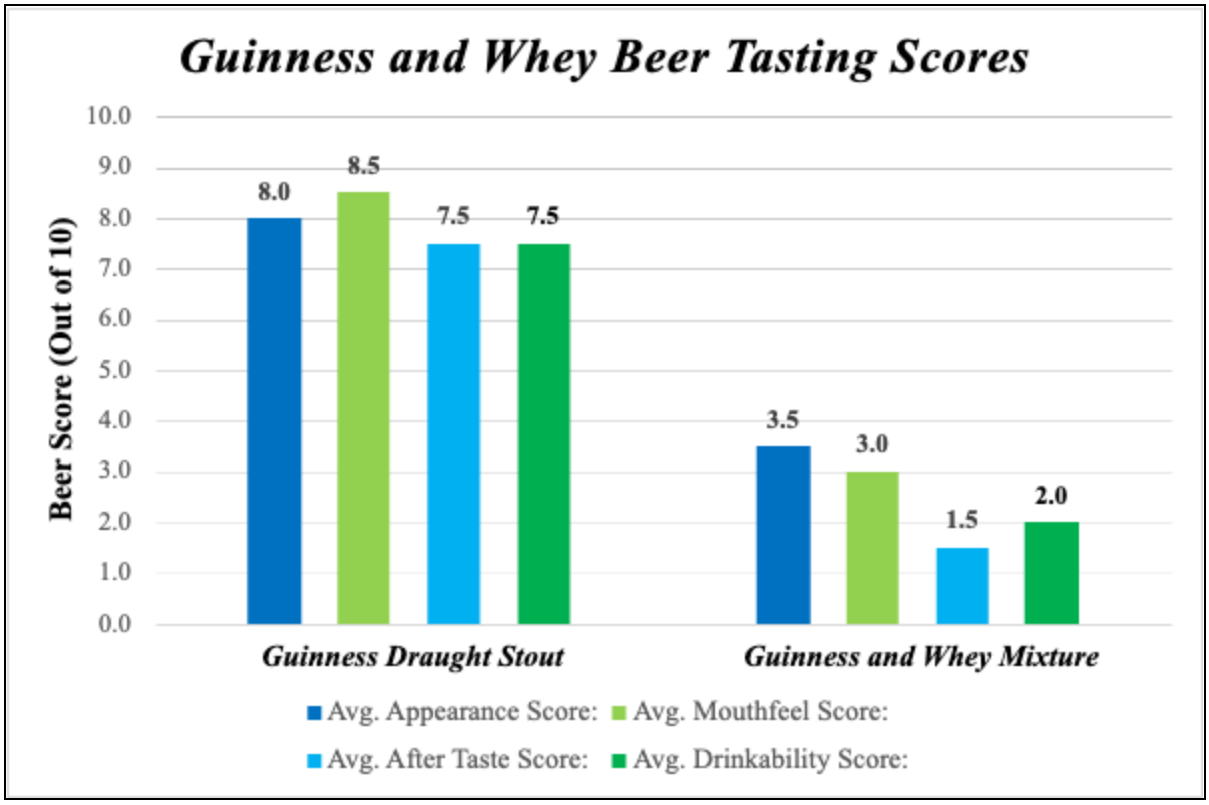
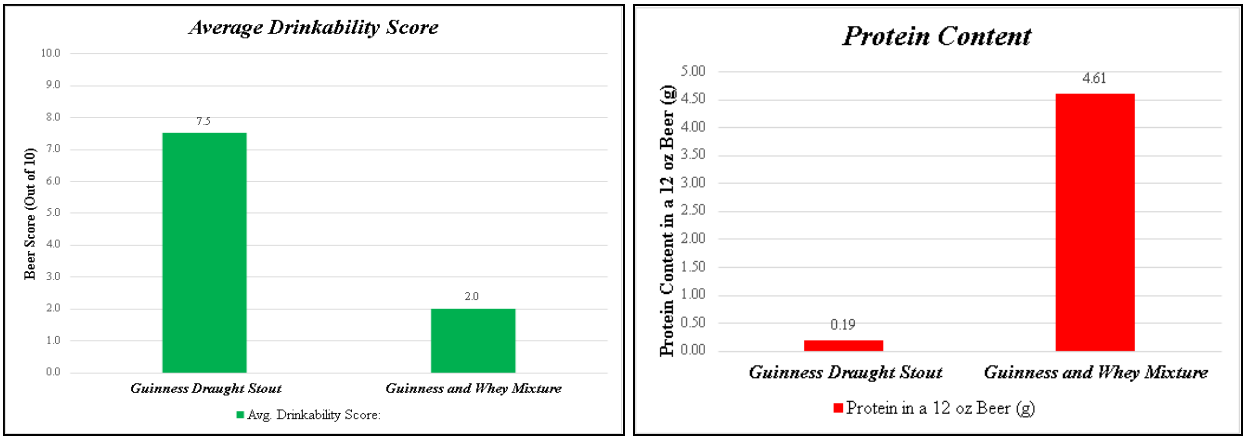


Figure 20: *Figure 20 shows the average appearance, mouthfeel, aftertaste, and drinkability of Guinness Draught Stout compared to the Guinness-Whey mixture.*

As can be seen in Figure 20, the average rating for drinkability of the whey-Guinness mixture was a 2 out of 10 and the aftertaste score was a 1.5. Both of these scores are dramatically lower than the scores given to the pure Guinness stout which led our group to believe that the Guinness-whey mixture would be unmarketable with the current proportions. The mouthfeel was also described as being chalky and chunky despite the small amount of whey added which led to the 3.0 rating out of 10.



Figures 21 & 22: *Figure 21 on the left shows a comparison of the average drinkability score for Guinness Draught Stout and the Guinness and whey mixture. Figure 22 shows a comparison for the amount of protein content in a 12 oz glass of these two beers.*

As can be seen in Figures 21 and 22, the Guinness and whey mixture has much more protein than the control Guinness despite having a much lower drinkability score. Although the Guinness and whey mixture has 4.61 grams of protein in a 12 oz beer, the drinkability score is so low that the amount of whey would have to be decreased to such an insignificant amount in order to make this beer drinkable.

Due to the solubility issues, extremely poor taste, and already low protein contents, the decision was made not to run further testing with lower concentration of whey protein. The likeliness of protein denaturing also ruled out the option of testing for the feasibility of adding whey to beer during the boil. After analyzing the results of the first mixture of whey and Guinness, our group concluded that adding whey protein to beer after fermentation is not a feasible method for increasing protein content in beer and that adding whey just before fermentation likely would not improve the overall taste of the beer. For the reasons outlined above, efforts were shifted towards increasing protein content in beer through the use of leucine and high-protein ingredients.

4.5 Testing Leucine

The feasibility of adding leucine to beer was tested both during the boil and after fermentation. For each of these two tests, the average appearance, mouthfeel, after taste, drinkability, and protein contents were measured.

4.5.1 Leucine Added During Boil

The first attempt at adding leucine to beer was by adding Nutricost L-Leucine powder to a scaled-down homebrew during the boil. Leucine was chosen for testing during the boil because it is not known to sublime until 145°C and doesn't denature until 293°C. This means that the supplement should be able to fully withstand the boil at 100°C. The recommended amount of

leucine powder for a half gallon of water was used in the brew because the goal was to produce a half gallon of beer.

Figure 23: *Figure 23 shows the leucine-stout mixture after the two-week fermentation.*

There were many issues with the leucine brew shown in Figure 23. Despite aiming to produce a half gallon of beer, only about a quarter of a gallon was left after the boil. As discussed in Section 4.2, this is due to the fact that our group did not take into account the difference in surface area of the pot when scaling down the brewing recipe. This miscalculation caused there to be approximately double the recommended concentration of leucine in the beer. Additionally, Figure 23 shows that some beer was found in the airlock. This likely means that too much yeast was added which caused the beer to bubble over and get stuck in the airlock. Since this over-bubbling means that additional beer was leaving the fermenter and not that air was entering the system, it was believed that this error did not have an impact on the resulting taste of the beer.

Following fermentation, the beer was taken to the lab to be tested for protein content. Upon mixing a simple of the beer with the Bradford Assay solution, it was noticed that the mixture did not turn very blue. After comparing the results of the leucine beer with the control batch, we found that the control beer had an average absorbance of 0.359 at a wavelength of 595 nm while the leucine beer only had an absorbance of 0.349. These absorbances both correspond to approximately 0.4 g of protein in 12 oz beer. The likely reason why the leucine beer appeared to have approximately the same protein content as the control beer is because the Bradford Assay

can not detect leucine. This is most likely because leucine is an essential amino acid and not a fully formed protein molecule. Despite still being beneficial to the human body in numerous ways, the molecules in the Bradford Assay reagent are not able to react with the leucine molecules and form the bright blue color which is necessary for protein quantification.

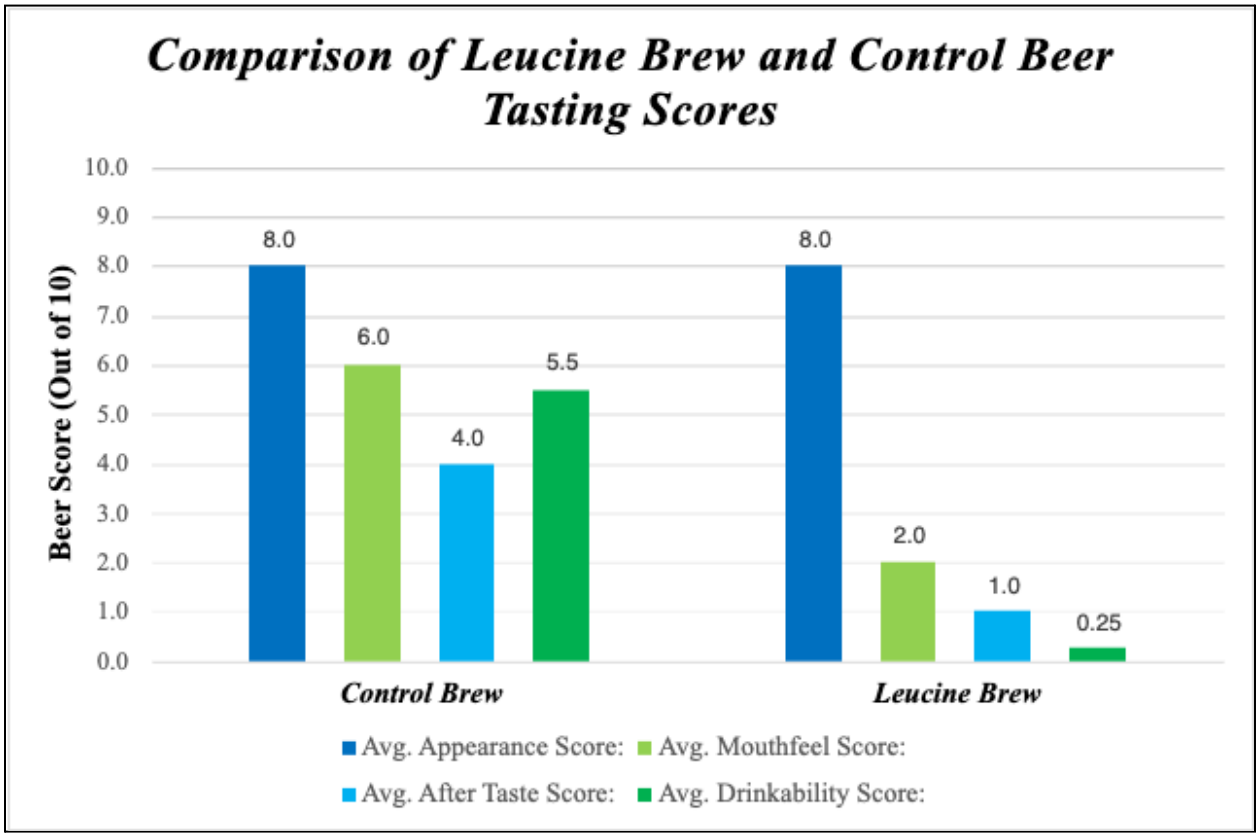


Figure 24: *Figure 24 shows the average appearance, mouthfeel, aftertaste, and drinkability of the leucine brew compared to the control brew.*

Figure 24 shows the leucine beer's taste testing results compared to that of the control batch. The average drinkability, mouthfeel and aftertaste scores for the brew containing leucine were significantly lower than the scores accumulated by the control brew. The team described the beer as having a drinkability score of 0.25 out of 10 and an aftertaste of 1 out of 10. The improper amount of water added to the boil is the most likely reason for the poor taste of the beer. This issue not only caused the concentration of leucine to be double the recommended amount on the package, but it also caused there to be twice as much liquid malt extract in the final brew. This excess malt is likely what contributed to the low mouthfeel score due to the syrupy texture. The appearance of the body of the beer did not seem to be noticeably different from that of the control batch.

Following the results of this test, our group made the decision to suspend further testing on adding leucine during the boil. This decision was made primarily for two reasons: First, since we did not have the resources or timeframe to develop a method to test the amount of leucine in

the beer, we felt that we should focus our efforts around protein that is quantifiable via the Bradford Assay. Second, even when taking into account the issue with the amount of water that boiled off during the brewing process, the taste of the beer was so revolting that we did not feel that this method could easily yield a drinkable beer.

4.5.2 Leucine Added After Fermentation

Although it was determined that the Bradford Assay does not account for leucine since it is an amino acid and not a complete protein, our group tested the feasibility of creating a drinkable beer by adding leucine to a beer post-fermentation. By adding leucine directly to Guinness we were able to make the assumption that all of the leucine being added to the beer was accounted for.

Due to the poor tasting beer in Section 4.4.1, only about 40% (0.5438 g) of the recommended amount of leucine was added to the 100 ml of Guinness. An image of the leucine-Guinness mixture can be seen in Figure 25 below.

Figure 25: *Figure 25 shows a mixture of 0.5438 g of L-Leucine powder and 100 ml of Guinness Draught Stout.*

As can be seen in Figure 25, the addition of leucine to Guinness does not have a significant impact on the color of the body of the beer; however, the mixture does develop a foamy white head. The head of unaltered Guinness is much less prominent and is a darker brown in color. The results of the taste test comparing the leucine-Guinness mixture to control Guinness can be seen in Figure 26 below.

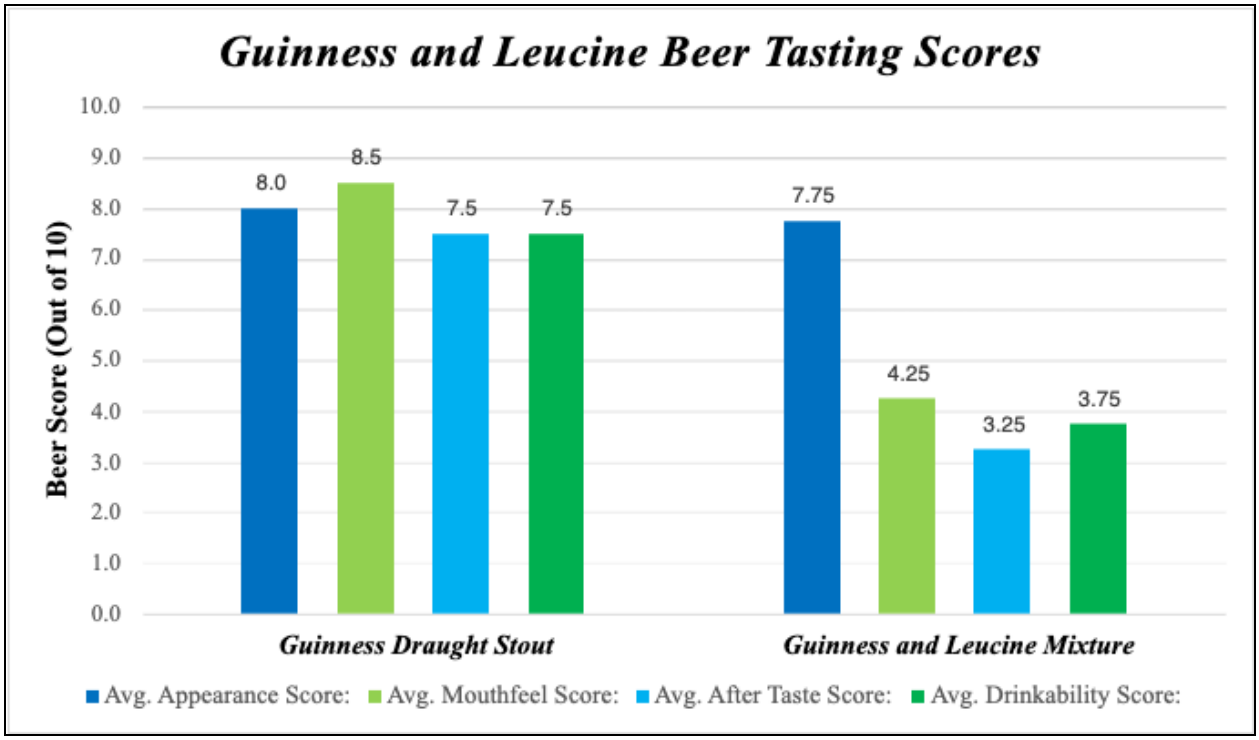


Figure 26: *Figure 26 shows the average appearance, mouthfeel, aftertaste, and drinkability of Guinness Draught Stout compared to the Guinness-Leucine mixture.*

As can be seen in Figure 26, the average rating for drinkability of the leucine-Guinness mixture is 3.75 out of 10 which is almost half of the 6.5 rating that unaltered Guinness received from our group members. Despite this relatively low rating, it is worth noting that 3.75 is significantly higher than the ratings from any of the previous whey or leucine tests. Group members rated the aftertaste of this beer at 3.25 and the mouthfeel at 4.25. All group members who tasted the beer described the mouthfeel as chalky which is likely due to the possibility of undissolved leucine powder in the beer.

From the results outlined above, our group was able to conclude that adding leucine powder to beer post-fermentation is more feasible than adding leucine during the boil or whey post-fermentation. Despite some slightly encouraging signs such as an unaltered body and relatively high taste rating by one group member, the decision was made to shift focus toward brewing with high-protein ingredients. This decision was made because our group felt that lowering the leucine concentration enough to improve the taste and mouthfeel of the beer would sacrifice the supplemental value of adding the leucine. Additionally, since the Bradford Assay is not capable of testing for leucine, there was no accessible way for our group to confirm the protein content in the beer.

4.6 High Protein Ingredients

In addition to introducing whey protein and leucine isolate, our group decided to test the feasibility of adding protein to beer by brewing with high-protein ingredients. The goal is that these ingredients will add protein and increase the flavor profile of the stout by introducing them during the boiling process. Having purchased five different ingredients to test both their effects on protein content and flavor, we created five different microbrews, along with a control, to test how each ingredient performs relative to each other. The tested ingredients included peanuts, peanut butter, quinoa, oats, and hot cocoa powder - all of which were pre-determined to be high in protein.

4.6.1 Adding High-Protein Ingredients During the Boil

To test the feasibility of adding high-protein ingredients to a beer during the boil, ½ gallon batches of beer with an additional cup of peanuts, hot cocoa powder, oats, peanut butter, and quinoa were brewed and their tastes and protein contents were measured. The resulting appearances of these brews plus the control batch can be seen in Figure 27 below.

Figure 27: *Array of each high protein ingredient brew showcasing, from left to right; control, peanuts, hot chocolate powder, oats, peanut butter, quinoa.*

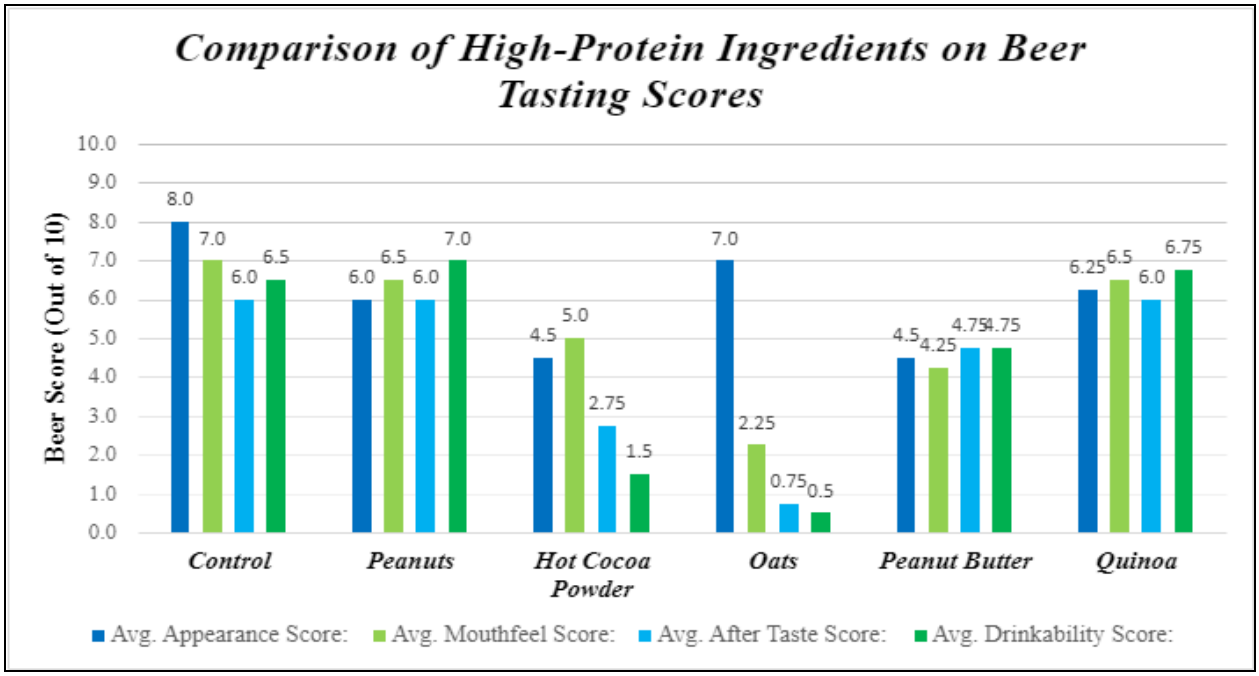


Figure 28: *Qualitative analysis for high protein ingredients added during the boil relative to a Control batch which was used as a baseline for the 4 categories.*

The peanut brew was unanimously regarded as the best tasting beer that was brewed using high-protein ingredients. The peanuts brought a uniquely recognizable flavor masking some of the malt flavor without overpowering the flavor profile. As seen in Figure 28 above, the appearance, mouthfeel and aftertaste were all very similar to the scores of the control brew. The only discrepancy comes from the lack of filtering performed to remove excess peanut pieces from the beer.

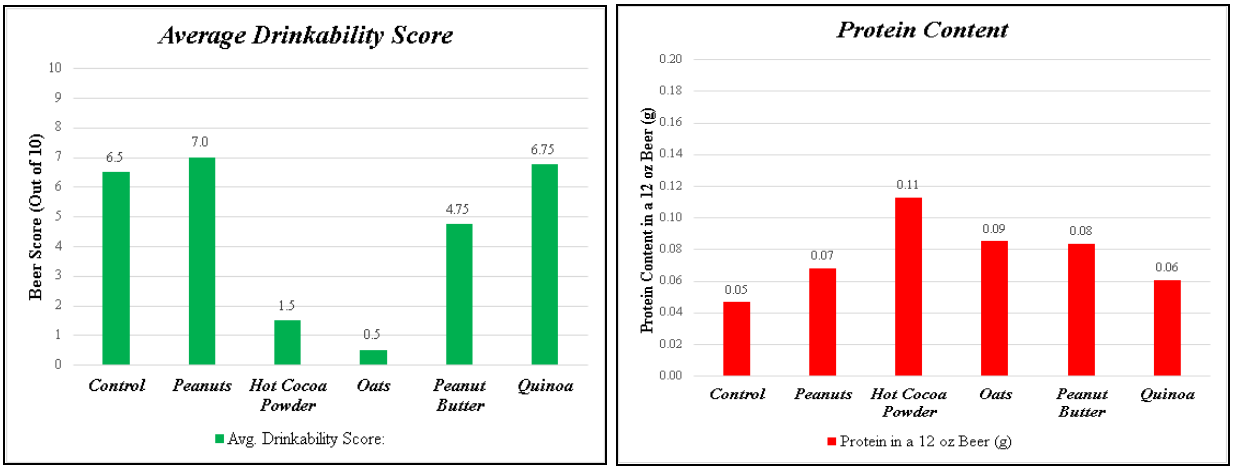
As depicted by the low scores the hot cocoa powder received in regards to appearance, mouthfeel, after-taste and drinkability, we concurred that it was one of the worst performing ingredients in terms of taste in comparison to the other ingredients tested. We believe this is attributed to the solubility of the hot cocoa powder. Its inability to completely dissolve into an ethanol-water solution left the beverage with a chalky/powdery feel, negatively affecting its scoring.

As is suggested by the exceptionally low scores that the oats received in regards to appearance, mouthfeel, after-taste and drinkability, which are presented in Figure 28, we can see that oats were the worst performing ingredient in terms of compromising the integrity of the beer when added during the brew. We believe this is due to the fact that oats absorb a lot of water, and their absorption rate increases at higher temperatures. Therefore, when the oats were able to process while the wort was at a rolling boil they absorbed a large portion of the water, leaving behind a significantly less amount of wort than expected. Due to the fact that we accidentally overlooked this property prior to adding the oats to the brew, the wort that remained in the pot after the hour brew contained a large amount of malt. This ultimately caused the brew to taste

very syrupy, which in turn negatively affected the score this ingredient received in regards to mouthfeel, after-taste and drinkability.

In terms of the peanut butter, although this ingredient had a very similar taste to the peanut brew (as was expected), it received lower scores among the team in terms of appearance, mouthfeel, after-taste, and overall drinkability in relation to the control brew as well as the batch made with peanuts. We suspect that this was due to the large amounts of oil peanut butter possesses. In terms of appearance, the oils in peanut butter negatively affected the score it received due to the fact they caused an unappealing layer to form on the surface of the final product. In addition, although much of the peanut butter eventually settled out during the fermentation period, a large amount of the particles remained in suspension creating a hazy tan color, which was not desired for the stout we sought to create. The suspended particles thought to be produced by the oils in peanut butter also caused the beer to have a thick mouthfeel, which negatively affected the score the brew received for that category along with the category related to its drinkability. Lastly, although the brew tasted similar to peanut brew upon consumption, the after-taste, which we believe was strongly influenced by the oils in the peanut butter, tasted rich and creamy, which was undesirable, as opposed to the burnt/roasted taste the peanut brew generated.

As for the brew that contained quinoa, this brew was unanimously regarded as one of the best beers that we brewed using high-protein ingredients. The reason why it acquired scores very similar to the control batch is due to the fact that the quinoa did not negatively affect the after-taste of the brew by adding unwanted flavor, nor did the introduction of this ingredient negatively affect the mouthfeel and therefore drinkability of the beer. In a sense, the quinoa had no substantial effect on these categories. As a result it received scores similar to those of the control batch in terms of mouthfeel, after-taste and overall drinkability. As for the appearance, it received a slightly lower score than the control brew because some of the particles remained in suspension creating a slightly lighter colored brew, which was “less” desirable then the color of the control brew.



Figures 29 & 30: *Figure 29 on the left shows a comparison of the average drinkability score for the 7 high protein ingredients added during the boil and the control. Figure 30 shows a comparison for the amount of protein content in a 12 oz glass of these 7 beers.*

The control brew from this batch had an average absorbance of 0.128, corresponding to a protein content of 0.0465 grams in a 12 ounce beer. In terms of taste, this beer was generally found to be less prefered than guiness, but still drinkable and acceptable as an adult beverage. This control beer had a smooth mouthfeel and a relatively pleasant aftertaste.

The peanut brew had an average absorbance of 0.168, corresponding to a protein content of 0.0663 grams in a 12 ounce beer. This initial peanut brew shows approximately a 40% increase in protein over the control brew.

The hot chocolate powder brew has an average absorbance of 0.222, corresponding to a protein content of 0.113 grams in a 12 ounce beer. The protein in this beer was the largest amount of any of the samples tested. The hot cocoa beer has a 120% increase in protein content over the control. This batch has the highest protein content which we can assume is due to it being in powder form, allowing for the entire substance to dissolve into solution. Unfortunately, it was voted as the second worst tasting batch. We decided to move on from this ingredient as it very clearly compromised the taste.

The oats brew has an average absorbance of 0.192, corresponding to a protein content of 0.0850 grams in a 12 ounce beer. The oats brew had an 80% increase in protein content over the control. Due to the poor taste of this beer and the relatively small added protein content, our group decided not to conduct any further testing on the feasibility of increasing protein content with oats.

The peanut butter brew has an average absorbance of 0.190, corresponding to a protein content of 0.0834 grams in a 12 ounce beer. The peanut butter beer has a 60% increase in protein content over the control. Due to the relatively low drinkability score and protein content when compared to other beers, peanut butter was determined not to be a feasible ingredient for infusing protein into beer.

The quinoa brew has an average absorbance of 0.156, corresponding to a protein content of 0.0605 grams in a 12 ounce beer. This beer has a 20% increase in protein content over the control. The quinoa had no noticeable effect on the taste of the beer; however, it also resulted in a very low increase in protein content. The lack of added protein content could be because quinoa tends to absorb liquid instead of allowing its protein content dissociate into solution. If a more effective way of extracting protein from quinoa is found, this ingredient could prove valuable in any brew to add protein without any change to the taste.

With all this in mind, we determined peanuts to be the most effective ingredient out of the handful that we tested. Although the protein content in each of these was less than we had hoped for, with the peanuts having the second lowest, they had the best taste. Adding 1 cup of peanuts in a half gallon batch was just a fixed amount to test these ingredients relative to each other. After deciding to move forward with peanuts as the "best" ingredient, we decided to test two more variables: increasing the amount of peanuts during the boil and adding peanuts during fermentation.

4.6.2 Increasing the Amount of High-Protein Ingredients During the Boil

Although it was determined that adding 1 cup of peanuts to a half gallon batch during the boil could not produce enough protein to market the beer as a "protein beer," the team tested whether increasing the amount of peanuts added would have an effect on protein content that would be substantial and effective. To test this variable, 3 cups of peanuts were added to the ½ gallon batch during the boil and the taste and protein content was measured.

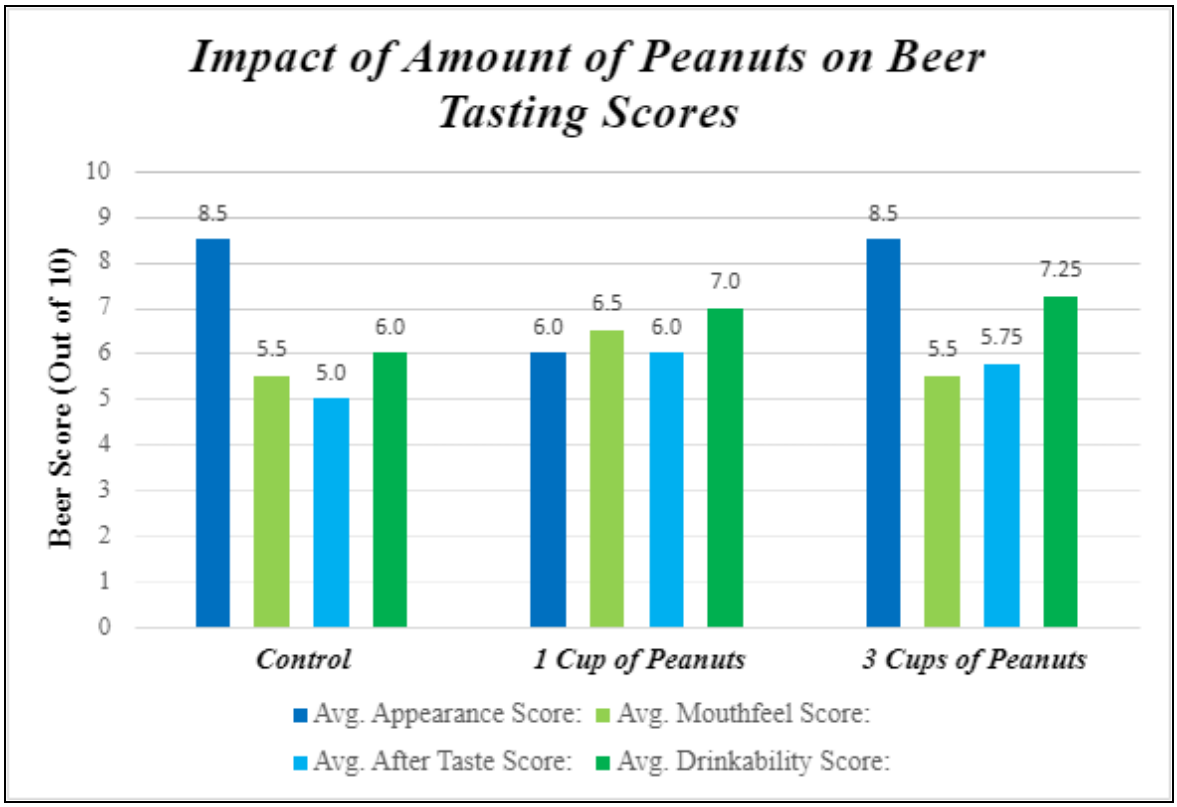
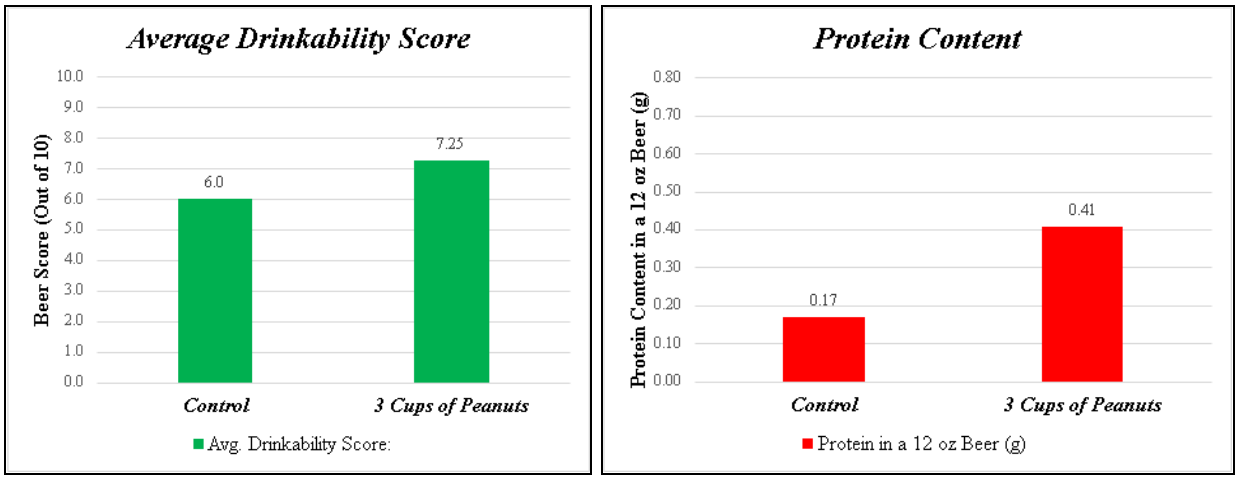


Figure 31: *Figure 31 shows the average appearance, mouthfeel, aftertaste, and drinkability of the stouts brewed with 1 cup of peanuts, 3 cups of peanuts and the control.*

As can be seen in Figure 31, the beers with 1 cup of peanuts and 3 cups of peanuts scored fairly similarly to each other and the control. It is worth noting that the data for "1 Cup of Peanuts" is the same as the peanuts brew from Section 4.6.1 and uses a different control recipe from the one depicted in this figure. The appearance of the brew with 3 cups of peanuts has a higher appearance score because the brew had a darker body. The drinkability score for the brew with 3 cups of peanuts was also the highest score out of the three beers because the peanut flavoring was very enjoyable.



Figures 32 & 33: *Figure 32 on the left shows a comparison of the average drinkability score for the stout with 3 cups of peanuts added during the boil and the control. Figure 33 shows a comparison for the amount of protein content in a 12 oz glass of these two beers.*

We also compared the drinkability scores and protein contents to observe the relationship between the increased amount of peanuts and the control. The brew with 3 cups resulted in an average absorbance of 0.358, which corresponds to a protein content of 0.41 grams of protein in a 12 ounce beer. Compared to the control batch, the brew with 3 cups of peanuts shows approximately a 140% increase in protein content. This increase is significantly more than the 40% increase that the original peanuts brew saw over its control batch. As seen in Figures 32 and 33, the brew with 3 cups of peanuts has a higher drinkability score and protein content than the control batch; however, a protein content of 0.41 g per 12 oz beer is still not enough to market this beverage as a "protein beer." Although this experiment shows that adding more high-protein ingredients leads to an increase in protein content, it would not be economically feasible to continue increasing the amount of peanuts used to obtain a beer with a significant amount of protein.

4.6.3 Adding High-Protein Ingredients After the Boil

The addition of peanuts added after the boil was also tested to determine if protein denaturing was affecting the protein concentration of the other high-protein ingredient beers that had been tested. The results of the taste testing scores for the beers with a cup of peanuts added during and after the boil are compared to a control in Figure 32 below.

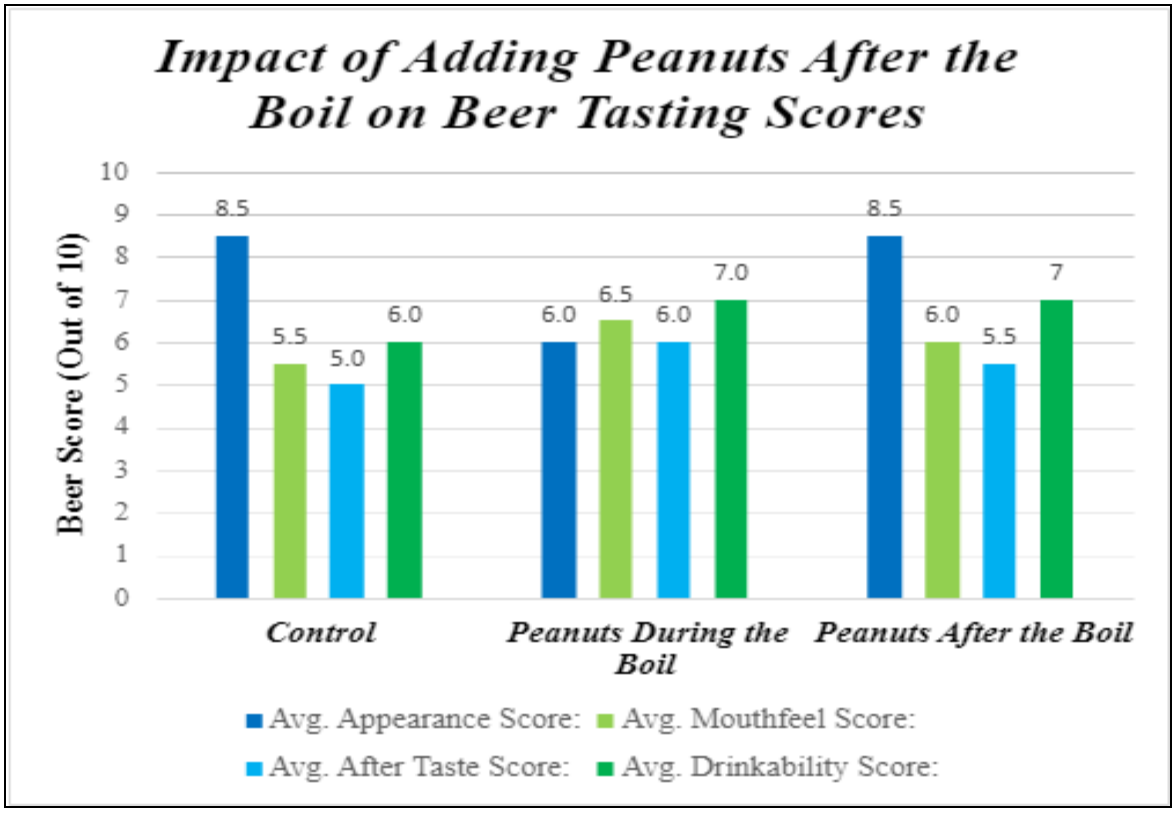
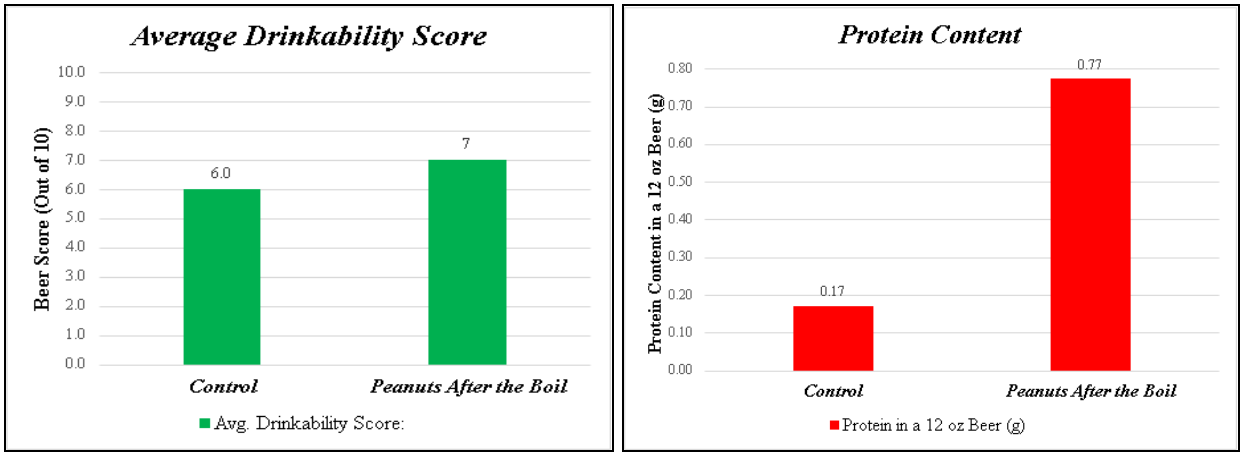


Figure 34: *Figure 34 shows the average appearance, mouthfeel, aftertaste, and drinkability of the stouts brewed with 1 cup of peanuts added during the boil, after the boil, and the control.*

As can be seen in Figure 34, the addition of peanuts during the boil or after the boil does not have much of an effect on the mouthfeel, after taste, or drinkability of the beer. The appearance of the beer with peanuts added during the boil was much darker which led to the lower score for appearance. Once again, this is likely due to the data for "Peanuts During the Boil" being the same as the peanuts brew from Section 4.6.1 which uses a different control recipe from the one depicted in this figure.



Figures 35 & 36: *Figure 35 on the left shows a comparison of the average drinkability score for the stout with peanuts added after the boil and the control. Figure 36 shows a comparison for the amount of protein content in a 12 oz glass of these two beers.*

The drinkability scores and protein contents of the batch with peanuts added after the boil are compared to the control in Figures 35 and 36. The batch with peanuts added after the boil had an average absorbance of 0.426, corresponding to a protein content of 0.77 grams in a 12 ounce beer. This batch shows approximately a 350% increase over the control which is significantly higher than the 40% for the original peanuts brew and 140% for the brew with 3 cups of peanuts. Although we knew some proteins would denature during the boil, we did not know which proteins would denature or how much of them. From this brew, it is clear that the use of high protein ingredients after the boil will result in the most additional protein to be added to the beer without compromising the taste of the beverage.

5 - Conclusions

Throughout this project our group was successful in completing the created objectives to determine which method is most feasible for adding protein to a craft beer. By researching online, a solution of Coomassie Blue G-250 was created and we were able to generate a Beer-Lambert Plot using a set of known protein solutions. This standard curve was then manipulated mathematically to easily determine the measured concentration of protein in a particular sample, based on the measured absorbance of the sample at 595 nm using a UV-Vis spectrometer.

Our group was also introduced into the art of craft brewing, which allowed us to complete our final goals of this project. Our initial brewing recipe was based on an online stout recipe for beginners. We were then able to use engineering principles to improve our process throughout the lifespan of the project. Starting with the basic recipe, we altered the hops, malt, water, and timing of each step in the process as we discovered ways to improve taste and create a better overall home-brewed beer. Additionally, our group was able to successfully scale down from a 1 gallon batch of beer to a ½ gallon batch of beer which necessitates finely tuning the ratio of ingredients rather than simply dividing all of them in half.

Using all of the initial data we collected for the standard curves and new brewing recipes, our group was able to evaluate whether or not we could create a high protein beer without compromising the taste. Our group found that we were able to introduce a high amount of protein into beer using whey powder, but we unanimously found that the beer tasted very chalky and was not feasible to be served to customers. Leucine performed similarly, in that it was still chalky; and with no method to test for its presence in the beverage, our group found it was unusable. Additionally, we were able to infuse a small amount of extra protein into a beer by using other high protein ingredients, the best tasting of which is peanuts. Despite these drinks tasting much better than the whey and leucine, there was not a sufficient amount of protein when tested to market it as a “protein beer.” Our group devised two additional methods to increase protein content using peanuts compared to the control brew. The base increase was about 40%. When we added 3 cups of peanuts instead of 1 cup, this number jumped up to about 140% increase. When peanuts were added after the boil and before fermentation, it increased to about 350%. This leads us to believe that a lot of the protein had denatured after being extracted from the peanuts during the boiling process.

Despite the lack of a final product that can be marketed as a “protein beer,” our group was able to complete our final objective to evaluate the performance of several methods to introduce protein into adult beverages. We were able to discover and identify the many drawbacks outlined above that arise from trying to create a beer with an increased protein content.

6 - Recommendations

Although our team objectives were completed successfully, our group has a multitude of recommendations that we believe can help Purgatory Brewing and any future brewers with continued research and experimentation for the creation of a protein beer. These recommendations come from our experiences and from continued research on the project.

Firstly, our team recommends that any continuation of this research should focus on the addition of amino acids into a beer as opposed to full protein molecules. This is based on our results using the Bradford Assay testing protocol. The Bradford Assay was very useful in determining the presence of full protein structures in solution, but was less useful at determining single amino acids. For example, our leucine trials showed a significant amount less protein than what we added. Since leucine was added post-boil, and leucine does not denature until higher temperatures, we know that it should have a higher absorbance. Upon further research, the downfall of using a Bradford Assay comes with quantifying amino acids in solution. Hence, a method to test for amino acid concentrations as well as full proteins should be investigated in order to retest for protein content. This would also indicate whether the discrepancies between lab tested protein values in Guinness and tabulated values of Guinness were caused by the protein testing protocol or another factor. Guinness reports having between one and two grams of protein in their beverages, which is about 1/5 to 1/10 of the concentration. Assuming this was the same for our 1 cup of peanuts after the boil, the protein content could jump up to 7.7 g, which is much higher and the feasibility of using it as a protein beer is much higher. Exploring the reason our group witnessed this accuracy could ensure future testing is more accurate and is accounted for in the results.

This leads us to the second recommendation our group has for future brewers involved in this project: research how to test for leucine specifically. Our results show very little for leucine, despite being one of the more promising methods to infuse protein into a beer pre-experimentation. Since the coomassie solution could not detect the leucine amino acids, the protein content was much lower than we expected. If we got accurate values for the protein content, our group feels comfortable saying it was one of the better brews we created, given that when we taste tested leucine did not add or detract the taste of the beer, nor did it cause changes to the texture like whey. Assuming a new method to test for leucine is determined, new combinations of high protein ingredients and flavors can be tested with the leucine additive to gauge the possibility if that could be a possible way to create a protein beer.

Our group also recommends that future groups steep grain in the beginning of the brewing process instead of using liquid malt extract. Our group utilized this method of introducing sugar to make the wort because it was quicker for our initial testing. Despite this method working, Purgatory Brewing does not use liquid malt extract, so to truly create a recipe that they can use, other ingredients must be used to ensure feasibility. Additionally, since our group was continuously improving our own recipe throughout the duration of the project, continuing to standardize the recipe will help make the final product consistent, and therefore

improve the feasibility of making a protein beer. Future groups can also combine different additive techniques. For example, if a group finds a way to test for leucine, combining a supplement and high protein ingredients to mask the taste would be a great way to introduce more protein and still have the beverage taste good.

Finally, our group believes that the beverages we made using peanuts would be a great way to add revenue. The peanut stout we created, even with the poor malt extract and overall average brewing techniques, tasted on par with most of the commercial beverages we tasted. Given the possibility of being a good tasting, protein-containing drink, this peanut stout would be a great stepping off point if Purgatory wants to work with another group to continue testing and determine if this beer can be created.

7 - Appendices

7.1 Appendix A: Calculations

Sample Calculation of converting an absorbance value to a protein concentration in a 100 µL:

With an example measured absorbance of 0.300, plug that value in for *y* in equation 1

$$y = 0.1061x + 0.1971 \qquad (1)$$

$$0.300 = 0.1061x + 0.1971, \quad x = 0.1029$$

This value is on the scale of the linearized graph. In order to undo that, you must mathematically undo the natural log function that was used to linearize the graph. In the same equation, a coefficient of 251 is used to scale the concentration from the diluted value which includes the 20 mL of water and 5 mL of Coomassie solution. The *x* value from equation one is now plugged into *x* in equation 2.

$$y = (251)e^{x} \qquad (2)$$

$$y = (251)e^{0.1029}, \quad y = 278.203$$

A measured absorbance of 0.300 represents a concentration of 278.203 µg/mL in a 100 µL sample. Then to get the amount of protein in a beer, µg/mL was converted to g/oz and multiplied by 12 oz for the amount in a 12 oz beer.

8 - Citations

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