THESIS

INFLUENCE OF ROASTED BARLEY ON QUALITY OF BEER

Submitted by

Dave Davis

Department of Food Science and Human Nutrition

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Master's Committee:

Advisor: Martha Stone

Jack Avens
Dale DeVoe

ABSTRACT

INFLUENCE OF ROASTED BARLEY ON QUALITY OF BEER

The research examined the influence of roasted barley content of beers on the quality of the resulting product. Recent researchers have indicated that moderate consumption can be a source of beer specific antioxidants, help reduce the risk of cardiovascular disease, and lower the occurrence of certain types of cancers. Beers were made with two-row malted and six-row unmalted roasted barley.

Brews contained varying levels of the unmalted six-row roasted barley with the balance of the grain bill composed of two-row malted barley. They were analyzed for total phenolic content, sensory properties, and the physical properties specific gravity, color, and calculated alcohol. Four test groups were prepared with four replications of each test group for a total of 16 brews. No significant differences ($P > 0.05$) were found between the test brews for total phenolics. Total phenolics ranged from 314.77 to 451.72 micromoles / 100 milliliters for the test brews. Using Kuskal-Wallis one-way analysis of variance, a significant difference was found (χ^2 =14.328, p = 0.00249) which demonstrated an increase in beer color as the percentage of roasted barley increased for the beer treatments. A significant difference was found in the organoleptic properties of the finished beers for total score, but not in individual categories. Perception of the bitter and burnt characteristics imparted by roasted barley appeared to increase as the percentage of roasted barley increased. This was not necessarily viewed as a detriment to the beer, but as an increase in complexity that added to the overall flavor and balance of the beer. There was a linear progression ($P > 0.05$) in the scores for aroma and appearance as the percentage of roasted barley increased. No significant differences ($P > 0.05$) were found in original or final specific gravity or within calculated alcohol values.

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CHAPTER I

INTRODUCTION

Beer has been a part of human diet and culture for thousands of years. Recent researchers have indicated that moderate consumption can be a source of beer specific antioxidants, help reduce the risk of cardiovascular disease, and lower the occurrence of certain types of cancers (Meister et al., 2000). Beer continues to be a favorite drink for many Americans, with almost 206 million barrels of beer produced in 2009 (Beer Institute, 2010). That equates to 227 twelve-ounce bottles annually for every person living in the United States and over 101 billion dollars in sales. Beer consumption is currently growing in the United States approximately 4% annually (Beer Institute, 2010).

Beer is the third most popular drink in the world after water and tea, and is the oldest, most popular alcoholic beverage (Nelson, 2005). In its simplest form, beer is an alcoholic beverage made from the fermentation of the wort, the liquid extracted from the mashing process during the brewing of beer, derived from the hydration and heating of malted barley. Hops are utilized to augment flavor by adding bitterness.

The number of breweries in the United States has steadily increased from a low of 89 in 1979 to over 2100 in 2012 (Brewers Association, 2012). While the number of large non-craft brewers has remained steady at about 20 over the past decade, the number of regional craft brewers, microbreweries, and brewpubs has been increasing at a rate of about 100 per year (Brewers Association, 2012). With this increase in the number of craft breweries over the past three decades, there has been an increase in the amount of beer available to the consumer.

Many of these new beer choices now offer possible health benefits outside of the traditional view of alcoholic beverages as a cardiovascular dilator. Hops, malts, and barley used

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in beer have all been studied as sources of antioxidants in beer throughout the brewing process. The objective of this study was to examine the effects of roasted barley on the antioxidant content of beer through total phenolic content. This study also examined common analytical and organoleptic measurements of finished beer due to effects of the brewing process, roasted barley, and grain composition. The null hypothesis is that there are no differences in effects of roasted barley on the total phenolic content or quality characteristics of beer.

CHAPTER II

LITERATURE REVIEW

Total Phenolics

Antioxidants are an important part of the human diet and their role in disease prevention

has been of particular interest in recent years. Antioxidants work to reduce substrates by

donating hydrogen or electrons. The compounds also have the ability to reduce molecular

oxygen levels, scavenge free radicals, and chelate prooxidative catalytic metal ions (Bright et al.,

1999). All are important functions either within the human body or in processed foods, or beer,

to act as a natural preservative and provide health benefits. Recent trends have moved

consumers away from added antioxidants like sulfites and ascorbic acid and to natural sources of

endogenous antioxidants, like those found in beer (Omwamba & Hu, 2009).

The primary sources of antioxidants in beer are from barley (Bamforth, 2002). Malted

and un-malted barley contain a wide range of antioxidants including polyphenols, reductones,

and melanoidins (Ghiselli et al., 2000). These compounds will be more concentrated in darker

barley and malt products such roasted barley and crystal malts (Omwamba & Hu, 2009). There

should be no difference in effects of roasted barley on the total phenolic content or quality

characteristics of beer.

Barley

Barley is the main brewing grain used in much of the world for beer production. While

wheat, corn, and rice are common adjuncts used in the modern industry, barley still makes up the

majority of the grain bill in even low price point brands. Special cultivars are used in beer

production and premium prices are paid for this barley over cultivars used in animal feeds. Two-

row and six-row are the varieties used in brewing, with six-row dominating in German style

lagers and two-row dominating in ale brewing (Lewis & Young, 2001).

Two-row barley has only the central spikelet as fertile, which results in the appearance of

two rows of kernels in the ear. In six-row barley, the central and the two lateral spikelets are

fertile resulting in the appearance of six rows of kernels in the ear. This difference results in

distinct physical and chemical properties between the two varieties (Lewis & Young, 2001).

Two-row barley typically results in a plumper kernel that has a larger endosperm and, therefore,

more starch and a higher yield in brewing. Six-row barley typically has a smaller kernel, but a

higher protein content and higher diastatic power.

Most barley destined for use in the brewing industry is malted to make it suitable for beer

production. Without malting, barley lacks the necessary enzymes, amino acids, color, flavor,

starch gelatinization, and dissolution properties to make a desirable product (Briggs, 2002). The

three steps to malting are steeping, germination, and drying. Barley has been shown to be a rich

source of antioxidants in its natural state and in altered states such as malted barley and roasted

barley (Bright et al., 1999). Most of the antioxidants in barley can be classified as phenolic

compounds. Of these, the free phenolics exist as flavanols and tocopherols and the bound

phenolics exist as phenolic acids, ferulic acid, and p-coumaric acid (Omwamba & Hu, 2009).

Steeping

The steeping phase of malting begins with cleaning and sorting barley before it enters the

steeping tanks, which are filled with fresh potable water. As the barley absorbs water, the

moisture content will rise from about 12% to 44%, respiration ensues and enzymes are activated

within the kernel that stimulate embryo development and chemical changes that are important to

the brewer (Lewis & Young, 2001). The initial embryonic growth is initiated by the steeping

and is due to the low amount of food that is available; the grain will secrete enzymes to utilize the stores of energy within the endosperm. This leads to the breakdown of the starch, protein, and the endosperm cell walls to provide initial energy (Briggs, 1998). This is not enough energy for the rapid growth that is occurring in the optimal conditions established by the maltster. The needs are met by the mobilization of the aleurone layer to produce enzymes from complex precursors and from endogenous amino acids (Bamforth, 2006). This action will lead to the further breakdown of the starch and protein contained within the kernel starting at the endosperm end and proceeding to the distal end, and from the outside of the kernel to the inside. During the process, the water in the tank is periodically overflowed and oxygen is pumped through the steeping bed. The process takes between forty and fifty hours and its completion is characterized when the coleorhizae is visible as a white dot penetrating through the husk (Kuntz & Bamforth, 2007).

Germination

The barley is then transferred to germination beds where it remains between three and five days to continue with the modification process (Blenkinsop, 1991). The kernel continues to have its starch and protein degraded. A small portion of the starch will be degraded to glucose to provide food for the germinating cell. The starch, which is present as amlyopectin and amylose, will be cleaved by the amylase enzymes (β, α) into shorter chains, which will be degraded later in the mashing of the grain (Kuntz & Bamforth, 2007). Also, during germination, the activity of certain endopeptidases will increase about twenty-fold and start to degrade the protein contained within the kernel into polypeptides (Bamforth, 2004). These polypeptides will become important contributors to factors such as head retention and foam formation in the finished beer. Other protein degrading enzymes activity also increase. Some of particular importance are the

carboxypeptidases, which will attack the protein where there is a free carboxyl group (Osman, 2003). These enzymes will liberate amino acids such as proline, which will be used by the yeast later in fermentation (Lekkas et al., 2009).

During the process, the bed is continually agitated to prevent bed compaction and the developing acrospires from growing together (Lewis & Young, 2001). The germination process is halted when the maltster determines that there is adequate modification to open up the starch reserves to the brewer without excessively modifying the grain and having loss of extract potential through protein and starch degradation (Lewis & Young, 2001).

Drying

The drying process is accomplished for preservation, flavor development, and to set the enzyme content of the malt. The maltster will take the moisture content from about 44% down to between 3 and 5%. Care has to be taken to avoid the application of high heat, as this will deactivate the enzymatic power of the malt making it useless to the brewer or leading to lower than desired yields within the brewing process. Many of the typical malt flavor attributes are developed with proper drying and kilning (Coghe et al., 2006).

Roasting

Roasted barley starts with cleaned and sorted six-row barley. The grain is roasted at temperatures of about 225 °C until the desired color is achieved (Lewis & Young, 2001). The maltster determines the endpoint of roasting by visually inspecting the barley. The grain still has extract potential of about 72%, but has a diastic power of zero since all of the enzymes that occurred in raw barley had been deactivated by the roasting process (Coghe et al., 2005).

The roasting process is responsible for the formation of many important contributors to flavor, aroma, and visual characteristics for many styles of beers throughout the world. The

roasting is also the source of the phenolic compounds p-hydroxy-benzaldehyde, gallic acid, and vanillic acid, which are responsible for much of the antioxidant capacity of roasted barley. Researchers showed that the concentration of these antioxidants can be optimized through the use of temperature, time, and amount of barley within the drum roaster (Omwamba & Hu, 2009).

Milling

The objectives of milling are particle size reduction and particle size control. To improve yield, the brewer wants to breakdown the endosperm to allow for proper starch dissolution and conversion, but must be careful not to breakdown the husk of the barley (Bamforth, 2000). Doing so would lead to poor extract recovery and increased astringency from the extraction of polyphenolic compounds within the husk (Lewis & Young, 2001).

Roller mills are typically used in brewing and can be simple single roller and plate to complex auto adjusting, multiple pass, six roll mills (Priest & Stewart, 2006). Some breweries also utilize wet mills that will lessen the importance of malt friability by hydrating the husk and allowing for finer milling without the husk being degraded (Kuhbeck et al., 2005). In multiple pass, multiple roller mills, screens are used to allow for an initial rough milling that will separate the husk and endosperm, with the husk passing through any additional milling and going to the mash tun. Smaller pieces such as parts of the endosperm will continue to go through finer milling to increase yield without disturbing the lautering process.

Mashing

Mashing is the process used to hydrate, mix, and steep the grain bill to allow for grain constituent solubilization and conversion. The mashing schedule and effectiveness will be dependent on the grain used and level of modification achieved in malting. Infusion mashing is the simplest and most common mashing technique used and was developed in the British Islands

(Arnold, 2005). The method uses a single temperature for the conversion and is effective with highly modified two-row barley with lower protein content versus six-row. The method may employ a mash-off step to set the carbohydrate profile and deactivate enzymes (Lewis & Young, 2001).

The main rest is between 60º and 70ºC to control the activity of the enzymes α and β-amylase, which will be responsible for the degradation of the starchy component of the barley (Bamforth, 2009). β-amylase catalyzes randomly and hydrolyzes the α 1-4 linkage of glucose molecules in the straight chained amylose and the branched amylopectin starch molecules of the malted barley, except near the α 1-6 branching points of the amylopectin. β-amylase is more active between 60˚ to 65˚C (Lewis & Young, 2001). The α-amylase will act structured, only catalyzing from the non-reducing end and cleaving off units of maltose, a disaccharide, and is active between 65˚ and 70˚C (Bamforth, 2009). The activity of α-amylase is halted at the branch points of the amlyopectin, which are α 1-6 linked (Edney et al., 2007). The results of the mash is a mixture of maltose from the activity of α amylase with the aid of β-amylase, which will increase the number of non-reducing ends, and a number of complex carbohydrates with varying degrees of branching created by both amylases (Lewis & Young, 2001).   The brewer dictates the mixture of resulting carbohydrates by controlling the temperature in a single step infusion. So for a more fermentable wort with less residual sweetness, such as in a Dry Irish Stout (Guinness), a brewer mashes grains at a lower temperature (approximately 62˚C), which results in more maltose creation and less dextrins (Lewis & Young, 2001). If a brewer desired to make a beer with more malty sweetness in the finished product, such as an English Brown Ale (New Castle), they would mash their grains at a higher temperature, (approximately 68˚C), to produce the extra dextrins (Lewis & Young, 2001).

Proteolytic enzymes are also active to a more limited extent during the early stages of the mash and cleave proteins and peptides to make essential nutrients for the yeast to use during various stages of their life cycle (Osman et al., 2002). Other important attributes are being transferred from the solid malt into the aqueous phase of the mash. These were the color and flavor constituents of the malt including the polyphenolic compounds that are a rich source of antioxidants (Bright et al., 1999).

Boiling

Boiling of the wort sterilizes and arrests enzyme activity, concentrates the wort, adds hop characteristics, precipitates protein, and boils off undesirable volatile compounds such as dimethyl sulfide (Lewis & Young, 2001). The most complex biochemical reactions of the whole brewing process take place inside the brew kettle and include redox and Maillard reactions.

The most obvious result of the 60 to 120 minute boils used by most brewers is the near sterilization of the product (Bamforth, 2000). Some thermopillic spores may survive, but they are of little consequence later in the fermentation or finishing processes. Boiling allows the brewer to dictate which microorganism uses the food they created during the mash, usually a monoculture of a specific species of *Saccharomyces cerevisiae*. During the boil, water is evaporated which concentrates the flavor and increases the specific gravity of the resulting beer.

The boil is used to introduce hop character in the form of flavor, aroma, and bitterness. Hops used in brewing are from the flower of the female *Humulus lupulus* plant. The bitterness from hops comes from the two types of resin that they contain, hard and soft (De Keukeleire, 2009). The main component of hop bitterness comes from the alpha acids humulone, cohumulone, and adhumulone that appear in the soft resin and make up between 3 and 15% of the total weight of the hop (De Keukeleire, 2009). These alpha acids will go through

isomerization during the boil to create the iso-alpha acids iso-humulone, iso-cohumulone, and iso-adhumulone that are the bittering compounds perceived in finished beer (Kappler et al., 2010). There are also beta acids that will isomerize during the boil, but contribute little to no bitterness due their poor solubility in wort (Fix, 2000). The solubility of alpha acids and their effect on finished beer bitterness is a function of the time that they are boiled. The longer an alpha acid is boiled, the more isomerization will occur and the more iso-alpha acid that will dissolve into the wort (Fix, 2000). In most cases, a bittering hop addition will be boiled between 60 and 90 minutes and approximately 40% of the total alpha acids contained in the hop will end up contributing bitterness to the finished product (Daniels, 2000).

Hops are also added to impart flavor and aroma to most beers. Some beer styles only have a bittering addition, but styles such as India Pale Ale or American Pale Ale have multiple hop additions throughout the boil. Hop flavor is imparted when the hops are boiled between about twenty and five minutes with any shorter boil resulting in hop aroma. This is due to the volatile nature of the compounds responsible for flavor. The flavor and aroma from hops comes from essential oils that makes up about 0.50% of the hops total weight (Fix, 2000). The major subcategories that make up the essential oils are hydrocarbons, oxygenated hydrocarbons, and sulfur-containing hydrocarbons (Power et al., 1913).

Hydrocarbons make up about 80% of the hop oils and are highly volatile and unlikely to remain unchanged if boiled (Fix, 2000). These compounds are most likely a major contributor to hop flavor and aroma associated with dry hopping, an addition of hops to secondary fermentation. The hydrocarbons will react with oxygen when the hops are added late in the boil to create the oxygenated hydrocarbons. These compounds, such as humulenol and caryophyllene, are responsible for many of the potent hop flavors associated with late hop

additions (Fix, 2000). The sulphur-containing hydrocarbons are seen as a negative contribution and have been described as cooked vegetable or rubber. They are in small amounts and are not considered a major factor (Lewis & Young, 2001).

Boiling also provides a good environment for reactions that aid in color development of the finished product. One major reaction is the Maillard Reaction in which reducing sugars such as maltose, glucose, or fructose and amino acids react with water to create N-substituted glycosylamine that will go through Amadori rearrangement (Hodge et al., 1972). The products of this rearrangement can go through various pathways to form a number of nitrogen-containing polymers called melanoidins, which will have a brown color (Kuntcheva & Obretenov, 1996). Carmelization also occurs if the sugars in the wort are subjected to temperatures of 200˚C or greater (Lewis and Young, 2001). This most likely occurs in a direct fire kettle at the area of greatest heat transfer and aids in darkening the beer and increasing the level of caramel flavor (Lewis & Young, 2001).

Boiling is also needed to coagulate proteins that were extracted from the barley during the mash and subsequent sparge and lautering. Coagulation is usually aided by the addition of a kettle coagulant such as carrageenan or other food gums that simply act as charged molecules that attract the proteins and polypeptides and drop them out of solution (Lewis & Young, 2001). The coagulation that occurs right after the boil is referred to as the hot break (Lewis & Young, 2001).

During boiling the alpha acids within the luplin glands of the hops, go through isomerization to produce iso-alpha acids, which are perceived as bitter by humans (Kappler et al., 2010). The iso-alpha acids are dissolved into the solution by a combination of the vigor and temperature of the boil. Hops also contribute flavor and aroma compounds from the essential

oils, but this is minimized due to the relatively long boiling time (45 minutes) and the volatile nature of the essential oils (Lewis & Young, 2001).

Cooling/Oxygenation

After the wort is boiled per recipe parameters, it is important to quickly cool while maintaining a high level of sanitation. Most brewers accomplish this through a plate and frame heat exchanger or some other type of wort chiller (Singh & Heldman, 2008).   On one side of the plate would be a cooling media such as chill water or glycol and on the other would be the product (Singh & Heldman, 2008). The unit would operate at a higher pressure on the product side to prevent contamination. The temperature on the outlet of the product side would depend on the desired fermentation temperature.

Oxygen is important in the pre-fermentation growth of yeast to get adequate numbers to ferment the beer. Before the yeast is pitched, the wort is oxygenated to provide the optimal conditions for the yeast to multiply by budding (Jones et al., 2007). This is accomplished through the use of an oxygenation stone while the wort is flowing from the wort chiller to the fermenter (Lewis & Young, 2001). The source of the oxygen must be microbiologically clean to prevent contamination.

Fermentation

After boiling the wort is cooled to fermentation temperatures, oxygenated, pumped into a clean and sanitized fermenter, and yeast is added (Lewis & Young, 2001). The two major types of beers, lagers and ales, are fermented with yeast from the *Saccharomyces* genus. Ales will use *Saccharomyces cerevisiae*, which ferment from the top of the vessel and will ferment at temperatures between 15.5˚ and 21.0˚C (Fix, 2000). Lagers use *Saccharomyces cervisiae vars*

uvarum, which ferment from the bottom of the vessel at temperatures between 4.5˚ and 13.0˚C and have the ability to ferment trisaccharides raffinose and melibiose (Fix, 2000).

During the fermentation process, the yeasts actively convert simple sugars created during the mashing process (maltose, glucose, maltotriose) to pyruvate and then to ethanol and carbon dioxide (Stanbury, 1999). The specific gravity of the wort will decrease as alcohol is formed (Fix, 2000). Many important contributors to flavor and aroma are by products of this metabolism (Coghe et al., 2005). The process begins with a lag phase. During this time yeast will utilize intercellular glycogen converted to glucose to fuel reproduction and increase cell counts (Verberlen et al., 2008). Adequate cell counts are important to control the over production of chemical compounds like diacetyl, which in low levels contribute a slight buttery flavor to the beer, but in high levels could lead to an overpowering and out of style flavor profile (Ramirez & Maciejowski, 2007).

The yeast cells then enter a respiration phase and begin uptake of oxygen from the wort. The yeast increases in numbers through cellular division and the phase ends with almost all oxygen in the wort being utilized. At this point true fermentation begins.

The yeast transport monosaccarhides via facilitated diffusion and disaccharides and trisaccharides through a permease system into the yeast cell (Raurio & Londesborough, 2003). Once inside the cell, the carbohydrates go through the biological metabolism known as the Embden-Myerhof-Parnas Pathway (EMP Pathway), which yields pyruvate, from multiple phosphorylations of glucose (Ramirez & Maciejowski, 2007). Pyruvate then goes through enzymatic decarboxylation and reduction to ethanol and carbon dioxide. During this process, many important flavor compounds are also formed including esters (Ramirez & Maciejowski, 2007).

Esters result from the reaction of acetyl-coenzyme A (CoA), alcohols, and andesine-triphosphate (ATP) (Meilgaard, 1975). The most common ester in beer is ethyl acetate, which is the reaction of CoA and ethanol, the most common alcohol in beer (Verstrepen et al., 2003). This compound in low levels results in a slight green apple flavor. Another important ester is isoamyl acetate, which gives a banana flavor and is a major contributor Belgian Dubbles and German Wheat beers (Quilter et al., 2003).

As the fermentation process nears an endpoint due to the depletion of fermentable sugars, the yeast begins to enter a sedimentation phase (Fix, 2000). This is important in the processing of beer since heavily yeasted beer is a strain on transport and filtration systems, and in the flavor maturation of the final product. The brewer monitors specific gravity during the fermentation process and moves the beer or yeast, depending on the fermenter type, when the beer has reached a terminal gravity. If the yeast were left in contact with the beer without an adequate food supply, the yeast would start a process known as autolysis, in which they begin to feed on themselves and produce off flavors that would have a detrimental effect on the final product (Ramirez & Maciejowski, 2007). Fermentation and aging lasts between 7 and 30 days for most beer styles and the product is ready for filtration, carbonation, and packaging.

Evaluation

While taste and aroma can be subjective, a trained human palate is one of the most common and best means of evaluation used in both the macro and micro-brewing industries (Coghe et al., 2004). Taste panels are set up to evaluate beer for meeting style guidelines, looking for off flavors, and to monitor the beer as it ages. As roasted barley levels increase in the grain bill, there are going to be distinct changes in the flavor profile of the beer. The color of the beverage will also increase as the levels of roasted barley and roasted malts increase.

CHAPTER III

MATERIALS AND METHODS

This study examined the antioxidant activity supplied by roasted barley as an ingredient in the brewing process. Beers were made with two-row malted and six-row unmalted roasted barley. They were analyzed for total phenolic content and physical properties specific gravity, color, and calculated alcohol. Four test groups were prepared with four replications of each test group for a total of 16 brews. Brews contained varying levels of the unmalted six-row roasted barley with the balance of the grain bill composed of two-row malted barley.

Total Phenolics

The beers were tested in the laboratory of the Department of Horticulture and Landscape Architecture for total phenolics using the Folin-Ciocalteau assay (Singleton et al., 1999). For the analysis, a standard curve was established by adding 0, 1, 2, 3, 5, and 10 ml of gallic acid stock solution into separate 100 ml flasks, which were then brought to volume with de-ionized water. The gallic acid stock solution was prepared by dissolving 0.500 g of dry gallic acid in 10-ml of ethanol in a 100-ml volumetric flask and diluting to volume with water. These solutions had a phenol concentration of 0, 50, 100, 150, 250, and 500-mg/L gallic acid, the effective range of the assay. From each flask, 20 μl were pipetted into a 3 ml glass cuvette where 1.58 ml de-ionized water and 100 μl of Folin-Ciocalteau reagent were added. The solution was mixed well and allowed to sit for four minutes. Earlier a standard sodium carbonate solution was prepared by dissolving 200 g of anhydrous sodium carbonate in 800 ml of water and the solution was brought to a boil. After cooling, a few crystals of sodium carbonate were added, and after 24 hr, the solution was filtered and water added to bring the volume to 1 L. To each cuvette, 300 μl of the sodium carbonate solution were added with the reagent and gallic acid solutions, and the mixture

was shaken to mix the contents. The cuvettes were held at 20°C for two hours then read to determine absorbance on a Spectronic 20D Spectrophotometer (Waltham, MA) at 765 nm. Plotting absorbance versus concentration established a standard curve and a linear regression was conducted to obtain a slope and intercept.

Samples were tested by pipetting 20 μl of each beer into a 3 ml glass cuvette to which 1.58 ml de-ionized water and 100 μl of Folin-Ciocalteau reagent were added. The solution was mixed well and allowed to stand for four minutes. Then 300 μl of sodium carbonate solution were added to the cuvette and the mixture was shaken. The cuvettes were held at 20°C for two hours then read to determine absorbance on a Spectronic 20D Spectrophotometer at 765 nm. The absorbance was entered into the equation developed with the standard curve and a total phenolic concentration as gallic acid was produced. This was converted to a total phenolic amount in mg/100ml of beer.

Barley

The grain used in the study was two-row malted and six-row roasted barley (unmalted) supplied by Austin Homebrew Supply (Austin, TX) that was acquired from Briess Malt and Ingredient Company (Chilton, WI). The two-row was Breiss's base two-row barley, which is commonly used in both micro-brewing and macro-brewing. The two-row malted barley went through Breiss' typical malting schedule for the production of base malts. The three-step process of steeping, germination, and drying takes the raw barley from the field to a product that is relatively stable and able to be transformed by talented brewers into beer. The six-row roasted barley went through a much simpler process than the malted barley. The six-row roasted barley started with raw barley that was cleaned, sorted, and roasted in a drum roaster. The grain was roasted at temperatures of about 225°C until the desired color was achieved. The maltster

determined the endpoint of roasting by visually inspecting the barley. The grain still had extract potential of about 72%, but diastic power of zero since all of the enzymes that occurred in raw barley had been deactivated by the roasting process.

Milling

The grain was milled using a Phil Mill 2 manufactured by Listermann Manufacturing Company (Cincinnati, OH). This was a single pass, single roller mill that was set at 1.0 mm for the experiment. Grain was visually inspected after milling to insure that the husk was still intact and endosperm was adequately milled to allow for good starch dilution and acceptable yield.

Mashing

A single infusion mash schedule was employed in this experiment due to its simplicity, commonality, and ease of controlling variables. A total of sixteen brews were made with four subgroups of four brews. The brews were made with varying levels of six-row roasted barley to test the grains' ability to influence overall antioxidant activity of the resulting beer. Brews with initial specific gravities of 1.040 were targeted and grain bills with 0.00, 3.33. 6.67, and 10.00% six-row roasted barley were used. The grain bills, mash, sparge water amounts are shown in Table 3.1.

All brews were mashed a 15.2 liter stainless steel combination mash and lauter tun manufactured at the Dairy Farmers of America plant in Fort Morgan, CO. The unit contained a false bottom manufactured by Listermann Manufacturing Company (Cincinnati, OH) and temperature monitoring devices manufactured by Tri Clover, which is a subsidiary of Alfa Leval Corporation (Lund, Sweden). Dairy Engineering Corporation (Arvada, CO) manufactured the outlet port from false bottom to brew kettle (Figure 3.1).

Figure 3.1 Brewing system used to produce beers

For all brews, 21.1°C milled grain was added to 1892 grams of 75.0°C water and stirred

until all grain was adequately wet and no clumping or dough balls were observed. The

Table 3.1. Grain bills, mash, and sparge water amounts used

	Grain Bills			
Test Brew % [a]	Barley		Mash water (g)	Sparge water (g)
	Two-row (g)	Six-row roasted (g)		
0.00	794.20	0.00	1892	2839
3.33	767.75	26.45	1892	2839
6.67	740.99	53.21	1892	2839
10.00	714.78	79.42	1892	2839

[a] Denotes the percentage of six-row unmalted roasted barley; remainder of the grain bill was two-row malted barley.

mash was covered and the temperature was raised by the application of direct heat using a

propane burner to 65.5°C. The mash was maintained at this temperature for 45 minutes via

direct heat and stirring. Temperatures were checked every five minutes and adjusted as needed.

Mash Off/Sparge

After the 45 minutes conversion, 2839 grams of 76.6°C water were added to the mash/lauter tun and the temperature was raised by the application of direct heat to 76.6°C to inactivate the enzymes, set the carbohydrate profile, and to increase extract yield from lautering a warmer mash. The wort was re-circulated through the mash bed to clarify the wort of particles such as husk and grain solids, which would lead to high levels of astringency if boiled in the brew kettle. Once the wort was determined clear by visual inspection, 2.8 liters of unhopped wort was collected into the brew kettle.

Boiling

The brew kettle was a 15.2-liter stainless steel kettle manufactured by Tramontina (Manitowoc, WI) and altered to include an outlet port manufactured by Dairy Engineering Corporation (Arvada, CO). Once the 2.8 liters of unhopped wort were collected in the brew kettle, it was brought to a boil and 6.0 grams of East Kent Golding hops (Kent, England) were added. Beers were designed with a target of 27 International Bittering Units (IBUs).

Whole hops were grown and pelletized in East Kent in England and obtained through Austin Homebrew Supply (Austin, TX). Alpha acids of the hops used in all brews were 6.2% (weight basis). The hops flavor is usually described as earthy and aroma is characterized as a candy-spicy-floral with mild pungency.

Thirty minutes into the boil, 6.0 grams of carrageenan or Irish moss were added to the kettle as a clarifying agent (Austin Homebrew Supply, Austin, TX). This special type of algae is a large electrostatic molecule that helps encourage protein coagulation and decreases the level of trub carried over into the fermenter, which could have adverse effects on fermentation and produce a cloudy final product. Trub is the coalescence of various solids that form during the

boil in the brewing process that consists of lipids, some protein, and vegetative hop compounds (Lewis & Young, 2001).

Cooling

After the forty-five minute boil (15 minutes after the Irish moss was added), the brew kettle was removed from heat and cooled with an immersion style heat exchanger to 21.1°C. The heat exchanger was built using 1.27 cm (½") copper coil. Once cool the hopped wort was tested for specific gravity and transferred to an 1890-milliliter (½ gallon) glass fermenter.

Aeration/Yeast Pitch

After transfer the hopped wort was shaken within the fermenter for 30 seconds to aerate the brew for better yeast performance. Actual fermentation is an anaerobic process. Once cooled and shaken, 2.0 grams of dried yeast was added to each fermenter. Safale S-04 dried yeast (Westport, MA) was used in the experiment. The yeast was a clean, fast fermenter that has good sedimentation, according to the manufacturer Crosby-Baker (Westport, MA).

Fermentation

The brews were allowed to ferment at 21.1°C (+/- 1.5°C) for ten days. After ten days the beers were sampled for specific gravity and organoleptically reviewed by the researcher and placed in brown 354 milliliter (12 ounce) glass bottles for preservation and shipment for analysis such as specific gravity, color, and calculated alcohol. The beers were only naturally carbonated during fermentation since carbonation would need to be removed for analysis and higher levels of carbonation can mask some flavor attributes.

Specific Gravity

All beers were analyzed for starting and final specific gravity and a calculated alcohol value was derived. Specific gravity was tested with a hydrometer and values were temperature

corrected. The final specific gravity samples were cooled to 15.55°C using a VWR (West

Chester, PA) water bath and measured with a hydrometer. Alcohol by weight was derived with

the following equation: alcohol by weight (ABW) = 76.08 (original specific gravity – final

specific gravity)/(1.775- original specific gravity).

Organoleptic

The beers were sensory analyzed organoleptically using the National Homebrew

Competition guidelines (Strong, 2008). The beers were compared for aroma, appearance, flavor,

mouthfeel, and overall impressions and scored on a 50-point scale. The scorecard used is in the

Appendix. All beers were compared to standards for pale ales and sampled at 7ºC (45ºF).

Color

The beers were color analyzed using the Standard Reference Method (SRM) for beer

color (Delang, 2008). A color guide was procured through Beer Color Laboratories (Carmel,

IN). Analyses were performed in approximately 80-footcandles of light with 1 cm of sample in a

standard American pint glass with a white background used on counter tops. The sample color

was visually compared with a reference color and the score noted. A picture of the reference

card is shown in Figure 3.2. The method is based on a reference chart that is standardized for

objective measurements made using a Spectrophotometer at 430 nm for beer samples in a 1 cm

cuvette. The SRM numeric value, an example, and the approximate color are shown in Figure

3.2.

Statistical Analysis

Four replications were produced for each treatment for a total of 16 test brews.

Treatments were the percentage of six-row roasted barley making up the grain bill with the
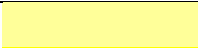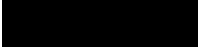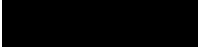
Standard Reference Method Numeric Value	Example	Beer color
2	Light Lager	
3	German Pilsner	
4	Pilsner Urquell	
8	Weissbier	
10	Bass pale ale	
17	Dark lager	
29	Porter	
35	Stout	
70	Imperial stout	

Figure 3.2 Beer color determined by Standard Reference Method

balance coming from two-row malted barley (Table 3.1). For each measurement (specific

gravity, color, organoleptic, and total phenolics) means and standard deviations were calculated

for the four test groups. One-way analysis of variance (ANOVA) was used to determine the

differences among treatments at a P-value of 0.05 for total phenolics and specific gravity.

Kruskal-Wallis one-way analysis of variance was used to determine the differences among

treatments at a P-value of 0.05 for color and organoleptic data. Kruskal-Wallis is a non-

parametric method for comparing more than two samples that are independent. The test does not

identify where the differences occur or how many differences actually occur. Calculations were

performed in Excel.

CHAPTER IV

RESULTS AND DISCUSSION

Color of beers is highly dependent on the malts used in their preparation. The use of

unmalted grains that have been subjected to roasting increased the color of the finished product

even when used in small proportions. In an initial series of tests, the influence of the level of

roasted barley on the color of the beer brewed was assessed. These beers were also tested for the

impact on sensory characteristics and for total phenolic content.

Total Phenolics

Total phenolics were determined using the Folin-Ciocalteau assay (Singleton et al.,

1999). Means and standard deviations were calculated for the data collected from total phenolic

analysis from the four test groups. One-way ANOVA was used to determine the differences

among treatments at $P \leq 0.05$. No significant differences were observed ($F = 0.97$, $p = 0.44$).

Total phenolic data are shown in Figure 4.1.

Bamforth and Omwamba have shown an established link between roasted malt and

barley use in brewing and the antioxidant capacity of the resulting beer. Total phenolics ranged

from 314.77 to 451.72 micromoles / 100 milliliters for the test brews in the current study. The

0% group had a range of 219.60 micromoles / 100 ml while the 10% group had a range of 295.32

micromoles / 100 ml. Using the same Folin-Ciocalteau assay, Dvorakova et al. (2008) found a

wide range of antioxidants in barley and the corresponding malt that could benefit human health.

Di Pietro and Bamforth (2010) examined the antioxidative properties of beer and found that

multiple assays would be needed to truly quantify beers antioxidative properties. In a 2002

study, Bamforth also demonstrated the primary sources of antioxidants in beer were from barley.

Ghiselli et al. (2000) demonstrated that malted and unmalted barley contain a wide range of

23
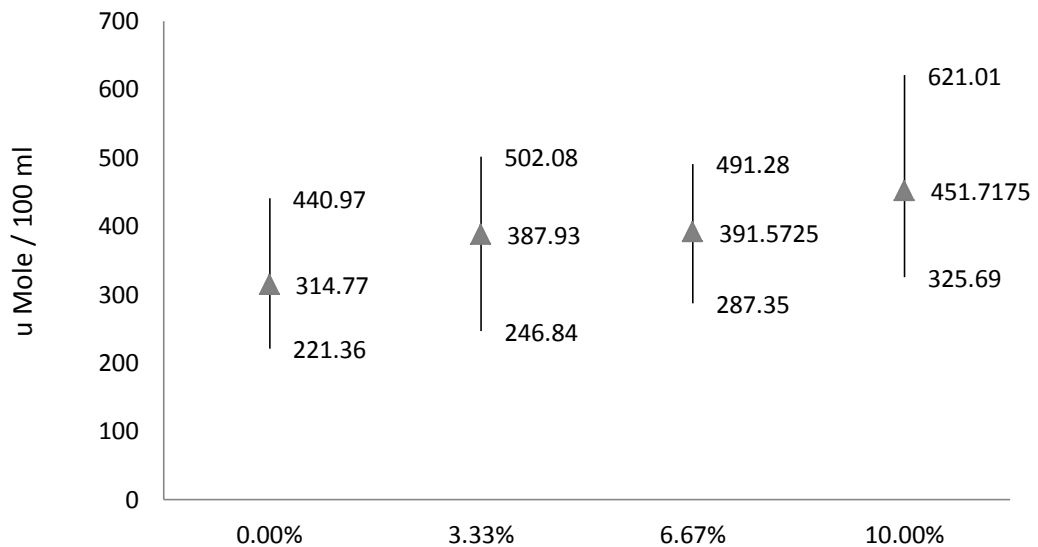
Figure 4.1 Total phenolic content of test beers

antioxidants including polyphenols, reductones, and melanoidins. Omwamba and Hu (2009)

showed that these compounds will be more concentrated in darker barley and malt products such

roasted barley and crystal malt, than in lightly toasted base malts.

Beer may never be a top source for antioxidants, but with optimized brewing practices

and ingredient selection, beer could help supplement the human diet to try and maximize

benefits. For the study, the test beers averaged 15.03 milligrams / 100 ml total antioxidant

activity as measured by the Folin-Ciocalteau assay. Using the same assay, Asami et al. (2003)

found that conventionally grown frozen strawberries contained 240 milligrams / 100 g. Lee at al.

(2001) found black and green tea, well respected sources of antioxidants, contained 124 and 165

milligrams / ml total antioxidants respectively.

Influence of Roasted Barley Level on Beer Color

Four treatments of beer were produced with levels of roasted barley ranging from 0 to

10% with the remainder of the grain bill comprised of two-row malted barley. All other

parameters for the brews were constant. Four replications of brews were produced. Beers were tested using the Standard Reference Method (SRM) for beer color (Delang, 2008). Testing was performed in approximately 80-footcandles of light with 1 cm of sample in a standard American pint glass with a white background used on countertops. Sample color was visually matched with a reference color and the score noted.

Mean color values of the 0, 3.33, 6.67, and 10% test brews were 4.75, 8.75, 12.00, and 19.75 standard reference methods (SRM) units, respectively. Colors of the test brews ranged from a light straw color typically of an American standard lager for the 0% test brew to a deep reddish black typical of a dry stout for the 10% test brew. Kruskal-Wallis one-way analysis of variance was used to determine if differences existed among treatments. A significant difference was found (χ^2 =14.328, p = 0.00249) which demonstrated an increase in color as the percentage of roasted barley increased for the beer treatments. The average SRM data for the test brews are in Figure 4.2. All of the test brews had colors that were in line with commercial examples with similar levels of roasted malts and barley and all were considered acceptable.

Other researchers found a positive relationship between an increase in the percentage of roasted malt and barley and an increase in color. In 2003, Coghe et al. found an increase in beer color as the proportion of roasted malt increased in the grain bill. The color of the malt and the beers produced from the roasted malt was directly tied to the temperatures used during roasting and the percentage of roasted malt making up the grain bill (Coghe et al., 2006). In the studies by Coghe et al. and the current study, an increase in beer color as measured in reference methods and a visual appearance of darker color were observed as the percentage of roasted malt

Beer Color

	0.00%	3.33%	6.67%	10.00%
■ Beer Color	4.75	8.75	12.00	19.75
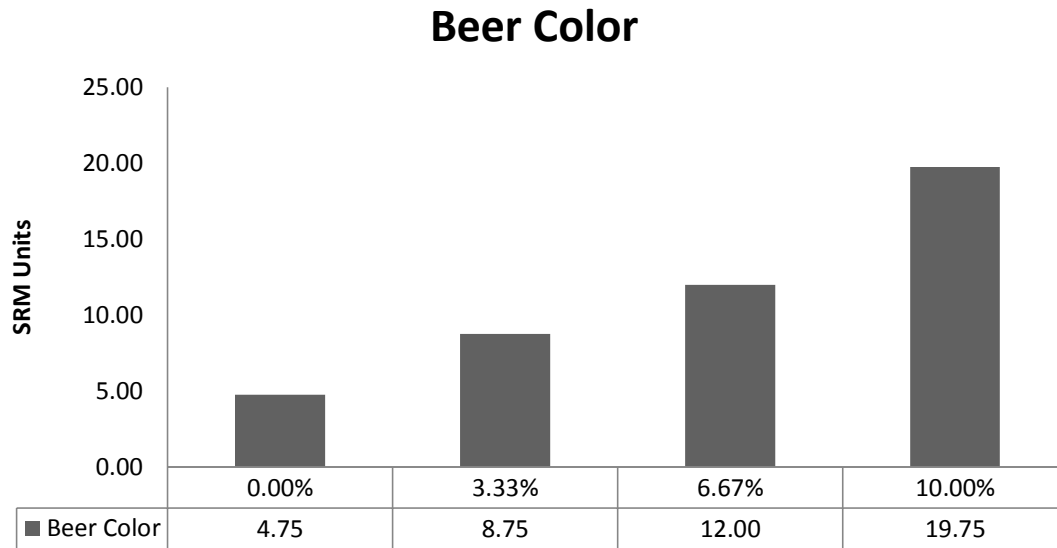
Figure 4.2 Beer color means as determined by the Standard Reference Method was increased.

Influence of Roasted Barley Level on Organoleptic Properties

The beers were reviewed organoleptically by the researcher using the National Homebrew Competition Guidelines (Strong, 2008). The beers were tested for aroma, appearance, flavor, mouthfeel, overall impressions and scored on a 50-point system. The total score is shown in Figure 4.3. Kruskal-Wallis one-way analysis of variance was used to determine the differences among treatments for each individual category at $P \leq 0.05$. The aggregate scores of the 0, 3.33, 6.67, and 10% test brews were 29.50, 30.50, 32.00, and 32.75, respectively. For aroma no significant difference was found ($\chi^2 = 6.19$, p = 0.10). For appearance no significant difference was found ($\chi^2 = 3.00$, p = 0.39). For flavor no significant difference was found ($\chi^2 = 2.00$, p = 0.57). For mounthfeel no significant difference was found ($\chi^2 = 2.14$, p = 0.54). For overall impression no significant difference was found ($\chi^2 = 4.89$, p = 0.18).

Organoleptic data for the test brews are shown in Figures 4.3 and 4.4. The addition of roasted barley did not significantly influence the sensory properties of the beer produced in individual categories. There were significant results as determined by the Kruskal-Wallis procedure on the total score ($\chi^2 = 8.38$, p = 0.038). In general, the perception of the bitter and burnt characteristics that roasted barley imparts increased as the percentage of roasted barley increased. This was not necessarily viewed as a detriment to the beer, but as an increase in complexity and added to the overall flavor and balance of the beer.

As seen with the data for color, as the roasted barley level increased there was an overall increase in SRM units and an increase in total score which indicated a product that could be viewed as a superior product by some consumers. Some of this perception would be subjective and based on the consumer preference for beer style, color and taste, but increased flavor and

	1
■ 0%	29.50
■ 3.33%	30.50
■ 6.67%	32.00
■ 10.00%	32.75
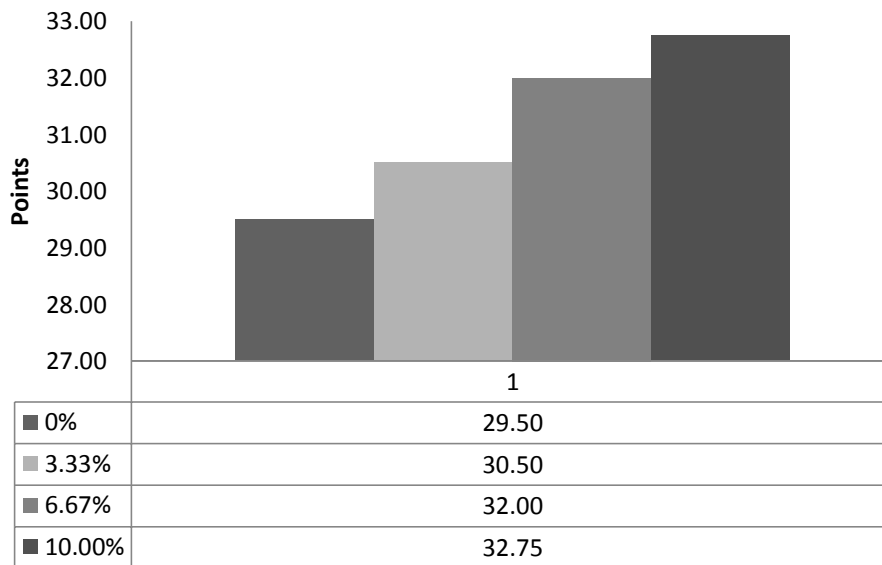
Figure 4.3 Total scores from organoleptic review

complexity would be a benefit in most structured taste panels. This increase in score can also be seen in aroma, appearance, and overall impression scores. Scores for flavor and mouthfeel did not have this trend.

Along with yeast and hops choices, the grains and their proportions used in a formulation have long dictated beer style and ultimately beer flavor along with the brewing practices.   In a 2004 study, Martens et al. found an increase in the bitter and burnt flavor perception as the color of the wort increased. They also perceived an increase in the sweet and husky flavors in lighter colored worts. With intermediate color, the caramel and bread flavors were more pronounced. Kuntcheva and Obretenov (1996) also observed an increase in caramel and other flavors associated with Maillard end products in finished beer as the level of roasted and caramel malts and barley was increased. In the organoleptic analysis of beer in this study, the level of perceived bitterness increased with roasted barley levels. The level of hops used in the study was constant for all brews, so an increase in α-acids from the hops was unlikely to contribute to this bitterness. The test brews in the study also demonstrated similar flavor profiles. The brews with lower levels of roasted barley demonstrated more husky and malt sweetness and the brews with higher levels of roasted barley demonstrated more burnt flavors.

	Aroma	Appearance	Flavor	Mouthfeel	Overall Impressions
■ 0%	8.00	2.00	10.25	3.00	6.25
■ 3.33%	8.50	2.25	10.00	2.75	7.00
■ 6.67%	8.75	2.50	11.25	2.75	6.75
■ 10.00%	9.25	2.75	10.25	3.00	7.50
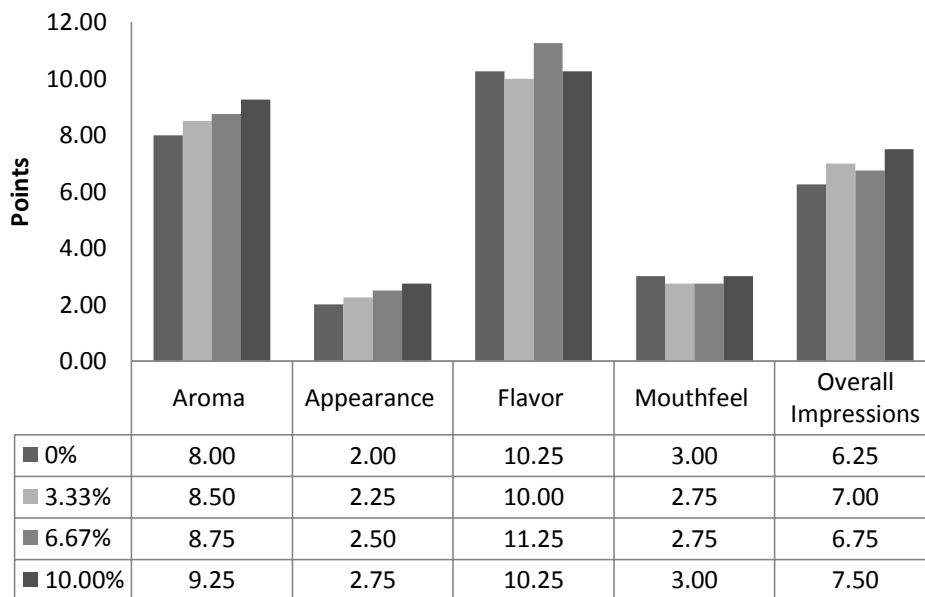
Figure 4.4 Category scores from organoleptic review

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Physical Characteristics

Means and standard deviations were determined from the four test groups for starting (SG) and final (FG) gravities measured with a hydrometer. Calculated alcohol by weight was obtained for each test brew with the equation ABW = 76.08(SG-FG)/(1.775-SG). Means and standard deviations for these calculated values were derived. One-way ANOVA was used to determine the differences among treatments at $P \leq 0.05$ for starting and final gravities. No significant differences were observed for starting gravity (F =1.41, p = 0.28). No significant differences were observed for final gravity (F =1.24, p = 0.34). The starting and final gravity data for the test brews are shown in Figure 4.5 and the calculated alcohol data are outlined in Figure 4.6. Starting gravities ranged from 1.0378 to 1.0400, while final gravities ranged from 1.0055 to 1.0070. Standard deviations for starting gravities for the 0, 3.33, 6.67, and 10% test brews were 0.002, 0.002, 0.002, and 0.001 respectively. Standard deviations for final gravities for the 0, 3.33, 6.67, and 10% test brews were 0.002, 0.001, 0.001, and 0.001 respectively. Calculated alcohol by weight ranged from 3.308 to 3.442 % with standard deviations for the 0, 3.33, 6.67, and 10% test brews were 0.049, 0.044, 0.047, and 0.034 respectively.

No significant differences in the physical properties of the test brews was a positive outcome to isolate the anitoxidant and sensory properties. Research by Coghe et al. (2005) showed a decrease in the attenuation as the percentage of roasted malts and barley was increased in the grain bills of beer. The effect was usually small and due to the lower levels of fermentable sugars in the roasted malts and barley. Blenkinsop (1991) also demonstrated a potential for wort differences when the percentage of roasted malts and barley were manipulated in the grain bills

	0.00%	3.33%	6.67%	10.00%
■ Starting Gravity	1.0400	1.0390	1.0395	1.0378
■ Final Gravity	1.0068	1.0070	1.0065	1.0055
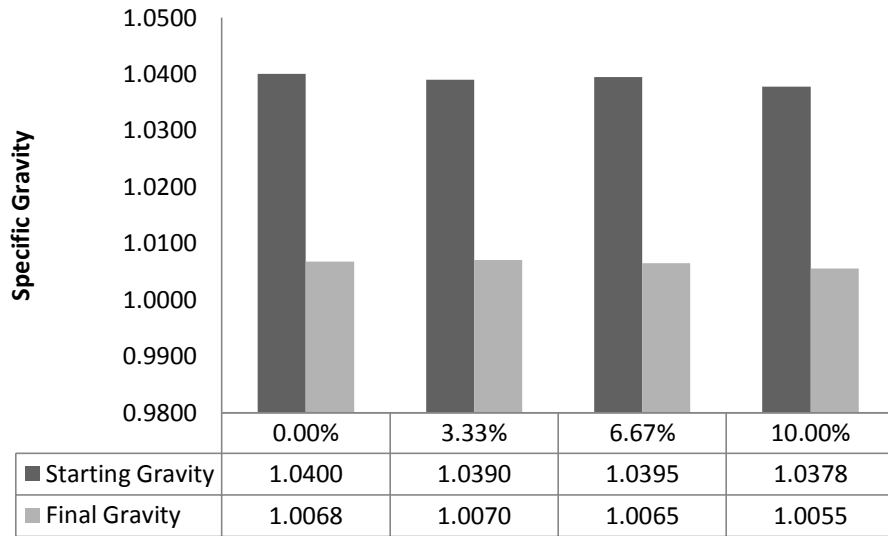
Figure 4.5 Mean starting and final specific gravities for test brews

	0.00%	3.33%	6.67%	10.00%
■ Calculated Alcohol	3.44	3.31	3.41	3.33
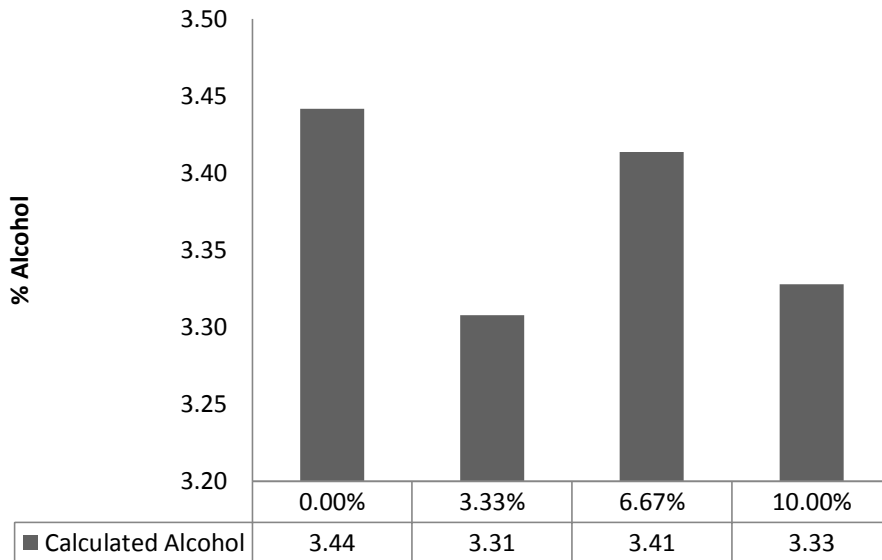
Figure 4.6 Percentage alcohol calculated from specific gravities

of beer, but this can also be controlled through the selected mashing profile that can effect the

level of fermentable and non-fermentable sugars created.

CHAPTER V

CONCLUSIONS

This research examined the influence of roasted barley levels on total phenolic content of beer to highlight the positive health benefits associated with moderate consumption of beer. No significant conclusions on total phenolic content can be drawn from this research ($P > 0.05$). Consumers will benefit equally from an antioxidant perspective in beers with little to no roasted barley content to beers with 10% roasted barley content.

As the proportion of roasted barley increased, there was an increase in final product color with the beers with higher levels of roasted barley demonstrating a darker color ($P < 0.05$). Researchers also found a significant differences in the total score organoleptic properties of the finished beers ($P = 0.038$). In general, the perception of the bitter and burnt characteristics that roasted barley imparts increased as the percentage of roasted barley increased. This was not necessarily viewed as a detriment to the beer, but as an increase in complexity and added to the overall flavor and balance of the beer. No significant differences were found in original or final gravity or within calculated alcohol.

The research indicated that beers brewed with roasted barley could be an important source of antioxidants through total phenolics for the human diet and more research is warranted. Beer continues to be an alcoholic beverage of choice for millions of Americans and getting Americans to make healthier choices could benefit the health of the individual and society as a whole. A future study with more replicates could help reduce variation and help determine significant differences that would allow for stronger conclusions.

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APPENDIX

BEER SCORESHEET

http://www.bjcp.org　　AHA/BJCP Sanctioned Competition Program　　http://www.homebrewersassociation.org

Judge Name (print) _____

Judge BJCP ID _____

Judge Email _____
Use Avery label # 5160

BJCP Rank or Status:
- ☐ Apprentice ☐ Recognized ☐ Certified
- ☐ National ☐ Master ☐ Grand Master __
- ☐ Honorary Master ☐ Honorary GM ☐ Mead Judge
- ☐ Provisional Judge ☐ Rank Pending

Non-BJCP Qualifications:
- ☐ Professional Brewer ☐ Beer Sommelier ☐ Non-BJCP
- ☐ Certified Cicerone ☐ Master Cicerone
- ☐ Sensory Training ☐ Other _____

Descriptor Definitions (Mark all that apply):
- ☐ **Acetaldehyde** – Green apple-like aroma and flavor.
- ☐ **Alcoholic** – The aroma, flavor, and warming effect of ethanol and higher alcohols. Sometimes described as *hot*.
- ☐ **Astringent** – Puckering, lingering harshness and/or dryness in the finish/aftertaste; harsh graininess; huskiness.
- ☐ **Diacetyl** – Artificial butter, butterscotch, or toffee aroma and flavor. Sometimes perceived as a slickness on the tongue.
- ☐ **DMS (dimethyl sulfide)** – At low levels a sweet, cooked or canned corn-like aroma and flavor.
- ☐ **Estery** – Aroma and/or flavor of any ester (fruits, fruit flavorings, or roses).
- ☐ **Grassy** – Aroma/flavor of fresh-cut grass or green leaves.
- ☐ **Light-Struck** – Similar to the aroma of a skunk.
- ☐ **Metallic** – Tinny, coiny, copper, iron, or blood-like flavor.
- ☐ **Musty** – Stale, musty, or moldy aromas/flavors.
- ☐ **Oxidized** – Any one or combination of stale, winy/vinous, cardboard, papery, or sherry-like aromas and flavors.
- ☐ **Phenolic** – Spicy (clove, pepper), smoky, plastic, plastic adhesive strip, and/or medicinal (chlorophenolic).
- ☐ **Solvent** – Aromas and flavors of higher alcohols (fusel alcohols). Similar to acetone or lacquer thinner aromas.
- ☐ **Sour/Acidic** – Tartness in aroma and flavor. Can be sharp and clean (lactic acid), or vinegar-like (acetic acid).
- ☐ **Sulfur** – The aroma of rotten eggs or burning matches.
- ☐ **Vegetal** – Cooked, canned, or rotten vegetable aroma and flavor (cabbage, onion, celery, asparagus, etc.)
- ☐ **Yeasty** – A bready, sulfury or yeast-like aroma or flavor.

Category # _____ **Subcategory (a-f)** _____ **Entry #** _____

Subcategory (spell out) _____

Special Ingredients: _____

Bottle Inspection: ☐ Appropriate size, cap, fill level, label removal, etc.

Comments _____

Aroma (as appropriate for style)　　　　　　　　_____ /12
Comment on malt, hops, esters, and other aromatics
_____

Appearance (as appropriate for style)　　　　　_____ / 3
Comment on color, clarity, and head (retention, color, and texture)
_____

Flavor (as appropriate for style)　　　　　　　_____ /20
Comment on malt, hops, fermentation characteristics, balance, finish/aftertaste, and other flavor characteristics
_____

Mouthfeel (as appropriate for style)　　　　　_____ / 5
Comment on body, carbonation, warmth, creaminess, astringency, and other palate sensations
_____

Overall Impression　　　　　　　　　　　　_____ /10
Comment on overall drinking pleasure associated with entry, give suggestions for improvement
_____

Total　　　　　　_____ /50

SCORING GUIDE		
Outstanding	(45 - 50):	World-class example of style.
Excellent	(38 - 44):	Exemplifies style well, requires minor fine-tuning.
Very Good	(30 - 37):	Generally within style parameters, some minor flaws.
Good	(21 - 29):	Misses the mark on style and/or minor flaws.
Fair	(14 - 20):	Off flavors/aromas or major style deficiencies. Unpleasant.
Problematic	(00 - 13):	Major off flavors and aromas dominate. Hard to drink.

Stylistic Accuracy
Classic Example ☐ ☐ ☐ ☐ ☐ Not to Style
Technical Merit
Flawless ☐ ☐ ☐ ☐ ☐ Significant Flaws
Intangibles
Wonderful ☐ ☐ ☐ ☐ ☐ Lifeless

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Figure A.1 Beer Scoresheet

Organoleptic Raw Data

Table A.1 Organoleptic Raw Data

Identity	Aroma (12 pts)	Appearance (3 pts)	Flavor (20 pts)	Mouthfeel (5 pts)	Overall (10 pts)	Total (50 pts)
0% #1	8	2	11	3	6	30
0% #2	7	2	11	3	7	30
0% #3	8	2	9	3	5	27
0% #4	9	2	10	3	7	31
0%	8.00	2.00	10.25	3.00	6.25	29.50
Standard Deviation	0.82	0.00	0.96	0.00	0.96	1.73
3.33% #1	8	2	11	2	6	29
3.33% #2	9	2	11	3	7	32
3.33% #3	9	2	8	3	8	30
3.33% #4	8	3	10	3	7	31
3.33%	8.50	2.25	10.00	2.75	7.00	30.50
Standard Deviation	0.58	0.50	1.41	0.50	0.82	1.29
6.67% #1	9	2	12	3	6	32
6.67% #2	9	3	11	3	7	33
6.67% #3	8	3	13	2	7	33
6.67% #4	9	2	9	3	7	30
6.67%	8.75	2.50	11.25	2.75	6.75	32.00
Standard Deviation	0.50	0.58	1.71	0.50	0.50	1.41
10.00% #1	9	3	11	3	7	33
10.00% #2	9	3	11	3	8	34
10.00% #3	10	3	9	3	7	32
10.00% #4	9	2	10	3	8	32
10.00%	9.25	2.75	10.25	3.00	7.50	32.75
Standard Deviation	0.50	0.50	0.96	0.00	0.58	0.96

Phenolic Raw Data

Table A.2 Phenolic Raw Data

Identity	Total Phenolics u Mole/ 100 ml
0% #1	306.65
0% #2	221.36
0% #3	440.97
0% #4	290.10
Average	314.77
Standard Deviation	91.88
Identity	Total Phenolics u Mole/ 100 ml
3.33% #1	246.84
3.33% #2	502.08
3.33% #3	479.96
3.33% #4	322.84
Average	387.93
Standard Deviation	123.35
Identity	Total Phenolics u Mole/ 100 ml
6.67% #1	315.58
6.67% #2	472.08
6.67% #3	491.28
6.67% #4	287.35
Average	391.57
Standard Deviation	104.98
Identity	Total Phenolics u Mole/ 100 ml
10.00% #1	325.69
10.00% #2	481.78
10.00% #3	621.01
10.00% #4	378.39
Average	451.72
Standard Deviation	130.15

Physical Characteristics Raw Data

Table A.3 Physical Characteristics Raw Data

Test Brew	Starting Gravity	Final Gravity	Calculated Alcohol
0% #1	1.038	1.005	3.407
0% #2	1.041	1.008	3.420
0% #3	1.039	1.006	3.411
0% #4	1.042	1.008	3.529
Average	1.040	1.007	3.442
Standard Deviation	0.002	0.002	0.058
Test Brew	Starting Gravity	Final Gravity	Calculated Alcohol
3.33% #1	1.037	1.005	3.299
3.33% #2	1.039	1.007	3.308
3.33% #3	1.039	1.008	3.204
3.33% #4	1.041	1.008	3.420
Average	1.039	1.007	3.308
Standard Deviation	0.002	0.001	0.088
Test Brew	Starting Gravity	Final Gravity	Calculated Alcohol
6.67% #1	1.039	1.006	3.411
6.67% #2	1.042	1.007	3.633
6.67% #3	1.038	1.007	3.200
6.67% #4	1.039	1.006	3.411
Average	1.040	1.007	3.414
Standard Deviation	0.002	0.001	0.177
Test Brew	Starting Gravity	Final Gravity	Calculated Alcohol
10.00% #1	1.038	1.006	3.303
10.00% #2	1.036	1.004	3.294
10.00% #3	1.038	1.006	3.303
10.00% #4	1.039	1.006	3.411
Average	1.038	1.006	3.328
Standard Deviation	0.001	0.001	0.056

Starting Gravity ANOVA

Table A.4 Starting Gravity ANOVA

SUMMARY

Groups	Count	Sum	Average	Variance
0	4	4.16	1.04	3.33333E-06
3.33	4	4.156	1.039	2.66667E-06
6.67	4	4.158	1.0395	3E-06
10	4	4.151	1.03775	1.58333E-06

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.1187E-05	3	3.73E-06	1.409448818	0.28806	3.4903
Within Groups	3.175E-05	12	2.65E-06			
Total	4.2937E-05	15				

Final Gravity
ANOVA

SUMMARY

Groups	Count	Sum	Average	Variance
0	4	4.027	1.00675	2.25E-06
3.33	4	4.028	1.007	2E-06
6.67	4	4.026	1.0065	3.33333E-07
10	4	4.022	1.0055	1E-06

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	5.1875E-06	3	1.73E-06	1.23880597	0.338701	3.4903
Within Groups	1.675E-05	12	1.4E-06			
Total	2.1937E-05	15				

Phenolic ANOVA

Table A.5 Phenolic ANOVA

SUMMARY

Groups	Count	Sum	Average	Variance
0	4	1259.088	314.7719	8442.16
0.0333	4	1551.72	387.9299	15215.07
0.0667	4	1566.282	391.5705	11020.21
0.1	4	1806.866	451.7165	16940.19

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	37703.47783	3	12567.83	0.973917	0.437124	3.4903
Within Groups	154852.9141	12	12904.41			
Total	192556.3919	15				

Color Kruskal-Wallis

Table A.6 Color Kruskal-Wallis

	Summary Statistics on Input Data				
	0	3.33	6.67	10	
Count of Observations:	4	4	4	4	Total Sample Size = 16
Sample Sum:	19	35	48	79	
Sample Mean:	4.75	8.75	12	19.75	
Sample Standard Deviation:	0.5	0.5	0.816497	1.258306	

	Summary Statistics on Ranks & Test Results				
	0	3.33	6.67	10	
Count of Observations:	4	4	4	4	Total Sample Size = 16
Rank Sum:	10	26	42	58	
Rank Average:	2.5	6.5	10.5	14.5	Overall Rank Average = 8.5
Number of Tie Series:	10				
Average Number of Ties by Series:	1.6				
H-statistic (Unadjusted):	14.11765				
H-statistic (tie-Corrected):	14.32836				
Significance Level:	0.05				
Critical Value:	7.814728				
P-value:	0.00249				

Aroma Kruskal-Wallis

Table A.7 Aroma Kruskal-Wallis

Summary Statistics on Input Data					
	0	0.0333	0.0667	0.1	
					Total Sample Size =
Count of Observations:	4	4	4	4	16
Sample Sum:	32	34	35	37	
Sample Mean:	8	8.5	8.75	9.25	
Sample Standard Deviation:	0.816497	0.57735	0.5	0.5	

Summary Statistics on Ranks & Test Results					
	0	0.0333	0.0667	0.1	
					Total Sample Size =
Count of Observations:	4	4	4	4	16
Rank Sum:	20	30	37	49	
					Overall Rank
Rank Average:	5	7.5	9.25	12.25	Average = 8.5
Number of Tie Series:	4				
Average Number of Ties by Series:	4				
H-statistic (Unadjusted):	4.919117				
H-statistic (tie-Corrected):	6.194444				
Significance Level:	0.05				
Critical Value:	7.814728				
P-value:	0.102524				

Appearance Kruskal-Wallis

Table A.8 Appearance Kruskal-Wallis

Summary Statistics on Input Data

	0	0.0333	0.0667	0.1	
					Total Sample Size =
Count of Observations:	4	4	4	4	16
Sample Sum:	8	9	10	10	
Sample Mean:	2	2.25	2.5	2.5	
Sample Standard Deviation:	0	0.5	0.57735	0.57735	

Summary Statistics on Ranks & Test Results

	0	0.0333	0.0667	0.1	
					Total Sample Size =
Count of Observations:	4	4	4	4	16
Rank Sum:	24	32	40	40	
					Overall Rank Average
Rank Average:	6	8	10	10	= 8.5
Number of Tie Series:	2				
Average Number of Ties by Series:	8				
H-statistic (Unadjusted):	1.941176				
H-statistic (tie-Corrected):	3				
Significance Level:	0.05				
Critical Value:	7.814728				
P-value:	0.391625				

Flavor Kruskal-Wallis

Table A.9 Flavor Kruskal-Wallis

Summary Statistics on Input Data					
	0	0.0333	0.0667	0.1	
Count of Observations:	4	4	4	4	Total Sample Size = 16
Sample Sum:	41	40	45	41	
Sample Mean:	10.25	10	11.25	10.25	
Sample Standard Deviation:	0.957427	1.414214	1.707825	0.957427	

Summary Statistics on Ranks & Test Results					
	0	0.0333	0.0667	0.1	
Count of Observations:	4	4	4	4	Total Sample Size = 16
Rank Sum:	31	29	45	31	
Rank Average:	7.75	7.25	11.25	7.75	Overall Rank Average = 8.5
Number of Tie Series:	6				
Average Number of Ties by Series:	2.666667				
H-statistic (Unadjusted):	1.808824				
H-statistic (tie-Corrected):	1.996753				
Significance Level:	0.05				
Critical Value:	7.814728				
P-value:	0.573081				

Mouthfeel Kruskal-Wallis

Table A.10 Mouthfeel Kruskal-Wallis

Summary Statistics on Input Data					
	0	0.0333	0.0667	0.1	
					Total Sample Size =
Count of Observations:	4	4	4	4	16
Sample Sum:	12	11	11	12	
Sample Mean:	3	2.75	2.75	3	
Sample Standard Deviation:	0	0.5	0.5	0	

Summary Statistics on Ranks & Test Results					
	0	0.0333	0.0667	0.1	
					Total Sample Size =
Count of Observations:	4	4	4	4	16
Rank Sum:	38	30	30	38	
					Overall Rank
Rank Average:	9.5	7.5	7.5	9.5	Average = 8.5
Number of Tie Series:	2				
Average Number of Ties by Series:	8				
H-statistic (Unadjusted):	0.705882				
H-statistic (tie-Corrected):	2.142857				
Significance Level:	0.05				
Critical Value:	7.814728				
P-value:	0.543291				

Overall Impression Kruskal-Wallis

Table A.11 Overall Impression Kruskal-Wallis

Summary Statistics on Input Data					
	0	0.0333	0.0667	0.1	
Count of Observations:	4	4	4	4	Total Sample Size = 16
Sample Sum:	25	28	27	30	
Sample Mean:	6.25	7	6.75	7.5	
Sample Standard Deviation:	0.957427	0.816497	0.5	0.57735	

Summary Statistics on Ranks & Test Results					
	0	0.0333	0.0667	0.1	
Count of Observations:	4	4	4	4	Total Sample Size = 16
Rank Sum:	22	36	30	48	
Rank Average:	5.5	9	7.5	12	Overall Rank Average = 8.5
Number of Tie Series:	4				
Average Number of Ties by Series:	4				
H-statistic (Unadjusted):	3.970588				
H-statistic (tie-Corrected):	4.891304				
Significance Level:	0.05				
Critical Value:	7.814728				
P-value:	0.179932				

Total Score Kruskal-Wallis

Table A.12 Total Score Kruskal-Wallis

Summary Statistics on Input Data

	0	0.0333	0.0667	0.1
Count of Observations:	4	4	4	4
Sample Sum:	118	122	128	131
Sample Mean:	29.5	30.5	32	32.75
Sample Standard Deviation:	1.7320508	1.290994	1.414214	0.957427

Total Sample Size = 16

Summary Statistics on Ranks & Test Results

	0	0.0333	0.0667	0.1
Count of Observations:	4	4	4	4
Rank Sum:	17.5	24.5	43	51
Rank Average:	4.375	6.125	10.75	12.75
Number of Tie Series:	7			
Average Number of Ties by Series:	2.285714			
H-statistic (Unadjusted):	8.0790			
H-statistic (tie-Corrected):	8.3874			
Significance Level:	0.0500			
Critical Value:	7.8147			
P-value:	0.0386			

Total Sample Size = 16

Overall Rank Average = 8.5