FILTRATION AND USE OF SILICA HYDRO GEL AND POLYVINYLPOLYPYRROLIDONE FOR REMOVAL OF HAZE-ACTIVE PROTEINS AND POLYPHENOLS IN BEER

by

Sarah M. Peterson

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Thesis Committee Members

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The Graduate School
University of Wisconsin-Stout
Menomonie, WI 54751

ABSTRACT

	Peterson	Sarah	M
(Writer)	(Last Name)	(First)	Initial

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There are several different types of haze that can be found in beer, but they are all caused by different reactions in the beer. The basis of this study was chill haze. Chill haze is caused by weak hydrogen bonding and hydrophobic interactions of haze-active proteins and haze-active polyphenols that complex to form a haze in the beer. It is unknown if there is a difference in haze-active and non-haze-active molecules, but studies conclude that the haze-active proteins contain a significant amount of proline, where the non-active proteins do not contain proline. The protein-polyphenol complexes that are formed are insoluble in beer thus causing the beer to appear cloudy. Many consumers will not drink beer that is cloudy, assuming that it is a microbial contamination, but most beers are pasteurized thus eliminating microbial contamination.

Silica hydrogel (SHG) and polyvinylpolypyrrolidone (PVPP) were used in five different combinations in the secondary filtration of beer to remove proteins and polyphenols. All other aspects of the brewing remained constant. Total polyphenols, total flavanoids, dissolved oxygen, initial haze, accelerated haze, and crude protein were measured in each variation. Based on the results of the project as well as the cost analysis of the silica hydrogel and polyvinylpolypyrrolidone it was determined that the combination of stabilizers that would decrease the amount of haze (with a reasonable increase in expense) in the product would be the combination of 15 lbs silica hydro gel per 100 barrels and 4 lbs polyvinylpolypyrrolidone per 100 barrels.

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CHAPTER I

INTRODUCTION

Jacob Leinenkugel, the son of a German brew master and brother to two others, first came to Wisconsin in 1866 and settled in Chippewa Falls. When he arrived he found many lumberjacks thirsty for good beer and in 1867 Jacob and his business partner John Miller brewed their first batch of Leinenkugel's beer at the Spring Brewery. Thirteen years later he was brewing 1,200 barrels of Leinenkugel's per year. In 1890 he renamed the brewery Jacob Leinenkugel's Spring Brewery and expanded the facility to include a new brew house, a three-story malt house and a barn to accomodate the horses that drew the Leinenkugel's delivery wagons. In 1899, Jacob died and son Matt stepped in as the second generation Leinenkugel to oversee the business. Since then Leinenkugel's has grown from one variety to seven and is now producing 250,000 barrels of beer per year (200 million barrels were produced in the United States in 2000) with shipments countrywide. Due to Jacob Leinenkugel's success, the Jacob Leinenkugel Brewing Comany is still in operation today along with one in Milwaukee, WI and one in Arizona. The Chippewa Falls plant employs about 65 people year round with a larger number of employees during the summer months. Each year the production is increasing and in 2002, the industry recorded its seventh straight year of growth, with shipments from breweries to wholesalers hitting an all-time high (Beer Serves America, 2003)

According to the 2003 Beer Industry Economic Impact Study, the beer industry contributed more than $144 billion per year to the United States economy and employed over 1.66 million people. In Wisconsin the brewing industry contributes 2,910 jobs (Beer

Serves America, 2003). There are currently over 3,500 different malt beverage brands produced in 1,800 breweries in the United States. Brewers in the United States currently export products to over 100 countries world wide (Beer Serves America, 2003). Not only do the breweries employ many people and contribute to the United States economy, they also produce over $27.5 billion in business, personal, consumption and excise taxes in the United States (Beer Serves America, 2003).

The current shelf life on Leinenkugel's Red Lager is three months. At the end of that time the beer is no longer clear resulting in a reduced shelf life of the product. Even though the beer is safe to consume in this hazy stage, most consumers will not drink it thinking that the haze is caused by microbial contamination. This is not the case at all since all bottled and canned beer is pasteurized and safe to drink even after the three months but will impart some degraded flavors.

Increasing amounts of haze formation in beer has been observed in recent years and is associated with the introduction of new varieties of malting barley (J. Buhrow, personal communication, April 12, 2003).These new varieties have been known to increase the amount of haze in the beer due to the presence of higher levels of haze-active protein and polyphenols in the barley. The haze-active proteins and polyphenols in beer can cause a haze to develop as time passes, so the control or elimination of this protein-polyphenol haze is the focus of this study.

Accompanyng the breeding and introduction of new varieties of malting barley has been a decline in crop yield to the extent that the United States can no longer produce enough barley for its brewing industry. The challenge to plant breeders at this time is to

find and develop new varieties of malting barley that have the same properties of the older varieties but with increased yield.

There are several ways that protein-polyphenol haze can be removed from beer. In this study, several combinations of silica hydrogel (SHG) and polyvinylpolypyrrolidone (PVPP) were studied to determine the most effective combination for removing the protein-polyphenol haze (colloidal haze) from beer.

CHAPTER II

REVIEW OF LITERATURE

The purpose of this study was to determine how different combinations of two colloidal stabilizers would affect the quality aspects of beer as well as to determine how each combination affected the amounts of protein, polyphenols and flavanoids in the product. Analyses were completed on replicates of five samples for each stabilizer combination added to beer. The effect of pasteurization on haze formation was also studied. To better understand how the colloidal stabilizers work in beer, the process of beer making and haze-formation must be understood.

Beer Production

There are three main parts of the brewing process; malting, mashing, and fermentation. During the malting process, barley, one of the raw materials of beer, is germinated to allow the release of amylolytic enzymes, that hydrolyze the starchy endosperm of the barley grain into soluble sugars (De Clerck, 1957/1994, p. 2). This step, also known as the germination step, is only done in larger breweries due to the high cost. Smaller breweries such as Leinenkugel's purchases pre-malted barley with certain specifications for the type of beer they wish to produce. The malted barley is then ground and extracted with hot water (De Clerck, 1957/1994, p. 2), and in many cases, adjuncts are used alongside the barley. At Leinenkugel's, corn grits are used as the adjunct and are added directly to the grist creating a mixture of crushed barley and adjuncts. After the starches in the malt are fully hydrolyzed by the amylolytic enzymes to

soluble simple sugars, the wort is separated from the malt (De Clerck, 1957/1994, p. 2). Wort is an aqueous mixture of soluble and suspended substances derived from the ingredient materials (Dougherty, 1983). The wort is then transferred into the kettle for boiling and hops addition, which imparts the characteristicbitter flavor and aroma of beer.

After boiling, the wort is cooled and pitched with yeast, thus starting the third step, fermentation. The pitched yeast metabolizes the fermentable sugars that were formed during mashing and catabolizes them to alcohol and carbon dioxide. Fermentation usually takes about one week (De Clerck 1957/1994, p. 2). Following fermentation, the beer is stored, filtered and bottled for shipping.

Malting

The malting process involves a controlled germination of barley that allows the starch to be converted to simpler sugars in preparation for their subsequent hydrolysis during the mashing phase of beer production. There are three stages of malting; steeping, germination, and kilning.

The components of a typical malthouse are depicted in Figure 1 with the exception of the kilning phase. In this figure, the kilns are shown as part of the germination beds. In 93% of malthouses in the United States, there are separate kiln beds that are shallower than the germination beds and have a smaller mesh for the bed floor (Adamic, 1983).

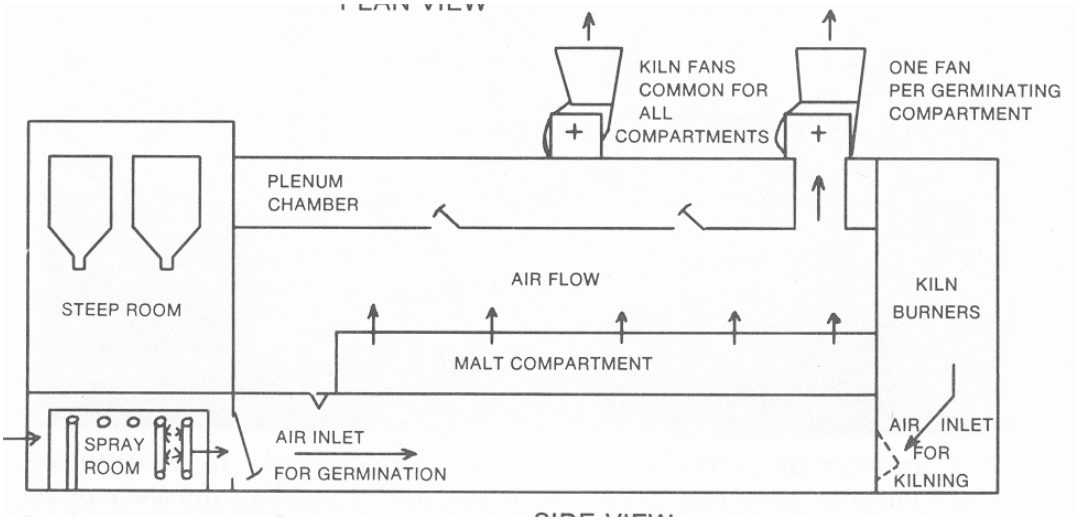


Figure 1. Diagram of typical malthouse.

From "The Practical Brewer: A Manual for the Brewing Industry," Edited by H.M. Broderick, 1983, p. 36. Copyright 1977 by Impressions Inc.

Steeping. In the steeping process, cleaned, graded barley is transferred from the storage elevator into steep tanks via conveyors. Then water, at temperatures of 5 to 18^{o}C, is added. Cool temperatures are used to avoid the growth of microorganisms. The moisture content of the barley increases from 11-13.5% to about 44-48% during the steeping. The time required for steeping will vary depending on the water temperature, kernel size, barley variety, and protein content of the kernel. The water in the steep tank is changed every six to eight hours and the water is constantly aerated because the barley kernels respire and use up the dissolved oxygen in the water. The steeping process is stopped when the chit, or rootlets are just ready to emerge. The water is drained from the tank and the moisture rich barley is transferred to the germination bed (Adamic, 1983).

Germination. The steeped barley is spread onto the pneumatic germination bed, a compartment with a perforated metal floor used for germination. Humidified inlet air at a

temperature of 11-16^{o}C is introduced to help maintain the temperature of the germinating grain at 16-21^{o}C. The humidified inlet air usually passes through the germination bed from under the grain as an updraft. As the germination of the barley continues, the respiration of the barley generates a considerable amount of heat thus necessitating a lower inlet air temperature. As the kernels grow, the rootlet masses need to be broken up and the beds need to be aerated. This is achieved by the use of counter-rotating helical turners that are automated to move one foot per minute. Large fans in the malthouse used to circulate the air cause some dehydration of the germinating kernels, so water is applied to the grain beds as a light mist. After four to six days the germinated malt is ready for kilning (Adamic, 1983).

Kilning. Depending on the type of malthouse, kilning can occur in one of two places. Kilning can be accomplished in the germination bed as in Figure 1 or in a separate kiln bed, which is most common in the United States. During kilning the germinated barley is placed in the kiln bed and hot air is passed through the kiln bed as in germination. This occurs in a stepwise fashion.

In the initial stage, the moisture is easy to remove. At this stage the temperature of the kiln inlet air is maintained at 49-60^{o}C while moisture in the malt is reduced from about 48% to approximately 23%. The second stage of kilning is much slower than the first because the remaining moisture is more difficult to remove. In the second stange of kilning the temperature of the inlet air is increased to about 71^{o}C,while the moisture in the malt is reduced to 12%. The final drying stage decreases the moisture to about 3.5% by increasing the kiln inlet air to 71-88^{o}C and reducing the airflow. When kilning is

complete, the dry malt is cooled to about 38^{o}C and cleaned of all roots and sprouts before it is stored. The malt is stored for at least three weeks before shipment to a brewer (Adamic, 1983).

Leinenkugel's brewing company orders pre-malted barley for their beer production. This allows for a more uniform malt in their product since all the malt has the same specifications for every brew. Because malting is an expensive, time consuming process, smaller breweries tend to purchase pre-malted barley to cut down on costs as well as time spent in the brewing process.

Wort Production

There are several steps in wort production that include mashing, lautering, and sparging.

Mashing. Mashing is the brewer's way of creating wort that will be fermented into beer. Wort is an aqueous solution of soluble and suspended substances derived from the raw ingredients such as barley and corn grits. Prior to mashing, the ingredients are delivered into the brew house via a conveyor to a grain weighing system that assures the proper formulation of malt and adjuncts is used. Once the ingredients are in the brewhouse they are milled using a five (or six) roller mill, that is often specially designed for a specific brewery based on the type of equipment being used. The three main objectives of milling are first and foremost to split the husk longitudinally to expose the endosperm of the kernel, to crush the entire endosperm to make the constituents accessible to enzymatic action, and to keep the amount of flour produced in the milling process to a minimum

thus keeping dough formation in the mash to a minimum. The adjuncts, mainly corn grits in this case are used without any milling (Dougherty, 1983).

Following milling, the actual mashing process can begin. Typically the type of mashing used in the United States is a double mash upward infusion system. This type of mashing process uses a cereal cooker, wherein the adjuncts are prepared by boiling. A separate mash mixer prepares a malt mash and then these two mashes are combined. The three main objectives of mashing are to dissolve any soluble substances in the ingredients, to make some insoluble substances soluble through enzymatic action, and to change the chemical structure of some of the constituents through a variety of enzymatic actions. Among the numerous enzymes involved in the mashing process are phosphatases, carbohydrases, proteases, and many others that play minor roles (Dougherty, 1983).

Phosphatases cause the hydrolytic release of phosphate ions from organic phosphates in the malt thus changing the pH to about 6 due to the mixture of K_2HPO_4 (pH = 8.4) and KH_2PO_4 (pH = 4.7). So that other enzymes can work, the pH needs to be decreased to between 5.2 and 5.7. The acidity of the mash is increased by the addition of calcium sulfate, $CaSO_4$. Calcium sulfate reacts with the alkaline potassium phosphate to create a precipitate of $Ca_3(PO_4)_2$. This adjusts the pH to the optimum range (Dougherty, 1983).

Alpha and beta amylase hydrolyze amylose and amylopectin from starch to dextrins and eventually to maltose. The beta amylase works to convert the starch to maltose and the alpha amylases work more effectively on the amylopectin to break it into dextrins and more slowly into maltose (Dougherty, 1983).

Proteases are proteolytic enzymes that break down large protein molecules into smaller polypeptides. There are three types of proteolytic activity that occurs in the mash; solubilization of insoluble proteins, parital digestion of soluble proteins, and continued breakdown of the soluble protein fractions into peptones that can ultimately be utilized by the yeast in fermentation (Dougherty, 1983).

The first step in the double mash upward infusion system is to mash-in (mix malt and adjunct with water) a small amount of crushed malt, generally 5% to 10% of the total ingredients, in hot water (100 to 122^{o}F) in the cereal cooker in a ratio of about 1 barrel water per 100 lbs malt. This is gently mixed for 15 to 30 minutes, to form what is called a peptonizing rest. The peptonizing rest, of soaked ground malt will not only liberate starches and proteins, but will also activate enzymes that released during malting. After preparation of the peptonizing rest, adjunct (corn grits) is added to the cooker with additional hot water at the same ratio as the malt. At this time heat is applied to the cereal cooker via its steam jacket to raise the temperature of the contents. As the temperature gradually increases cornstarch granules absorb water and swell causing the mash to thicken to a gel consistency, thus termed gelatinization of the starch. When the temperature increases to approximately 185^{o}F, liquefaction occurs. Liquifation is the result of alpha amylase action.. It occurs when the water-rich starch granules break open and the starch molecules mix freely throughout the mash. After this happens, the temperature is increased to boiling and the grist is boiled for about 10 minutes (Dougherty, 1983).

The preparation of this malt mash (malted barley) occurs at the same time as adjunct preparation. The malt is introduced into the mash mixer with hot water to begin

the peptonizing rest. In the malt mash, a protein rest is also included along with the peptonizing rest. The high temperatures (100-122^{o}F) allow the proteolytic enzymes to break down the larger molecular weight proteins into smaller more soluble polypeptides. At the end of the protein rest, the grist boil should be complete so the adjunct from the cereal cooker can be added to the mash mixer. Immediately following the transfer, the grist is mixed with the malt in the mash mixer creating the optimum temperature for the malt enzymes alpha-amylase and beta-amylase to convert amylose and amylopectin into fermentable sugars, usually maltose, some unfermentable sugars and some larger unfermentable dextrins. Once the conversion is complete, the temperature of the mash is increased to a mashing-out temperature of 167-170^{o}F, denaturing the enzymes that were used earlier in the mashing process thus rendering the mash ready for lautering or filtration (Dougherty, 1983).

Lautering. Lautering occurs in the lauter tun, a large stainless steel vessel that is equipped with a lautering machine to mix the contents and to separate grain hulls from the wort, equipped with water jets for sparging and equipped with a wort collection system in the bottom of the vessel. The lautering machine (Figure 2) consists of lautering blades or rakes, which are thin knife-like blades placed about 6 inches apart on the arms (Figure 3). The arms rotate about an axis in the center of the lauter tun (Dougherty, 1983).

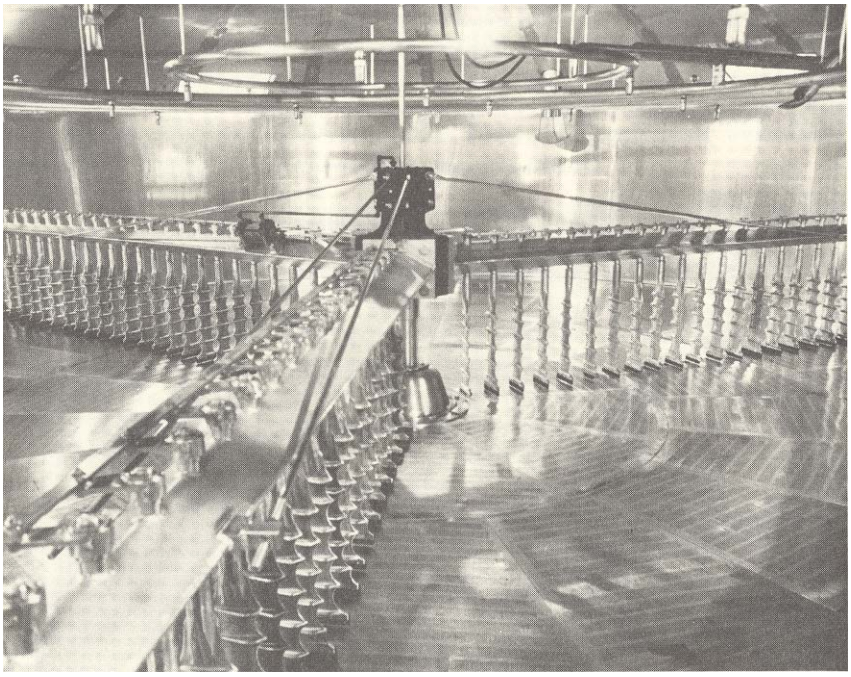


Figure 2. Interior of the lauter tun showing the lauter machine.

From "The Practical Brewer: A Manual for the Brewing Industry," Edited by H.M. Broderick, 1983, p. 88. Copyright 1977 by Impressions Inc.

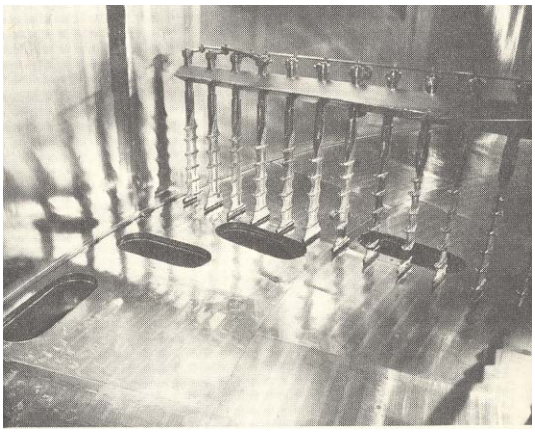


Figure 3. Lauter machine blades

From "The Practical Brewer: A Manual for the Brewing Industry," Edited by H.M. Broderick, 1983, p. 92. Copyright 1977 by Impressions Inc.

The water jets, or sparging system, emanate from a system of concentric pipes suspended just below the ceiling of the tub. The system is equipped with spray nozzles

directed downward to deliver sparge water over the entire surface of the mash bed in the lauter tun. Lautering is the brewer's term for the separation of the grain solids from the wort. This occurs immediately after the mashing-out process in the mash-mixer. The grist is transferred slowly into the preheated lauter tun that contains a small amount of water on the bottom of the tank. The water in the tank acts as a cushion and prevents the mash from impacting on the bottom. The mash is distributed evenly over the bottom of the lauter tun with the help of the lautering machine. When the mash is completely transferred into the lauter tun and properly leveled, the wort is circulated through the grain bed that has formed on the bottom of the tank. This helps to form a filter bed and creates a clearer wort with the help of the lautering machine. Once the filter bed is properly established, the first wort can be drawn out of the lauter tun by a siphon system and diverted into the kettle. As the first wort is drawn off, sparging begins. Sparging is done to dilute and reduce the viscosity of the first wort encouraging more rapid flow through the grain bed. The sparge water is maintained about 3-4 inches above the grain bed at all times until sparging is complete to assure an uninteruppeted flow of wort through the grain bed. Sparging is finished when the final volume of the wort plus the volume of the first wort is appropriate for the kettle. During sparging, the lautering machine is run on its lowest speed (in decreasing positions) to allow sparge water to run through the grain bed, as well as to keep the grain bed from compacting. Once the sparge is complete, the water is turned off and the dilute wort is collected until the kettle has been filled. Spent grains are removed from the lauter tun with the help of the lautering machine which sweeps out the grains (Dougherty, 1983).

Wort Boiling and Hops Addition

The next step in brewing is to boil the wort. The four principal goals of boiling the wort are to stabilize the wort, to enhance flavor development, to concentrate the wort, and to aid in spent hop removal (Hudston, 1983).

Wort Stabilization. Stabilization includes four areas: biological, biochemical, colloidal, and flavor stability. Biochemical stabilization deactivates the remaining enzymes, including alpha- and beta-amylase thus preventing any unwanted carbohydrate breakdown from occurring later in the brewing process. Colloidal stabilization is brought about by the high heat of the boil that coagulates unstable proteins causing them to precipitate. Precipitation of these proteins is expedited by the high movement of the boil, and decreasing pH from reactions with calcium and phosphate, as well as the action of reducing agents in the wort (Hudston, 1983).

Flavor Development. Enhancement of flavor development is the second goal of the wort boil. There are a few different ways by which flavors can develop in beer. The first and foremost is the introduction of flavors from the hops. Flavors can also be developed by evaporation of volatile flavor compounds derived from the barley and the malting process, but can only occur in an open kettle. Kettle additives also modify the flavor of wort. The addition of salt produces a more full flavored beer as do sugars, syrups, and caramel colors to the wort as it is being transferred to the kettle, but it is the addition of hops that has the most influence on flavor development. Hops are added to the kettle about 30 to 45 minutes before strikeout or the emptying of the kettle. The flavor most

associated with hops is the bitterness caused by iso-alpha acids that are released during the boiling of the hops in the wort. Flavor also comes from the isomerization of alpha acids from the hop resin (Hudston, 1983).

Concentration. Concentration of the wort during the wort boil removes excess water that may have been used during the mash and sparge. By concentrating the wort, the required specific gravity of the product is achieved fairly quickly. Sometimes, brewers will fill the kettle to a set volume and then concentrate it to a pre-established set volume, before they strike the kettle, but this requires that the yield of materials, evaporation rates and times are kept constant to create a constant specific gravity product. A final blending will even out any differences in the specific gravity of the wort (Hudston, 1983).

Hop Removal. The form of hops used in the kettle, will determine how the hops need to be removed. Whole hops are removed from the wort by directing the wort though a series of screens. At Leinenkugel's, pelletized hops are used. These are formed from ground hops that are pressed together. In order to remove the hop pellets from the beer, a whirlpool tank is used (Strauss, 1983). In this tank suspended particles of hops migrate to the bottom and center of the whirlpool creating a trub cake as the hot wort rotates in the vessel (Strauss, 1983). The wort starts to rotate as it is being pumped into the tank from the kettle. The wort stays in the whirlpool for a set amount of time before the wort is removed and cooled, but the wort temperature is not allowed to drop below 82^{o}C (Strauss, 1983). The “clear” wort is drawn out of the whirlpool with a draw-arm positioned just above the trub cake (Strauss, 1983). The wort is finally cooled via a plate

type cooler that consists of a stainless steel frame that holds many stainless steel recessed plates pressed tightly together. This allows the wort and cooling medium to pass each other in a counter flow manner in shallow layers between the adjoining plates (Strauss, 1983). Once the wort is cooled it can be fermented. The entire brewing process, from mashing to cooling, takes about two hours at Leinenkugel's.

Fermentation

Fermentation is dependent on four basic parameters including the wort composition, which are the nutrients for the yeast, the yeast itself, and the processing conditions such as time, temperature, volume, pressure, agitation, and vessel shape and size (Knutsen, 1983).

Wort Composition. The wort composition will influence the degree of fermentation due to the nutrients available, the pH of the wort as well as the degree of aeration and the temperature of the wort. The carbohydrates that are fermentable by the brewer's yeast include isomaltose, panose, isopanose, maltotriose, and maltotetraose. The alpha-glucans or dextrins and the beta glucans (gums and pentosans) are largely unfermentable by the brewer's yeast, but will contribute to the viscosity of the wort. Nitrogen content is also a concern for the yeast. At levels less than 150 ppm nitrogen, the wort will be insufficient in yeast nutrition, mainly free amino acids and peptides. Yeast also needs a small amount of ergosterol or another unsaturated lipid or it will autolyze, but this is rarely a problem in wort. Lastly the yeast needs many vitamins, mainly B-vitamins, and minerals that are

found in the wort in amounts in excess of what the yeast need for proper growth (Knutsen, 1983).

Yeast Types. Each brewery uses it's own strain of yeast for beer production. The strains are chosen by their biochemical and their physical behavior, which will determine the fermentation pattern used by the yeast. The pitching of yeast, the amount added, and the condition of the yeast will greatly affect how the yeast ferments the wort (Knutsen, 1983).

Fermentation Process. The fermentation starts when the wort leaves the cooler at about 8 –10^{o}C and is aerated to about 8 ppm dissolved oxygen. Once the wort is aerated, the yeast is pitched (yeast added to the wort). The pitched yeast must be clean and sanitary to prevent the introduction of wild yeast or bacteria into the wort. After about ten to twenty hours the wort is covered by white foam changing to creamy-white thick foam as time passes. At the end of the fermentation, 8-10 days, the yeast will flocculate and settle to the bottom of the tank, leaving a "green beer" to be stored. Following the fermentation, the beer is transferred to the storage tank and stored at low temperatures, 30^{o}F (Knutsen, 1983).

Cellar Operations

Cellar operations include actions and processes that occur subsequent to primary fermentation but before packaging. The four major activities in cellar operations include

carbonation, clarification, chillproofing/stabilization, and flavor maturation. The most important of these activities is chillproofing/stabilization.

Carbonation. The carbonation of beer at Leinenkugel's occurs as the beer is traveling from the storage tank to primary filtration a process known as in-line carbonation. As the beer is being transferred though a line, CO_2 is injected into the beer through a fritted stainless steel diffuser until the appropriate amount of CO_2 is present in the beer. The diffusers create very small bubbles of CO_2 that go into solution easily as long as the beer is not saturated. This procedure is highly accurate and can be continuously measured (Coors, 1983).

Clarification. Clarification at Leinenkugel's is accomplished by primary filtration. Following fermentation, the beer is very turbid. Clarification can be accomplished by various methods including gravity sedimentation, fining, centrifugation, and filtration. Of these methods, filtration is the only one that gives the clarity required in the marketplace. Filtration is the only clarification method used at Leinenkugel's and will be the only clarification method discussed here. There are three common methods used for filtration; filtration through kieselguhr (diatomaceous earth), sheet filtration, and pulp filtration. Of these, Leinenkugel's employs kieselguhr filtration.

Kieselguhr or diatomaceous earth is the skeletal remains of microscopic plants which were deposited on ocean floors and lake bottoms during the Miocene Period, 21 million years ago. Today it is mined from chalk-like deposits, ground to a powder,

calcined, to destroy any organic matter, and sterilized. Figure 4 shows the typical kieselguhr filtration system (Coors, 1983).

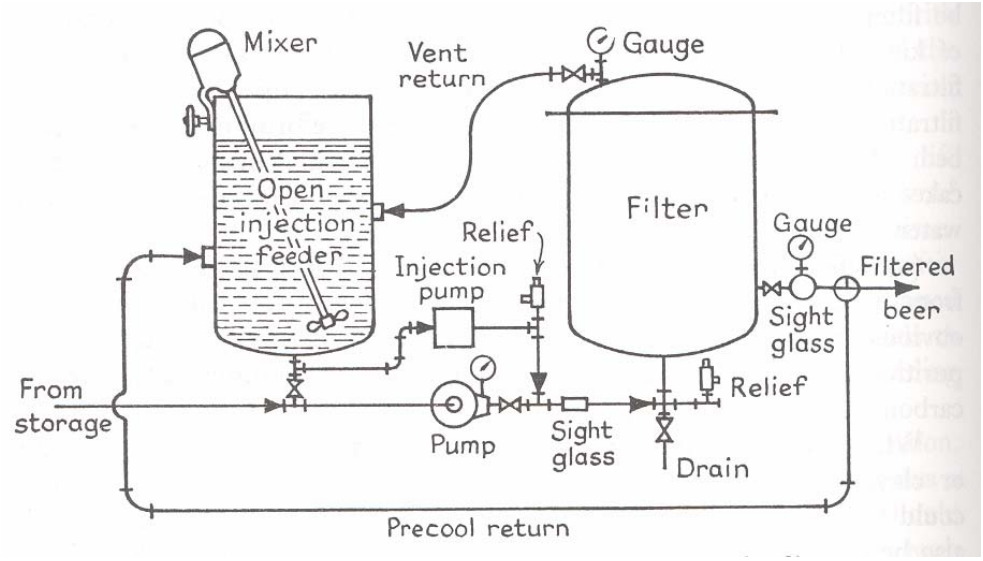


Figure 4. Diagram of a typical wire mesh kieselguhr filter
From "A Textbook of Brewing," By Jean De Clerck, 1994, p. 464. Copyright 1957 by Chapman & Hall LTD

An aqueous slurry of diatomaceous earth (kiesleguhr) is prepared in an open injection feeder that is mixed constantly with a propeller to keep the diatomaceous earth in suspension. This slurry is then circulated through the filter and back to the open injection feeder system (De Clerck, 1994). At first the diatomaceous earth will pass through the wire mesh or filter septum, but with continued circulation, a pre-coat of diatomaceous earth about 1 mm thick is formed on the septum (De Clerck, 1994). When the liquid from the open injection feeder becomes clear after passing through the filter, an adequate pre-coat layer has been formed. This forms a rigid but porous filter cake that can sieve out particulate matter in beer, but may allow some yeast through, thus creating the need for secondary filtration (De Clerck, 1994). At this point the beer can be passed through the filter which now contains the pre-coat layer of diatomaceous earth. A small

amount of diatomaceous earth is injected into the beer before it reaches the filter and will be deposited on the pre-coat that continuously renews the filter-bed (De Clerck, 1994). Due to continuous renewal of the filter-bed, the filters can only run for about 8 to 12 hours before the filter cake becomes too thick and exceeds the physical space allotted for the filter. Figure 5 shows the filter septum with the pre-coat and filter cake on vertical filters. This is how the primary filtration is completed at Leinenkugel's. The next step in the filtration process is the secondary filtration, which is also known as the chillproofing or stabilization treatment (Coors, 1983).

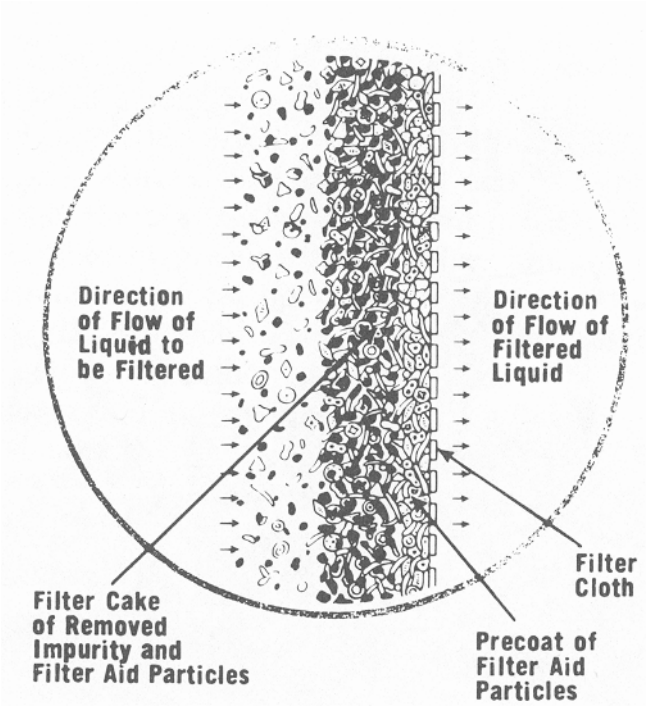


Figure 5. Filter Septum with the pre-coat and filter cake.
From "The Practical Brewer: A Manual for the Brewing Industry," Edited by H.M. Broderick, 1983, p. 237. Copyright 1977 by Impressions Inc.

Chillproofing/Stabilization. Chillproofing or stabilization is the secondary filtration process that is used to remove an insoluble colloidal haze that can form due to a chemical

reaction of protein with polyphenols. This colloidal haze may be soluble at temperatures above 20^{o}C, but becomes insoluble at refrigeration temperatures. The three ways that the colloids can be stabilized is by the use of proteolytic enzymes (papain), tannic acid, or adsorbents. Each of these stabilizers will be discussed following the discussion of haze formation in beer.

Stabilization is employed immediately following the primary or diatomaceous earth filtration. As the primary filtered beer is pumped back to storage tanks, a slurry of polyvinylpolypyrrolidone (PVPP) and silica hydrogel (SHG) is injected into the beer. These stabilizers are left in contact with the beer for about 24 hours to effectively adsorb polyphenols and proteins respectively. The stabilizers are then removed from the beer by secondary filtration, which uses the same methods as primary filtration, with the exception of a finer ground diatomaceous earth pre-coat (J Buhrows, personal communication April 22, 2003). The filtered, stabilized beer is then pumped into government tanks for storage until bottling (Coors, 1983).

Flavor Maturation. Very little is known about the chemical and biochemical reactions that take place as green beer ages to produce a quality mature product, but it is well known that these transformations are temperature dependent and usually require yeast activity. The three principal changes that occur during flavor maturation are a reduction in concentration of hydrogen sulfide, acetaldehyde and diacetyl, all of which are products of yeast fermentation. Hydrogen sulfide is transformed, by yeast, to sulfate, which has no influence on the beer flavor. Hydrogen sulfide may also be removed when the CO_2 from the fermentation is removed since hydrogen sulfide is highly volatile. Acetaldehyde, the

biochemical precursor of ethanol, can be reduced by conversion to alcohol during maturation and can also be reduced by decreasing the fermentation temperature, decreasing the pH of the wort, and by reducing the sulfite levels of the wort. Diacetyl concentrations can be reduced by yeast to 2,3-butanediol which has a higher flavor threshold by increasing the agitation during fermentation (Coors, 1983). The finished beer, in the government tanks is now ready to be packaged, pasteurized, and shipped to the consumers.

Types of Beer Haze

One of the most obvious quality defects found in beer is cloudiness or haze (Sheehan, 1999) which can limit the shelf-life of the product since consumers expect beer to be clear (Siebert, 1996b). During long-term storage, products that were haze-free at packaging may develop haze, which is a persistent problem in the brewing industry (McMurrough, 1999). Several types of haze can be found in beer. The smaller particles causing haze are considered "invisible" haze or "pseudo-hazes" (Bamforth, 1999). Pseudo-haze consists of very small particles not visible to the naked eye that cause the light to scatter (Bamforth, 1999). Pseudo-haze may be attributed to particles of residual starch, pentosans from wheat-derived adjuncts, oxalate from calcium-deficient worts, beta-glucan from inadequately modified malt, and carbohydrate and protein damaged by yeast (Bamforth, 1999). A second type of haze is caused by low molecular weight polyphenols cross-linked with protein by weak interactions, such as hydrogen bonds (Bamforth, 1999) and is the most frequent cause of haze development in beer (Siebert, 1998). Many details of this haze have yet to be disclosed by literature, but there are

effective ways of delaying the onset of this haze (McMourrough, 1999). Protein-polyphenol haze is the subject of this study since as little as 0.1% of the total nitrogen in beer is sufficient to produce protein-polyphenol haze (McMurrough, 1999).

Protein-Polyphenol Hazes

Beer contains proteins and polyphenols that may combine to form a colloidal suspension that scatters light presenting a cloudy appearance. Protein-polyphenol haze is thought to be the biggest problem in beer clarity (Siebert, 1996a). This colloidal haze is often formed during cold fermentation and storage and is usually removed during clarification. However, the reactions between proteins and polyphenols can continue after filtration if sufficient quantities remain after filtration (Coors, 1983). Siebert and colleagues (1996a) analyzed beer haze material and found it to contain as much as 80% carbohydrates. Since there is no mechanism for haze to develop caused by carbohydrate, it is believed that the carbohydrate found in the haze is entrained or coprecipitated with the proteins and polyphenols (Siebert, 1996a). It was also noted by McMurrough, Madigan, Kelly, and O'Rourke (1999) that typical protein-polyphenol haze consisted of about 50% protein and 25% polyphenol and the remaining 25% from polysaccharides and metals. Protein-polyphenol haze may form when low molecular weight polyphenols cross-link with a protein though a weak interaction creating a phenomenon called chill haze. McMourrough, Madigen, Kelly, and O'Rourke (1999) believe the force of attraction between polphenols and proteins are through bidentate hydrogen bonding between the ortho-dihydroxyhphenolic groups and -keto-amide groups of polypeptides (protein). This haze appears when beer is chilled to 0^{o}C, but returns to solution when

beer is warmed to 20°C and particles range in size from 0.1 ③m to 1.0 ③m (Bamforth, 1999). This type of haze is extremely important to control as it may induce a permanent haze in the beer. Permanent haze is created when polyphenols polymerize and interact with the protein in the beer and describes any haze in the product at temperatures above 70°C (Guzman, 1999). Permanent haze particles are much larger than chill haze ranging in size from 1 ③m to 10 ③m (Bamforth, 1999) and it's formation can be accelerated in the presence of oxygen and metal ions (Guzman, 1999). Polyphenol polymerization is promoted by oxidation, which in part may be catalyzed by enzymes such as polyphenol oxidase (also known as laccase and peroxidase), but can also occur during the heat-up phase of the wort boiling (Bamforth, 1999). Polyphenol polymerization may not be the only way to create a permanent haze. It is observed that even though most beer is packaged with very low levels of oxygen resulting in low polyphenol oxidation levels, it can still become hazy (Bamforth 1999). It was suggested that some haze may be created by the interaction of aldehydes including acetaldehyde to form different species of polyphenols that cross-link with proteins (Bamforth, 1999). According to the literature, this cross-linking can be accomplished with one o-dihydroxybenzene group on the polyphenol, but with a greater number of o-dihydroxybenzene groups there is additional cross-linking (Siebert, 1996a). If the protein concentration increases but the polyphenol concentration remains the same, the amounts of haze formed will first increase, peak, and then decline. The same is true when the polyphenol levels increase with a constant protein level (Siebert, 1997a). This makes it difficult to study samples with unknown and variable levels of protein and polyphenols (Siebert, 1997a).

Alcohol, pH, free amino acid, and metal content may influence protein-polyphenol haze formation as well. The amount of alcohol in the beer will change the hydrophobic bonding between the proteins and the polyphenols due to the lesser polarity of ethanol than water. This was exhibited to a small extent in model studies completed by Siebert and collogues in 1996(a). In the same study, a model system was set up to test the effect of pH on haze formation. This model concluded that there was decreased haze formation at a lower pH value with a maximum haze formation at pH 4.2 (Siebert, 1996a). When looking at the complexes of tannic acid and gelatin, pH is one of the most important environmental factors (Van Buren, 1969). The optimum pH for tannic acid precipitation with gelatin was pH 4.7, which is slightly below the isoelectric point of gelatin (Van Buren, 1969). When free amino acid content was considered there was no effect on the amount of free amino acids in the haze formation (Siebert, 1996a). Metal content was studied in the same model studies by Siebert, Carrasco, and Lynn (1996a). They concluded that the effect of metals on protein-polyphenol haze formation could best be explained by the catalytic action of the metals on the polymerization of the polyphenols.

Mechanism of Protein-Polyphenol Haze and Adsorbent Action. The mechanism of protein-polyphenol haze is not fully understood (Kaneda, 1990), but there are predictive models that suggest a mechanism for haze formation and the action of stabilizers, SHG and PVPP to adsorb proteins and polyphenols, respectively. Protein-polyphenol complexes are held together by weak intermolecular forces such as hydrogen bonds and hydrophobic interactions. These bonds, that form upon cooling and are broken upon

warming creates chill haze (Siebert, 1996b). In protein-polyphenol complexes, the polyphenolic compounds act as a bridge between adjacent protein molecules (Siebert, 1996b). A diagram of a model for protein-polyphenol interactions proposed by Siebert and colleagues (1996b) is shown in Figure 6.

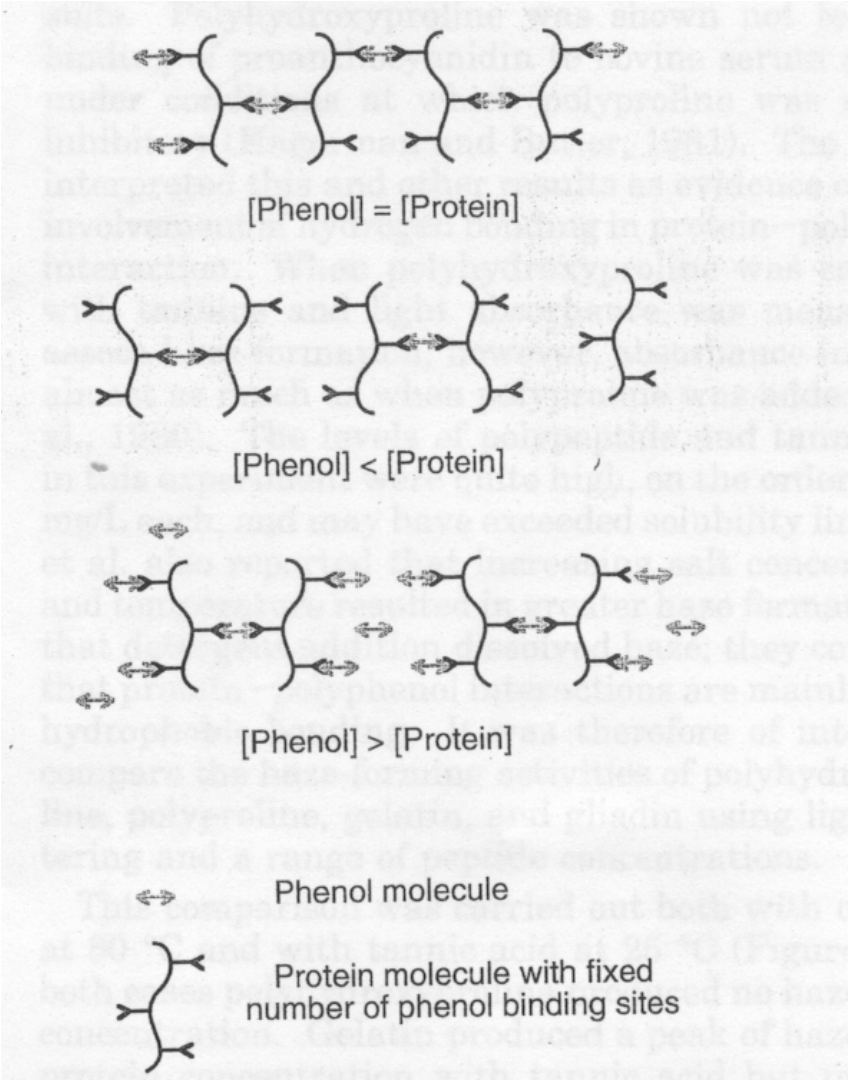


Figure 6. Model for protein-polyphenol interaction.

From "Comparison of Polyphenol Interactions with Polyvinylpolypyrrolidone and Haze-Active Protein," K. Siebert & P. Lynn, 1998, *Journal of the American Society of Brewing Chemists.* 56(1), 24-31.

In Figure 6, each polyphenol has a fixed number of binding ends (shown as two) and each protein also has a fixed number of polyphenol binding sites (shown as three). If the number of polyphenol ends is equal to the number of protein binding sites the largest network of proteins and polyphenols should be produced thereby creating the largest amount of haze (Siebert, 1996b). If the amount of protein is in excess relative to the polyphenols, as is usually the case with beer, then each polyphenol molecule can bridge two protein molecules, making it unlikely that an extended network of bridges would be formed between the protein and polyphenol molecules (Siebert, 1996b). If the number of polyphenol molecules significantly exceed the number of haze-active protein molecules, all of the haze-active protein binding sites would likely be bound to polyphenols making it unlikely that an extended network of bridges between haze-active protein and polyphenol molecules would be created (Siebert, 1996b). The fixed number of binding sites on haze-active protein is thought to be the accessible prolines, which are attachment ports for the polyphenols (Siebert, 1998).

There are two possible mechanisms for PVPP adsorption of haze-active polyphenols. Figure 7 shows a mechanism, in which PVPP binds to the same sites of the haze-active polyphenols that would be bound by haze-active protein molecules during haze formation in beer. According to this model PVPP molecules are specific for haze-active polyphenols suggesting that PVPP would be quite efficient as a haze preventative (Siebert, 1998). In a second possible mechanism for PVPP adsorption of haze-active polyphenols, the PVPP binds to a different part of the polyphenol molecule making PVPP non-specific for haze-active polyphenols (Siebert, 1998). Bonds formed between the PVPP and polyphenols in both models would involve hydrogen bonds between phenolic

hydroxyls of these two molecules and hydrophobic interactions between the aromatic ring of the polyphenol and the PVPP ring, or some combination of these (Siebert, 1998). In beer, most of the haze active polyphenols are attached to the haze-active protein and are not accessible to the PVPP. Consequently, only about 50% of the polyphenols could be removed (Siebert, 1997b). Since only a small amount of haze-active polyphenols can be removed from beer, SHG would likely be needed to remove haze-active proteins from solution.

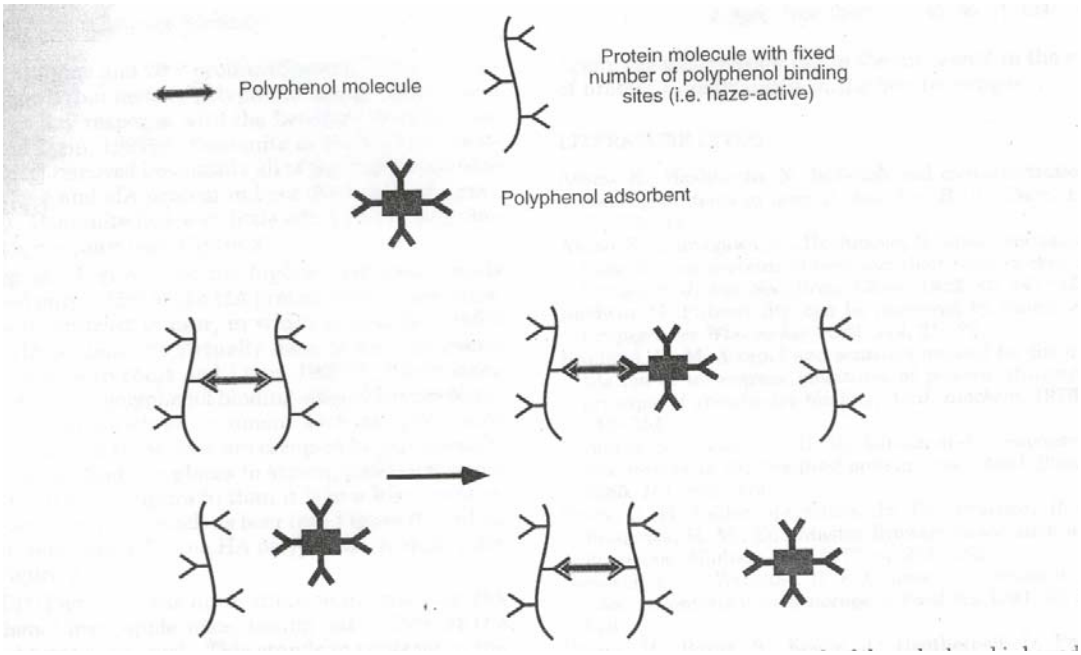


Figure 7. PVPP adsorption of beer haze-active polyphenols: PVPP is specific for haze-active polyphenols.

From "Comparison of Polyphenol Interactions with Polyvinylpolypyrrolidone and Haze – Active Protein," K. Siebert & P. Lynn, 1998, *Journal of the American Society of Brewing Chemists.* 56(1), 24-31.

When looking at the adsorption mechanism for haze-active proteins and SHG, the SHG will only bond to proteins with open proline sites, thus being specific for haze-

active proteins (Siebert, 1997b). Figure 8 shows a proposed mechanism in which silica adsorbs protein/polyphenol in beer complexes in beer.

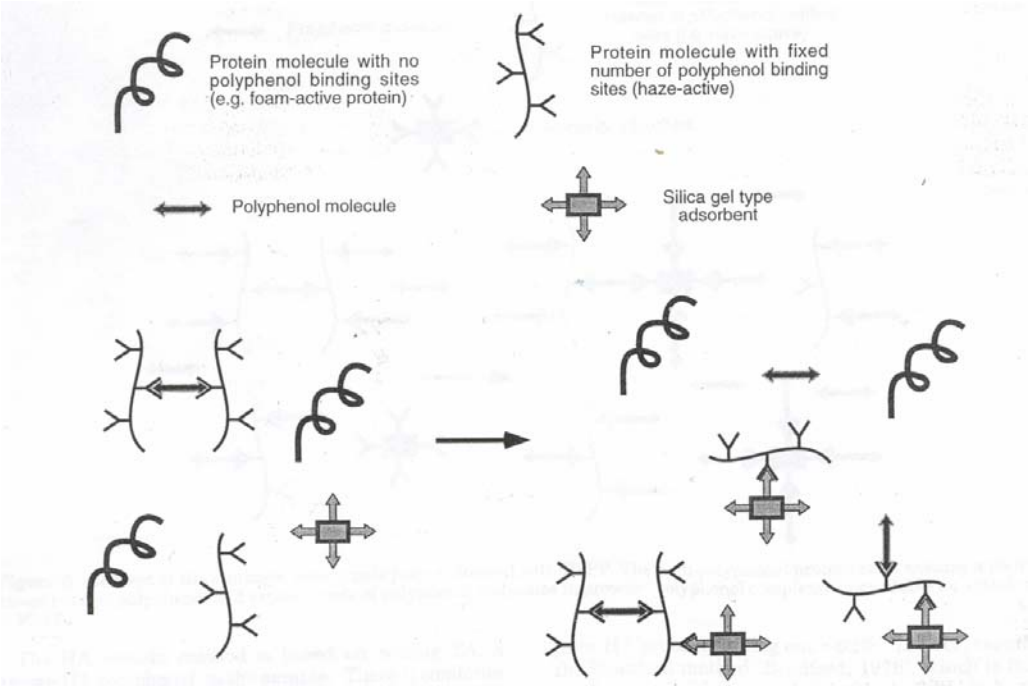


Figure 8. Mechanism of silica adsorption in high protein/polyphenol situation in beer. From "Mechanisms of Adsorbent action in Beverage Stabilization," K. Siebert & P. Lynn, 19997b, *Journal of Agriculture and Food Chemistry.* 45, 4275-4280.

In high protein/polyphenol beer, SHG binds to unoccupied proline residues in the haze-active proteins, but will not bind with proteins lacking proline (Siebert, 1997b). Consistent with the proposed mechanisms of adsorption of proteins and polyphenols to the SHG and PVPP, foam-active proteins and unbound polyphenols remain dissolved in the beer (Siebert, 1997b).

Haze-Active Proteins. According to research compiled by Charles Bamforth (1999), as little as 2 mg protein/liter is required to induce a haze of 1 EBC (equivalent to 69

Formazin Turbidity Units). This is interpreted as a very slight haze just below clear (Bamforth, 1999). The EBC method of haze measurement "involves the measurement of light scattering at an angle of 90^{o} to the incident beam that is calibrated with formazin standards" (Bamforth, 1999). EBC is a European scale that is not used as often as the Formazin Turbidity Units (FTU). One EBC is the same as 69 FTU with the scale of Formazin Turbidity being 200 FTU or below clear, 300 to 400 FTU slightly hazy and an FTU above 450 hazy. An FTU above 250 is noticeable to the consumer and thus becomes a defect (Compton, 1983). Due to the fact that most beers contain 0.5% protein, there is more protein in the beer than is needed to form a haze (Bamforth, 1999).

Many studies have concluded that there is not a clear separation between haze-active and foam-active proteins, but there is some controversy over this issue. Only about one third of the polypeptides in beer are actually involved in haze formation (McMourrough, 1999). According to a study by Asano and colleagues (1980), there was not a distinction between haze-active and foam-active proteins. They found that hordeins, a prolamin or alcohol-soluble protein that is rich in proline, were first to interact with polyphenols, but albumin-derived and globulin-derived polypeptides also create chill haze. In 1996(a, c), Siebert, Carrasco and Lynn found when working with model systems that haze-active proteins contained a significant amount of proline, up to 20 mol%, and proteins that lacked proline formed little or no haze when polyphenols were added indicating that a protein that contains proline is more likely to be haze-active. According to other studies, polypeptides with an isoelectric point between 3 and 5 were haze-active and that haze-forming proteins could be differentiated from foaming proteins based on differences in their isoelectric point, with acid proteins being haze formers

(Bamforth, 1999). It is thought that the foam-active proteins originate in the albumin fraction of the barley. The activity of foam-active proteins is attributed to the interaction of side chain amino groups of the protein with isoalpha acids, resulting in nonpolar complexes that are surface active thus creating a highly stable foam (Siebert, 1997c). Hordein has been known to be the source of haze-active proteins due to the high proline content (Siebert, 1997c). This matches many of the findings in literature that haze-active and foam-active proteins are different, but there are many studies to support the fact that there is no way to differentiate between haze-active and foam-active proteins. One study concluded that hydrophobic amino acids will interact with polyphenols to form haze, however hydrophobidicity is also a key in foaming properties of protein resulting in a crossover between haze-active and foam-active proteins (Bamforth, 1999), however it is generally accepted that haze active proteins are hydrophilic and foam-forming proteins are hydrophobic (Guzman, 1999). Proteins in beer are not easily characterized because chemical and biological modifications occur during processing and fermentation resulting in mixtures of polypeptide fragments (Sheehan, 1999; McMourrough, 1999).

Haze-Active Polyphenols. Polyphenols (Figure 9) are in a group of molecules called phenolics, which indicates that they possess phenol-like structural characteristics. Polyphenols are molecules that contain two or more phenol-like rings (Bamforth, 1999).

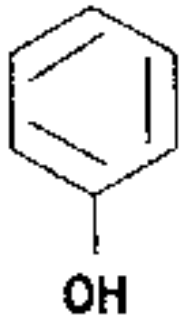


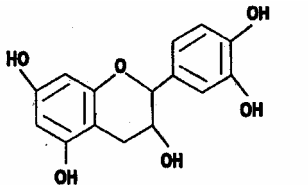
Figure 9. Structure of a phenol group.

From "Beer Haze," C. Bamforth, 1999, *Journal of the American Society of Brewing Chemists*. 57(3), 81-90.

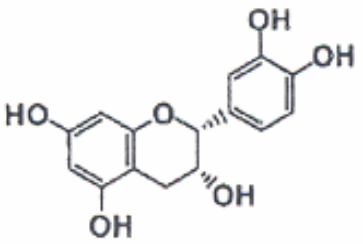
The term polyphenol includes flavanols, a monomeric species often represented by catechin; flavanoids, oligomers of flavanols; proanthocyanidin, which polymerizes in the presence of oxygen to anthocyanidin; and tannoids, intermediates of tannins (polymers of flavanoids) (Bamforth, 1999). Some of these structures can be seen in Table 1.

Table 1.

Structures of important polyphenols found in beer.

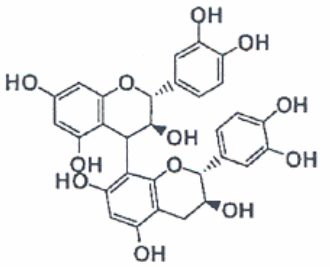
Important proanthocyanidin monomers	
(+)-Catechin*	

(-)-Epicatechin*

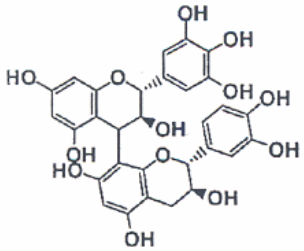


Important proanthocyanidin dimers in beer

Procyanidin B3*

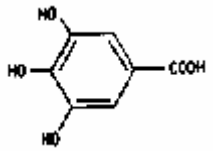


Prodelphinidin B3*

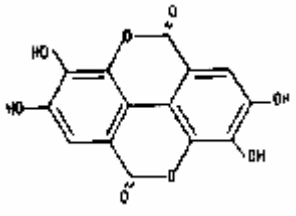


Structures of Some Tannins (Not necessarily in beer)

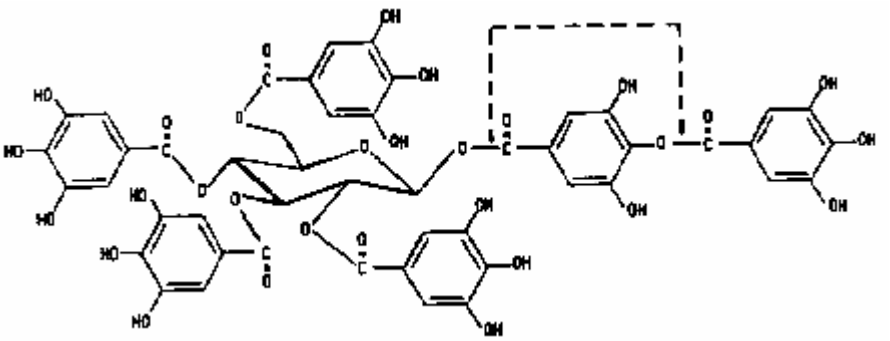
Gallic acid**



Ellagic acid**



Gallotannin**



* From "Comparison of Polyphenol Interactions with Polyvinylpolypyrrolidone and Haze –Active Protein," K. Siebert & P. Lynn, 1998, *Journal of the American Society of Brewing Chemists.* 56(1), 24-31.
**From www.ansci.cornell.edu/plants/toxicagents/tannin/chemical.html

Tannins, which are low molecular weight, can co-exist in beer with proteins, but tannins of larger molecular weight may bind with protein to form soluble and insoluble complexs (Bamforth, 1999; Van Buren, 1969). Representative tannin structures can be seen in Table 1, but may not be found in beer. Tannin-protein complexes are held together by hydrogen bonds linking phenolic hydroxyl groups of the tannins to the carbonyl groups of the protein peptide bonds (Van Buren, 1969). The most active haze-forming polyphenols are the proanthocyanidins, dimers of catechin and epicatachin (Bamforth, 1999). Haze formation is increased by oxidation, especially if beer is subjected to high temperatures even if oxygen is not present (Kaneda, 1990). McMurrough and colleagues (1995) found through gas chromatography that there was a selectivity of adsorption of haze-active proteins to proanthocyanidins rather than the catechins. Oxygen and acetaldehyde to some extent, polymerize the proanthocyanidins thereby increasing their ability to form protein-polyphenol haze (Kaneda, 1990). According to McMourrough (1999), the polyphenols in the beer most associated with haze formation are barley flavanoids that were modified during the brewing process to

form substances with unknown structures. Flavanoids consist of two aromatic rings linked by a C_3 unit (Doner, 1993). It is believed that at least two phenolic groups on an aromatic ring are required for the polyphenol to participate in haze formation (Doner, 1993). Flavanoids, especially catechin, are good examples of polyphenol containing compounds with multiple hydroxyl groups possibly making it more susceptible to oxidation (Bamforth, 1999). It is interesting to note that the level of catechin decreases with storage due to its conversion to tannoids thus reducing the amount of chill haze formed (Bamforth, 1999). A beer that is proanthocyaidin free will not throw a haze (Siebert, 1996a). It has been shown that proanyhocyanidins are the most effective at protein binding, but unfortunately, is not commercially available and is difficult to synthesize. As a result catechin and tannic acid are commonly used in model studies and laboratory analysis (Siebert, 1996a).

Polyphenols are derived from both malt and hops, although some researchers believe that polyphenols from hops do not play a role in the protein-polyphenol haze while other researchers claim that malt and hop polyphenols are identical in their haze inducing capabilities (Bamforth, 1999). Hops contain 2-4% polyphenols on a dry weight basis, but the concentration of malt derived polyphenols will vary depending on the variety of barley used for malting (Bamforth, 1999). Winter varieties contain higher levels of polyphenols than do spring varieties and American brewers tend to use winter varieties thus resulting in a higher polyphenol content in the malt (Bamforth, 1999). Heat produced during the mashing process of malt, can promote precipitation of haze-active protein-polyphenol complexes as well as oxidation of the polyphenols resulting in an increased polymerization of the polyphenols (Bamforth, 1999).

Stabilization Treatments

A holistic approach must be used when considering stabilization of beer; both the raw materials and their processing can have a significant effect on the amount of haze precursors, proteins and polyphenols, in beer (Bamforth, 1999). Stabilization will only delay the formation of haze in the product (Siebert, 1996a). There are three strategies currently in use to stabilize the beer; the removal of proteins, the removal of polyphenols, and the removal of a proportion of both. There is no "prescription" for the correct type and appropriate concentration of stabilizers to use in order to effectively remove the haze-forming constituents from beer (McMourrough, 1999). Proteins and polyphenols contribute to the flavor, taste, and foam (Kaneda, 1990) in beer but to remove protein-polyphenol haze brewers must treat the beer with stabilizers. These stabilizers must remove one or both of the haze-sensitive constituents and their effectiveness depends on their capacity to specifically target the "haze sensitive" protein in beer (Sheehan, 1999). Prior to the use of stabilizers, diatomaceous earth is used as a coarse filter as discussed and allows the stabilizers to work more efficiently by removal of any large particles left in the beer.

The bonds that hold the haze together are relatively weak in strength thus requiring low-temperatures to be used as a pre-requisite for the removal of the haze (Bamforth, 1999). In order to determine the appropriate stabilization treatment for the product in question, it is important to make sure that the "filtration system is properly set up in respect to the filter aid selection, dosing rate, lowest possible temperature, minimum oxygen pick-up, and other procedures" (Bamforth, 1999).

There are four main substances the brewer can use to remove the constituents of haze; papain, tannic acid, silica hydrogels, and polyvinylpolypyrrolidone (Bamforth, 1999). Each of these methods are dependent on their ability to specifically target the haze sensitive proteins or polyphenols without affecting the beer foam quality or the taste (Sheehan, 1999).

Papain. Papain was one of the first haze-preventative stabilizers used in the brewing industry. It is no longer used because of its preference for hydrolyzing peptide bonds involving hydrophobic amino acids, reduced the foaming qualities of beer (Bamforth, 1999). Papain is a proteolytic enzyme from the plant *Carica papaya* (Bamforth 1999). This stabilizer was used by adding it to beer on the transfer to maturation. The enzyme progressively loses its activity during storage and pasteurization (Bamforth, 1999). Since papain makes it into the bottle, residual papain may progressively affect the foaming qualities of the beer, by reducing the foam stability (Bamforth, 1999) and a foam-stabilizer should be used to offset this damage (Siebert, 1997c). Because papain is a non-selective proteolytic enzyme with a negative effect on foaming research is being conducted to find a proteolytic enzyme that is sufficiently specific to attack only haze-active proteins (Siebert, 1997c). This would require a unique site in the haze-active protein that is lacking in foam-active protein and an enzyme that specifically cleaves at this site (Siebert, 1997c). If a protease were found that could cleave specifically next to the selected amino acid and would be active at the pH of beer, it would be of interest to try to evaluate its effectiveness in reducing protein-polyphenol haze (Siebert, 1997c).

Tannic Acid. Tannic acid is used as a specific precipitant of haze-active proteins in beer, but has been found to decrease concentration of the foaming polypeptides in some cases (Bamforth, 1999). Because the molecular structure of tannic acid is similar to that of polyphenols, it can precipitate proteins that otherwise react with the polyphenols in the beer (Bamforth, 1999). The resulting precipitate settles to the bottom of the storage tank and is removed by either decantation or subsequent filtration (Coors, 1983). Tannic acid in not currently used in stabilization treatments in most breweries due to the high cost of handling the precipitate (Coors, 1983).

Silica Hydrogels. Silica hydrogels (94% SiO_2) are one of the most common stabilization treatments used in the brewing industry today. These substances absorb haze-active proteins with very little effect on the foam-active proteins (Siebert, 1998; Siebert 1997c). Siebert and Lynn (1997c) stated that silica gel binds to proline residues in the proteins, the same residues that polyphenols attach to, to create haze (Siebert, 1997c).

Features of the hydrogels important for selection are the pore size of the particles and the surface area of the particle (Bamforth, 1999). It has been found that the best pore size for the hydrogel is 3-12 nm and that anything larger than this will result in a loss of head retention. Hydrogels with a lower particle size, have a proportionately larger surface area, with an associated increase in the adsorption rate (Bamforth, 1999; Siebert, 1997c). Research has shown that to increase or prolong the shelf life of beer, the brewer should use hydrogels by one of two methods available. The first is to use low-permeability gels in the storage tanks and the second is to use larger quantities of high-permeability gels (Bamforth, 1999). Newer types of silica hydrogels have been

developed with high stabilization efficiency that can increase the shelf-life of beer to as much as two years, but having a reduced permeability they need to be added to the storage tank (Bamforth, 1999). Some research indicates that a combined filtration with silica hydrogels can create savings in the cost of raw materials, waste disposal costs, total process costs, filter life, and a reduced risk of iron pick-up (Bamforth, 1999). The use of silica hydrogels will depend greatly on the product being developed as well as the shelf life required (Bamforth, 1999).

Silica hydrogel works by preferentially removing haze-forming proteins from beer. Silica hydrogel recognizes and interacts with the same sites on the proteins as polyphenols (Bamforth, 1999), but may not work as efficiently in high polyphenol products (Siebert, 1998). It is still unknown if the polyphenols and the hydrogels compete for the same sites on the haze-active protein. If this is the case, then high levels of polyphenols in beer will inhibit the use of silica hydrogels making the co-treatment of silica hydrogels and polyvinylpolypyrrolidone the best stabilization technique (Bamforth, 1999).

Polyvinylpolypyrrolidone (PVPP). PVPP was introduced for commercial use as an adsorbent of beer phenolics as early as 1961 and is now widely used to prolong the stability of beers against the formation of haze (McMurrough, 1995). PVPP, a neutral polyamide, adsorbs polyphenols in the beer, especially the flavanoids and tannins, two of the more common polyphenols in beer (Siebert, 1998) leaving nothing for the protein to interact with (Coors, 1983). PVPP is water-soluble and hydrophilic and will bind water through hydrogen bonding (Doner, 1993). The affinity of PVPP increases as the number

of phenolic hydroxy groups increase,as more groups are available for hydrogen bonding (Doner, 1993). Siebert, Carrasco, and Lynn (1996a) state that the structure of PVPP strongly resembles the structure of polyproline and that it will bind polyphenols in the same way proteins rich in proline will bind with polyphenols. Figure 10 shows the partial structures of polyproline and PVPP. It is interesting to see that both substances have five-membered, saturated, nitrogen-containing rings and amide bonds with no other functional groups (Siebert, 1998).

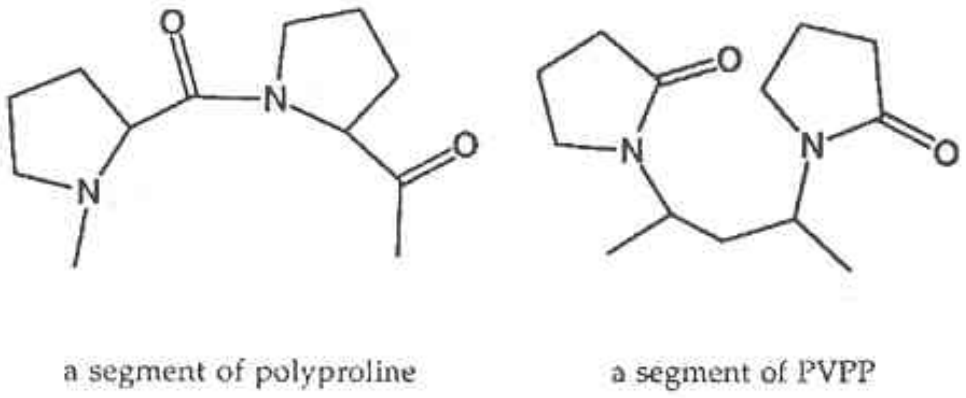


Figure 10. Comparison of partial structures of polyproline and polyvinylpolypyrrolidone. From "Comparison of Polyphenol Interactions with Polyvinylpolypyrrolidone and Haze – Active Protein," K. Siebert & P. Lynn, 1998, *Journal of the American Society of Brewing Chemists.* 56(1), 24-31.

According to literature from BASF, the manufacturer of PVPP, the use of PVPP is an appropriate method of stabilization of beer due to its ability to extend shelf-life, give a constant quality product, and give colloidal stability under extreme climatic conditions. This is only possible with the homogeneous treatment of PVPP in the tank, otherwise substantially different levels of polyphenols will be seen in different packages (Bamforth,

1999). The use of PVPP may be viewed as inappropriate due to the removal of potential antioxidants as well as the removal of polyphenols that are needed for beer body, but overall the general conclusion is that the levels of polyphenols present in most beers are too low to make a contribution to the mouthfeel (Bamforth, 1999). PVPP treatment may also impact the flavor due to the proposal that some polyphenolic compounds may influence the development of stale, oxidized flavors in beer (McMurrough, 1995). One added benefit of the use of PVPP is that it promotes foam stability by taking out polyphenols that may otherwise interact with foaming polypeptides (Bamforth, 1999).

CHAPTER III

METHODOLOGY

Beer Production

Leinenkugel's Red Lager was the beer of interest in this study. The beer was produced per protocol for the plant with the ingredients being barley, corn grits, and hops. The formulation is held in strict confidence by the manufacturer. To ensure that the product was the same throughout, six batches were produced from the same raw materials. The six batches were then put into three fermentation tanks where the yeast was injected and the wort fermented for 8 to 10 days. Once fermentation was complete, the contents of all three fermentation tanks were mixed together in one storage or ruh tank and stored to complete the aging process. Once the aging was complete, the lager was filtered through a primary filter containing diatomaceous earth and then separated into five secondary ruh tanks. The beer was stored and then filtered a second time, the variable, into five government tanks from which the lager was bottled. The control, primary filtered beer was taken from the primary filtration storage tank prior to the secondary filtration. Figure 11 shows the flow of the raw materials through the plant and the steps used to make the beer.

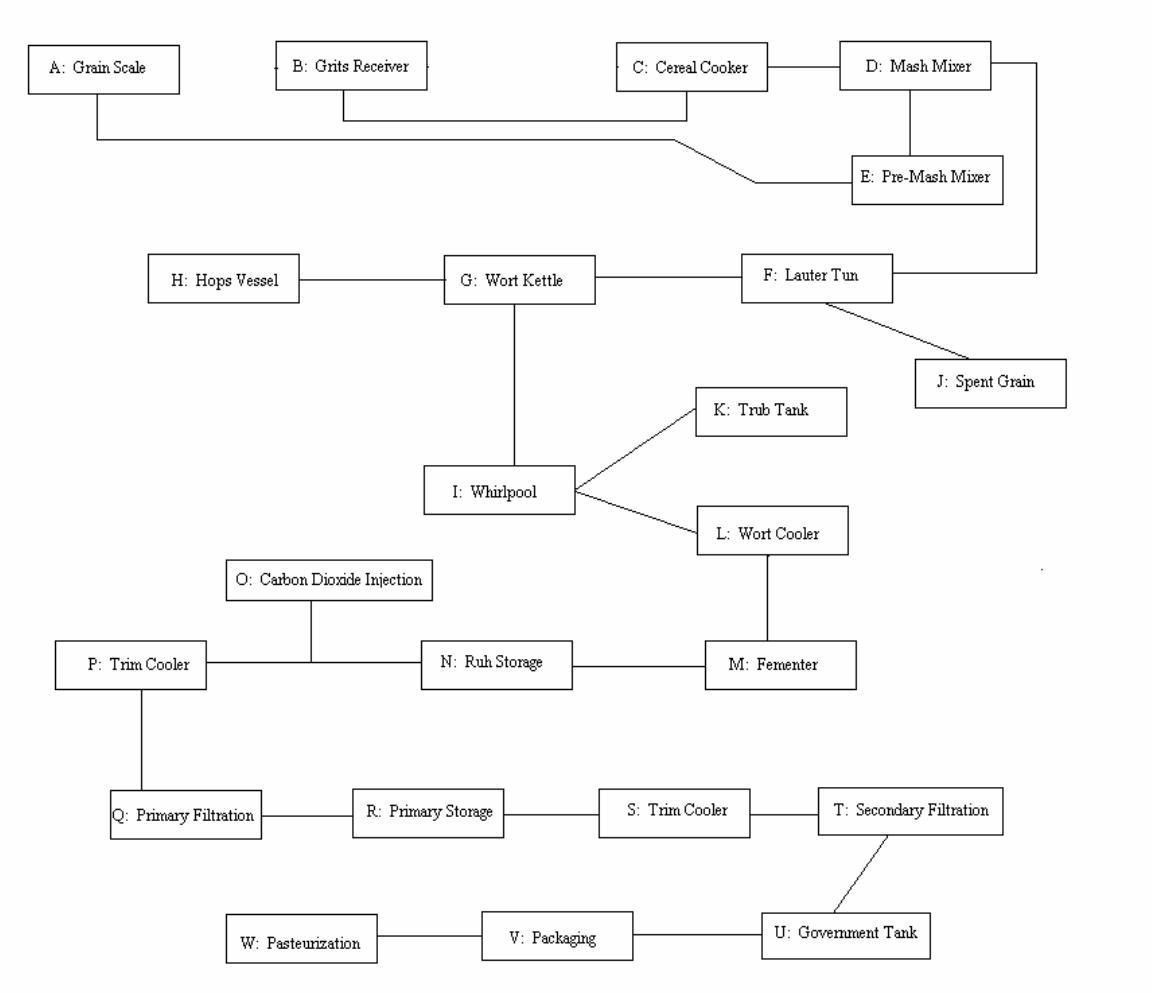


Figure 11. Flow chart of raw materials through the plant in beer processing

Secondary Filtration

The primary filtration was completed at the brewery prior to primary storage with the use of diatomaceous earth. The diatomaceous earth filters out any large particles left in the lager following production. The secondary filtration was completed at the brewery per protocol with six different combinations of filtering aids for the variables. The control for this project was lager that only received primary filtration. The five variations

of filtering aids are ratios of polyvinylpolypyrrolidone (PVPP) and silica hydrogel (SHG) at mixtures shown in Table 2. Once the lager was filtered, it was stored in government tanks (holds finished beer) until packaging.

Table 2.

Secondary Filtration Aids

Silica Hydrogel (SHG)	Polyvinylpolypyrrolidone (PVPP)
0 lbs/100 barrels	0 lbs/100 barrels
10 lbs/100 barrels	8 lbs/100 barrels
10 lbs/100 barrels	5 lbs/100 barrels
15 lbs/100 barrels	4 lbs/100 barrels
15 lbs/100 barrels	3 lbs/100 barrels
20 lbs/100 barrels	0 lbs/ 100 barrels

The combinations of secondary filtration aids were set-up at the brewery by the brewmaster based upon recommendations from the manufacturers of each filtrant and was explained in Chapter 2.

Filtering was completed at the brewery only after the beer was chilled. The beer was pumped from the ruh tanks through a tubular heat exchanger to chill the beer. This precipitates coarse, high molecular weight proteins. Once the beer was properly chilled, the diatomaceous earth slurry was injected into the beer, which was then passed through a filtering system that removed the diatomaceous earth as well as any precipitates,

including yeast. At this point the PVPP and SHG slurries were injected into the beer as it traveled to the secondary ruh tanks. The resulting mixture was allowed to stand for twenty-four hours. After this rest period, the beer containing the filtering aids was pumped through the filtering system again to remove the SHG and PVPP and pumped directly into the government tanks for bottling.

The bottles were filled per protocol at the plant using the bottle filler. This allowed more control of the amount of air in the bottles and simulates the packaging of beer at the plant. Nine filled bottles from each variation were pulled from the line at random times during the filling to provide a representative sample of the entire tank of beer. These were the unpasteurized samples. The rest of the bottles were allowed to go through the low temperature long-time pasteurizer. At the completion of this process, nine bottles from each pasteurized variation were randomly pulled from the line.

Sample Preparation

To ensure that there was a representative sample for each test, the following procedure was completed in Science Wing 313, chemistry lab to produce samples that would be used in all tests with the exception of the dissolved oxygen and the physical stability tests. The latter were measured separately at the plant.

From each set of nine bottles, three of the bottles were opened and mixed in a 2000-mL beaker (six bottle were reserved for physical stability). The beer mixture was then placed into three different 500-mL suction flasks equipped with rubber tubing connected to the laboratory vacuum system. The flasks were stoppered and shaken to remove most of the carbonation through the rubber tubing. Once the foaming subsided,

the tubing was attached to the vacuum system and the vacuum turned on and off alternately with gentle swirling to ensure none of the sample went into the tubing. Once degassed, the flasks were again mixed in the beaker and poured back into the bottles for storage and stored in the cooler.

Dissolved Oxygen Test

Dissolved oxygen (DO) was measured in the government tank (package release tank) with the use of the Orbisphere, Series 3650 Micro O_2 Logger, per Operators Manual directions. The Orbisphere is a readout instrument with an oxygen sensor in a flow chamber. To test the dissolved oxygen, the Orbisphere was connected to the zwickle (sampling spout). The spout was opened to allow a small trickle to flow through Orbisphere. Once the beer was flowing through the Orbisphere, the instrument was turned on. The reading (ppm) was taken after the value stabilized. This was repeated 5 times on each tank. The dissolved oxygen content of unfiltered beer could not be read due to the high amounts of solids such as yeast and grain particles left in the lager.

The Orbisphere works on the concept of electrophoresis. The sample in the flow cell comes in contact with an oxygen-permeable membrane. The oxygen passes into the chamber containing a gel in which electrical conductivity is measured. DO is inversely proportional to conductivity. (Orbisphere 3560 Micro O_2 Logger Operators Manual, n.d.). Prior to the measurement of dissolved oxygen, the Orbisphere was calibrated and the membrane and seals were replaced.

Color

Color was measured using two different spectrophotometers to compare a single beam instrument to a double beam instrument. The first method was with the use of the Shimadzu UV-Visible Spectrophotometer equipped with a sample sipper system and flow cell, model UV mini-1240 120V, that is currently in use at the Leinenkugel Brewery. The Shimadzu has a wavelength range of 190 to about 1100 nm with a spectral bandwidth of 5 nm. The accuracy of the Shimadzu is ±1.0 nm with a repeatability of ±0.3 nm. It has single beam optics with less than 0.05% stray light. The light sources are a Deuterium lamp as well as a 20W tungsten halogen lamp that uses a concave holographic grating monochromator with a silicon photodiode (Shimadzu Operators Manual, n.d.).

In this method, the wavelength was set at 430 nm and the absorbance was zeroed using distilled water as the blank. Each sample was degassed and equilibrated at room temperature prior to measurement of absorbance. The sampling tube was inserted into each sample and the pump activated to draw sample into the spectrophotometer flow cell. Absorbance was displayed on an LCD backlit screen (Shimadzu Operators Manual, n.d.). Between each sample distilled water was passed through the system to rinse the flow cell.

A Varian Cary UV/Visible Spectrophotometer was the second instrument used to measure absorbance. This is a double-beam instrument equipped with both a deuterium lamp and a tungsten halogen lamp.

The wavelength was again set at 430 nm and the absorbance was zeroed using distilled water in a 10-mm cuvette in the reference cell as well as the sample cell. The samples were degassed and equilibrated at room temperature prior to taking

measurements. Distilled water was left in the reference cell and the samples were measured in a matched cuvette. The sample cuvette was rinsed with distilled water several times between readings. This procedure was repeated five times for each sample.

Protein (Percent by Weight)

A micro kjeldahl apparatus was used for the measurement of nitrogen. The digestion procedure was a modification of the American Society of Brewing Chemists standard procedure. The distillation procedure was taken from the Operators Manual for the Labconco Rapid Still I. Two samples from each filtration were digested allowing for four distillations from each digested sample for a total of eight readings from each sample (each digestion was diluted to 100 mL). These results were averaged.

Prior to digestion of the samples each of the Labconco 100-ml digestion flasks was washed with nitrogen free soap and rinsed well with distilled water. The flasks were placed in the Lindberg/ Blue mechanical convection oven (Model #MO1450A) for an hour or until they were dry. They were allowed to cool to room temperature and the weight was measured and recorded. A milligram balance was used to measure 25.00 g of degassed beer at 20^{o}C in a clean, dry digestion flasks to which 2 ml of 96% H_2SO_4 was added and allowed to concentrate into a syrupy consistency. To the digestion flask, 10 g of powdered K_2SO_4 was added as well as 0.3 g powdered TiO_2, 0.3 g powdered $CuSO_4$, 25 mL 96% H_2SO_4, and a small amount of paraffin wax (to reduce foaming). This was gently swirled to mix the contents of the digestion flasks. The flasks were placed in an inclined position on the Labconco Micro-Kjeldahl (Model #60300-00) digestion rack and heated at the heat control setting of 4 until the frothing stopped. At this point the heat

was slowly increased to the heat control setting of 7 to bring the acid to a brisk boil. They were allowed to boil until the solution cleared. Once cleared, the flasks were allowed to boil for an additional 30 minutes. The flasks were removed, and allowed to cool to room temperature overnight. Once cooled the samples were diluted to the mark (100 mL) with distilled water and mixed well. This was again cooled to room temperature.

Prior to the distillation of the samples, the steam reservoir of the Labconco Rapid Still I (Model #65000) distillation unit was filled 2/3 full with deionized water and the heating element turned on with the heat control set at 9. The water was allowed to come to a boil and the condenser water was turned on. At this point the sample addition funnel was filled with distilled water and the stopcock opened allowing the water into the mixing chamber. The mixture was boiled for 4-5 minutes to make sure the chamber and condenser were clean. The water was aspirated from the mixing chamber and the steam reservoir was refilled. Once the steam reservoir water returned to a boil, the digestion flask was mixed well and 20.0 mL of the digested sample was placed in the sample addition funnel. The stopcock was opened allowing the sample to slowly enter the mixing chamber. A 50 mL sample of saturated boric acid was placed in a small beaker and 4 drops of methyl red/bromcresol green added. The beaker was placed under the condenser tube with the end of the tube fully submerged in the acid. The sample addition funnel was rinsed multiple times with 4 mL of distilled water. All rinses were added to the mixing chamber. The sample addition funnel was used to introduce 20 mL of 40% NaOH into the mixing chamber. Distillation was allowed to proceed for nine minutes. Following this time period, the boric acid beaker was removed from beneath the

condenser tube and the sample removed by aspiration. The sample addition flask was again filled with distilled water and allowed to enter the mixing chamber and aspirated to rinse remnants of the old sample. The distillate was titrated with 0.100 N HCl until the color of the sample returned to the pink/red color. At this point the amount of HCl (mL) was recorded and the calculation was completed to determine the amount of protein in the sample. The procedure was repeated until all the samples were completed

$$\text{Sample effective weight} = \frac{(\text{Weight of sample (g)}) \times (\text{Sample Aliquot (mL)})}{100}$$

$$\%\ \text{Protein} = \frac{(\text{mL HCl}) \times (\text{N HCl}) \times (0.014) \times (100) \times (6.25)}{\text{Sample Effective Weight}}$$

The calculations were completed using the equations above where 0.014 is the milliequivalent weight of nitrogen and 6.25 converts percent nitrogen to percent protein.

Total Polyphenols

The American Society of Brewing Chemists procedure for total polyphenols is accepted as a standard procedure in the brewing industry (ASBC Methods of Analysis 1987, Beer-35). Carboxymethylcellulose reagent was prepared by placing 500 mL of distilled water in an Erlenmeyer flask and adding 10.0 g pure CMC and 2.0 g disodium ethylene diamine tetraacetate to it. This was stirred for about 2-3 hours using a stir bar or until all the solid was in solution. The solution was quantitatively transferred to a clean 1-L volumetric flask and diluted to volume with distilled water. Ferric reagent was prepared by mixing 21.845 g of green ammonium ferric citrate with 78.155 g of water in an Erlenmeyer flask to make a 3.5% Fe solution or a 21.8% $FeNH_4C_6H_6O_7$ solution.

Dilute aqueous ammonia was prepared by mixing 1 volume of concentrated ammonia with 2 volumes of distilled water.

The polyphenol procedure consisted of pipetting 10.00 mL of degassed beer and 8.00 mL of the CMC reagent into five 25 mL volumetric flasks with ground glass stoppers. 0.5 mL of ferric reagent was added and mixed, followed by 0.5 mL of dilute ammonia. This was adjusted to 25.00 mL by adding distilled water. The solutions were mixed and allowed to equilibrate for 10 minutes. The blank was made by mixing 10.00 mL of degassed beer, 0.5 mL dilute ammonia, and 8.00 mL of CMC reagent in a 25-mL volumetric flask. The solution was made to volume by adding distilled water and mixed. This solution was allowed to equilibrate for 10 minutes. The Varian-Cary spectrophotometer was turned on and set to a wavelength of 600 nm. It was zeroed using distilled water in both the reference cell and the sample cell. Once the equilibration was complete, the blank was placed in the sample cell with the distilled water still in place in the reference cell. The absorbance was measured against the distilled water. The blank was removed from the sample cell, the sample was placed in the sample cell and the absorbance was measured, five times, against the distilled water. This was repeated until all five samples were read. The absorbances were recorded for each sample and the average was calculated using the five readings. The absorbance of the blank was subtracted from the sample absorbance and multiplied by 820, which is the inverse of the slope of the standard curve, to give the concentration of the polyphenols in mg/L. To compensate for the dilution of the beer to 25.00 mL, the concentration was multiplied by a dilution factor of 2.50.

Flavanoids

The American Society of Brewing Chemists procedure for flavanoids ((+)-catechin) is accepted as a standard procedure in the brewing industry (ASBC Methods of Analysis 1987, Beer 36). A standard curve was created using the method of standard addition. The ASBC method for flavanoid tests for (+)-catechin and is an indicator of the amount of flavanoids in the beer. Catechin was used as the added standard in all samples analyzed. The Varian-Cary spectrophotomer was set up to read a wavelength of 640 nm and was zeroed using distilled water in both the reference cell and the sample cell. Chromagen solution was prepared by mixing 125 mL of concentrated hydrochloric acid with 350 mL of methanol. The solution was cooled to room temperature and 500 mg p-dimethylaminocinnamaldehyde added. This was diluted with methanol to a final volume of 500 mL in the volumetric flask. The solution was wrapped in tinfoil and placed in a cool dark place for storage. The beer was diluted by pipetting 10.0 ml of the degassed beer into five 100-mL volumetric flasks. To the first flask there was no added (+)-catechin. In the four remaining flasks, 1.00 mL, 2.50 mL, 5.00 mL and 10.00 mL (+)-catechin were added to make the solutions. Each was labeled according to the amount of added internal standard: 0.00 mg/L, 10.0 mg/L, 25.0 mg/L, 50.0 mg/L, and 100.0 mg/L. Each of the flasks was diluted to 100 mL with distilled water. Using a pipette 1.00 mL of each standard and 5.00 mL of chromagen reagent were added to a 50-mL beaker. This was mixed and, following a 10-minute equilibration, transferred to a 10 mm cuvette. The absorbance was read at 640 nm against a distilled water reference. The blank was

prepared by mixing 1.00 mL of distilled water with 5.00 mL of chromagen reagent. Following a 10 minute equilibration, was transferred to a 10-mm cuvette and absorbance read at 640 nm. The absorbance of the prepared blank was subtracted from the standards' absorbance. Five absorbances were taken on each standard and prepared blank. Once the difference was calculated, the average was calculated. The flavanoids content was determined on a Beer's law plot and interpolating the concentration of the (+)-catechin equivalents (x-axis) as the absolute value of the x-intercept of the standard curve. This value was multiplied by 10.0 as a dilution factor.

Physical Stability (Chill Haze)

The physical stability of beer is more commonly known as the permanent haze if completed on warm samples, or chill haze if measurements are taken from cold samples. Chill haze testing is completed by subjecting the sealed bottles to extremely high temperatures as well as extremely low temperatures. This is the most widely used method for testing for the tendency to form haze (McMourrough, 1999). Turbidity instruments project a light beam into the sample and the light scattered by solids in suspension, is picked up by photo cells (Hahn, 1983).

Three unpasteurized samples and three pasteurized samples of each filtering variation were tested. Three of the six bottles were chilled to 0^{o}C for 24 hours. After 24 hours the bottles were opened and the contents poured into the test bottle for the measurement of the turbidity. Turbididty was measured by placing the test bottle in the turbidometer and measuring the turbidity in Foramzin turbidity units. This was the initial haze. The accelerated haze was determined using the three remaining bottles. These

were placed in an upright position in a 60^{o}C convection oven for 1 week. At the end of the week the bottles were removed and allowed to sit at room temperature for 12 hours to cool. The bottles were then placed in the 0^{o}C cooler for 24 hours. Immediately following the chill, bottles were opened and the contents poured into the test bottle for measurement of the turbidity as described above.

Statistical Analysis

The statistical analysis was completed using Microsoft Excel. Single factor analysis of variance was completed on each test to determine if the varying amounts of PVPP used in each sample had a significant effect ($p \geq 0.05$). Fischer's Least Significant Difference (LSD) was then used to determine which samples were different. This was completed after arranging the values in descending order and assigning letter subscripts based on the LSD value. Once the subscripts are determined, the data values are rearranged in the original order thus the subscripts are out of order. To determine if the pasteurization had a significant effect ($p \geq 0.05$) Student's t-test (Paired two sample for equal means) was used. This determined if in each filtration there was a significant difference between the pasteurized sample and the unpasteurized sample.

CHAPTER IV

RESULTS AND DISCUSSION

Determination of Beer Components

The purpose of this study was to determine how different colloidal stabilizers would affect quality aspects of the beer as well as to determine how each combination affected the amounts of protein, polyphenols and flavanoids in the product. The analysis was completed in replicates of five samples on each stabilizer combination. The effect of pasteurization was also studied.

Determination of Color. The absorbance was measured using both the Shimadzu and the Varian-Cary Spectrophotometers at 430 nm. Since most of the laboratory work was completed at the University of Wisconsin-Stout, the data collected from the UW-Stout owned Varian-Cary spectrophotometer was used. The results were compared to the measurements taken at the brewery using the Shimadzu spectrophotometer, but there was little difference between the absorbances measured, thus only the results from the Varian-Cary spectrophotometer will be discussed here. The data from the Shimadzu spectrophotometer is shown in Appendix A and the data from the Varian-Cary spectrophotometer is shown in Appendix B. According to quality standards set up at the brewery, the absorbance values must be between 1.5748 AU and 1.7323 AU to be within specification limits. Since the absorbance of coarse filtered beer was well above the upper limit the values were not included in the statistical analysis. There was significant

difference ($p \geq 0.05$) between all the filtering aids in both pasteurized and unpasteurized beer as seen in Table 3.

Table 3.

Color Measurements from the Varian-Cary Spectrophotometer (λ = 430nm) using Varying Levels of Stabilizers

SHG (lbs/100bbls): PVPP (lbs/100bbls)	Mean (AU)* Unpasteurized	Mean (AU)* Pasteurized
20:0	$1.6212 \pm 0.0002_{e}$	$1.7048 \pm 0.0002_{c}$
15:3	$1.666 \pm 0.0018_{c}$	$1.6902 \pm 0.0012_{e}$
15:4	$1.7128 \pm 0.0002_{b}$	$1.7037 \pm 0.0003_{d}$
10:5	$1.6331 \pm 0.0004_{d}$	$1.7399 \pm 0.0004_{b}$
10:8	$1.7585 \pm 0.0009_{a}$	$1.7679 \pm 0.0009_{a}$

Note. Means in a column with different subscripts are significantly different ($p \geq 0.05$) by Fisher least significant difference test (LSD = 0.0012).

*Uncertainties represent standard deviations from the mean of five measurements.

The effect on the absorbance measurement from the pasteurization on each filtering/stabilizer treatment was analyzed by comparing the pasteurized sample to the unpasteurized sample in a paired two-sample for equal means t-test. The results showed that there was a significant difference ($p \geq 0.05$) due to the pasteurization on all stabilizer treatments and can be seen in Table 4.

Table 4.

Means Comparing the Effect of Pasteurization on Color Measured on the Varian-Cary Spectrophotometer (λ = 430nm)

SHG (lbs/100bbls): PVPP (lbs/100bbls)	Means (AU)* Unpasteurized	Means (AU)* Pasteurized
20:0	$1.6212 \pm 0.0002_{b}$	$1.7048 \pm 0.0002_{a}$
15:3	$1.6660 \pm 0.0018_{b}$	$1.6902 \pm 0.0012_{a}$
15:4	$1.7128 \pm 0.0002_{a}$	$1.7037 \pm 0.0003_{b}$
10:5	$1.6331 \pm 0.0004_{b}$	$1.7399 \pm 0.0004_{a}$
10:8	$1.7585 \pm 0.0009_{b}$	$1.7679 \pm 0.0009_{a}$

Note. Means in the same row that are followed by different subscripts are statistically different ($p \geq 0.05$) by paired two sample for equal means t-test.

*Uncertainties represent standard deviations from the mean of five measurements.

Absorbance readings at 430 nm were found to be significantly different when stabilizer treatment and pasteurization were considered. The stabilizers altered the appearance of color in both the pasteurized and unpasteurized samples. The absorbance readings were expected to decrease with an increasing amount of PVPP (J. Buhrows, personal communication, April 12, 2003), but this expected trend was not observed. In this case, the color decreased, increased and then decreased again. A build up of diatomaceous earth on the filters during primary filtration may have caused the change in color

intensity. This problem is seen in all of the Leinenkugel's products due to the filtering system used in the plant.

At the beginning of the day, when the primary filtration starts there is a small layer of diatomaceous earth pre-coat on the filter. As the beer flows into the filters, a small amount of diatomaceous earth is injected into the beer. As the day and the filtration continues, the layer of diatomaceous earth increases on the filter, and may have an effect on the color of the eluting beer in primary filtration. Due to this phenomenon in the primary filters it is difficult to determine if the difference in color was due to the filtering aids or the diatomaceous earth build-up. Since there is no way to determine when each of the batches of beer was filtered it is difficult to determine the cause of the color differences in the product.

This phenomenon is not seen in secondary filtration when dealing with SHG and PVPP since both these stabilizers/filtering aids work by adsorption of proteins and polyphenols, respectively. The adsorption of the proteins and polyphenols is dependant on the temperature of the beer when the filtering aids are injected as well as contact time (J. Buhrows, personal communication, April 12, 2003).

Total Polyphenols. Polyphenols are mainly stabilized/filtered with the use of PVPP. As the amount of PVPP injected into the beer increases, it is expected that the total polyphenol content will decrease. PVPP is removed from the product via filtration and the adsorbed polyphenols with it. Measurements of the total polyphenols in unpasteurized beer (Table 5) showed that there was no significant difference ($p \geq 0.05$) between coarse filtered beer and beer filtered with only 20 SHG. This is reasonable since

there was no increase in the amount of PVPP in the beer. There was also no significant difference ($p \geq 0.05$) between unpasteurized beer filtered with 15:4 SHG:PVPP and unpasteurized beer filtered with 10:5 SHG:PVPP. In all other filtering combinations of SHG:PVPP there was a significant difference ($p \geq 0.05$) in total polyphenol content in the unpasteurized beer. When looking at the pasteurized beer samples, there was a significant difference ($p \geq 0.05$) in total polyphenol content between all the samples except for the samples filtered with 10:5 SHG:PVPP and 10:8 SHG:PVPP which were found to have no significant difference and can be seen in Table 5. When the PVPP was increased to 8 lbs/100 barrels from 5 lbs/100 barrels in the pasteurized beer, there was an apparent increase in the total polyphenol content measured, but the change was not statistically significant ($p \geq 0.05$). One phenomenon not studied was if there was a point in which added amounts of stabilizer would no longer affect the polyphenol content. In a study completed by Siebert and Lynn, (1997c) it was observed that the polyphenol content rapidly declined to about one half of the original amount with lower PVPP treatments, but when higher increments of PVPP were added, there was little additional change in the polyphenol content. For example, in their study, PVPP was added from 0 g/L to 5 g/L. The amount of haze-active polyphenols showed a decrease when up to 2 g/L PVPP was added and then leveled off if more PVPP was used (Siebert, 1997c). This phenomenon was not seen in the unpasteurized beer samples in this study as shown in Table 5. The samples filtered with 10:8 SHG:PVPP actually showed a significant decrease in the polyphenol content when compared to the samples filtered with 10:5 SHG:PVPP.

Table 5.

Total Polyphenol Measurements Using Varying Amounts of Stabilizers

SHG (lbs/100bbls): PVPP (lbs/100bbls)	Mean (mg/L)* Unpasteurized	Mean (mg/L)* Pasteurized
Coarse	737 ± 23 $_{a}$	758 ± 34 $_{a}$
20:0	732 ± 28 $_{a}$	690 ± 9 $_{b}$
15:3	641 ± 16 $_{b}$	658 ± 15 $_{c}$
15:4	589 ± 17 $_{c}$	609 ± 25 $_{d}$
10:5	578 ± 19 $_{c}$	549 ± 14 $_{e}$
10:8	538 ± 16 $_{d}$	577 ± 7 $_{e}$

Note. Means in a column with different subscripts are significantly different ($p \geq 0.05$) by Fisher least significant difference test (LSD = 27).

*Uncertainties represent standard deviations from the mean of five measurements

The effect of pasteurization on the total polyphenol content of beer (Table 6) was studied as well. It was shown that there was a significant difference ($p \geq 0.05$)in the total polyphenols in beer due to pasteurization in the samples stabilized/filtered with 20:0 SHG:PVPP and the samples stabilized with 10:8 SHG:PVPP. There was no significant difference ($p \geq 0.05$) due to pasteurization in all other stabilizer/filtering treatments. The total polyphenol data can be seen in Appendix C. - Total Polyphenol Measurements in Pasteurized and Unpasteurized Beer using Varying Amounts of Stabilizers.

Table 6.

Means Comparing the Effect of Pasteurization on Total Polyphenols

SHG (lbs/100bbls): PVPP (lbs/100bbls)	Means (mg/L)* Unpasteurized	Mean (mg/L)* Pasteurized
Coarse	737 ± 23 $_{a}$	758 ± 34 $_{a}$
20:0	732 ± 28 $_{b}$	690 ± 9 $_{a}$
15:3	641 ± 16 $_{a}$	658 ± 15 $_{a}$
15:4	589 ± 17 $_{a}$	609 ± 22 $_{a}$
10:5	578 ± 19 $_{a}$	549 ± 14 $_{a}$
10:8	538 ± 16 $_{b}$	577 ± 8 $_{a}$

Note. Means in the same row that are followed by different subscripts are statistically different ($p \geq 0.05$) by paired two sample for equal means t-test.

*Uncertainties represent standard deviations from the mean of five measurements

Total Flavanoids. Flavanoids are just one of the many polyphenols that are thought to cause haze in beer. This is not the most haze active, but is the one that is commonly measured due to the ease of the test as well as the availability of the indicator (+)-catechin to use in standard curves. This test is actually the measurement of (+)-catechin equivalents and the results are reported in mg/L of (+)-catechin equivalents. This test was run only once for the measurement of total flavanoids due to the use of the method of standard addition. The results shown are interpolated from the standard curve produced for each sample. There was a total of 12 standard curves made using the sample as the

base of the curve and can be seen in Appendix D. The values shown here are the interpolated values (multiplied by 10 for the dilution factor) from each of the standard curves.

Table 7.

Total Flavanoid Measurements Using Varying Amounts of Stabilizers.

SHG (lbs/100bbls): PVPP (lbs/100bbls)	Flavanoids (mg/L) Unpasteurized	Flavanoids (mg/L) Pasteurized
Coarse	380	210
20:0	250	200
15:3	270	150
15:4	180	130
10:5	190	210
10:8	130	170

The PVPP stabilizer works by adsorbing the polyphenols in the beer and their removal when the PVPP gets filtered out of the beer. Consequently, with increasing amounts of PVPP added to beer there should be a reduction in the amount of flavanoids remaining in the beer after filtration. This is seen in the first four samples, but when the level of PVPP increases over four pounds/100 bbls, there is no longer a decrease in the total flavanoids content in beer. In a study by McMurrough, Madigan, Kelly, and O'Rourke (1999), the total flavanoids did not decrease in linear proportion to the amount of PVPP used, which also occurred in this study. This was also seen in the total

polyphenol content measurements and is consistent since, the family of polyphenols contains flavanoids.

The amount of dissolved oxygen (Table 8) will also affect the polyphenol and flavanoid content of the beer since the oxygen will polymerize the polyphenols and flavanoids thus not allowing the PVPP to adsorb them as effectively. The amount of dissolved oxygen in the sample stabilized with 10:8 SHG:PVPP was about double the amount in the other five stabilizer treatments and could account for the increased amount of flavanoids.

Dissolved Oxygen. The dissolved oxygen values were determined at the brewery by the use of the Orbisphere Micro O_2 Logger. Five measurements were taken on samples from each of the filtration/stabilization treatments in the unpasteurized beer. Measurements were not taken on the pasteurized beer since the dissolved oxygen would be the same as in the unpasteurized beer. Table 8 shows the average dissolved oxygen measurement in beer samples along with the standard deviation of the mean. The actual measurements can be seen in Appendix E – Dissolved Oxygen Measurements in Unpasteurized Beer with Varying Amounts of Stabilizers.

Dissolved oxygen was measured to make sure that the processing of the beer was in control at the brewery. Dissolved oxygen will not be affected by the different filtering aids, but will affect the development of haze in the product due to oxygen interaction with the polyphenols. As seen in Table 8, the amount of dissolved oxygen is about the same in all the batches, with the exception of the beer filtered with 10:8 SHG:PVPP. This variation is not caused by the filtering aid, but was caused by something that

occurred during the processing of this batch of beer. One possible reason for the higher dissolved oxygen content is that when the product was being transferred between tanks in the brewery, backpressure in the tank may not have been correct. This would leave some oxygen in the tank and result in higher dissolved oxygen values.

Table 8.

Dissolved Oxygen Measurements on Varying Levels of Filtering Aids in Unpasteurized Beer

SHG (lbs/100bbls): PVPP (lbs/100bbls)	Mean (ppm)*
Coarse	0.039 ± 0.001
20:0	0.049 ± 0.001
15:3	0.039 ± 0.001
15:4	0.048 ± 0.002
10:5	0.038 ± 0.003
10:8	0.091 ± 0.002

* Uncertainties represent standard deviations from the mean of five measurements.

Initial and Accelerated Haze. The haze measurements were completed on six bottles of beer per filtration for both pasteurized and unpasteurized samples. Three of the bottles were used for the initial chill haze test and the other three were used for the accelerated chill haze test. The raw data for the initial haze measurements are found in Appendix F and accelerated haze measurements in Appendix G. Initial haze was measured after a 24 hour chill at 0^{o}C while the accelerated chill haze was measured after a 1 week incubation at 60^{o}C followed by 24 hours of chill at 0^{o}C. This mimics the effect of a three-month

shelf life. The means and the standard deviations of haze measurements of the mean are shown in Table 9 for unpasteurized beer and Table 10 for pasteurized beer.

Table 9.

Haze Measurements using Varying Levels of Filtering Aids in Unpasteurized Beer

SHG (lbs/100bbls): PVPP (lbs/100bbls)	Initial Haze Mean (FTU)*	Accelerated Haze Mean (FTU)*
Coarse	331± 46 $_a$	>800 $_a$
20:0	114 ± 2 $_b$	>800 $_a$
15:3	120 ± 1 $_b$	>800 $_a$
15:4	121 ± 1 $_b$	627 ± 58 $_b$
10:5	100 ± 1 $_b$	615 ± 13 $_b$
10:8	99 ± 1 $_b$	460 ± 34 $_c$

Note. Means in a column with different subscripts are significantly different ($p \geq 0.05$) by Fisher least significant difference test (Initial Haze LSD = 34; Accelerated Haze LSD = 50).

*Uncertainties represent standard deviations from the mean of three measurements

There was no significant difference ($p \geq 0.05$) in initial haze due to the varying levels of filtering aids with the exception of coarse filtered beer, which was significantly different from all other samples when single factor analysis of variance was used to determine mean differences. When looking at the accelerated chill haze there was no

significant difference between the unpasteurized samples that were coarse filtered, the unpasteurized samples that were filtered with 20:0 SHG:PVPP and the unpasteurized samples filtered with 15:3 SHG:PVPP. There was also no significant difference ($p \geq 0.05$) between the unpasteurized samples filtered with 15:4 SHG:PVPP and the unpasteurized samples filtered with 10:5 SHG:PVPP, but the beer filtered with 10:8 SHG:PVPP was significantly different from all other samples when the accelerated haze was measured in the unpasteurized beer (Table 9).

Table 10 shows the results of initial and accelerated chill haze on pasteurized beer based on single factor analysis of variance. The initial haze in the samples filtered with 20:0 SHG:PVPP and samples filtered with 15:3 SHG:PVPP showed no significant difference ($p \geq 0.05$) as well as no significant difference between the samples filtered with 15:3 SHG:PVPP and 15:4 SHG:PVPP, but there was a significant difference ($p \geq 0.05$) between the samples filtered with 20:0 SHG:PVPP and the sample filtered with 15:4 SHG:PVPP. The coarse filtered and the pasteurized filtered beer with 10:8 SHG:PVPP had initial hazes that were significantly different ($p \geq 0.05$) from all other samples. It is interesting to note that the sample filtered with 20:0 SHG:PVPP and the sample filtered with 10:5 SHG:PVPP showed no significant difference ($p \geq 0.05$).

When looking at accelerated haze measurements, there is no significant difference ($p \geq 0.05$) between the samples that were coarse filtered, the samples filtered with 20:0 SHG:PVPP and the samples filtered with 15:3SHG:PVPP. There was however a significant difference ($p \geq 0.05$) between all other filtrations in the pasteurized beer.

Table 10.

Haze Measurements using Varying Levels of Filtering Aids in Pasteurized Beer

SHG (lbs/100bbls): PVPP (lbs/100bbls)	Initial Haze Mean (FTU)	Accelerated Haze Mean (FTU)
Coarse	516 ± 14 $_{a}$	>800 $_{a}$
20:0	110 ± 1 $_{cd}$	>800 $_{a}$
15:3	117 ± 2 $_{bc}$	>800 $_{a}$
15:4	122 ± 3 $_{b}$	600 ± 10 $_{c}$
10:5	103 ± 3 $_{d}$	657 ± 25 $_{b}$
10:8	99 ± 6 $_{e}$	459 ± 33 $_{d}$

Note. Means in a column with different subscripts are significantly different ($p \geq 0.05$) by Fisher least significant difference test (Initial Haze LSD = 12; Accelerated Haze LSD = 31).

*Uncertainties represent standard deviations from the mean of three measurements

Accelerated haze mimics the haze formation in beer with a three month shelf life, thus consumers will only see the equivalent of an accelerated haze if they are consuming an outdated bottle of beer. Most other haze the consumers see is attributed to initial haze. The turbidometer used in this study is only sensitive enough to measure an FTU up to 800. On an FTU scale, a consumer will detect a haze at any measurement in excess of 250 FTU. None of the filtering aid/stabilizer treatments used in this study achieved accelerated haze measurements as low as 250 FTU however; the filtering combination that first showed a significant difference ($p \geq 0.05$) was filtered with 15:4 SHG:PVPP.

The effect of pasteurization on haze formation was also studied using a paired two-sample for equal means t-test. When looking at the initial haze, there was significant difference ($p \geq 0.05$) between the coarse filtered beer and all other stabilizer variations with no difference between all other stabilizer variations (Table 11). When looking at the result of the accelerated haze test, there was no significant difference ($p \geq 0.05$) due to pasteurization in all stabilizer variations studied (Table 12).

Table 11.

Means Comparing the Effect of Pasteurization on Initial Haze

SHG (lbs/100bbls): PVPP (lbs/100bbls)	Unpasteurized (FTU)	Pasteurized(FTU)
Coarse	331 ± 46 $_b$	516 ± 14 $_a$
20:0	114 ± 2 $_a$	110 ± 1 $_a$
15:3	120 ± 1 $_a$	117 ± 2 $_a$
15:4	121 ± 1 $_a$	122 ± 3 $_a$
10:5	100 ± 1 $_a$	103 ± 3 $_a$
10:8	99 ± 1 $_a$	99 ± 6 $_a$

Note. Means in the same row that are followed by different subscripts are statistically different ($p \geq 0.05$) by paired two sample for equal means t-test.

*Uncertainties represent standard deviations from the mean of three measurements

Table 12.

Means Comparing the Effect of Pasteurization on Accelerated Haze

SHG (lbs/100bbls): PVPP (lbs/100bbls)	Unpasteurized (FTU)	Pasteurized(FTU)
Coarse	>800 a	>800 a
20:0	>800 a	>800 a
15:3	>800 a	>800 a
15:4	627 ± 58 a	600 ± 10 a
10:5	615 ± 13 a	657 ± 25 a
10:8	460 ± 34 a	459 ± 33 a

Note. Means in the same row that are followed by different subscripts are statistically different ($p \geq 0.05$) by paired two sample for equal means t-test.

*Uncertainties represent standard deviations from the mean of three measurements

Crude Protein. The crude protein was measured in samples of beer using the Kjeldahl method of analysis. In previous studies completed for Leinenkugel's, the red beer was shown to contain about 0.5% protein by weight. Such a small amount of protein is not only difficult to measure by methods currently used in the food industry but a decrease in protein concentration upon removal of a portion of that protein would be even harder to detect. Nevertheless, such an incremental change in protein concentraion could be significant in reducing the amount of haze in beer (Sheehan, 1999). It was noted by Siebert and Lynn (1997a) that most protein analysis methods do not give information about the haze formation in the product since there is no accurate method for measuring the small amount of protein present in the beer. For example, the Coomassie blue dye

binding method is biased in favor of peptides rich in basic and aromatic amino acids, known to be proportionately low in beer (Siebert, 1997a). Since the concentration of protein in beer didn't appear to decrease in a regular fashion with increasing amounts of silica hydrogel (SHG) added, the change in protein concentration may have been too small to be detected by the analytical procedure or errors were made in the application of that method. Table 13 shows the average protein concentration in samples of beer treated with various combinations of stabilizers, whther pasteurized or unpasteurized. The raw data for Table 13 can be seen in Appendix H. Single factor analysis of variance was used to compare the means of the protein concentrations in each of the various beer samples. No significant difference ($p \geq 0.05$) was observed in the protein concentrations of samples of pasteurized, coarsely filtered beer and beer filtered with 15:3 SHG:PVPP. Beer filtered with 10:5 SHG:PVPP and 10:8 SHG:PVPP also showed no significant difference ($p \geq 0.05$) in protein concentration. There was significant difference ($p \geq 0.05$) in percent protein between all other samples due to the change in the filtering aids.

Among the unpasteurized beer samples, there was no significant difference in the protein concentration ($p \geq 0.05$) between coarsely filtered beer, beer filtered with 15:4 SHG:PVPP and beer filtered with 10:8 SHG:PVPP. There was a significant difference ($p \geq 0.05$) between all other unpasteurized samples due to the change in filtration.

Table 13.

Protein Measurements Using Varying Levels of Filtratnts

SHG (lbs/100bbls): PVPP (lbs/100bbls)	Mean (%)* Unpasteurized	Mean (%)* Pasteurized
Coarse	.491 ± .029 $_{b}$	.534 ± 0.00 $_{a}$
20:0	.409± 0.018 $_{d}$	.490 ± 0.010 $_{c}$
15:3	.540 ± 0.00 $_{a}$	.540 ± 0.010 $_{a}$
15:4	.485 ± 0.022 $_{b}$	.526 ± 0.017 $_{b}$
10:5	.460 ± 0.008 $_{c}$	.461 ± 0.009 $_{d}$
10:8	.486 ± 0.010 $_{b}$	.466 ± 0.010 $_{d}$

Note. Means in a column with different subscripts are significantly different ($p \geq 0.05$) by Fisher least significant difference test (LSD = 0.026 Unpasteurized; 0.016 Pasteurized).

* Uncertainties represent standard deviations from the mean of four measurements.

Table 14.

Means Comparing the Effect of Pasteurization on Protein Measurements

SHG (lbs/100bbls): PVPP (lbs/100bbls)	Mean (%)* Unpasteurized	Mean (%)* Pasteurized
Coarse	.491 ± .0290 $_b$	.534 ± 0.00 $_a$
20:0	.409± 0.018 $_a$	.490 ± 0.010 $_a$
15:3	.540 ± 0.00 $_a$	.540 ± 0.010 $_a$
15:4	.485 ± 0.022 $_a$	.526 ± 0.017 $_a$
10:5	.460 ± 0.008 $_a$	.461 ± 0.009 $_a$
10:8	.486 ± 0.010 $_a$	.466 ± 0.010 $_b$

Note. Means in the same row that are followed by different subscripts are statistically different ($p \geq 0.05$) by paired two sample for equal means t-test.

*Uncertainties represent standard deviations from the mean of four measurements.

The effect of pasteurization on the protein concentration in beer samples treated with various stabilizers was evaluated using a paired two-sample for equal means t-test. The results showed that there was no significant difference ($p \geq 0.05$) in protein concentration due to the pasteurization of coarsely filtered beer and the beer treated with 10:8 SHG:PVPP. All other filtering combinations showed a significant difference ($p \geq 0.05$) due to pasteurization and can be seen in Table 14.

CHAPTER V

RECOMMENDATIONS

The problem investigated was the removal of protein/polyphenol haze found in Leinenkugel's Red Lager after an accelerated shelf-life study by the use of secondary filtration within the processing of the beer. It is already known that the amount of haze that could potentially precipitate out of the beer may depend on what filtering aids/stabilizers are being used in the stabilization/filtration step during the brewing of the beer. In this study, the stabilizers silica hydrogel (SHG) and polyvinylpolypyrrolidone (PVPP) were explored to determine the best combination of the two stabilizers. The stabilizers were used in the following combinations: None (Primary filtration only), 20 lbs SHG/100 bbls to 0 lbs PVPP/100 bbls (20:0), 15 lbs SHG/100 bbls to 3 lbs PVPP/100 bbls (15:3), 15 lbs SHG/100 bbls to 4 lbs PVPP/100 bbls (15:4), 10 lbs SHG/100 bbls to 5 lbs PVPP/100 bbls (10:5), and 10 lbs SHG/100 bbls to 8 lbs PVPP/100 bbls (10:8).

Recommendations Based on Determination of Beer Components

The beer was produced at the brewery under normal brewing and storage conditions as any other beer would be produced with the only change being the change in the stabilizer used. The beer was packaged and samples were taken from the line, nine samples before the pasteurizer and nine samples following pasteurization for each stabilizer treatment. The methods of analysis used are accepted as the standard methods in the brewing industry by the American Society of Brewing Chemists. The absorbance (color), total polyphenols, total flavanoids, dissolved oxygen, initial haze, accelerated

haze, and crude protein were measured in each treatment. The results of these tests were used to determine which stabilizer treatment would most effectively remove chill haze in Leinenkugel's red lager.

Only the pasteurized beer is used for the recommendations of this study since all bottled and canned beer is pasteurized. The dissolved oxygen of the beer was measured to make sure that the levels of dissolved oxygen in the product were within the control limits at the brewery. When the level of dissolved oxygen exceeds the control limits, there will be more haze in the product due to the polymerization of the polyphenols in the beer. This decreases the ability of the stabilizers to remove the proteins and polyphenols due to their change in structure. All the beer produced had levels of dissolved oxygen that were within the brewery limits with the exception of the stabilizer treatment of 10:8. This treatment had twice the amount of dissolved oxygen observed in the other samples, which may have altered the results of this stabilizer treatment.

When looking at color, there were statistically significant differences between all the samples due to the small variations in the measurements, but the difference in the absorbances that were measured were so small that no human would be able to determine the small differences in the color with the naked eye when two samples are compared. Differences in absorbance must exceed 0.1 AU for humans to detect a difference in color intensity and all measurements were within 0.06 AU of one another. Therefore, the absorbance measurements will not be used in recommending a solution to the haze problem since the color intensity was not affected by the different stabilizer treatments.

Total polyphenol content was measured in all the stabilizer treatments. The treatments are listed in descending order with the first stabilizer treatment containing the

highest level of polyphenols in the beer: coarse filtered, 20:0, 15:3, 15:4, 10:8 and 10:5, but the 10:5 and 10:8 showed no significant difference in the amount of polyphenol content based on analysis of variance tests completed on the data. This shows that the best filtration to use to decrease the polyphenol content would be the treatment of 10:5 or 10:8 since there was no significant difference in the polyphenol content between them.

Total flavanoid content was measured in all the stabilizer treatments with the beer filtered with 15:4 having the lowest amount of flavanoids measured. The remaining stabilizer treatments in descending order of total flavanoid content are coarse filtered, 20:0, 10:5, 10:8, 15:3 and finally 15:4. Since flavanoids are a precursor to the anthocyanidins, it is important to remove as many flavanoids as possible without removing the foam-active polyphenols. When only looking at the flavanoid content, it is best to use the stabilizer treatment of 15:4 to achieve the lowest possible flavanoid content.

The initial haze measurements of all the stabilizer treatments were below 250 FTU, with the exception of the coarse filtered beer. A haze of 250 FTU is the minimum level of haze needed in the beer for the average consumer to be able to detect the presence of haze in the product. The levels of initial haze in the beer in each stabilizer treatment in descending order is coarse filtered, 15:4, 15:3, 10:5, 20:0, and 10:8. Based on these results the best stabilizer treatment to reduce the amount of initial haze would be 10:8. The initial haze is the amount of haze left in the product following the stabilization of the beer and the accelerated haze mimics the haze present in beer that is three months old. This time limit is important as it is the current shelf life of beer. When the accelerated haze was determined in the beer with the different stabilizers, it was found

that the beer filtered with 10:8 had the least amount of accelerated haze with 15:4 coming in second. The difference in accelerated haze between them is about 150 FTU and 10:5 was only 50 FTU higher than the 15:4 treatments. The other three treatments were all equal in the amount of haze present. Based on these results the best treatment to reduce accelerated haze would be the stabilizer treatment of 10:8 then 15:4.

The protein concentrations in the beer samples were difficult to measure due to the lack of highly sensitive methods to determine the amount of protein in beer. The protein levels in beer require a very sensitive method of measurement. There are many methods that could be used, but none of them were sensitive enough to measure the small amounts of protein in the beer. Since the Kjeldahl method was the accepted method by the American Society of Brewing Chemists this was the chosen method. Even with the lack of sensitivity, differences in protein values were seen. The stabilizer treatments used in this study are listed in descending order of protein concentraion: Coarse filtered, 15:3, 15:4, 20:0, 10:5, and 10:8. The treatments of coarse filtered beer and the treatment of 15:3 and the treatments of 10:5 and 10:8 showed no significant difference in protein concentraion as determined by analysis of variance. Thus based on these results the treatment most effective in protein removal was the treatments of 10:5 and 10:8.

Based on the determination of beer components, the treatment that seemed to be most effective in all tests was the beer treated with 10 lbs SHG/100 bbls and 8 lbs PVPP/100 bbls. This treatment was the most effective at removing polyphenols, initial haze, accelerated haze and protein from the beer. The next best choice would be the beer treated with 15 lbs SHG/100 bbls and 4 lbs PVPP/100 bbls since it was the second most effective in all the same categories and was the most effective at removal of total

flavanoids. Based on these results, I would recommend the treatment of 10:8 and 15:4 for the removal of haze in Leinenkugel's Red Lager.

Recommendations Based on a Price Comparison of SHG and PVPP

The recommendations based on the determination of beer components must be examined from the standpoint of the cost difference for each stabilization treatment. The stabilizers evaluated in this study are currently being used at the brewery in the amounts of 15 lbs SHG/100 bbls and 3 lbs PVPP /100 bbls. The cost of these materials are currently $0.54 per pound of SHG and $7.50 per pound of PVPP. Since the brewery is currently using the treatment of 15:3, the difference in cost per bottle per pound of PVPP increase was determined. To increase the amount of PVPP by 1 lb/100 bbls would amount to about $0.005 per bottle. This doesn't seem like much, but it would amount to almost $19,000 for the entire year if all the beer produced in the plant used the same treatment. Based on cost, the recommendation for the stabilizer treatment that is most effective in reducing beer components of concern as well as based on the cost, is the treatment of 15 lbs SHG/100 bbls and 4 lbs PVPP/100 bbls.

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APPENDICES

APPENDIX A

Color Measurements from the Shimadzu Spectrophotometer (λ = 430nm) using Varying Levels of Stabilizers in Pasteurized and Unpasteurized Beer

	Unpasteurized					Pasteurized				
	SHG(lbs/100bbls): PVPP(lbs/100bbls)					SHG(lbs/100bbls): PVPP(lbs/100bbls)				
Replicates	20:0	15:3	15:4	10:5	10:8	20:0	15:3	15:4	10:5	10:8
1	1.5574	1.6583	1.6055	1.5732	1.6276	1.5889	1.6457	1.6323	1.6165	1.6213
2	1.5559	1.657	1.611	1.5779	1.6323	1.5898	1.6465	1.6315	1.6189	1.6213
3	1.5567	1.6559	1.6071	1.576	1.6291	1.5906	1.6449	1.6339	1.6165	1.622
4	1.5583	1.6535	1.6094	1.5732	1.6275	1.5906	1.6472	1.6323	1.6378	1.6228
5	1.5567	1.6583	1.6062	1.5787	1.6268	1.5889	1.6465	1.6323	1.6181	1.6203
Average	1.557	1.6566	1.6078	1.5758	1.6287	1.5898	1.6462	1.6325	1.6216	1.6215
Std. Dev.	0.0009	0.002	0.0023	0.0026	0.0022	0.0009	0.0009	0.0009	0.0091	0.0009

APPENDIX B

Color Measurements from the Varian-Cary Spectrophotometer (λ = 430nm) using Varying Levels of Stabilizers in Pasteurized and Unpasteurized Beer

	Unpasteurized						Pasteurized					
	SHG (lbs/100bbls): PVPP (lbs/100bbls)						SHG (lbs/100bbls): PVPP(lbs/100bbls)					
Replicates	Coarse	20:0	15:3	15:4	10:5	10:8	Coarse	20:0	15:3	15:4	10:5	10:8
1	1.8100	1.6210	1.6649	1.7129	1.6328	1.7583	1.8225	1.7047	1.6892	1.7039	1.7393	1.7666
2	1.8029	1.6212	1.6646	1.7128	1.6326	1.7576	1.8194	1.7045	1.6892	1.7040	1.7397	1.7674
3	1.8124	1.6214	1.6644	1.7129	1.633	1.7580	1.8223	1.7049	1.6897	1.7036	1.7402	1.7682
4	1.8130	1.6213	1.6676	1.7125	1.6332	1.7587	1.8233	1.7051	1.6913	1.7032	1.7402	1.7687
5	1.8085	1.6213	1.6683	1.7128	1.6337	1.7600	1.8235	1.7049	1.6918	1.7038	1.7401	1.7684
Average	1.8093	1.6212	1.666	1.7128	1.6331	1.7585	1.8222	1.7048	1.6902	1.7037	1.7399	1.7679
Std. Dev.	0.0040	0.0002	0.0018	0.0002	0.0004	0.0009	0.0016	0.0003	0.0012	0.0003	0.0004	0.0009

APPENDIX C

Total Polyphenol Measurements in Pasteurized and Unpasteurized Beer using Varying Amounts of Stabilizers

	Unpasteurized						Pasteurized					
	SHG (lbs/100bbls): PVPP (lbs/100bbls)						SHG (lbs/100bbls): PVPP (lbs/100bbls)					
Replicates	Coarse	20:0	15:3	15:4	10:5	10:8	Coarse	20:0	15:3	15:4	10:5	10:8
1	768	707	650	583	577	536	753	681	679	611	547	566
2	731	716	612	563	571	537	761	692	654	633	547	572
3	707	713	651	604	562	563	742	698	662	632	541	584
4	732	770	645	595	570	518	721	678	637	576	573	581
5	748	755	648	601	611	537	812	697	660	594	539	582
Average	737	732	641	589	578	538	758	690	658	609	549	577
Std. Dev.	23	28	16	17	19	16	34	9	15	25	14	7

APPENDIX D

Standard Curves and Data of Total Flavanoid Tests

Data from the test for total flavanoids is shown in Table 1D. There were three readings taken from each standard addition as well as the blank. The data shown in Table 1D is the average once the blank was subtracted from the reading. These are the values used in the standard curves as shown in Figures D1 through D12.

Table 1D.

Measurements used for the Standard Curves

	Concentration of Spiked (+)-Catechin				
	0 mg/L (AU)	10 mg/L (AU)	25 mg/L (AU)	50 mg/L (AU)	100 mg/L (AU)
Coarse Pasteurized	0.1142	0.1405	0.2129	0.3291	0.5856
Coarse Unpasteurized	0.1442	0.1742	0.2321	0.3229	0.5098
20:0 Pasteurized	0.1308	0.1536	0.2412	0.3799	0.6666
20:0 Unpasteurized	0.0882	0.1268	0.1886	0.2783	0.4592
15:3 Pasteurized	0.0882	0.1025	0.1686	0.2888	0.5278
15:3 Unpasteurized	0.1021	0.1403	0.2006	0.2803	0.4828
15:4 Pasteurized	0.0666	0.1181	0.1917	0.3198	0.5763
15:4 Unpasteurized	0.1109	0.1301	0.5423	0.6352	0.852
10:5 Pasteurized	0.0723	0.1107	0.1752	0.2783	0.4484
10:5 Unpasteurized	0.0686	0.1031	0.169	0.2571	0.4412
10:8 Pasteurized	0.0594	0.0918	0.1593	0.2348	0.4147

The standard curves were made by plotting the concentration of spiked (+)-catechin on the x-axis and the absorbance reading on the y-axis. Figure D1 through Figure D12 show the standard curves. The amount of (+)-catechin present in the sample is an interpolated value where the standard curve intersects the x-axis when the absorbance is equal to zero. The interpolated value was then multiplied by a dilution factor of 10 to give the actual amount of (+)-catechin present in the sample.

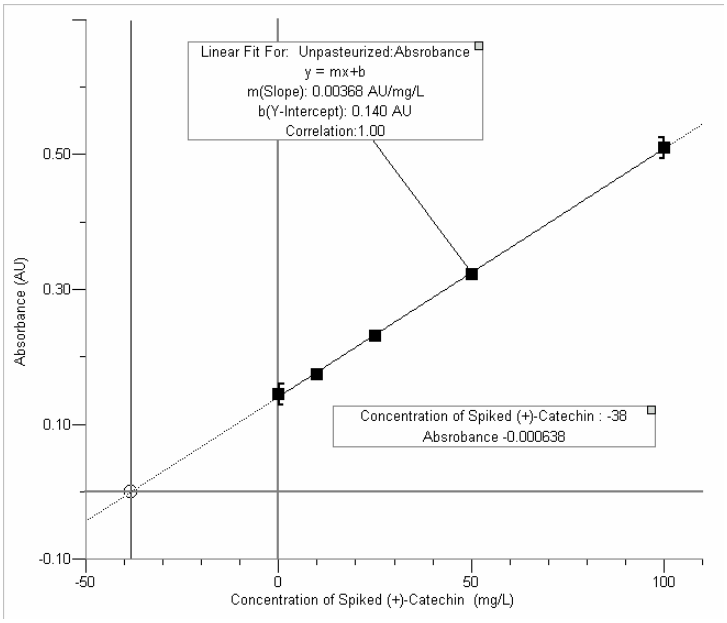

Figure D1. Concentration of (+)-catechin in unpasteurized beer coarse filtered.

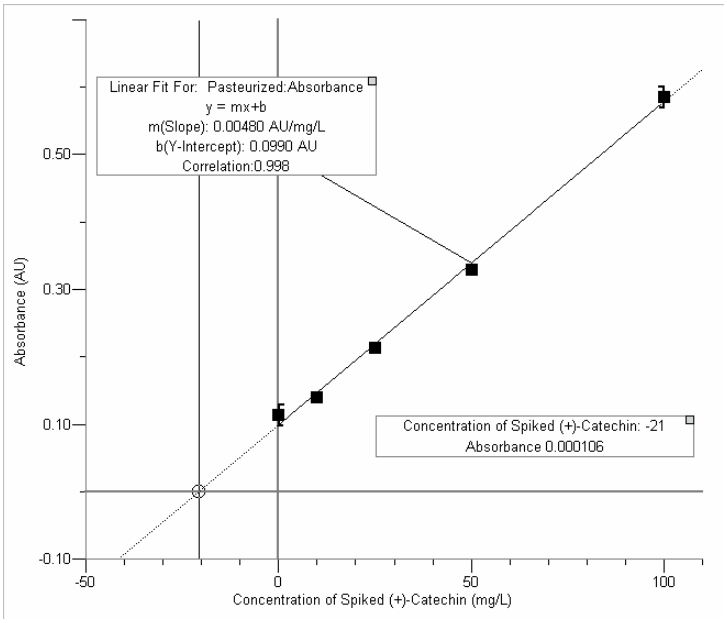

Figure D2. Concentration of (+)-catechin in pasteurized beer coarse filtered.

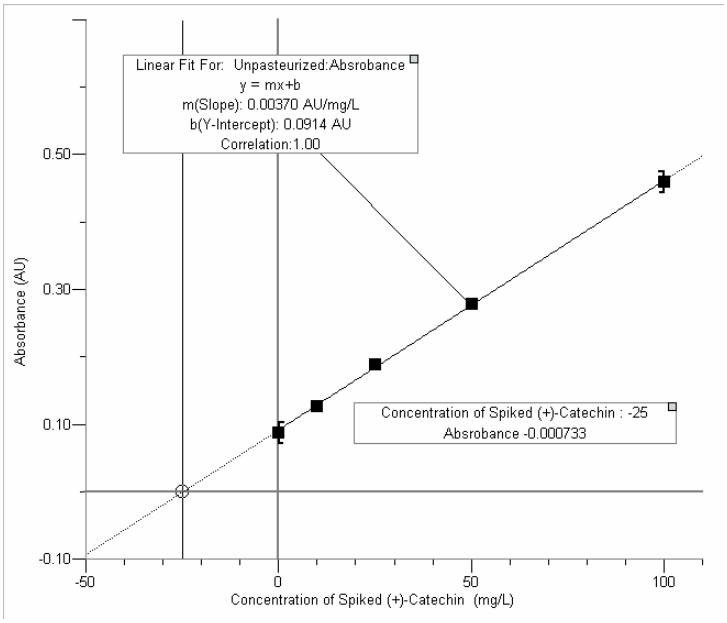

Figure D3. Concentration of (+)-catechin in unpasteurized beer filtered with 20 lbs SHG/100 bbls.

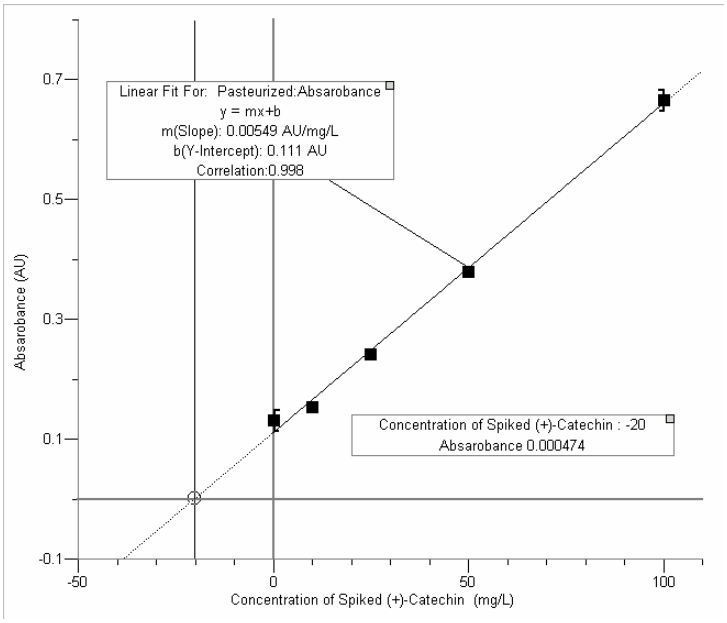

Figure D4. Concentration of (+)-catechin in pasteurized beer filtered with 20 lbs SHG/100 bbls

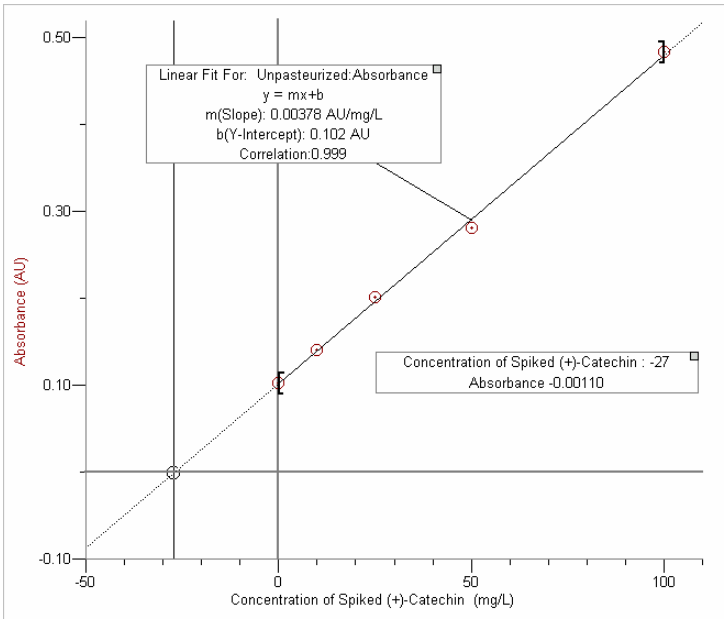

Figure D5. Concentration of (+)-catechin in unpasteurized beer filtered with 15 lbs SHG/100 bbls and 3 lbs PVPP/100 bbls

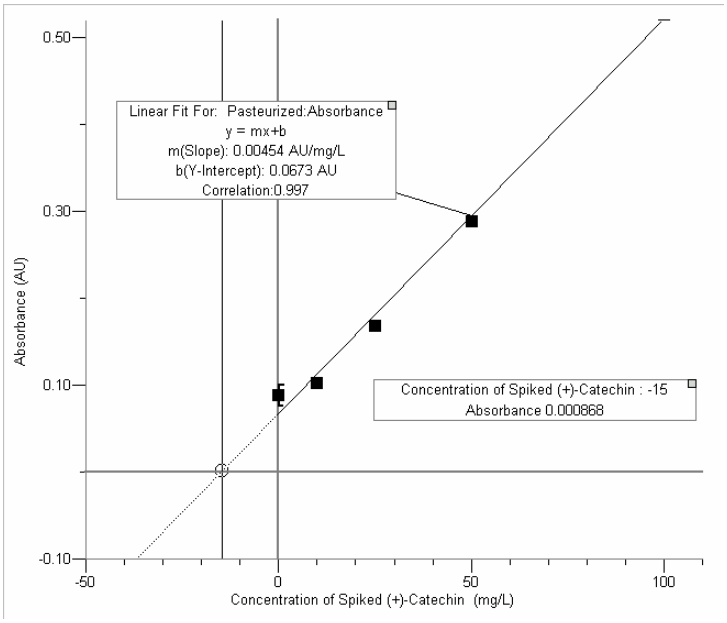

Figure D6. Concentration of (+)-catechin in pasteurized beer filtered with 15 lbs SHG/100 bbls and 3 lbs PVPP/100 bbls.

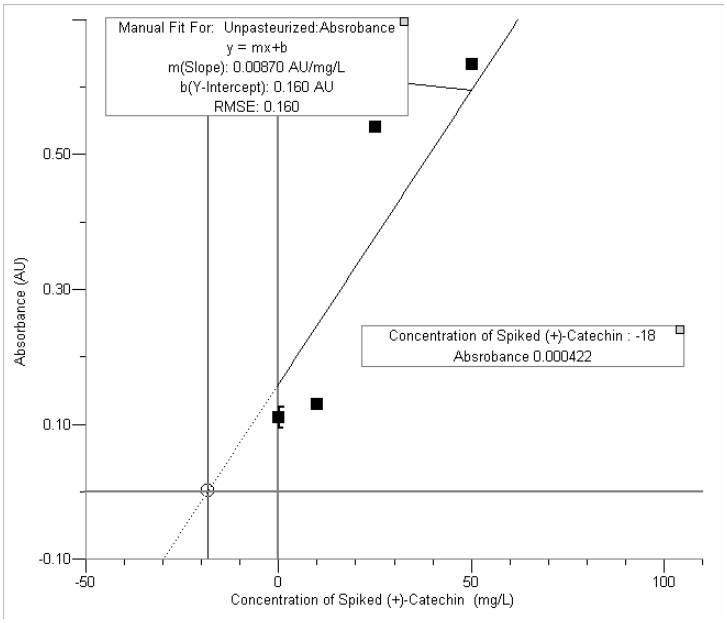

Figure D7. Concentration of (+)-catechin in unpasteurized beer filtered with 15 lbs SHG/100 bbls and 4 lbs PVPP/100 bbls.

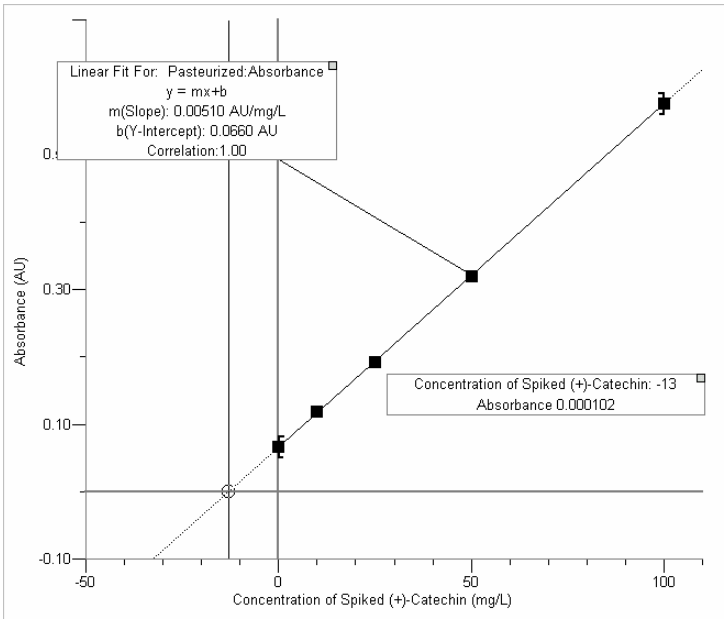

Figure D8. Concentration of (+)-catechin in pasteurized beer filtered with 15 lbs SHG/100 bbls and 4 lbs PVPP/100 bbls.

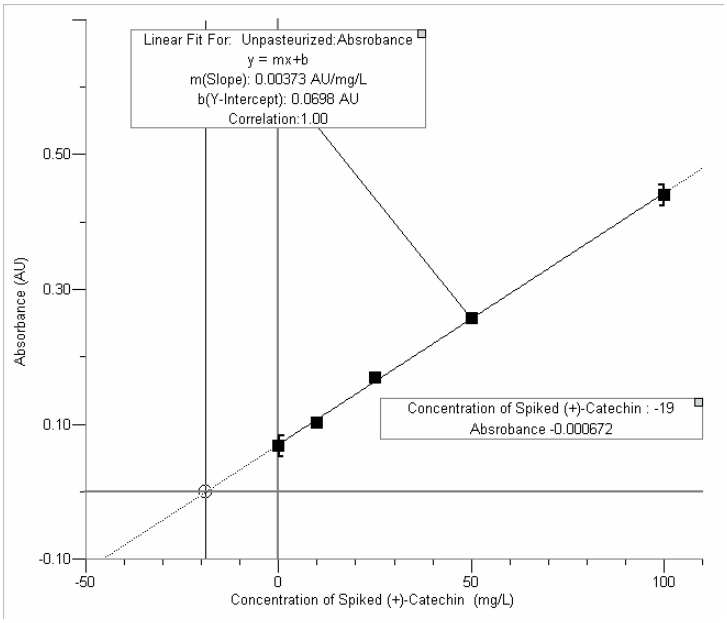

Figure D9. Concentration of (+)-catechin in unpasteurized beer filtered with 10 lbs SHG/100 bbls and 5 lbs PVPP/100 bbls.

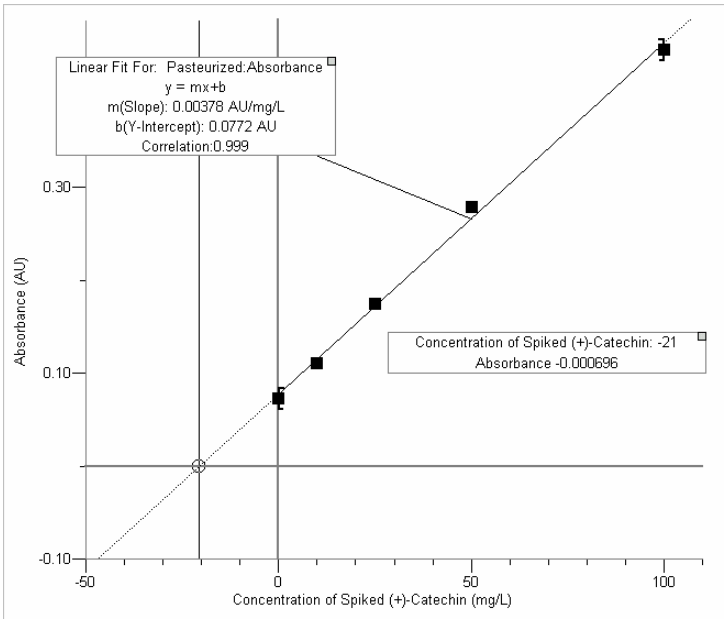

Figure D10. Concentration of (+)-catechin in pasteurized beer filtered with 10 lbs SHG/100 bbls and 5 lbs PVPP/100 bbls.

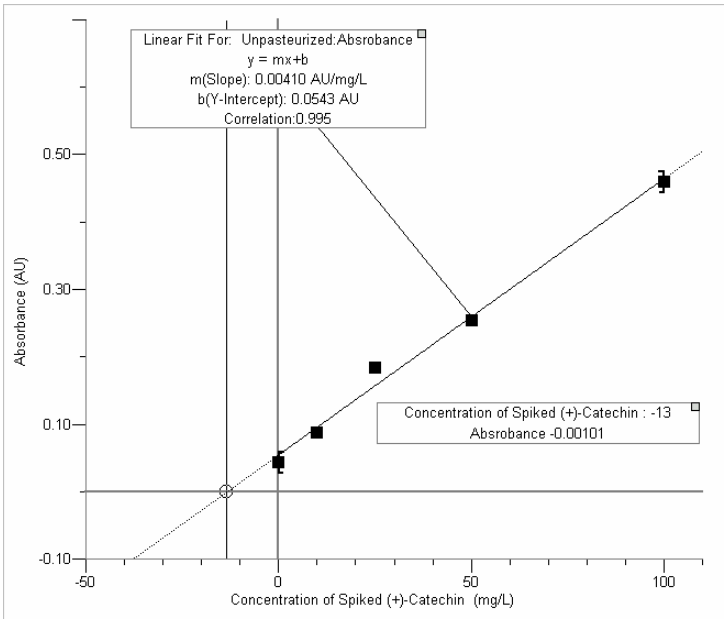

Figure D11. Concentration of (+)-catechin in unpasteurized beer filtered with 10 lbs SHG/100 bbls and 8 lbs PVPP/100 bbls.

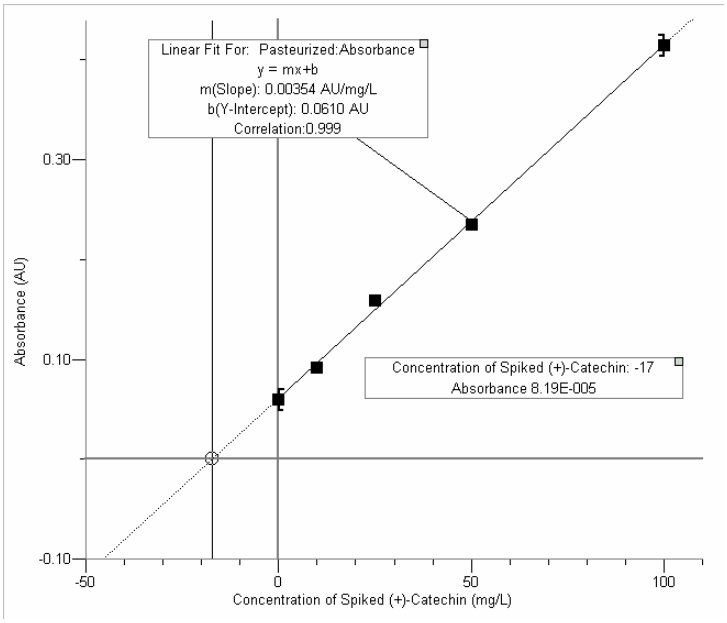

Figure D12. Concentration of (+)-catechin in pasteurized beer filtered with 10 lbs SHG/100 bbls and 8 lbs PVPP/100 bbls.

APPENDIX E

Dissolved Oxygen Measurements in Unpasteurized Beer with Varying Amounts of Stabilizers

	SHG (lbs/100bbls): PVPP (lbs/100bbls)					
Replicates	Coarse	20:0	15:3	15:4	10:5	10:8
1	0.038	0.049	0.039	0.048	0.04	0.091
2	0.039	0.05	0.041	0.044	0.038	0.093
3	0.039	0.048	0.038	0.049	0.033	0.089
4	0.04	0.05	0.04	0.05	0.038	0.088
5	0.038	0.047	0.039	0.048	0.041	0.092
Average	0.039	0.049	0.039	0.048	0.038	0.091
Std. Dev.	0.001	0.001	0.001	0.002	0.003	0.002

APPENDIX F

Initial Haze Measurements Using Varying Amounts of Stabilizers in Pasteurized and Unpasteurized Beer.

	Unpasteurized						Pasteurized					
	SHG (lbs/100bbls): PVPP (lbs/100bbls)						SHG (lbs/100bbls): PVPP (lbs/100bbls)					
Replicates	Coarse	20:0	15:3	15:4	10:5	10:8	Coarse	20:0	15:3	15:4	10:5	10:8
1	288	115	120	121	101	100	528	110	116	122	100	105
2	326	112	120	121	100	100	500	110	116	120	105	98
3	380	116	119	122	99	98	520	110	119	125	105	93
Average	331	114	120	121	100	99	516	110	117	122	103	99
Std. Dev.	46	2	1	1	1	1	14	0	2	3	3	6

APPENDIX G

Accelerated Haze Measurements Using Varying Amounts of Stabilizers in Pasteurized and Unpasteurized Beer.

	Unpasteurized						Pasteurized					
	SHG (lbs/100bbls): PVPP (lbs/100bbls)						SHG (lbs/100bbls): PVPP (lbs/100bbls)					
Replicates	Coarse	20:0	15:3	15:4	10:5	10:8	Coarse	20:0	15:3	15:4	10:5	10:8
1	800	800	800	560	600	442	800	800	800	610	660	440
2	800	800	800	660	625	440	800	800	800	590	680	440
3	800	800	800	662	620	500	800	800	800	600	630	498
Average	800	800	800	627	615	460	800	800	800	600	657	459
Std. Dev.	0	0	0	58	13	34	0	0	0	10	25	33

APPENDIX H

Protein Measurements in Pasteurized and Unpasteurized Beer with Varying Amounts of Stabilizers

	Unpasteurized						Pasteurized					
	SHG (lbs/100bbls): PVPP (lbs/100bbls)						SHG (lbs/100bbls): PVPP (lbs/100bbls)					
Replicates	Coarse	20:0	15:3	15:4	10:5	10:8	Coarse	20:0	15:3	15:4	10:5	10:8
1	.504	.512	.540	.454	.456	.477	.554	.499	.548	.548	.466	.457
2	.521	.474	.540	.507	.473	.495	.554	.481	.531	.513	.448	.474
3	.487	.495	.540	.489	.456	.495	.554	.481	.548	.513	.466	.474
4	.452	.474	.540	.491	.456	.477	.554	.499	.531	.531	.466	.457
Average	.491	.409	.540	.485	.460	.486	.534	.490	.540	.526	.461	.466
Std. Dev.	.029	.018	0	.022	.008	.010	0	.010	.010	.017	.009	.010