

# **Spectrophotometric Analysis of Beer and Wort**

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## **Abstract**

A representative sampling of beers and laboratory worts were measured spectrophotometrically. The applicability of the Bouguer-Lambert-Beer law to beers and worts without visible turbidity, the independence of cell position for measurement of bright beers, the adequacy of the SRM/EBC color indices to accurately characterize the color of beer and wort, the verity of Linner's observation of linear with wavelength log Absorbance spectra, and the dimensionality of beer spectra are examined. It was concluded that SRM/EBC color does not accurately characterize the color of beer, that the Bouguer-Lambert-Beer law does apply to beers free from visible turbidity, that the cell position does not materially affect the measurement of non-turbid beers (but does so for turbid beers), and that Linner's observation also applies reasonably (but not perfectly) well to beers and worts. It is tentatively concluded that beer spectra are two-dimensional in log Absorbance space.

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*To the memory of Doc.*

# 1. Introduction

For better or worse, the first attribute upon which a beer is judged by consumers (or prospective consumers) is its appearance. Key aspects of a beer's appearance are:

- Head texture and retention
- Clarity/Turbidity
- Color

This report addresses color.

The ability of a brewer to predict and control the color of a beer is important to the success of their product for a number of reasons. Consumers have been conditioned to associate, sometimes inaccurately, beer color with certain flavor profiles. In both amateur and professional competitions, beers are judged against a set of established criteria for their particular styles, and one of those criteria is color. [3] Finally, a beer's color sets some expectations regarding other of its sensory attributes. For example, one does not expect to encounter roastiness in a straw-colored beer. If roastiness is perceived in such a beer, those tasting it may assume something is wrong with the beer.

Chris Swersey, competition manager of the Beer World Cup and Great American Beer Festival competitions, writes:

Beer color is definitely considered during judging. It's the first parameter that a judge considers, by default – after all, the first sip is with your eyes...



Each beer style has a range of acceptable colors. Beers that are only moderately out of range might still advance in a multiple round competition, but would probably not win a gold medal. In a single round competition, a beer with good color might be among the last beers standing, a beer with less than acceptable color would not. [11]

Medals won at these competitions can be a significant part of a professional brewer's resumé, just as quality publications are to an academic. Therefore, it is in a brewer's interest to get the color correct.

## **1.1. Quantification of Beer Color**

Beer and wort (unfermented beer) color is currently assessed according to two scales: the Standard Reference Method (SRM<sup>1</sup>), principally in North America, and the European Brewing Convention (EBC) in the remainder of the world. Both are based on spectrophotometry, using the Absorbance (relative to that of a blank cell containing distilled water) of a sample of the beer or wort at a wavelength of 430 nanometers. The two scales differ primarily in path length: SRM color is based (after considering the multiplicative factors) on an effective 5 inch path length, while EBC color is based on an effective 25 centimeter path length.

### **1.1.1. Dimensionality of Beer Color**

Color is three dimensional, embracing Hue (described with words such as "Red" and "Purple"), Lightness (or Value, described with words such as "Light" and "Dark"), and Chroma (described with words such as "Pale" and "Vivid"). The SRM and EBC methods for quantification of beer color are one dimensional. How adequately do they represent the actual color of beers? I shall examine a sufficient condition for this to be

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<sup>1</sup>In literature, "SRM" appears with a degree symbol, as in "45 °SRM." Because it is an absolute measure (water, a colorless "beer" with no extract, has zero SRM), I have dropped the use of the degree symbol, except when used in quotations.

true; if this sufficient condition is satisfied, there is no question that the proposition holds. I shall also examine a necessary condition; if a necessary condition is not satisfied, the proposition does not hold. Once formulated, these conditions can guide the experimental portion of the investigation and assist in the formulation and testing of hypotheses.

### **Sufficiency of SRM/EBC Beer Color Measurements**

A *sufficient* condition for the SRM and EBC beer color measurements to completely describe the actual color of beer under a given illuminant is satisfied if beer spectra are one-dimensional in some invertible transform of spectral transmittance. The dimensionality of the spectra in this yet-to-be specified space may be established using Singular Value Decomposition (SVD) or the essentially equivalent Principal Component Analysis (PCA).

A *necessary* condition for SRM/EBC beer color measurements to completely embody the color of beers is that any dimensions above the first which are not metameric to black under a specified set of viewing conditions be relatable to the first through some non-linear transformation. For example, if two eigenvectors permit reconstruction of the tristimulus values of beers to within some specified tolerance, and the second extracted dimension is the cube root of the second, then it may be concluded that the SRM/EBC color does completely specify the color of the beers.

#### **1.1.2. Quantification of Beer Color in the CIELAB Color Space**

Following suggestions made in the early 1990s by Sharpe, Garvey, and Pine [2] and Smedley, [10] both the EBC [13] and the ASBC

## 1.2. The Bouguer-Lambert-Beer Law

A fundamental physical model for transmission was first formulated by Bouguer, improved by Lambert, and generalized by Beer. It is known by various permutations of the names of these three investigators. In this report, we use the names of all three in the order in which they made their contributions.

The Bouguer-Lambert-Beer law may be stated mathematically:

$$T_{\lambda} = \exp(-k_{\lambda} \cdot c \cdot Z) \quad (1.1)$$

where

$k_{\lambda}$  is the extinction coefficient spectrum of the material [typical units:  $\text{cm}^2/\text{mol}$ ];

$c$  is the concentration of the material [ $\text{mol}/\text{cm}^3$ ];

$Z$  is the optical path length through the material [cm]; and

$T_{\lambda}$  is the transmission spectrum of the material [ $\cdot$ ].

Eq (1.1) may be written in terms of Absorbance, where Absorbance is the negative common logarithm of transmittance:

$$A_{\lambda} = \ln(10) \cdot k_{\lambda} \cdot c \cdot Z = A_{1,1,\lambda} \cdot c \cdot Z \quad (1.2)$$

where

$A_{1,1,\lambda}$  is the Absorbance spectrum of the material at unit concentration and path length,

with  $A_{1,1,\lambda} = -\log_{10}(k_{\lambda} \cdot 1 \text{ mol}/\text{cm}^2)$ ; and

$A_{\lambda}$  is the Absorbance spectrum of the material.

This model is the solution of the first-order linear differential equation:

$$dT_{\lambda} = -T_{\lambda} \cdot k_{\lambda} \cdot c \cdot dz \quad (1.3)$$

where  $dz$  is the thickness of an elementary sub-layer of the material, subject to the initial condition  $T_\lambda|_{c,z=0} = 1$ . In words, rate at which the flux of photons at wavelength  $\lambda$  changes is proportional to the photon flux at that wavelength, the molar absorption coefficient at that wavelength, and the concentration of the absorbing material. The model assumes a homogeneous medium which does not scatter light.

### 1.2.1. Applicability of the Bouguer-Lambert-Beer Law to Beer

It has been suggested in craft-brewing professional literature that dark beers in particular do not obey the Bouguer-Lambert-Beer law. [5, 7, e.g.] Daniels writes:

The problem, ultimately, is that – incredible as it may seem – beer does not always follow Beer’s Law... . Over the years, several authors have asserted that beer obeys Beer’s Law. Unfortunately, these studies appear to have examined a very limited portion of the beer color universe. It appears that Beer’s Law does hold for beers with a final color of less than 5 or perhaps 10 °SRM. [5]

The implication is that the Bouguer-Lambert-Beer law does not apply to beers darker than this (5 SRM is typical of a normal-gravity light-colored lager; 10 SRM is a pale amber). One of the motivations for this investigation was to examine this assertion. To do this, a concentration series of some dark beers may be measured spectrophotometrically.

### 1.2.2. Causes of Failure of the Bouguer-Lambert-Beer Law

There are several reasons why the Beer-Bouguer-Lambert law will fail. These include:

*Scatter of light by the material* — The differential equation (1.3) does not account for light scattered within the material. If there is significant scatter, models such as those developed by Kubelka and Munk should be used.

*Stray light within instrument* — If light can reach the detector in the instrument without having to pass through the sample, it will limit the reported Absorbance. For example, if the instrument has 0.1 percent stray light, the maximum Absorbance it will be able to report, without careful compensation, will be about 3.0. Consider a solution which does in fact obey the Bouguer-Lambert-Beer law, and has a measured spectral Absorbance of 2.0. If the concentration were doubled, the measured spectral Absorbance will be no greater than 3.0 – somewhat less than the 4.0 predicted by the Bouguer-Lambert-Beer law.

*Multiple reflections within the material* — If all or a portion of the flux must traverse the material more than one time because of internal reflection at the material boundaries, the optical path length will be longer. The mean number of internal reflections will depend on the change in refractive index, which varies with wavelength because of dispersion, and the absorption spectrum of the material (lower Absorbance will permit a larger number of internal reflections).

*Non-homogeneous medium, in combination with other factors* — If other factors which cause the Bouguer-Lambert-Beer law to fail are present, their effect may be amplified by heterogeneity of the material.

Scattering is caused by a discontinuity in refractive index. The refractive index of beer is approximately 1.34 and of carbon dioxide, approximately 1.0. Bubbles of carbon dioxide gas within a beer sample create discontinuities in refractive index and scattering; the ASBC cautions against these: “Gas bubbles in beer during color measurement can cause appreciable error in photometer readings. Take care to avoid them by using well-decarbonated beer and by making readings quickly.” [1, Beer 10-B]

De-gassing procedure is described in Method Beer-1A, Preparation of sample for chemical and physical analysis. In essence, the sample is permitted to come to nearly room temperature, placed in an oversize flask, and agitated until it appears to be completely decarbonated. [1, Beer 1-A]

### **1.3. Measurement Geometry and Scatter**

Because scatter is one cause of failure of the Bouguer-Lambert-Beer law, it shall be measured in this study. Transmission spectrophotometry may be performed using a number of different arrangements of illuminating light source, sample, and collection/measurement. Two which shall be exercised in this study are termed "Total Transmittance" and "Regular Transmittance." [8] Both yield the same results for materials which do not scatter light; in the presence of scatter, the Total transmittance should be greater than Regular. The difference between the two spectra (Total minus Regular transmittance is termed "Diffuse" transmittance) may be used to quantify a material's scattering ability.

When a material scatters light, it causes photons to change direction. Non-scattering materials do not; photons are either absorbed or allowed to continue in the same direction. The Regular transmission measurement counts only those photons which pass through the material virtually undeviated (within a narrow angle from the axis). The Total transmittance measurement also includes photons which have been scattered into the hemisphere on the opposite side.

### **1.4. The Linner Observation and the Linner Hue Index for Caramel**

Linner, who was employed by a manufacturer of caramel coloring, analyzed log Absorbance spectra of numerous caramel colors. He made an interesting observation: the log Absorbance spectra of caramel colors were essentially linear, at least in the central 100 nanometer band of light. Different caramel colors had different log Absorbance spectra, but they tended to vary in only two ways. The overall height of a log Absorbance spectrum indicates the tinctorial strength of the caramel color, while the slope indicates the hue. The slopes were always negative (caramels are warm-colored); slopes closer to zero (larger in value but smaller in magnitude) indicated caramels which were less red than slopes further from zero (smaller in value but larger

in magnitude). The tinctorial strength was already well known, but the Hue aspect was not. Linner suggested a “hue index,” based on the slope of the log Absorbance spectrum. [9]

In this report, liquids whose log Absorbance spectra are linear in the visible region are referred to as “Linner Solutions.”

Linner’s proposal became a trade standard in the caramel color industry. It is computed as:

$$H_L = 10 \cdot [\log_{10} A_{510} - \log_{10} A_{610}] \quad (1.4)$$

Linner’s observation implies that the spectra of solutions will be two-dimensional within the spectral region over which they comply with the observation. The space in which they may be resolved linearly (i.e., using PCA or SVD) will be log Absorbance space. Compliance with Linner’s observation is a sufficient condition for the dimensionality to be 2, not a necessary condition. There are conditions under which Linner’s observation is not satisfied, but the log Absorbance spectra will still be two-dimensional.

For solutions which obey the Bouguer-Lambert-Beer law, mixing occurs in Absorbance space. Unfortunately, this means that the log Absorbance spectra of mixtures of solutions with different Linner Hue Indices will not be linear; its second derivative with respect to wavelength will be positive. A discussion of this is in Appendix B. The deviation from linear log Absorbance spectra is expected to be greater when the SRM color contributions of the differently-hued components are equal, such as in a Scottish Ale whose SRM color would be roughly half if a small amount of roast barley were omitted. Nevertheless, if Linner’s observation holds reasonably well for beers and worts, it will greatly simplify the measurement, analysis, and (perhaps most importantly) the communication and specification of beer and wort color in a manner which includes hue.

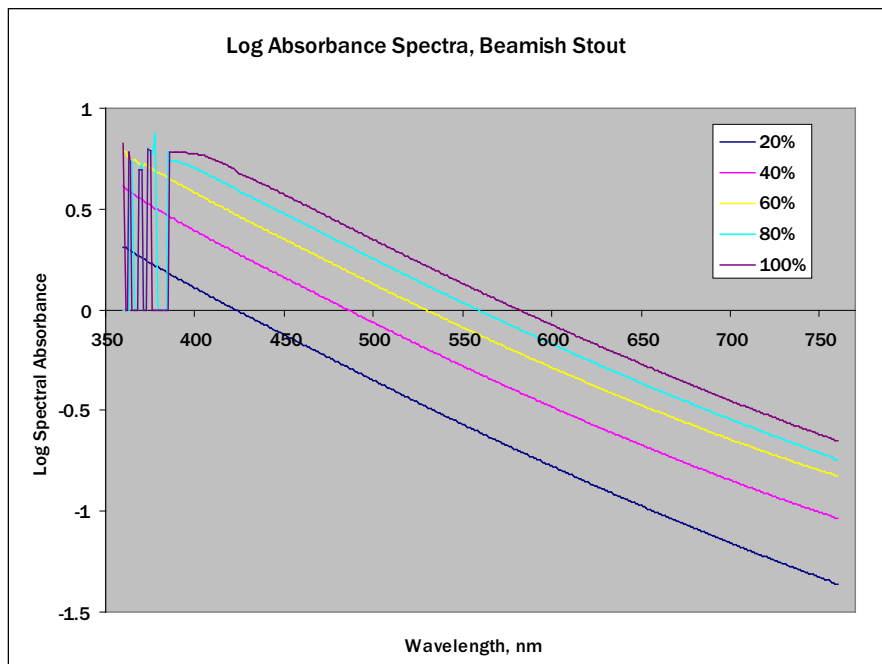


Figure 1.1.: The log Absorbance spectra of Beamish Stout at five different concentrations, showing that Linner's observation applies reasonably well (though not perfectly) to this beer. The jaggedness at the lower wavelengths is attributable to measurement noise; it occurs only at the highest concentrations (80% and 100%) for the very shortest wavelengths in the visible spectrum.



## 2. Orthogonal Bases for Beer and Wort Spectra

Once a collection of beer and wort spectra have been assembled, it will be more useful if it is summarized in some coherent manner. One of the first things one may do when faced with such a collection is compute the average spectrum. However, this presents some choices. Should the average transmittance spectrum be computed? Or should the average of the Absorbance spectra be computed, instead?

Rather than simply taking the average, which can very nicely summarize the multivariate data set as a whole, the technique of Principal Component Analysis (PCA, or the equivalent Singular Value Decomposition, SVD) shall be employed. This technique not only summarizes multivariate data sets in a manner more general than the mean (indeed, the mean is often the first piece of information contained in the summary), but often permits reasonably accurate reconstruction of each multivariate observation (in this case, a spectrum) using a reduced number of parameters.

Some familiarity with PCA/SVD is assumed in this report. Readers unfamiliar with it are referred to texts, such as Ted Jackson's excellent *User's Guide to Principal Components*.<sup>1</sup>

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<sup>1</sup>Jackson, J Edward, *A User's Guide to Principal Components*, ISBN 0471622672. New York: Wiley, 1991.

## 2.1. Data centering/standardization choices in PCA/SVD

When performing PCA/SVD, one customarily faces three options regarding the data being analyzed. These options are:

1. The data are not centered or standardized; the eigenvectors are those of the auto-inner product matrix
2. The data are centered by subtracting from each observation the mean observation (vector); the eigenvectors are those of the covariance matrix
3. The data are standardized by subtracting the mean vector and dividing by the standard deviation; the resulting eigenvectors are those of the correlation matrix.

Jackson discusses the relative merits of these options, the latter two in particular. In short, the first is most appropriate when a linear system is felt to apply, the second is probably most frequently used in general, and the third is popular when the variates are of different types or cover vastly different ranges. Each choice may be justified in the current investigation, though not to equal extents.

Absorbance spectra of liquids which obey the Bouguer-Lambert-Beer law and contain a small number (relative to the number of wavelengths at which the spectra are measured) of absorbing species will be linear in the concentrations of each. The eigenvectors, in the absence of error, will be linear combinations of the Absorbance spectra of the absorbing species, and the PCA scores will be the “concentration” of each of these linear combinations. Further, if transmission spectra are analyzed using this option, the tristimulus values of each sample, under a given combination of observer and illuminant, may be computed as a matrix product of the PCA scores and the tristimulus values of the eigenvectors. Thus, compelling cases may be made for the first choice, while custom favors the second.

## 2.2. Raw data choices

Another question is, “Should the PCA/SVD be performed in transmission space, Absorbance space, or log Absorbance space?” Again, arguments may be made for each. Having the eigenvectors in transmittance space would be most convenient (for tristimulus integration, as mentioned above), but has the potential to introduce negative transmittances in the reconstructed spectra. Absorbance space would be the most robust for mixtures of a small number of absorbing species with different Absorbance spectra, as it is the space which is linear in concentration when the Bouguer-Lambert-Beer law applies. If Linner’s Observation holds for beers and worts, log Absorbance would be preferred, as it posits that the spectra are linear with respect to wavelength, and would require, at most, two eigenvectors.

A final option which will be considered in this study is based on log Absorbance. If SRM or EBC color rating is specified, the Absorbance at 430 nanometers is fixed. If the log Absorbance at 430 nanometers is subtracted from the entire log Absorbance spectrum, the result will be orthogonal to the SRM/EBC color rating. This is appealing, as it permits maximum compatibility with existing methodology. This space shall be termed “SRM-Excess Log Absorbance” in this investigation.

Three different data centering/standardization options have been suggested for the PCA/SVD process, and four different raw data types. If all are combined factorially, there would be 12 different combinations of these two factors. However, because the variance at 430 nanometers is zero with the SRM-Excess Log Absorbance raw data option (and can be expected to be very small in the vicinity of this wavelength), it is not recommended that these data not be standardized. This leaves 11 combinations.

Rather than attempt to eliminate one or more on theoretical bases, they shall all be exercised. For a given number of vectors, the space and normalization option combination considered “best” will be the one which produces the smallest 90th percentile  $\Delta E_{94}^*$  (2 degree observer, 1931 observer, 1 cm path length) between measured and re-

constructed spectra for an ensemble of beers and worts.

### 3. Hypotheses

1. (Dependence of cell position for turbid beers) The total transmittance of beers and worts which are visually turbid will be higher than the regular transmittance.
2. (Independence of cell position for transparent beers) The total and regular transmittance of beers and worts which exhibit no visual turbidity will be equal.
3. (Applicability of the Bouguer-Lambert-Beer law) The Absorbance of aqueous solutions of beers and worts which exhibit no visual turbidity will be proportional to their concentration.
4. (Single dimensionality of beer transmission spectra) The transmission spectra of a representative sampling of beers and worts may be characterized by a single dimension, the SRM/EBC color measurement.
5. (Adequacy of Hue Index) The log Absorbance spectra of beers and worts may be adequately characterized by two dimensions, SRM/EBC color and Linner Hue Index.
6. (Applicability of Linner hypothesis to beers) The log Absorbance spectra of beers and worts will be linear with respect to wavelength over the range of 400 to 700 nanometers.

## 4. Experimental

The experimental portion of this investigation was focused on three aspects of beer color. First, it was necessary to know if beers exhibited appreciable scatter. To check this, a sampling of beers were measured in both Total and Regular transmission modes. Secondly, it was necessary to determine whether dark beers obeyed according to the Bouguer-Lambert-Beer law. This was checked by measuring concentration series for dark beers. Finally, it was desired to start building a database of beer and malt (more precisely, laboratory wort produced from malt) spectra, and explore their dimensionality. The specific methodology is discussed in this chapter.

### 4.1. Transmission Measurements on the Macbeth CE7000

Most transmission spectra were performed on the Macbeth Color Eye 7000 spectrophotometer at the Munsell Color Science Laboratory at RIT. This instrument was selected because it enabled the measurement of both Total and Regular transmittance spectra. All measurements in this instrument were performed using a cell 1 cm in thickness, roughly 3 cm wide and 3 cm high. Special cell holders were constructed from wood and painted matte black, one for each position (Total and Regular). Only the holder being used was inside the instrument while a measurement was being performed.

Large area apertures were used for both reflection port and collection optics; the UV filter was set to “exclude,” while the specular component was included. A piece of pressed polyfluoroethylene (PTFE) was placed at the reflection port.

The cell was filled with distilled water and placed in the Regular transmittance position. The instrument was then calibrated using its automatic procedure. The water blank was then measured in this position and the average of at least five measurements were saved to disk. It was then positioned in the Total transmittance position, and the process repeated. Samples were then ready to be measured.

After removing the previous contents, the cell was carefully rinsed twice with the next solution to be measured, then filled. Any drops on the outside were wiped carefully with a lint-free tissue, while bubbles inside the cell were removed with a pipette. The cell was then placed in the instrument in the Regular (and, if needed, Total) transmittance positions and measured. At least five measurements were averaged together by the instrument before they were saved to disk.

When the measurements were complete, the cell was rinsed thoroughly with distilled water, its outside gently dried with a lint-free tissue, and allowed to air dry on its side on a clean lint-free tissue.

After making the measurements, the transmittance spectra were saved to a tab-delimited text file for analysis. In addition to providing the raw data for the hypothesis testing and the PCA/SVD dimensionality analysis, the SRM color and Linner Hue Index of each beer was computed. The Regular-mode spectra were used exclusively for the PCA/SVD analyses and the computation of SRM color and Linner Hue Index.

#### **4.1.1. Practice with Food Dyes**

Measurements were first conducted on solutions of vegetable dyes (ordinary grocery store food dyes) as a check of the instrument and procedures. A concentration series was prepared for each: one, two, and five drops were added to sufficient water to produce 25 ml of solution in a volumetric flask.

Upon analysis, it was discovered that the log Absorbance spectra in the concentration series were not displaced in log Absorbance from one another, but became progressively flatter at the top as the concentration was increased. This was noticeable at

spectral Absorbances of 2.0 and greater. This effect was attributed to the instrument's handling of signals close to the dark current level. This meant that beers darker than 18 or 20 SRM would need to be diluted before measurement.

## **4.2. Other Spectrophotometers**

Two other spectrophotometers were used in this investigation. Both are double beam instruments, which, though less convenient, have some advantages over single-beam instruments such as the Macbeth CE7000. Of principal concern in this application is the ability to attenuate the reference beam (with a diluted sample of the same beer or wort) which enables longer integration times and greater sensitivity.

The first of the two additional spectrophotometers was a Varian Cary 5000 spectrophotometer, used to measure a few very dark samples. Unfortunately, the scan speed was too fast (hence the integration time was too short) for the darkest samples. The second was a Shimadzu UV-2100 spectrophotometer, which was noisier than the Cary, but did permit a sample as dark as 58 SRM to be measured undiluted. However, special techniques were necessary.

### **4.2.1. Measuring Dark Samples**

In order to measure a very dark sample without dilution, the following procedure was used. First, water blank was measured against another water blank in the reference position. Next, a dilution was prepared which has a maximum spectral Absorbance of less than 2.0 (for the Shimadzu; 3.5 would probably be appropriate for the Cary). The bandpass of the instrument was increased to 2 nm, the scan speed was set to minimum, and the diluted version was measured against a water blank. The carefully measured diluted version then replaced the blank cell in the reference beam. A less-diluted version was then measured. The process was repeated until the undiluted version was measured.



The measured transmittance spectra of all but the first dilution were then corrected using the following formula:

$$T_{i\lambda} = \tilde{T}_{i\lambda} \frac{T_{i-1\lambda}}{T_{0\lambda}} \quad (4.1)$$

where:

$T_{i\lambda}$  is the spectral transmittance of the  $i$ th dilution;

$\tilde{T}_{i\lambda}$  is the raw uncorrected spectrum of the  $i$ th dilution;

$T_{i-1\lambda}$  is the spectral transmittance of the  $i - 1$ th dilution; and

$T_{0\lambda}$  is the spectral transmittance of the water blank.

(The use of a water blank in the sample beam is probably unnecessary in a double beam instrument. Look into this.)

### **4.3. Preparation and Measurement of Beers**

#### **4.3.1. Degassing of Beers**

In order to be measured, the beer samples would have to be at room temperature and free of bubbles. *ASBC Methods of Analysis* provides a procedure, "Preparation of Sample for Chemical and Physical Analysis." [1, Beer 1-A] It was easily adapted to the conditions under which this study was performed. Samples of room-temperature beer were opened the day before. Approximately 50 ml of beer was placed in a 355 to 475 ml clean, dry clear glass bottle, shaken gently to remove most gas, and capped. These were stored at room temperature overnight.

#### **4.3.2. Dilution of Samples**

Samples which required dilution were identified by eye. The author is an experienced beer judge, and was able to assess visually when a beer was darker than 18 SRM.

All dilutions were performed by adding a volume of beer or wort using a volumetric pipette to a volumetric flask, and bringing the total volume up to the flask's rated volume with distilled water. The solution was gently mixed before measurement.

Typically, a 50 ml volumetric flask was used. This permitted more than sufficient volume to rinse the inside of the cell twice before filling it. The amount of dilution was judged by eye; if the diluted version appeared too dark, a less concentrated new dilution was then prepared. Volumetric pipette with capacities of 20, 10, 5, and 2 ml were used; these produced concentrations of 0.40, 0.20, 0.10, and 0.04. Only rarely were other concentrations used, usually to produce concentration series for evaluation of the Bouguer-Lambert-Beer law.

#### **4.3.3. Beers Measured**

The beers measured and included in the analysis appear in Table 4.1. Also included is the style to which each beer was classified by this author.

#### **4.4. Production and Measurement of Laboratory Worts**

The contribution of each malt to the spectrum of a finished beer should be a function of the spectrum of a laboratory wort produced from each malt. An assortment of malts were generously provided by the Briess Malt and Ingredients Company of Chilton, WI. The *ASBC Methods of Analysis* provides "Extract," which includes the procedure for production of a laboratory wort from a malt. [1, Malt 4] It was adapted to the equipment and resources available. The procedure which was eventually arrived at was:

- 400 ml of distilled water were placed in a 500 ml Erlenmeyer, a magnetic stir bar added, and the flask placed on a stirring hotplate.
- The stirring hotplate was turned on.

Beer Name	Style Category
Two Pete's CAP	Classic American Pilsner
Dundee's Pale Bock	Maibock/Hellerbock
Dundee's Amber Lager	American Amber Lager
Beck's Oktoberfest	Oktoberfest/Märzen
Burgerbräu Alte Weisse	Dunkelweizen
Trögenator	Doppelbock
Püterscheins Dunkelweizen	Dunkelweizen
Beamish Stout	Dry Irish Stout
Victory Storm King	Russian Imperial Stout
Kasteel Bruin	Belgian Dark Strong Ale
Phin & Matt's	Special Bitter
Cameron's Long Leg	English Pale Ale
Stoudt's Blonde Double Mai Bock	Maibock/Hellerbock
Flying Dog Heller Hound	Maibock/Hellerbock
Saranac Pale Ale	English-style Pale Ale
Boland Brothers Porter	Robust Porter
Boland Brothers Irish Red	Irish Red Ale
Spaten Optimator	Doppelbock

Table 4.1.: The beers measured and included in the analysis, together with their style as determined by an experienced, certified beer judge. Twelve of BJCP style categories 1-19 are represented.

- 24 to 26 g of the malt to be tested was balanced, ground in a small electric coffee grinder, and discarded into a large beaker through a funnel.
- 50.5 g of the malt to be tested was balanced. 24-26 g were placed in the coffee grinder.
- The grinder was operated in three short bursts of approximately one second, one second apart.
- The ground malt was placed in the flask on the hotplate using the funnel.
- The remainder of the 50.5 g sample of malt is ground as above, and introduced to the beaker.
- Clinging dust is cleaned from the grinder and funnel.
- The top of the flask was covered with aluminum foil, and a thermometer inserted through the cover.
- The temperature of the mash was brought within 0.5 °C of 40.0°C.
- The mash was held within 0.5 °C of 40.0°C for 30 minutes.
- The temperature of the mash was raised to within 0.5 °C of 70.0°C over the course of 25 minutes, using a rate of as close to 1°C per minute as possible.
- The mash was held within 0.5 °C of 70.0°C for 60 minutes.
- The flask was removed from the hotplate, the magnetic stir bar retrieved with a piece of clean martensitic stainless steel, and the top of the flask covered with a clean piece of foil.
- The following day, approximately 150 ml of the liquid above the turbid mass in the flask was poured or pipetted into a clean beaker.

- Approximately 1/2 g Sparkloid, a fining agent, was added as a filter aid, and the mixture stirred.
- A piece of filter paper was placed in a 6 cm diameter Büchner funnel, which was then placed in a clean 500 ml filter flask.
- A small amount of the wort sample was poured into the Büchner funnel, and the filter paper allowed to adhere to the perforated surface of the Büchner funnel.
- Approximately half the wort sample was gently poured into the Büchner funnel, and a vacuum pump attached to the flask was turned on.
- The wort sample was recycled until visibly clear.

If the malt had a diastatic power (ability to convert starch into sugars and dextrins) lower than 40 Lintner, approximately 1/2 g amylase enzyme, procured from a homebrew shop, was added to the mash at the start of the ramp-up to 70.0°C.

Establishing this procedure was one of the more difficult aspects of this study. Particularly troublesome was the clearing of the wort sample. Even so, one wort sample, produced from Carapils (R) malt, refused to clear, even after filtration using a 0.8 micrometer syringe filter (discarding the first 10 ml through the filter). It was extremely viscous and difficult to filter. This was not in hindsight unexpected, as the purpose of this malt is to increase the body and mouthfeel of a beer.

The laboratory worts were measured using the same procedures as for the beers.

#### **4.4.1. Malts from which Laboratory Worts were produced**

Laboratory worts were produced from the following malts, all from Briess:

- Two Row Brewers' Malt
- Pale Ale Malt
- Vienna Malt

- Bonlander (R) Munich Malt
- Aromatic Malt
- Special Roast
- Carapils (R)
- Caramel 20L Two-Row
- Caramel 40L Two-Row
- Caramel 60L Two-Row
- Caramel 80L Two Row

According to lot-specific analyses provided by the manufacturer, the color ratings of these malts (i.e., the color rating of a laboratory wort produced from these malts) ranged from 1.7 to 85. It was felt that these products formed a representative sampling of products used in beers, save two very dark ones: roast barley and black malt. Time precluded their inclusion before completion of this report; the author would like to include them (and others) as well.

The spectrum of the wort produced from Carapils (R) malt was not included in the analysis because of the clarity issues mentioned earlier.

## 5. Results, Discussion, and Conclusions

### 5.1. Scattering

Only beers and worts which exhibited visible turbidity had spectra which differed in the Total and Regular measurement modes. Typical of the comparisons for the bright beers was that obtained for the Kasteel Bruin, a sweet Belgian Dark Strong Ale. The Absorbance spectra of this beer, measured by both methods, appears as Figure 5.1. The two spectra are nearly coincident, evidence of low scatter.

Only one sample had visible turbidity and was excluded from the dimensionality analysis. Its Absorbance spectra, measured according to the Regular and Total methods, appears as Figure 5.2. It was an old sample which had been stored for several weeks while awaiting resolution of an issue with the spectrophotometer, so it is not identified by name (nor does it appear in Table 4.1). Accordingly, Hypotheses 1 and 2 are accepted.

Surprising was the clarity of the two dark wheat beer (Dunkelweizen) samples. Both had been stored aseptically for several years, which permitted the yeast to settle. The yeast sediment was not stirred up while pouring the samples.

### 5.2. Applicability of the Bouguer-Lambert-Beer Law

Figures 5.3 and 5.4 show the conformance of these beers with the Bouguer-Lambert-Beer law. Other beers which were measured at several different concentrations exhibited similar conformity. A possible exception was the visibly turbid beer mentioned

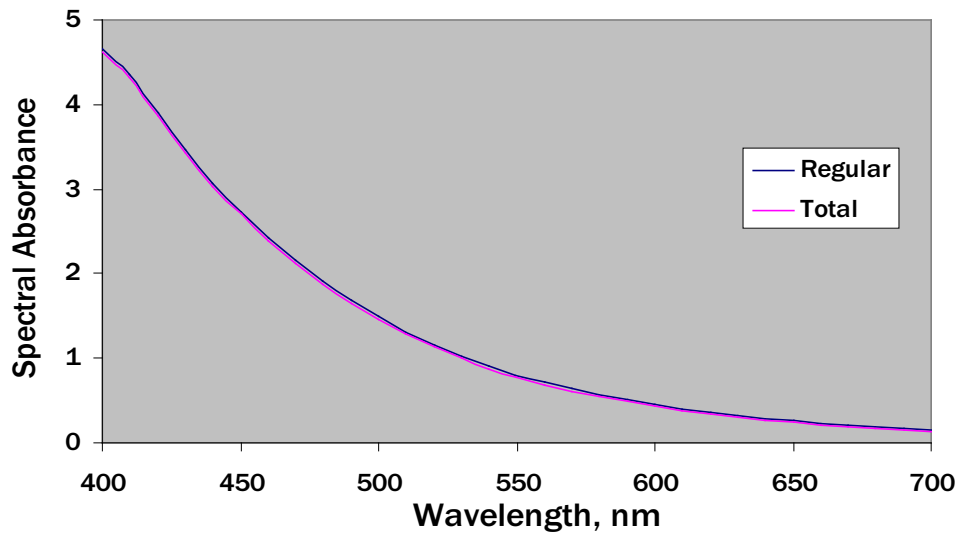


Figure 5.1.: The Absorbance spectra for the Kasteel Bruin beer, measured in both Total and Regular modes. That the two spectra are nearly identical indicates low scattering. (Because this plot is in Absorbance, the Regular spectrum should appear above the Total, which it does, however slightly.)

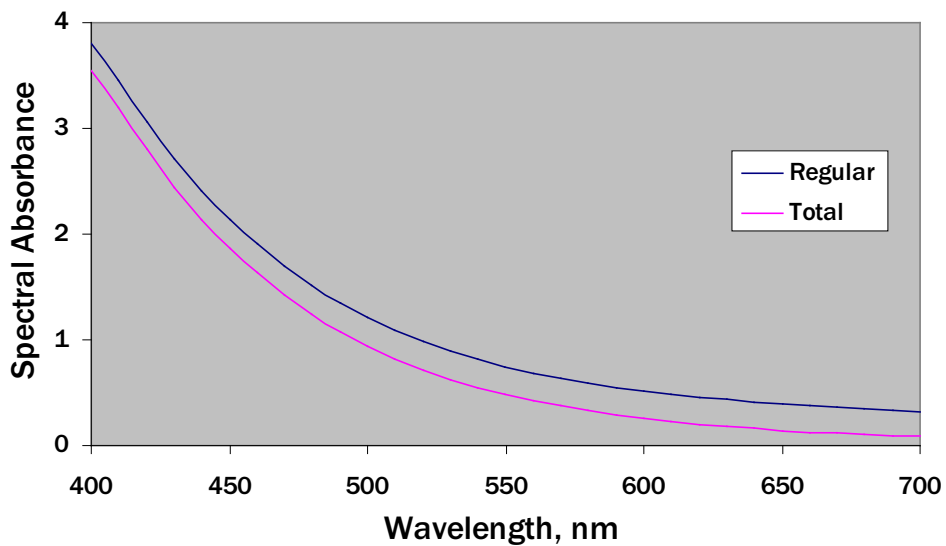


Figure 5.2.: The Absorbance spectra for a visibly turbid beer, measured using the Total and Regular modes. This beer was excluded from the dimensionality analysis, as it was not representative.



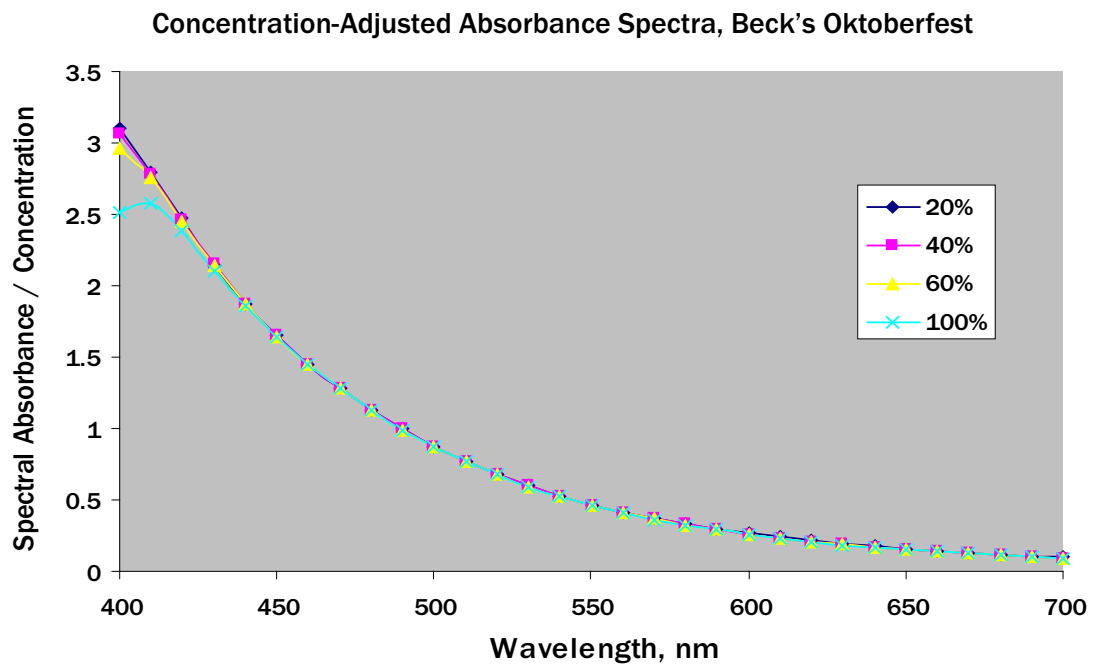


Figure 5.3.: The Absorbance spectra of Beck's Oktoberfestbier at four different concentrations, after dividing spectral Absorbance by concentration. This illustrates the applicability of the Bouguer-Lambert-Beer law to this beer. The falloff of the spectrum of the 100% concentration at 400-420nm (and of the 60% at 400nm) is attributable to the non-linearity of the Macbeth Color Eye 7000 spectrophotometer at spectral Absorbances above approximately 2.

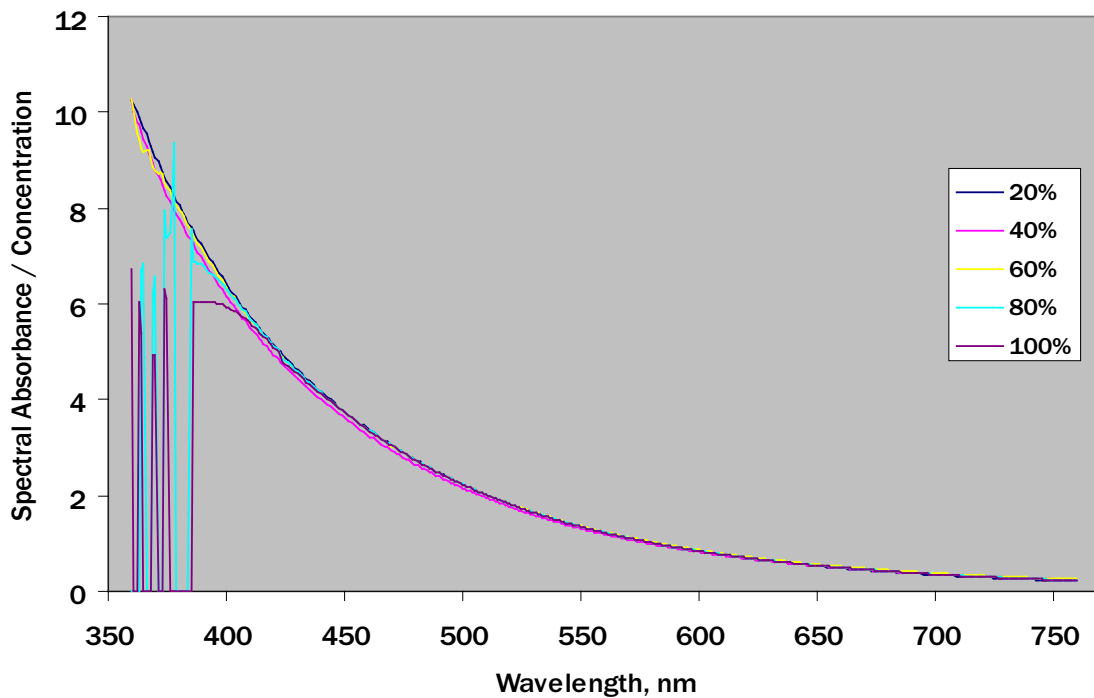


Figure 5.4.: The concentration-adjusted Absorbance spectra of five concentrations of Beamish Stout, measured in a double-beam instrument. The coincidence of these spectra, except at the very lowest wavelengths for the two highest concentrations, demonstrates that this beer conforms to the Bouguer-Lambert-Beer Law.

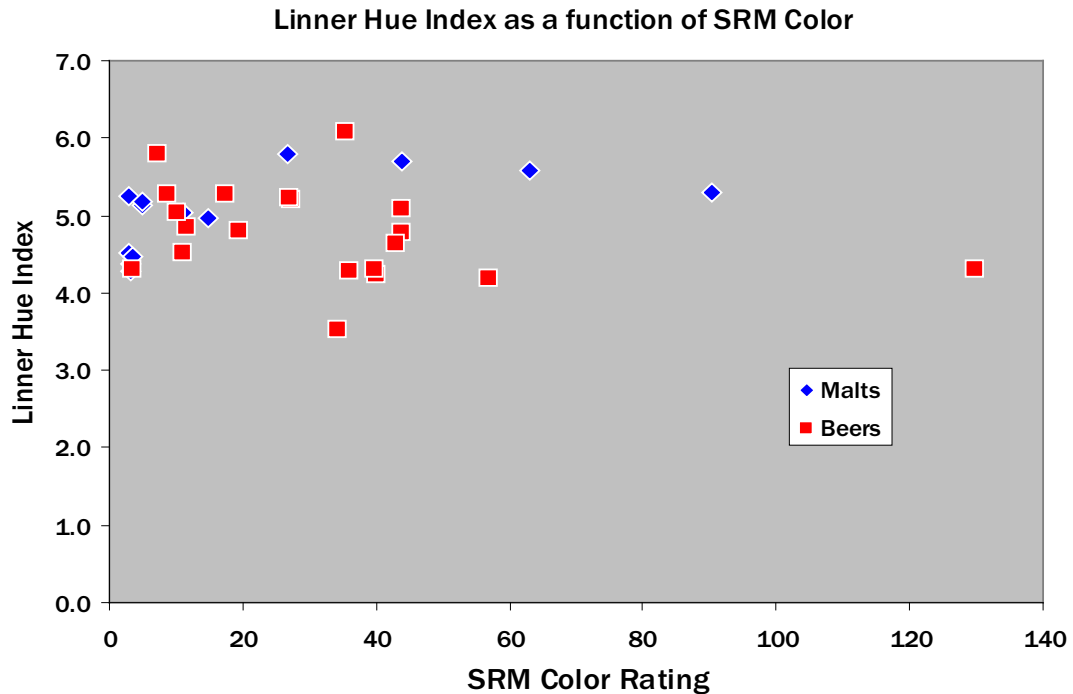


Figure 5.5.: The Linner Hue Index is plotted as a function of the SRM Color Rating for the beers and malts analyzed in this study. While it is useful to know that the Hue Index seems to fall in the same range cited for caramel colors, there is no obvious relationship between the Hue Index and SRM Color Rating.

in the previous section; over a doubling of concentration a four percent decrease in spectral Absorbance was noted.

Hypothesis 3 is accepted, and it is concluded that the Bouguer-Lambert-Beer law applied to the beers and worts in this study which lacked visible turbidity.

### 5.3. Dimensionality of Beer Spectra

It had been established earlier that beer spectra conform reasonably well, though not perfectly, to Linner’s model of linear log Absorbance spectra. For beer spectra to be one-dimensional, there must be a relationship (perhaps non-linear) between SRM color (which quantifies absorption in the complementary band) and Linner’s Hue Index

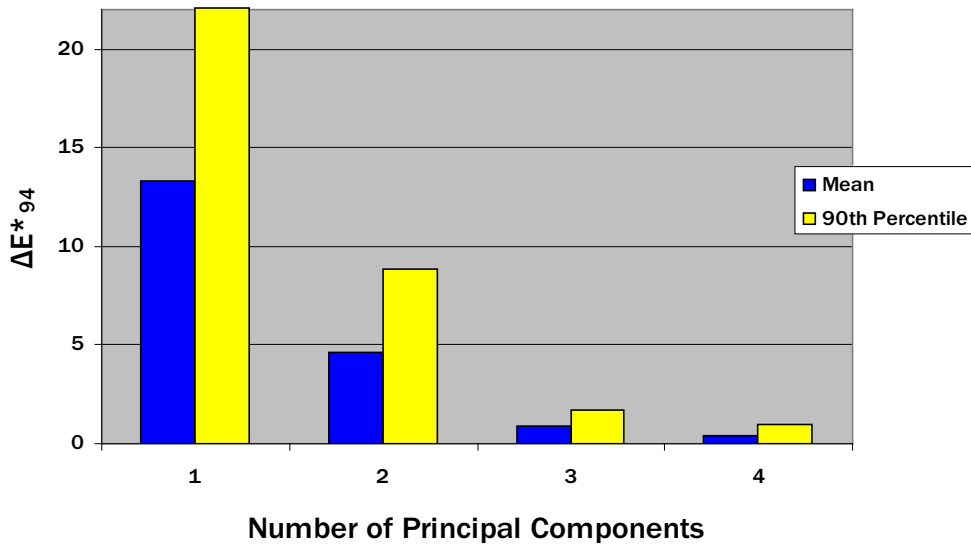


Figure 5.6.:  $\Delta E_{94}^*$  as a function of the number of Principal Components. Both the Mean and 90th percentile statistics were averaged across the 11 analyses run for each.

(which speaks to the ratio of Green to Red absorption). The SRM color values and Linner Hue Indices were computed for the beers and worts mentioned earlier, and appear graphically as Figure 5.5. It is not possible to compute one quantity from the other with reasonable accuracy. Accordingly, Hypothesis 5 is rejected, and it is concluded that SRM color alone does not completely specify or determine the color of the beers and worts in this study.

### 5.3.1. Principal Component Analyses

(Okay, the “statistics with a shovel” approach maybe was a bad idea. Now I have to deal with it with a shovel. I’m sorry.)

Overall measures of reconstruction accuracy from the Principal Component Analyses appear in Figures 5.6, and 5.7, and 5.8.

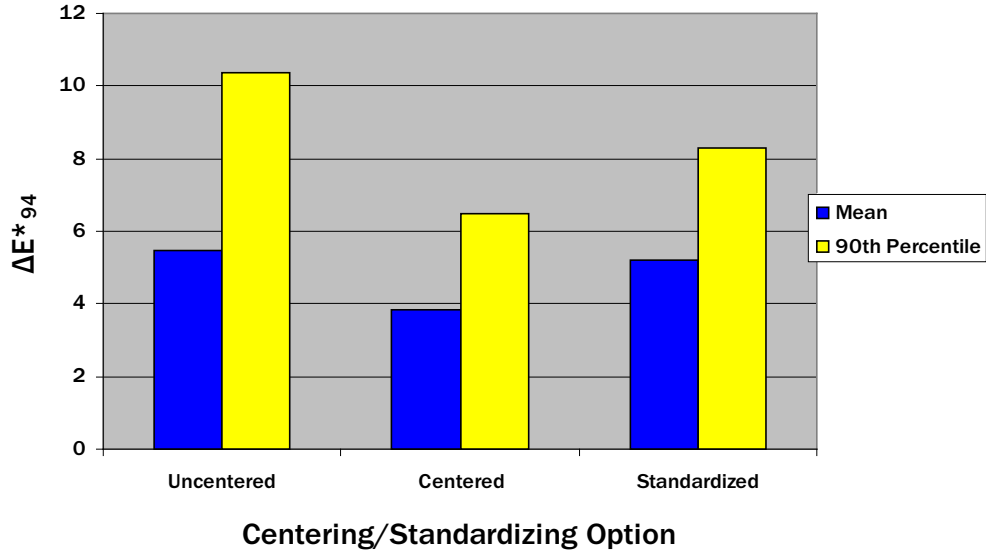


Figure 5.7.: The error of the reconstructed transmittance spectra as a function of the Centering/Standardization option. As in Figure 5.6, the Mean and 90th percentile statistics are averaged among each of the analyses run for each.

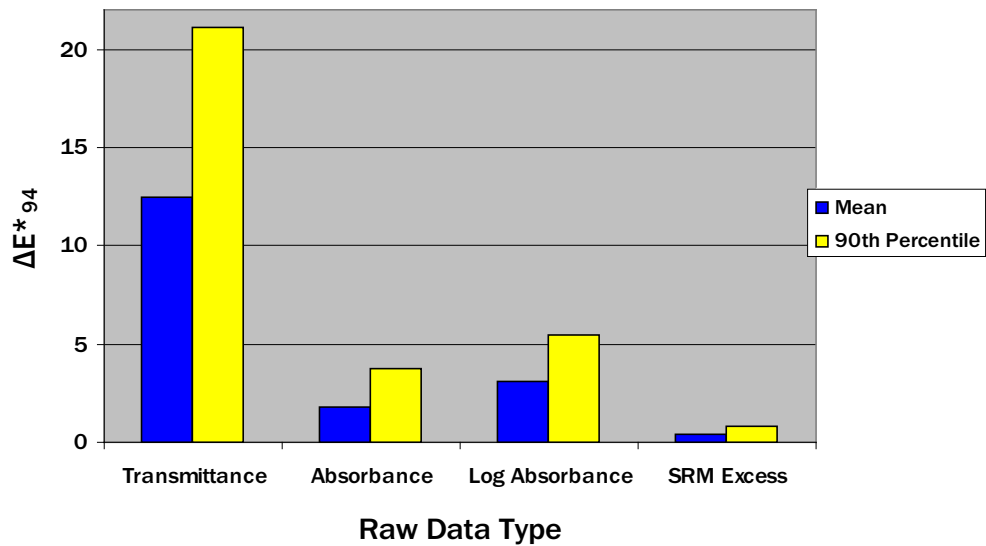


Figure 5.8.: The effect of raw data type (Transmittance, Absorbance, log Absorbance, or SRM Excess) on the error of the reconstructed spectra is illustrated here. Mean and 90th Percentile statistics are averaged among each of the analyses run for each.

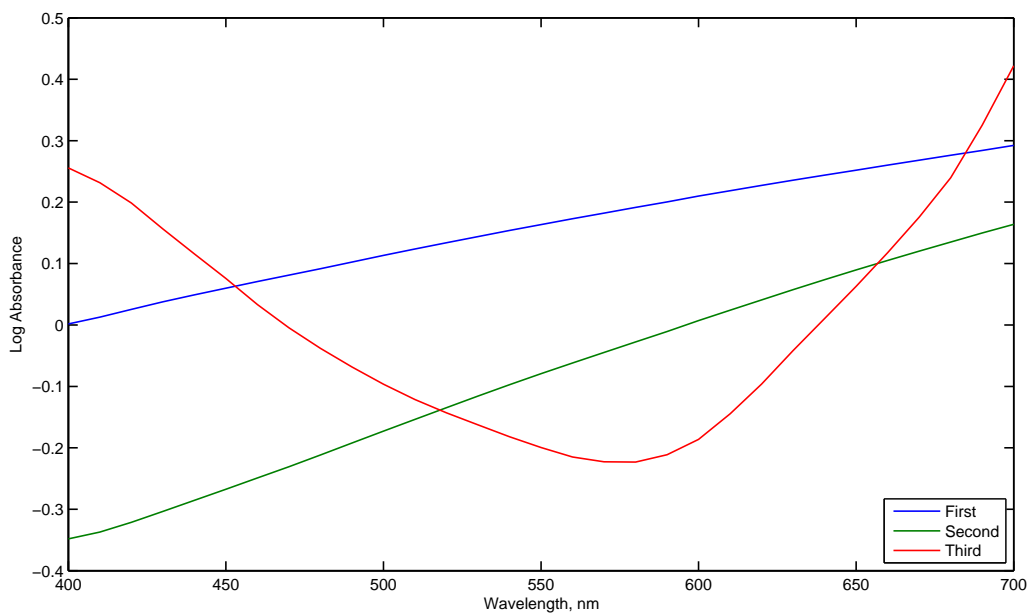


Figure 5.9.: The first three Principal Components from the Uncentered analysis of Log Absorbance spectra. The first two components, accounting for 99.98 percent of the squared Log Absorbance, are essentially linear with respect to wavelength.

### 5.3.2. Examination of a Specific PCA Result

If Linner's observation applies to beers and worts, the log Absorbance spectra should be of dimension 2, and the first two eigenvectors should be lines (or at least resolve into a pair of lines through rotation of axes) when plotted against wavelength. The latter seems reasonable, as borne out by Figure 5.9, which shows that the first two eigenvectors are essentially linear. Further, they account for 89.14 and 10.84 percent of the total squared log Absorbance (a cumulative total of 99.98 percent). However, reconstructing log Absorbance spectra may not translate as favorably into reconstructing CIELAB coordinates. For this analysis, the mean  $\Delta E_{94}^*$  was 0.78, and the 90th percentile was 1.49. (Adding the third eigenvector improved the figures to 0.42 and 0.85, respectively). If this is sufficient accuracy, one may accept Hypotheses 5 and 6. One must keep in mind that beer is rarely viewed in layers 1 cm thick in real life; the error for more typical thicknesses (about 5 cm) would be higher.

This specific analysis was selected for more detailed examination because it performed better than the others based on log Absorbance at the two-vector level. Because the SRM-Excess method assumes that the SRM color has already been accounted for, it would be necessary for the reconstructions from a single principal component in this space to be sufficiently accurate to use it to accept Hypothesis 5, and that one vector would have to be linear with respect to wavelength in order for it to support Hypothesis 6. Both the linearity and the reconstruction accuracy were not as good as for the uncentered log Absorbance analysis mentioned above.

## 6. Future Work

I have learned much during this investigation. Often, when in a situation like this, one learns how little one actually knows. This certainly applied here, and I want to learn (and do) more! Some things to consider:

- Expand the database of spectra, and publish it on the web
- Develop a modern color working standard which beer judges can use to learn beer color more accurately
- Re-run the PCA reconstruction accuracy evaluations using a 4 or 5 centimeter path length
- Test the hypothesis that SRM color is proportional to extract concentration (mass per volume) as the sparge progresses. If true, this would mean a relatively simple color prediction model should work accurately (see the next chapter for the development of such a model)
- For the same number of eigenvectors, the PCAs using Absorbance were often about as accurate (sometimes even more accurate) than those which used log Absorbance, in spite of the linearity of the log Absorbance spectra. The effect of taking the second logarithm on spectral weighting could be investigated (one log should be near optimal weighting)



## 7. Models for Prediction of Beer and Wort Color

### 7.1. Malt Color Units (MCUs)

This method is frequently used by small scale brewers, either on its own, or as a starting point in color prediction. It is easily calculated if one knows the grain bill, the color rating of each grain, and the wort or beer volume: [6]

$$C_{MCU} = \frac{1}{V} \sum_{all\ grains} w_i \cdot C_i \quad (7.1)$$

where

$V$  is the volume of wort or beer, in gallons;

$w_i$  is the weight of grain  $i$ , in pounds;

$C_i$  is the color rating of grain  $i$ , in SRM; and

$C_{MCU}$  is the computed Malt Color Units, in SRM-pounds per gallon (MCUs).

This simple formula certainly seems appealing. It seems reasonable to expect that the color of a beer produced from a single grain will be proportional to the color rating of the grain, proportional to the amount of that grain employed, and inversely proportional to the volume of beer. Unfortunately, it is based on a number of unreasonable assumptions. First, it seems to assume that the mash and lauter process is as efficient as

was the mash and lauter upon which each grain's color measurement was based. Secondly, it appears to assume that the mash from which each grain's color is estimated uses one pound of grain per gallon of liquor. Thirdly, it precludes any color pickup during processing. Relative to these:

1. Grain color is based on 100 percent efficiency; some light absorbing material will be retained in the grains at the end of the sparge.
2. Grain color is assessed from a laboratory mash using 50 grams of grain in 400 ml of water; this is equivalent to approximately 1.05 pounds per gallon, rather than the one pound per gallon implied above.
3. Processing, particularly the boil, will affect the color of the wort and the subsequent beer.

Based on these reasons, one would expect the MCU calculation to underestimate the color of darker beers and worts. The experience of many who have used MCUs directly as a prediction of color is consistent with this expectation. A more sophisticated prediction model is necessary.

## 7.2. A New Model

A new model for beer and wort color is presented here. It is based on the MCU calculation, but includes two factors and one term which account for things which the MCU calculation does not. This model is:

$$C = C_p + \frac{\eta}{1.05\text{lb/gallon}} C_{MCU} \quad (7.2)$$

where:

$C_p$  is the color pick-up introduced by processing, in °Lovibond;

$\eta$  is the mash and lauter efficiency, a unitless decimal fraction.

The quotient in Eq (7.2) accounts for two factors. The first, in the numerator, is the efficiency of the mash and lauter process, which the raw MCU calculation assumes to be unity. The second, in the denominator, is the ratio between the liquor-to-grist ratio used in the laboratory mash to that assumed by the MCU calculation. There is also an additive term which permits correction for color increase during processing.

The new model makes a few assumptions:

1. The ratio of color to extract concentration is constant throughout the lauter.
2. Extraction efficiency is the same for all grains.
3. Color pickup during processing is independent of pre-processing color.

The verity of each assumption can influence the accuracy of the new model.

## Acknowledgments

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Ms Bernadette Wasdovitch and Mr Bob Hansen of Briess Malt and Ingredients Company, for their generous donation of malt and extract samples;

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Dr Anthony Vodacek of RIT's Carlson Center for Imaging Science, for loan of equipment and use of the Shimadzu spectrophotometer;

Mr Jim McDermott of the Rohrbach's Brewing Company, for providing filtering aids, and for agreeing to participate in what has become follow-on portion of this investigation.

## A. Older Methods of Color Determination

### A.1. Visual Comparison with Diluted Iodine Solutions

The oldest method for quantifying the color of beer and wort included here is based on a solution of iodine. As described by De Clerk, a 1/10 Normal (N/10) solution of iodine was prepared by making a saturated solution of 25 grams of potassium iodide, adding 12.69 grams crystalline iodine, mixing well, and adding water to make 1000 milliliters. [De Clerck 2:243-244] This stock solution was stored in a brown bottle because it was photoreactive. The color was quantified as the number of milliliters of the N/10 iodine solution which needed to be added to 100 milliliters of water to produce the closest color match to that of the beer or wort in a similar container. [12, p 332]

This method has the advantage of relying on equipment normally found in even a basic chemical laboratory. Iodine solutions are routinely used in brewing to check starch conversion and for other purposes, so this would have been available to those working in a brewing or malting laboratory – it used an off-the-shelf reagent.

A disadvantage of this method is that the hue of the iodine solution is different from that of most beers and worts. This is especially noticeable for pale beers and worts; De Clerk relates: "... in pale worts, especially, the colours of the iodine solution and of the sample do not match satisfactorily and the determination becomes difficult."

### A.1.1. Interpretation of values on this scale

When reading historical texts, it would be helpful to be able to interpret values on this scale into a modern color scale. Because the iodine solution is transparent, the Bouguer-Beer-Lambert may be presumed to hold if the spectrophotometer has no significant stray light, and the internal reflections are not significant. The first step in such a translation would be to determine the normality of the iodine solution.

Suppose a volume  $v$  of  $N/10$  iodine solution in 100 milliliters of water produces the closest color match to a beer or wort sample in identical containers. The normality,  $N$ , of the matching iodine solution will be a first order rational function in  $v$ :

$$N = \frac{1}{10} \cdot \frac{v}{100ml + v} \quad (\text{A.1})$$

The Absorbance of the iodine solution at 430 nanometers will be proportional to its normality  $N$ . This implies the following relationship between the SRM and iodine color scales:

$$SRM = \frac{k \cdot v}{100ml + v}$$

De Clerck reports a conversion: “one degree Lovibond multiplied by the factor 0.086 is equivalent to the colour of a decinormal iodine solution.” [12, p 333] Presumably, the path length would be 12.7 millimeters, the thickness of the cell used in the Lovibond Tintometer. The statement could be interpreted that 0.086 milliliters of  $N/10$  iodine in 100 ml water would measure one °Lovibond. This interpretation would place the start of the standard Brand color scale, based on 0.14 milliliters of  $N/10$  iodine in 100 milliliters of water, at about 1.6 °Lovibond. This seems quite reasonable as the palest standard, but it would be nice to verify this.

## **A.2. The Brand Scale**

A refinement of the iodine procedure was devised by Brand. A mixture of dyes was prepared which more closely matched the hue of beers and worts than the iodine solution. Its concentration, however, was selected so that it closely resembled the N/10 iodine solution. Standard dilutions were then prepared, ranging from 0.14 to 6.00 milliliters of stock solution to 100 milliliters of water; one then reported the interval into which the beer or wort fell. [12, p 333]

Some of these dyes are no longer readily available, so it would be difficult to use this technique today. However, other dyes could be substituted.

### **A.2.1. Interpretation of values on this scale**

Because the concentrations of the Brand dyes in the stock solution were selected so the Brand and iodine scales would be equivalent, historical figures on this scale should be interpreted in the same manner as for the iodine scale above.

## **A.3. Visual Colorimetry**

The Lovibond Tintometer was used to assess beer and wort color, first in Britain, then in the United States. A special set of colored filters, Lovibond Series 52, were used. [4] These were identified by the equivalent amount of Lovibond Yellow units; fixed amounts of Lovibond Red and Blue were used for each scale value; the scale was one-dimensional. In continental Europe, colored filters were produced which matched the color of the standard dilutions of the Brand dye mixtures (at some specified thickness, under some illuminant). Discs containing these filters were employed in visual colorimeters, and the result was expressed as with the standard dilutions of Brand dyes (which are equivalent in strength to that of the N/10 iodine solution) in Europe. [12]

In 1953, the ASBC adopted the Standard Reference Method (SRM) for quantifying

beer color, which brings us to the present day.



## B. Nonlinearity of Log Absorbance Spectra of Admixtures of Two Solutions With Linear Log Absorbance Spectra

Consider two solutions which obey the Bouguer-Lambert-Beer law and Linner's observation over our region of interest. Their log Absorbance spectra will be linear with wavelength. Assume they have two different Linner Hue Indices,  $H_{L1}$  and  $H_{L2}$ , respectively. An admixture of the two is produced, using a volume fraction  $c$  of the first, and  $(1 - c)$  of the second. The Absorbance spectrum of the admixture,  $A_{m\lambda}$ , will be a linear combination of the Absorbance spectra of the constituents,  $A_{1\lambda}$ , and  $A_{2\lambda}$ , respectively:

$$A_{m\lambda} = c \cdot A_{1\lambda} + (1 - c) \cdot A_{2\lambda} \quad (\text{B.1})$$

in accord with the Bouguer-Lambert-Beer law. If the log Absorbance spectrum of the admixture is linear in wavelength, its second derivative with respect to wavelength will be zero. We determine the conditions under which this is so. First compute the first derivative of log Absorbance:

$$\begin{aligned} \frac{\partial \log A_{m\lambda}}{\partial \lambda} &= \frac{c \cdot \frac{\partial A_{1\lambda}}{\partial \lambda} + (1 - c) \frac{\partial A_{2\lambda}}{\partial \lambda}}{A_{m\lambda} \cdot \ln 10} \\ &= - \frac{c \cdot H_{L1} \cdot A_{1\lambda} + (1 - c) \cdot H_{L2} \cdot A_{2\lambda}}{1000\text{nm} \cdot A_{m\lambda}} \end{aligned} \quad (\text{B.2})$$

making use of a property of solutions which obey Linner's observation and which stems from the definition of the Linner Hue Index:

$$\frac{\partial A_\lambda}{\partial \lambda} = -\frac{\ln 10}{1000\text{nm}} H_L \cdot A_\lambda \quad (\text{B.3})$$

The second derivative is obtained by differentiating Eq (B.2) with respect to wavelength. After simplification, it yields:

$$\frac{\partial^2 \log A_{m\lambda}}{\partial \lambda^2} = \frac{\ln 10}{10^6 \text{nm}^2} c \cdot (1 - c) \cdot A_{1\lambda} \cdot A_{2\lambda} \cdot (H_{L1} - H_{L2})^2 \quad (\text{B.4})$$

### **B.1. Conditions for an admixture of two Linner Solutions to also be a Linner Solution**

For  $0 < c < 1$ , and with positive spectral transmittances less than unity (implying both  $A_{1\lambda}$  and  $A_{2\lambda}$  both positive), we observe that the second derivative given in Eq (B.4) is positive definite. It can assume values of zero only when  $c = 0$ ,  $c = 1$ ,  $A_{1\lambda} = 0$ ,  $A_{2\lambda} = 0$ , or when  $H_{L1} = H_{L2}$ . The first two conditions occur when the admixture is an improper one – produced entirely from one or the other parent solution. The third and fourth conditions occur when one of the solutions has an Absorbance spectrum of zero (i.e., is colorless, like water). (It is apparent that a dilution of a Linner solution will not only be a Linner solution itself, but will have the same Hue Index.) The fifth condition applies when the two solutions have the same Hue Index. Therefore, it may be concluded that an admixture of two Linner solutions will also be a Linner solution only when one or more of the following conditions are satisfied:

- The admixture is composed entirely of one of the solutions;
- One of the solutions does not absorb light; or
- The two solutions have the same Linner Hue Index.

## C. Glossary

**Absorbance** The negative of the common logarithm of the fraction of light transmitted by a sample.

**ASBC** American Society of Brewing Chemists, a professional society founded in 1934, and currently located in St Paul, MN.

**Beer** A fermented cereal-based beverage, usually bittered with hops.

**Conversion** The hydrolysis of starch (large molecule carbohydrates) into sugars (small molecule carbohydrates).

**EBC** European Brewing Convention, a professional organization founded in 1946 and currently located in Zoeterwoude, The Netherlands.

**Efficiency** In brewing, the ratio of extract in a wort to the extract contained in the grains used in the mash.

**Extract** The dissolved solids component of wort, or a concentrated wort popular with homebrewers for the production of beer without mashing grains.

**Hops** A plant, *Humulus lupulus* or the cone-like bracts of the female plant used in brewing to provide bittering, other flavors, and aromas to beer.

**Lauter** The process of draining extract-bearing liquor from a mash (which may be accompanied by sparging, *q.v.*), or a vessel in which this process is conducted.

**Malt** Cereal grains which have been permitted to germinate, modifying some of the proteins and synthesizing enzymes which break down starch into simple sugars, then dried.

**Mash** An infusion of ground malt (and possibly other cereals) with warm water to effect conversion of starch into sugars; the process of conducting such an infusion.

**Modification** A transformation of proteins and, to a lesser extent, carbohydrates, during germination to forms needed by the nascent plant.

**Sparging** Rinsing a mash with warm water to glean some of the extract (*q.v.*) retained inside the grains.

**SRM** Standard Reference Method; a spectrophotometer-based technique for quantification of the tinctorial strength of beer. Adopted in 1953 by the American Society of Brewing Chemists.

**Yield (Extract)** The ratio of extract in a wort to the mass of grains mashed.

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