COMPARISON OF A PORTABLE BEER MEASURING DEVICE AGAINST STANDARD METHODS;

AND

BEER RECIPE DESIGN: RATIOS AND BOTTLE CONDITIONING

A Project Paper

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ABSTRACT

The basis of a consistent product is the standardization of its production process. Understanding and controlling production parameters is paramount to achieving high quality that can be replicated and scaled. The production of beer has benefited from myriad of technological advantages throughout history to achieve qualitative consistency and extended shelf life. Inventions such as the microscope offered new tools for brewers to determine the root cause of changes in the product. Similarly, existing technologies have been optimized to achieve higher accuracy, reduced form-factor, and increase their overall capabilities. While large-scale brewing operations are outfitted with specialized equipment for this task, small and medium craft breweries often rely on external services to measure beer properties due to the economic entry barrier of specialized machinery and labor. This study compares the performance of a portable full-range spectrophotometric device against specialized hardware using standard methods on testing beer color, alcohol by volume (ABV), bitterness, and total sugars. As these parameters vary by beer style, two different brands of three different beer styles were analyzed. When measuring color using CIELab, the portable unit yielded a higher standard deviation than standard methods. When measuring Alcohol by Volume, all methods yielded results within regulatory standards allowance. When measuring bitterness, the portable unit followed similar ordinal behavior to spectrophotometry with a higher standard deviation. Total sugar measurement results were inconclusive due to experimental challenges.

Beer recipe formulation is often referred to as a combination of art and science. Brewing apprenticeships require on-the-job training and continued education to understand the underpinnings of a successful beer recipe. Using component ratios in the malt bill, yeast pitching, water chemistry, and hops additions can be a useful tool for brewers.

Bottle conditioning or refermentation during packaging is a traditional method of carbonating at the packaging step, leveraging the carbon dioxide produced by yeast during fermentation and reducing the need for extraneous CO_2. Several sources of sugar have been utilized by brewers for this purpose, normally in the form of a liquid solution. This work explores the usage of different sugar sources and their potential implications on refermentation and flavor.

BIOGRAPHICAL SKETCH

Christian Joel Mercado Acevedo started his pursuit of knowledge in the Puerto Rico public education system, where he attended a STEAM magnet high school (i.e., CROEM) and decided to pursue engineering. He earned a Bachelor of Science degree in Industrial Engineering at the University of Puerto Rico, Mayagüez campus in 2009. During his undergraduate studies, Christian participated in design projects (i.e., SAE mini-Baja) and further developed leadership skills as president of the American Society for Quality (SAE) student chapter. While doing undergraduate research under the tutelage of Dr. Mauricio Cabrera-Rios, Christian used his technical skills to build a statistical research laboratory. After graduating, Christian worked at Pfizer Pharmaceuticals developing digital production tracking dashboards. These experiences set the foundation that started his career in the technology consulting industry. Christian found his passion for beer and completed a certificate in Business of Craft Brewing from Portland State University in 2015. After attending the Advanced Brewing Technology course offered by Cornell University in 2015, Christian decided to further his studies in Food Science and Technology by pursuing a Master of Food Science degree, specializing in fermented foods and beverages.

DEDICATION

To my parents Elba and Efraín for always encouraging my pursuit of knowledge.

To my wife Mariely for galvanizing me to follow my passion.

To my dog Cali for making my life better.

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CHAPTER 1
COMPARISON OF A PORTABLE BEER MEASURING DEVICE AGAINST STANDARD METHODS

1.1. Introduction

Small to medium scale brewers often lack the infrastructure and budget to have an in-house analytical laboratory to assess beer quality. This represents a capability limitation to reveal underlying causes for potential beer defects and maintain control of key qualitative metrics. The solution often lies in hiring independent laboratories to process samples or leveraging the infrastructure of larger breweries in their network. Relying on external sources to process samples can present logistical challenges and introduce points of failure that can result in less reliable data and flawed conclusions. With the combination of analytical hardware technology and software solutions, smaller and more affordable devices have been recently reaching the market. These measuring devices represent an opportunity for craft brewers to maintain tight production controls without the delayed results and logistical variables associated with third-party analysis. This project compares a portable, battery-operated, full-range spectrophotometric device against specialized hardware using standard methods on testing beer color, alcohol by volume (ABV), bitterness, and total sugars. Additionally, a technical and user-experience (UX) assessment is included to identify strengths and potential areas of improvement of the device.

1.2. Methods and Materials

1.2.1. Sample Preparation

To assess the performance of the portable device against established methods and equipment three beer styles were selected due to their popularity, distribution, and characteristics: Pilsner, American IPA, and American Porter. For each beer style, two different brands were selected. The experiment was run twice, each time using three beers (one per style) in triplicates. A 150 ml

sample from a single bottle was decanted to a vacuum flask and decarbonated using a water vacuum while simultaneously agitating on an orbital platform shaker (Flask Dancer, Boekel Scientific, Pennsylvannia, USA) for 15 min at 200 RPM.

1.2.2. Color

Color can be measured using several standard methods like Standard Reference Value (SRM), European Brewery Convention (EBC), and CIELab. The latter is preferred due to its discriminatory power (SMEDLEY, 1995). CIELAB uses a three-coordinate system to determine color. L* measures lightness, a* measures red-green, and b* measures blue-yellow. Prepared samples were transferred to 50 ml centrifuge tubes and centrifuged at 6,000 RPM (Eppendorf, 5810 R, Hamburg, Germany) for 5 min to separate suspended particulates. Porter samples were diluted with deionized water to 1:5, 1:10, and 1:20 until a turbidity test was passed. The samples' colors were analyzed with a Colorimeter (HunterLab, UltraScan VIS, Virginia, USA), Spectrophotometer (Molecular Devices, SpectraMax Plus 384, California, USA), and a portable spectrophotometer (DNA Phone, Smart Analysis, Parma, Italy).

1.2.3. Alcohol by volume

Prepared samples were split three ways and Alcohol by Volume (ABV) was tested using three methods: gas chromatography (Agilent Technologies, 6890N, Delaware, USA) using method ASBC BEER-4D (ASBC, 2011a), near-infrared spectroscopy (Anton Paar, Alcolyzer for Wine, Graz, Austria), and an enzyme analyzer/spectrophotometer (DNA Phone, Smart Analysis, Parma, Italy).

1.2.4. Bitterness

Bitterness is measured in International Bitterness Units (IBU) using the ASBC method BEER-23A (ASBC, 2011b). One IBU is equated to 1 mg/l of isomerized alpha acid in beer. Prepared samples

were centrifugated at 2,000 G (Eppendorf, 5810 R, Hamburg, Germany) for 5 minutes. Samples' bitterness was analyzed with a Spectrophotometer (Molecular Devices, SpectraMax Plus 384, California, USA) following ASBC method BEER23-A, and a portable spectrophotometer (DNA Phone, Smart Analysis, Parma, Italy).

1.2.5. Total Sugar

Total sugar for this study is defined as the cumulative concentration in grams per liter (g/l) of glucose, fructose, sucrose, and maltose in each beer sample. Total sugar was measured using High-performance liquid chromatography (Prominence, Shimadzu, Kyoto, Japan) and a portable spectrophotometer (DNA Phone, Smart Analysis, Parma, Italy). No standard ASBC method for measuring these sugars in beer was identified. A wine method fom the Cornell AgriTech Wine Analytical Laboratory that measures glucose, fructose, and sucrose was adapted to include maltose. To add maltose to the method, standard calibration solutions of 10 g/l, 5 g/l, 1 g/l, 0.5 g/l, 0.1 g/l, and 0.05 g/l were prepared and measured using HPLC (Prominence, Shimadzu, Kyoto, Japan) with a Rezex™ ROA-Organic Acid H+ 300 X 7.8 mm column with LC guard column 50 X 7.8 mm at 45°C with a 0.5 ml/min flow rate. A calibration curve was built from the resulting values, yielding a limit of detection of 0.028 g/l, and a limit of quantification of 0.028 g/l (R^2=1) (**Error! Reference source not found.**).

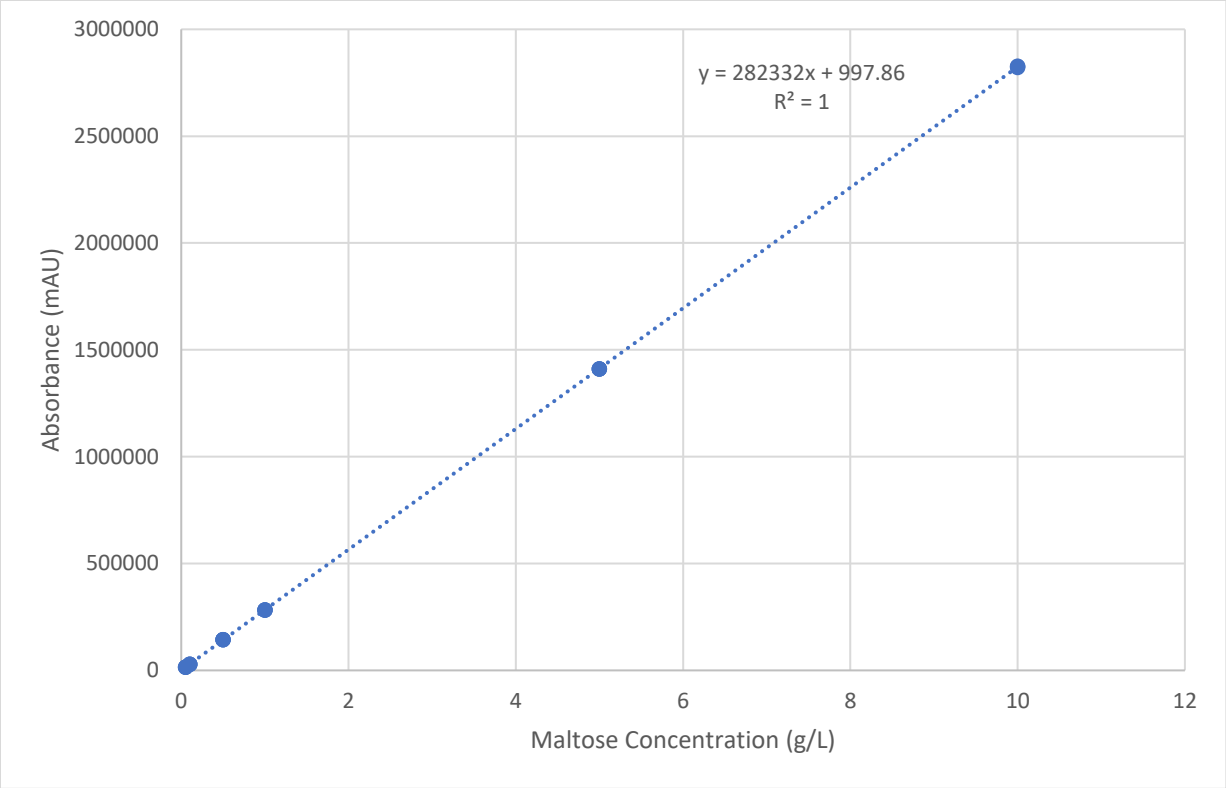

Figure 1. Maltose standard curve for the analysis of sugars in beer by HPLC.

Once a satisfactory calibration curve was created, the samples were measured alongside two standard solutions: a noise standard containing water and invertase, and a sugar standard containing 1 g/l each of glucose, fructose, sucrose, and maltose (4 g/l total).

1.2.6. Statistical Analysis

Analysis of variance (ANOVA, α=0.05), and all pairs Tukey-Kramer Honest Significant Test (HSD) (α=0.05) was performed on the resulting data sets using JMP Pro 15 (SAS Institute, North Carolina, USA).

1.3. Results and Discussion

1.3.1. Color

When comparing device performance on color measurement using color CIELab parameters L, a & b; colorimeter readings yielded a lower average standard deviation (L=0.62, a=0.09, b=0.41) followed by spectrophotometer (L=0.41, a=0.49, b=8.4), followed by portable spectrophotometer (L=5.2, a=4.4, b=7.6) (Table). Standard methods yielded a higher precision than the Smart Analysis. Smart Analysis reported values closer to colorimeter 61% of the time using Tukey's HSD test (Table). Spectrophotometer reported values closer to colorimeter 39% of the time. Higher standard deviation values were observed for Porter-style beers when using the Smart Analysis (Table , Figure , Figure , Figure). This may indicate dark-colored beers may pose a challenge to the device.

Table 1. Average standard deviation of color parameters by CIELab in beer by device.

					Average Std Dev By device		
		L	a	b	L	a	b
Device	**Beer**	**Std Dev**	**Std Dev**	**Std Dev**			
Colorimeter	Porter 1	0.27	0.19	0.52	0.62	0.10	0.41
	Pilsner 2	0.75	0.03	0.17			
	IPA 1	0.62	0.02	0.11			
	Porter 2	0.01	0.05	0.02			
	Pilsner 1	1.48	0.12	1.42			
	IPA 2	0.59	0.19	0.21			
Smart Analysis	Porter 1	18.39	10.75	28.90	5.21	4.36	7.61
	Pilsner 2	1.32	0.11	0.39			
	IPA 1	0.74	0.14	0.75			
	Porter 2	7.75	15.01	13.39			
	Pilsner 1	2.12	0.13	0.38			
	IPA 2	0.96	0.06	1.86			
Spectrophotometer	Porter 1	0.98	1.76	1.62	0.41	0.49	8.37
	Pilsner 2	0.11	0.07	0.72			
	IPA 1	0.35	0.13	29.23			
	Porter 2	0.58	0.55	0.88			
	Pilsner 1	0.13	0.13	0.46			
	IPA 2	0.31	0.29	17.28			

Table 2. Detailed statistics summary for CIELab parameters (α=0.05) for beer color evaluation.

		L*				a*				b*			
Beer	**Device**	Mean	Std Dev	P value	Tukey	Mean	Std Dev	P value	Tukey	Mean	Std Dev	P value	Tukey
Porter 1	Colorimeter	35.69	0.27	0.0401	AB	34.81	0.19	0.3384	A	59.60	0.52	0.1569	A
	Smart Analysis	32.67	18.39		A	27.05	10.75		A	53.70	28.90		A
	Spectrophotometer	59.58	0.98		A	28.41	1.76		A	82.92	1.62		A
Pilsner 2	Colorimeter	96.29	0.75	0.0697	A	-2.10	0.03	0.2959	A	32.91	0.17	<.0001	A
	Smart Analysis	94.40	1.32		A	-2.18	0.11		A	22.25	0.39		B
	Spectrophotometer	96.15	0.11		A	-2.08	0.07		A	17.15	0.72		C
IPA 1	Colorimeter	89.56	0.62	0.0003	B	-0.10	0.02	<.0001	A	38.15	0.11	0.8169	A
	Smart Analysis	89.73	0.74		B	-1.23	0.14		B	32.81	0.75		A
	Spectrophotometer	93.43	0.35		A	-2.44	0.13		C	41.67	29.23		A
Porter 2	Colorimeter	0.07	0.01	0.002	B	0.49	0.05	0.0077	B	0.12	0.02	0.0002	B
	Smart Analysis	9.67	7.75		B	24.14	15.01		A	16.69	13.39		B
	Spectrophotometer	23.67	0.58		A	34.63	0.55		A	59.39	0.88		A
Pilsner 1	Colorimeter	93.87	1.48	0.3613	A	-1.24	0.12	<.0001	A	31.46	1.42	<.0001	A
	Smart Analysis	93.07	2.12		A	-1.83	0.13		B	27.39	0.38		B
	Spectrophotometer	94.96	0.13		A	-2.48	0.13		C	22.46	0.46		C
IPA 2	Colorimeter	85.41	0.59	0.0002	B	4.04	0.19	<.0001	A	77.71	0.21	0.0041	A
	Smart Analysis	86.63	0.96		B	0.52	0.06		B	41.75	1.86		B
	Spectrophotometer	90.75	0.31		A	-1.70	0.29		C	34.94	17.28		B

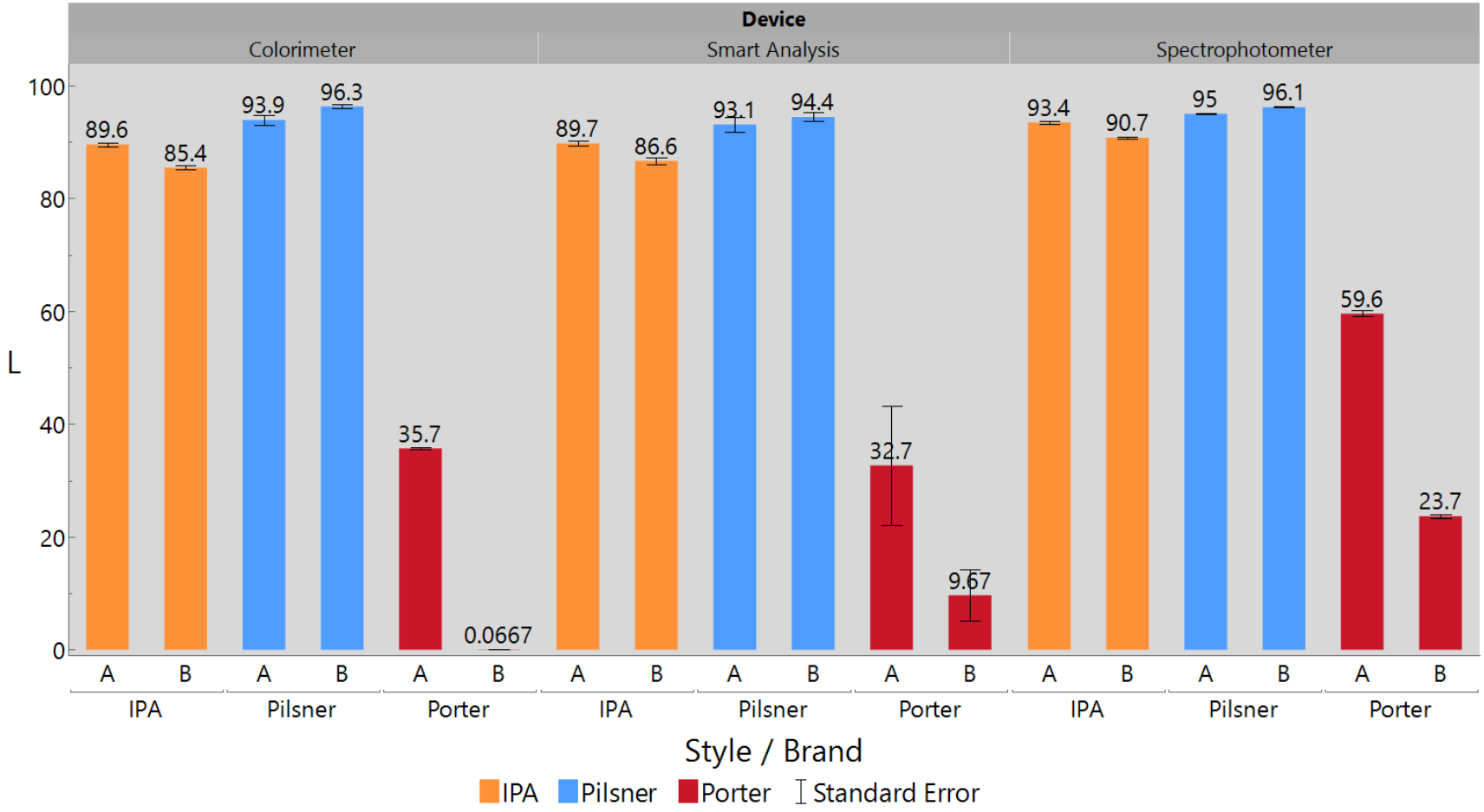

Figure 2. Evalaution of beer color by CIELab L value by device. Error bars indicate standard error.*

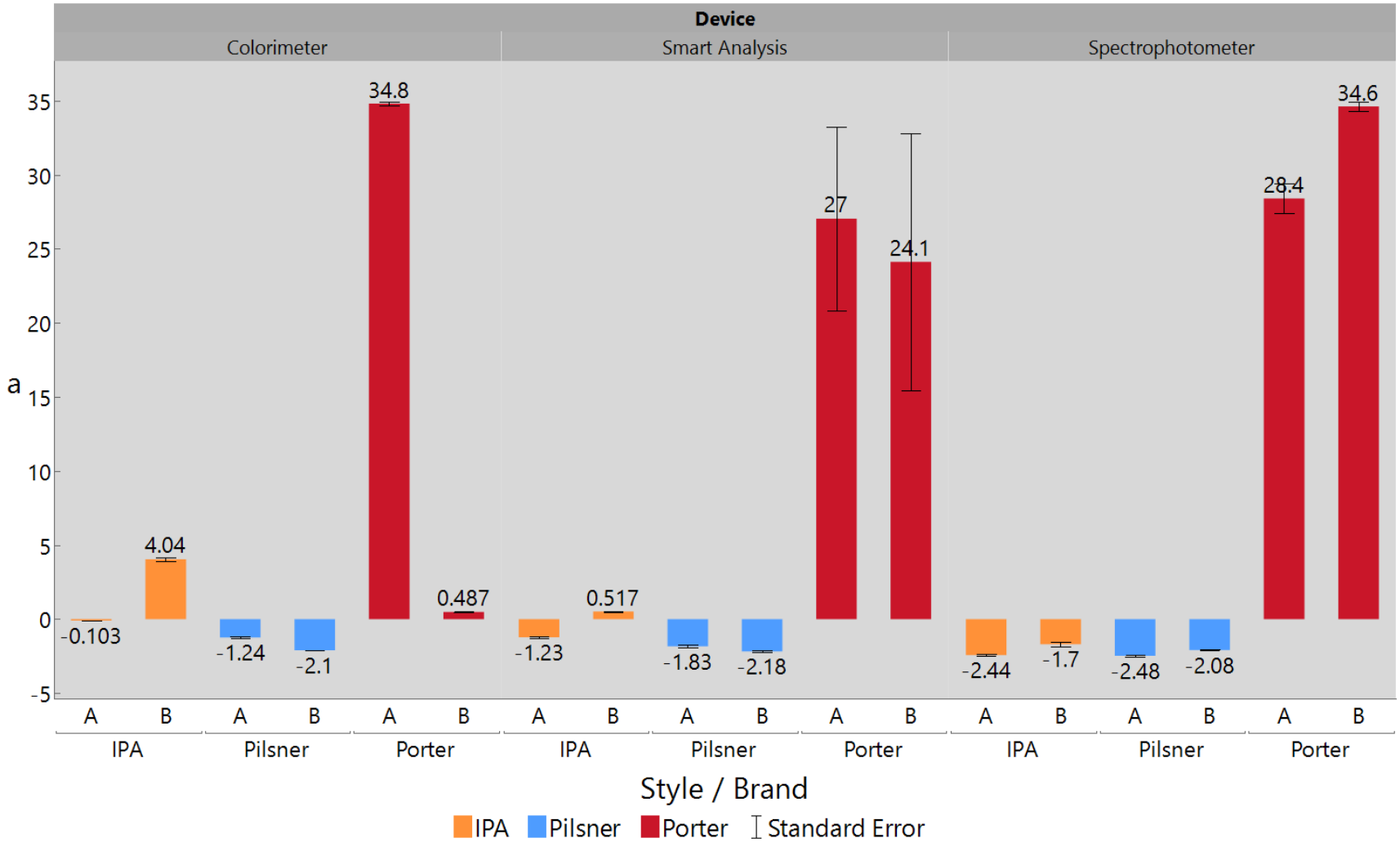

Figure 3. Evaluation of beer color by CIELab a value by device. Error bars indicate standard error.*

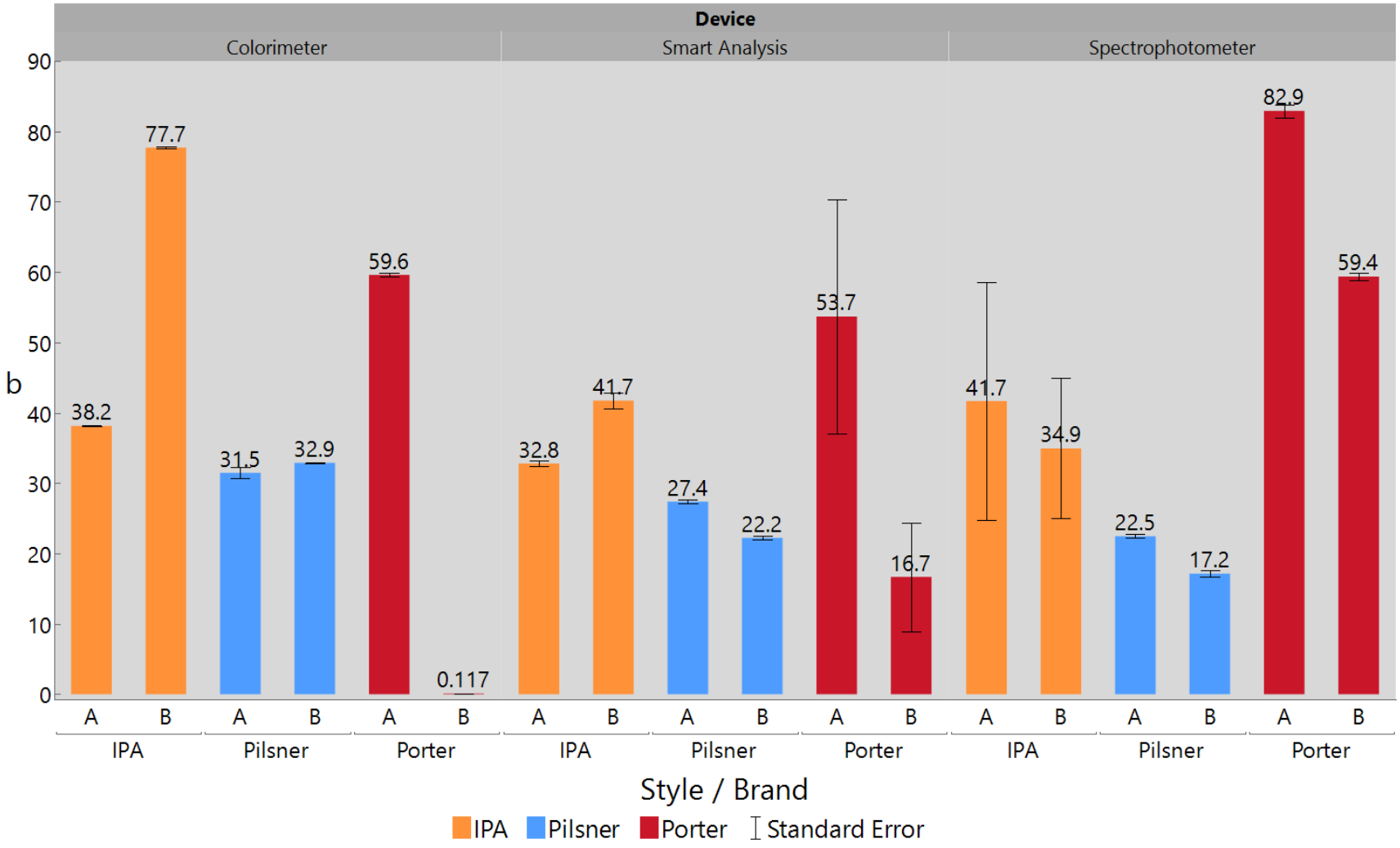

Figure 4. Evaluation of beer color by CIELab b value by style/brand and device. Error bars indicate standard error.*

1.3.2. Alcohol by volume

When comparing device performance on measuring ABV, Gas Chromatography (GC) yielded a lower average standard deviation (0.02% ABV), followed by Alcolyzer (0.15% ABV), followed by Smart Analysis (0.17% ABV) (Table). The Alcolyzer was within CG accuracy in 50% of cases using regulatory tolerance on ABV (TTB, 2018). Smart Analysis was within GC accuracy in 67% of cases using regulatory tolerance on ABV (Table).

While Alcolyzer and Smart analysis devices are not approved for determining ABV for beer label purposes, both devices performed within the regulatory boundaries for ABV. It should be noted that the GC method used in this study is TTB approved.

Table 3. Evaluation of alcohol content (v/v) in beer based on instrumental device (red values indicate P < 0.05).

Beer	Device	Mean	Std Dev	Std Error	P value (α=0.05)	Tukey
	Alcolyzer	4.28	0.06	2.47		A
	GC	4.40	0.02	2.54		A
Pilsner 1	Smart Analysis	3.94	0.09	2.28	0.0002	B
	Alcolyzer	5.11	0.11	2.95		B
	GC	6.02	0.03	3.48		A
IPA 1	Smart Analysis	6.11	0.11	3.53	< 0.0001	A
	Alcolyzer	5.75	0.03	3.32		AB
	GC	5.68	0.02	3.28		B
Porter 1	Smart Analysis	5.85	0.08	3.38	0.0216	A
	Alcolyzer	4.19	0.09	2.42		C
	GC	5.19	0.02	3.00		A
Pilsner 2	Smart Analysis	4.87	0.15	2.81	< 0.0001	B
	Alcolyzer	6.22	0.03	3.59		A
	GC	7.17	0.03	4.14		B
IPA 2	Smart Analysis	7.03	0.10	4.06	< 0.0001	A
	Alcolyzer	6.26	0.58	3.62		A
	GC	6.51	0.01	3.76		A
Porter 2	Smart Analysis	6.13	0.52	3.54	0.6018	A

Table 4. Average standard deviation and error by device for alcohol content in beer.

Device	*Avg. Standard Deviation*	*Avg. Standard Error*
Alcolyzer	0.15	3.06
GC	0.02	3.37
Smart Analysis	0.17	3.26

1.3.3. Bitterness

Bitterness results were compared by using analysis of variance (ANOVA) and all pairs Tukey-Kramer Honest Significant Test (HSD).

Table shows a summary of basic statistics for both devices. Overall, spectrophotometer readings had a lower standard deviation and standard error than Smart Analysis. The average standard error for Smart Analysis was 2.77, and 0.64 for spectrophotometer readings using ASBC BEER-23A (

Table). 60% of Smart Analysis IBU standard deviation values were below the human detection threshold of isomerized alpha acids in beer of 7.1 mg/l (Kolpin and Shellhammer, 2009) compared to 100% of the spectrophotometer readings. Bitterness measurements in both devices followed the same ordinal behavior.

It should be noted that the reagents used for determining bitterness were part of a pre-production reagent kit not yet available in the market. The results from this study indicate that further work should be done to refine this reagent kit in a way that minimizes user-introduced variance.

Table 5. Bitterness statistical summary from the analysis of beer by different (red values indicate P < 0.05).

Beer	Device	Mean	Std Dev	Std Err	P value (α=0.05)
Pilsner 1	Smart Analysis	29.42	8.48	4.9	0.3883
	Spectrophotometer	24.67	0.76	0.44	
IPA 1	Smart Analysis	35.99	1.79	1.03	0.0048
	Spectrophotometer	29.5	0.87	0.5	
Porter 1	Smart Analysis	24.68	1.54	0.89	0.5246
	Spectrophotometer	25.5	1.32	0.76	
Pilsner 2	Smart Analysis	24.26	6.61	3.82	0.0203
	Spectrophotometer	10	0.5	0.29	
IPA 2	Smart Analysis	46.86	1.56	0.9	0.2543
	Spectrophotometer	44.5	2.65	1.53	
Porter 2	Smart Analysis	29.41	8.82	5.09	0.0277
	Spectrophotometer	46.67	0.58	0.33	

Table 6. Average standard deviation and error per device for bitterness analysis in beer.

DEVICE	AVG. STD. DEV.	AVG. STD. ERR.
SMART ANALYSIS	4.80	2.77
SPECTROPHOTOMETER	3.51	2.03

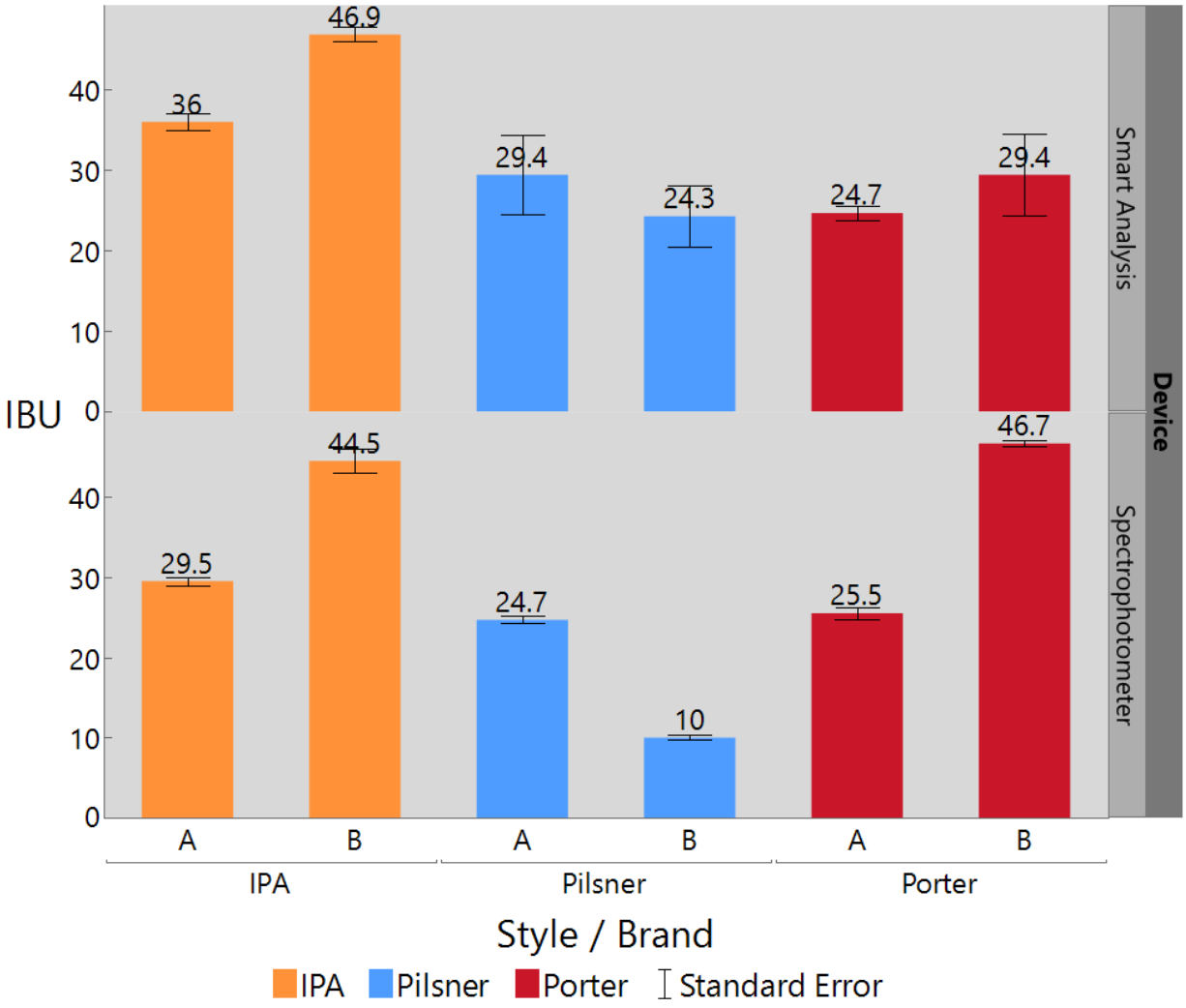

Figure 5. Bitterness in beer by style/brand and device. IBU = International Bitter Units.

1.3.4. Total Sugar

Total sugar in beer was compared by using analysis of variance (ANOVA) with α=0.05. One hundred percent of tests yielded statistically different values between Smart Analysis and HPLC (Table 2). A single outlier value was identified and omitted using the Cauchy distribution outlier detection function in JMP Pro 15. This difference may be explained by insufficient reagent volumes included in the kit used.

HLPC total sugar standard 4 g/l solutions yielded an average reading of 6.5 g/l, whereas the background standard yielded an average reading of 2.23 g/l of total sugar. These results may

indicate that readings using HPLC may overestimate total sugar. Furthermore, subtracting the background standard average value from the sugar standard yielded an average reading of 4.25 g/l, potentially still overestimating total sugar. Experimental design refinement and adding a third measuring method is suggested. Additionally, a different column may be required avoid the separation of glucose into monosaccharides (Jurková, Olšovská and Čejka, 2018).

Table 2. Statistical summar of total sugar in beer by device (outlier included, red values indicate P < 0.05)

Beer	Device	Mean	Std Dev	Std Err	P value (α=0.05)
Pilsner 1	Smart Analysis	9.03	0.27	0.19	0.0392
	HPLC	9.53	0.02	0.01	
IPA 1	Smart Analysis	12.46	0.58	0.34	0.0001
	HPLC	17.37	0.13	0.07	
Porter 1	Smart Analysis	1.79	0.05	0.03	< 0.0001
	HPLC	7.51	0.02	0.01	
Pilsner 2	Smart Analysis	2.61	0.04	0.02	< 0.0001
	HPLC	11.49	0.08	0.04	
IPA 2	Smart Analysis	12.46	0.58	0.34	< 0.0001
	HPLC	17.37	0.13	0.07	
Porter 2	Smart Analysis	4.29	0.15	0.08	< 0.0001
	HPLC	9.69	0.01	0.01	

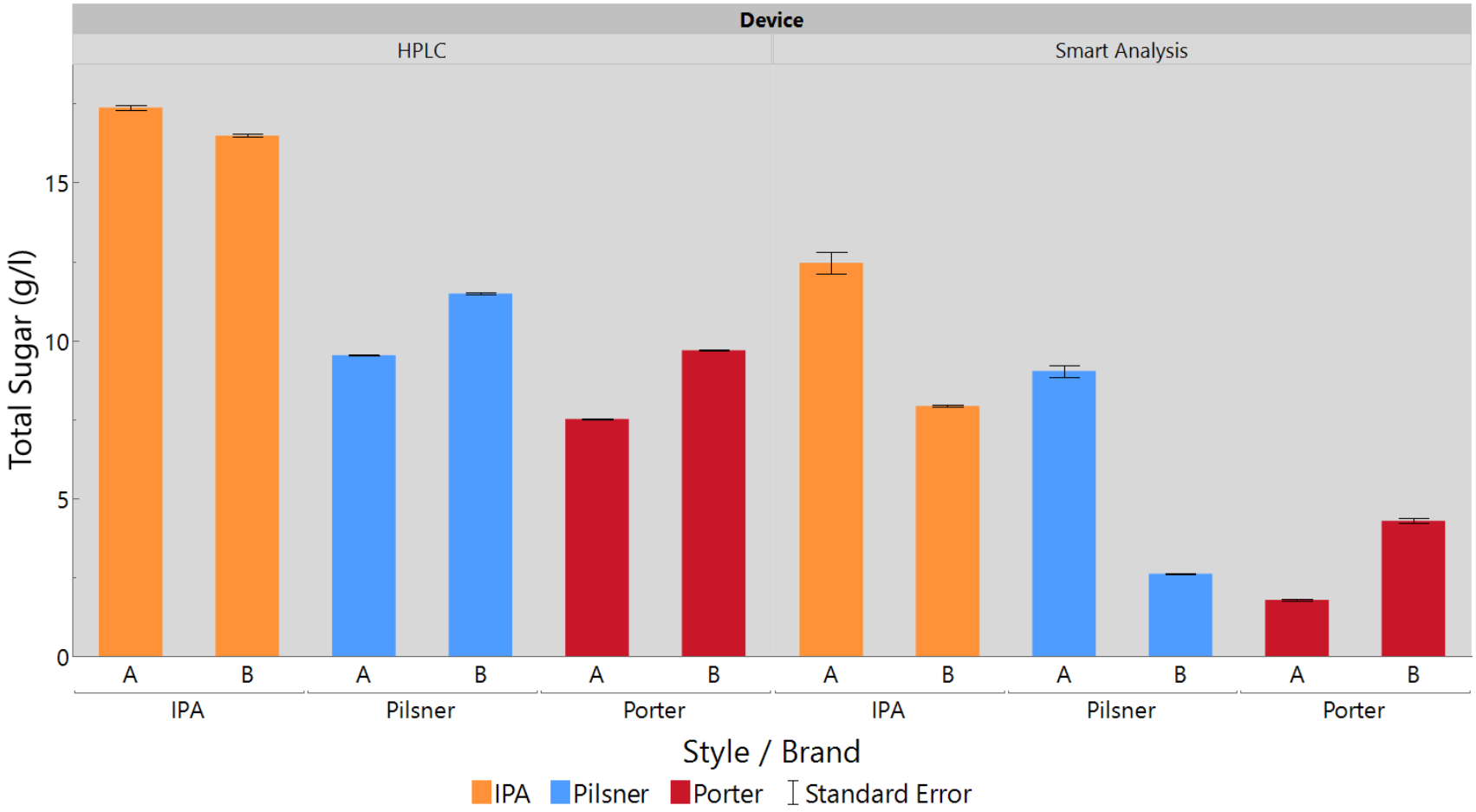

Figure 6. Total sugar by device and beer/brand (outlier included).

1.4. Conclusions

The DNA Phone Smart Analysis device provides the ability to measure a relatively wide array of beer properties when compared to dedicated equipment. The methods developed for this device utilize small reagent and sample volumes, and multiple preparation steps that can introduce variability when in use by untrained individuals. However, considering the unit cost is a fraction of specialized laboratory equipment and labor, it may provide small and medium breweries visibility over previously unmeasured quality performance indicators. This newly acquired visibility can be used to monitor process variations and provide customers with a more consistent product. Furthermore, the portability provides an in-situ measurement that can reduce reaction time and potentially lower operational costs to breweries by allowing for shorter reaction times to process deviations.

Results for color measurement in Pilsner and IPA beers styles were within expected values, while for Porter beer style, an increased standard error was observed even at a 1:20 dilution. Refinement of the color method is suggested for dark-colored beers. Additionally it is suggested that the manufacturer of the Smart Analysis integrates a turbidity test as part of color measurement to minimize operator-introduced variation. Alcohol by volume (ABV) measurements performed as expected and within 0.3% difference from gas chromatography (GC). Improvements are needed for bitterness measurement reagent kit to provide results with lower standard error.

CHAPTER 2
BEER RECIPE DESIGN: RATIOS AND BOTTLE CONDITIONING

2.1. Introduction

Beer brewing has been referred to as both an art and a science (Hardwick, 2006). Starting as an agricultural product that leveraged harvest bounty, beer has served in many cultures as a way to foster creativity. Creating a beer recipe is an iterative process that involves knowledge of the brewing process, raw materials, and consumer preferences. For this reason, brewing beer has historically been performed by individuals that invest a considerable amount of time to understand the process and materials required for making good quality beer. Although homebrewing has been popularized since the 1970s, recipe design remains obfuscated. A useful tool when designing beer recipes is the understanding of ratios between ingredients. By understanding how ingredient ratios vary across beer styles, a brewer can have a frame of reference to create new recipes.

Carbonation is one of the main characteristics of the beer. Various methods of beer carbonation have been used throughout history. Cask ales use a secondary fermentation in the cask, primed by the addition of sugar and oftentimes fining agents to aid in clarity (Oliver, 2013). Bottled conditioned ales are primed with a sugar solution, and sometimes yeast, to start a secondary fermentation in the bottle once capped. This sugar solution can be unfermented wort, dextrose, sucrose, malt extract, or other sources of sugar that yeast can metabolize. While various types of sugars can be leveraged to achieve the task of carbonation, the concentration of fermentable sugars in the priming solution must be considered to achieve a consistent carbonation level (measured in volumes of CO_2).

This review aims to collect and democratize a series of ratios that can help in the design of beer recipes; as well as describe the process of bottle-conditioning.

2.2. Ratio-based recipe design

2.2.1. Beer styles

Classification has been used as a way to describe and differentiate beer. Although not the first person to separate the libation into groups, the English writer Michael Jackson coined the term *beer styles* (Cornell, 2011) as a way to differentiate beers based on origin and sensory evaluation. Organizations like the Beer Judge Certification Program (BJCP) categorize beers into styles to aid in the evaluation of beer for competition purposes. Beer styles capture the essence of a beer at a point in time and are subject to change as consumer preferences vary. This information is crucial to understanding how beer evolves and to more accurately recreate its organoleptic properties. By analyzing the ratios of components in beer, recipe building can be simplified.

2.2.2. Ratios

Yeast Pitching

Building a beer recipe often starts by selecting a style. Although not a requirement, beer styles can help narrow the scope of ingredients and methods to be used. One of the main defining characteristics of a beer style is yeast selection (Palmer, 2017). Higher yeast pitch rates (inoculum size) can impact fermentation power, alcohol, and off-flavor compounds production (Verbelen *et al.*, 2009); the consensus is a pitch rate of one million cells per milliliter of wort per every degree Plato, with slight variations based on the yeast species (0.75 for ales, 1.5 for lagers) (White and Zainasheff, 2010). Higher gravity worts may require an elevated pitch rate (Suthko, Vilpola and Linko, 1993).

Malt Bill

In simplified terms, beer is the fermented product of the sugars extracted from cereals, most notably malted barley. The malts and cereals used in a beer recipe are known as grain bill, or grist once ground (Oliver, 2013). The percentual contribution of every grain type in the grist can thus be used to determine a ratio. A traditional Czech Pilsner can have a base malt to crystal malt ratio of 9:1, whereas a German wheat beer can have a 1:1 ratio of malted barley to wheat. Grist ratios are also suggested by malt manufacturers to ensure proper enzymatic saccharification during mashing, and to avoid specialty malts from overpowering the malt bill. These ratios provide a starting point for the brewer when designing a beer recipe. It is advised to then perform a sensory analysis on the resulting beer and adjust the ratios to achieve the desired malt character.

Mashing is the process of steeping the grist in water to extract sugars aided by enzymes present in malt. The water used to perform the mashing operation is known as liquor. Although more pertinent to process than to recipe, the liquor to grist ratio in the mash can have sugar extraction efficiency and fermentability implications (Fox, 2016). A liquor to grist ratio between 2:1 and 7:1 is recommended, depending on the scale of brewing operations (Muller, 1991; Palmer, 2017)

Bitterness to Gravity

Hops are the strobiles of the climbing perennial plant *Humulus lupulus*. Hops contain resins and essential oils that confer flavor qualities to beer. The resins present in hops are classified as α-acids and β-acids, and account for approximately 15% of the hop weight. The boiling process isomerizes the α-acids, making them bitter and soluble in the wort (Hieronymus, 2012). The bitterness provided by the iso-α-acids (and other compounds) balances the sweetness of the wort. The bitterness to sweetness ratio may be difficult to calculate as different worts have different saccharide compositions. However, the initial specific gravity of the wort (gravity units, GU) can

be used as an indicator of sweetness, as a linear relationship exists between the two (Palmer, 2017). Bitterness is measured using Bittering Units (BU) and is calculated by measuring the absorbance of beer at 275 nm using a spectrophotometer multiplied times fifty (ASBC, 2011b). There are several methods for estimating BU when designing a beer recipe (e.g., Tinseth, Noonan, Daniels, Garetz, Rager, Mosher, etc), each with its advantages and disadvantages for different beer styles. Comparing BU to GU as a ratio provides an estimation of the balance of bitterness to sweetness in the resulting beer. An IPA with 60 BU and an initial specific gravity of 1.060 will therefore have a BU:GU ratio of 60:60 or 1:1, whereas a Märzen will typically have a BU:GU ratio of 20:60 (i.e., 20 IBU, 1.060) or 1:3.

Water Chemistry

Water is the main component in beer by quantity. Beer styles have been historically optimized for their local ingredients, including water. The water of Burton upon Trent in Staffordshire, England has been emulated by many when designing variations on India Pale Ale (English IPA). The reason for this water's fame is the high levels of hardness and alkalinity. Water hardness is determined by the amount of calcium and magnesium ions in solution (Palmer, 2017). Alkalinity is mainly determined by the amount of dissolved carbonate species in water (Palmer and Kaminski, 2013). There are six ions of interest for brewers when assessing brewing water: Calcium, Magnesium, Total Alkalinity as $CaCO_3$, Sulfate, Chloride, and Sodium. Furthermore, the ratio between some of these ions can enhance certain organoleptic properties of the beer. The main ratio of interest for this review is the sulfate to chloride ratio. Chloride promotes a full mouthfeel that favors malt sweetness, whereas sulfate can aggrandize the perception of bitterness and dryness commonly found in highly hopped beers (Holbrook, 2020). Thus when designing a malt-forward beer, such as a Bock, the ratio should favor chloride (eg. 1:2), and when designing an American IPA the ratio

should favor sulfate (e.g. 4:1). It should be noted that the total amount of these ions dissolved in water must also be taken into consideration to avoid off-flavors. Chloride levels above 250 ppm can lend a salty character. Sulfate levels above 400 ppm can make the resulting beer astringent (Palmer and Kaminski, 2013).

2.3. Bottle conditioning

2.3.1. Background

Beer bottle conditioning came from the wine industry in the 19th century (Štulíková et al., 2020). Since then, the beer industry has experienced significant growth and new techniques have been developed and adapted to carbonate beer. Bottle conditioning is a traditional method of carbonating beer at packaging, by promoting a secondary fermentation in the bottle. However, bottle conditioning is not limited to glass bottles. There are commercial examples of can-conditioned craft beer that utilize the same mechanism of secondary fermentation in packaging.

2.3.2. Conditioning factors

Carbonation is one of the main elements that impact the mouthfeel of beer (Langstaff and Lewis, 1993). Carbonation has a chemesthetic impact on flavor perception that can be described in terms of its tactile and trigeminal sensations (Lawless and Heymann, 2010). Several factors impact the level of carbonation in a bottle-conditioned beer. Remaining fermentable sugars, temperature, alcohol level (ABV), and yeast viability and vitality after primary fermentation. For a given beer recipe, the difference in initial and final specific gravity, or density, can be used as a method to estimate yeast sugar uptake during fermentation. This uptake is the amount of sugar consumed by the yeast, which can vary based on the sugar composition of the beer matrix and the attenuation of the yeast strain used for fermentation. Temperature can have a multivariate effect on carbonation. The solubility of CO_2 produced by yeast during refermentation will vary based on temperature.

Yeast can metabolize different compounds at different fermentation temperatures. This can impact the organoleptic characteristics of the resulting beer. High alcohol concentration can stress yeast and affect its performance during bottle conditioning. Yeast viability is the percentage of living yeast cells in a population (Kwolek-Mirek and Zadrag-Tecza, 2014). Yeast vitality is defined as the physiological capabilities of yeast. Yeast vitality can have an impact on beer flavor stability (Guido *et al.*, 2004). By taking yeast viability and vitality into consideration when calculating the amount of yeast needed for bottle conditioning, a more informed decision can be made, and higher repeatability can be achieved.

2.3.3. Sugar sources

Bottle conditioning of beer can be achieved through various mechanisms. If terminal specific gravity (i.e., Terminal gravity) for a beer recipe is known with a high degree of certainty, a brewer can choose to package beer before terminal gravity is achieved, thus allowing for fermentation to continue in the bottle. Alternatively, a sugar solution can be added before packaging to encourage refermentation. Like in the case of sparkling wine, a *liqueur de tirage* (a sugar solution containing nutrients) can be added alongside a yeast inoculum to increase the likelihood of a successful refermentation (Ivit and Kemp, 2018). The sugar solution can be made by dissolving crystallized sugars in water (e.g., sucrose, fructose, maltose, dry malt extract, etc.) or by using an alternate liquid like fruit juice that contains a suitable level of fermentable sugars. Fruit juices such as cherry or grape can impart both fermentable sugars and a desirable fruit character to the resulting beer, where stylistically applicable. In this case, the sugar composition of the juice should be taken into consideration to achieve suitable carbonation in the beer. When a sugar source contains non-fermentable sugars, it will affect the perceived sweetness and body of the beer.

While optimal carbonation level varies by beer style, 2.5 volumes of CO_2 is often used for American Pale Ale (i.e., APA). In order to achieve this level of carbonation using monosaccharides (e.g., glucose, fructose), 6.3 g/L of monosaccharides are required (Palmer, 2006).

2.4. Conclusions

While beer recipe formulation requires ingredients and process knowledge, the usage of component ratios can guide the brewer in this process. The ratios presented in this review aim to facilitate recipe formulation without disregarding basic principles such as human sensory thresholds and safety.

Bottle conditioning with young beer or dextrose (glucose) is commonplace for brewers of diverse backgrounds. By changing the sugar source and understanding its saccharide composition, a brewer can introduce new flavors during packaging and modulate the perceived sweetness of the resulting libation. The brewer can introduce different yeast strains to attain a different character. Furthermore, yeast nutrients can be incorporated during this phase to supplement yeast nutrient needs during bottle conditioning.

WORKS CITED

ASBC (2011a) ‘ASBC Alcohol method’, *ASBC Methods of Analysis*, pp. 1–8. doi: 10.1094/ASBCMOA-Beer-4.

ASBC (2011b) ‘Beer Bitterness’, *ASBC Methods of Analysis*, (reagent 1), pp. 1–10. doi: 10.1094/asbcmoa-beer-23.

Cornell, M. (2011) ‘Michael Jackson and beer styles’, *Journal of the Brewery History Society*.

Fox, G. (2016) ‘Infrared spectral analysis of sugar profiles of worts from varying grist to liquor ratios using infusion and ramping mash styles’, *Journal of the Institute of Brewing*, 122(3), pp. 437–445. doi: 10.1002/JIB.341.

Guido, L. F. *et al.* (2004) ‘The impact of the physiological condition of the pitching yeast on beer flavour stability: an industrial approach’, *Food Chemistry*, 87(2), pp. 187–193. doi: 10.1016/J.FOODCHEM.2003.10.033.

Hardwick, william a (2006) *Handbook of Brewing*, *Handbook of Brewing*. doi: 10.1201/9781420015171.

Hieronymus, S. (2012) *For The Love of Hops - The Practical Guide to Aroma, Bitterness and the Culture of Hops*. Brewers Publications.

Holbrook, C. J. (2020) ‘Brewhouse operations’, *The Craft Brewing Handbook*, pp. 65–109. doi: 10.1016/B978-0-08-102079-1.00003-5.

Ivit, N. N. and Kemp, B. (2018) ‘The impact of non-Saccharomyces yeast on traditional method sparkling wine’, *Fermentation*. Multidisciplinary Digital Publishing Institute, p. 73. doi:

10.3390/fermentation4030073.

Jurková, M., Olšovská, J. and Čejka, P. (2018) ‘Determination of Sugars and Saccharides in Beer’, *KVASNY PRUMYSL*, 64(2), pp. 58–64–58–64. doi: 10.18832/KP201812.

Kolpin, K. M. and Shellhammer, T. H. (2009) ‘The human bitterness detection threshold of Iso-α-acids and tetrahydro-iso-α-acids in lager beer’, *Journal of the American Society of Brewing Chemists*, 67(4), pp. 200–205. doi: 10.1094/ASBCJ-2009-0706-01.

Kwolek-Mirek, M. and Zadrag-Tecza, R. (2014) ‘Comparison of methods used for assessing the viability and vitality of yeast cells’, *FEMS Yeast Research*, 14(7), pp. 1068–1079. doi: 10.1111/1567-1364.12202.

Langstaff, S. A. and Lewis, M. J. (1993) ‘THE MOUTHFEEL OF BEER — A REVIEW’, *Journal of the Institute of Brewing*, 99(1), pp. 31–37. doi: 10.1002/J.2050-0416.1993.TB01143.X/FORMAT/PDF.

Lawless, H. T. and Heymann, H. (2010) *Sensory Evaluation of Food*. New York, NY: Springer New York (Food Science Text Series). doi: 10.1007/978-1-4419-6488-5.

Muller, R. (1991) ‘THE INFLUENCE OF GRAIN COMPONENTS ON INFUSION MASH PERFORMANCE’, *Journal of the Institute of Brewing*, 97(2), pp. 93–100. doi: 10.1002/J.2050-0416.1991.TB01056.X.

Oliver, G. (2013) *The Oxford Companion of Beer*. Oxford University Press. Available at: https://www.oxfordreference.com/view/10.1093/acref/9780195367133.001.0001/acref-9780195367133.

Palmer, J. J. (2006) *How to Brew: Everything You Need To Know To Brew Beer Right The First*

Time: John J. Palmer, Brewers Publications. Available at: https://www.amazon.com/How-Brew-Everything-Great-Every/dp/1938469356 (Accessed: 24 February 2021).

Palmer, J. J. (2017) *How to brew : everything you need to know to brew great beer every*. 4th edn.

Palmer, J. and Kaminski, C. (2013) *Water: A Comprehensive Guide for Brewers, I.*

SMEDLEY, S. M. (1995) 'DISCRIMINATION BETWEEN BEERS WITH SMALL COLOUR DIFFERENCES USING THE CIELAB COLOUR SPACE', *Journal of the Institute of Brewing*, 101(3), pp. 195–201. doi: 10.1002/J.2050-0416.1995.TB00862.X.

Štulíková, K. *et al.* (2020) 'Bottle Conditioning: Technology and Mechanisms Applied in Refermented Beers.', *Beverages*, 6(3), pp. 1n-1n. doi: 10.3390/BEVERAGES6030056.

Suthko, M. -L, Vilpola, A. and Linko, M. (1993) 'PITCHING RATE IN HIGH GRAVITY BREWING', *Journal of the Institute of Brewing*, 99(4), pp. 341–346. doi: 10.1002/J.2050-0416.1993.TB01173.X.

TTB (2018) *TTBGov - Beer Beverage Alcohol Manual: A Practical Guide*. Available at: https://www.ttb.gov/beer/beverage-alcohol-manual (Accessed: 24 September 2021).

Verbelen, P. J. *et al.* (2009) 'Impact of pitching rate on yeast fermentation performance and beer flavour', *Applied Microbiology and Biotechnology*, 82(1), pp. 155–167. doi: 10.1007/S00253-008-1779-5/FIGURES/6.

White, C. and Zainasheff, J. (2010) 'Yeast : the practical guide to beer fermentation', p. 304.