085

WORLD BREWING CONGRESS 2016

Update: Solid Phase Extraction of Isomerized Alpha Acids in Beer and Subsequent Spectrophotometric Measurement

Scott Lafontaine[a], Philip Wiestock[b], Thomas H. Shellhammer[a]

a. Department of Food Science Oregon State University, Corvallis, OR 97333

b. Department of Food Technology and Food Chemistry, Technische Universität Berlin, Seestraße 13, 13353 Berlin, Germany

World Brewing Congress
August 13-17, 2016
Sheraton Downtown Denver
Denver, CO 80202, U.S.A.

Introduction and Background

Commonly used methods for determining bitterness/iso-α-concentration in beer:

1. International bitter units (IBU), ASBC method
   - Extraction in isooctane
     IBU = Abs_{275nm} * 50
2. Iso-Alpha-Acid (IAA, archived method)
   - Extraction in isooctane → extraction in acid methanol → transfer in alkaline methanol
     IAA = (Abs_{255nm} x 96.15) + 0.4]*3
3. Iso- α-acid concentration via HPLC
   - Solid phase extraction (SPE) → high performance liquid chromatography → compare peak area to known standard

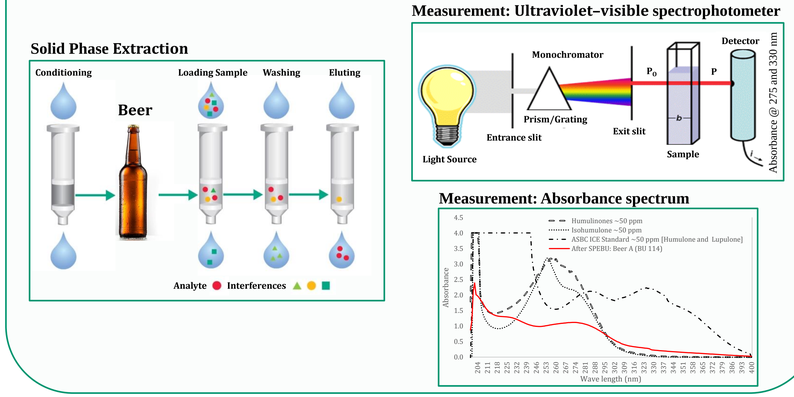
Issues with existing bitterness methodology:

- IBU utilizes solvent (isooctane) that is difficult to dispose of
- IBU result is noisy, particularly for dry-hopped beers
- IAA is complicated, time-consuming and tedious to perform
- HPLC equipment is expensive

Development of solid phase extraction bitterness unit (SPEBU) method:

- Developed in 2011 by Wiestock et al.[1] to overcome issues with existing bitterness methodology and as an alternative to IBU and/or HPLC
- After two external ring studies (spring 2011 and spring 2013) reproducibility and repeatability statistics were determined to be to high and the ASBC Technical committee recommended the method be optimized

Figure 1: Outline of SPEBU technique



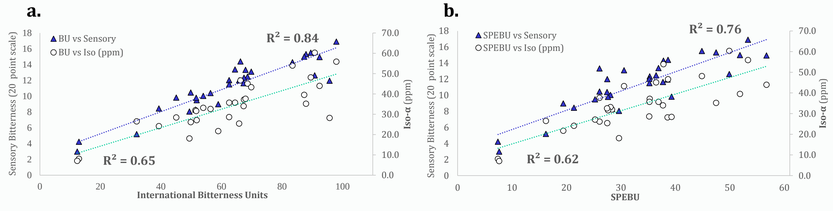
Methodology

Table 1: SPEBU method breakdown and optimization evolution

	Round 1 Method (Spring 2011)	Round 2 Method (Spring 2013)	Optimization Current Iteration
Solid Phase Packing Material	C-8	C-8	*C-18*
Pre-condition Steps			
Pre-Wash A:	2mL MeOH	2mL MeOH	*3mL MeOH*
Pre-Wash B:	4mL MilliQ H_20	4mL MilliQ H_20	*3mL MilliQ H_20*
Sample Loading	2mL of acidified beer	2mL of acidified beer	2mL of acidified beer
Sample Wash			
Wash A:	10 mL H_20 + 200 µl conc. H_3P0_4		*5 mL H_20 + 200 µl conc. H_3P0_4*
Wash B:	5mL 60/40 MeOH + 200 µl conc. H_3P0_4	5mL 60/40 MeOH+ 200 µl conc. H_3P0_4	4mL 60/40 MeOH+ 200 µl conc. H_3P0_4
Sample Elute	3x 3mL MeOH + 100 µl conc. H_3P0_4	2x 4.5mL MeOH+100 µl conc. H_3P0_4	2x 4.5mL MeOH+100 µl conc. H_3P0_4
Syringe filter	0.45µm PVDF	0.45µm PVDF	*0.45µm GHP (polypropylene)*
Measurement	Absorbance @ 275nm	Absorbance @ 275(IAA) 360(AA)	Absorbance @ 275(IAA) 360(AA)
IAA Calculation	SPEBU = Abs@275nm * 75	SPEBU = Abs@275nm* 119-Abs@360*228	*SPEBU = Abs@275nm*65-Abs@330*82*

*Proposed method changes are indicated in *Red Italics*

Figure 2: **a.** Sensory bitterness and iso-α concentration vs IBU **b.** Sensory bitterness and iso-α concentration vs SPEBU



- 30 unique beers (ranging from 12 BU to 96 BU) were used to optimize the SPEBU method
- Figure 2b indicates that sensory bitterness and iso-α concentrations correlate moderately well with IBU (R^2 = 0.84 and 0.65, respectively)
- Figure 2c indicates that sensory bitterness and iso-α concentrations also correlate moderately well with the optimized SPEBU method (R^2 = 0.76 and 0.62, respectively)
- Taking the results in Figures 2b and 2c together, the SPEBU method is a comparable alternative the IBU method

Discussion and Results

Table 2: SPEBU optimization OSU internal repeatability and reproducibility comparison

Statistical Summary of Results[a]								
Sample Pair	**No. of Labs**	**Grand Mean**	**Repeatability**			**Reproducibility**		
			Sr	**cv_r**	***r*95**	**S_R**	**CV_R**	**R_{95}**
IAA								
B1/B2	20	20.3	1.28	6.3	3.58	7.03	34.67	19.69
A1/A2	20	30.8	2.45	7.96	6.87	6.25	20.29	17.51
C1/C2	16	34.0	1.94	5.69	5.42	7.44	21.89	20.84
D1/D2	20	71.8	3.06	4.27	8.58	17.8	24.79	49.84
SPEBU Ring Study First Round (Spring 2011)[2]								
C/C_1	16	8.69	0.52	6.03	1.47	1.59	18.23	4.44
A/A_1	16	36.6	1.68	4.58	4.69	3.46	9.46	9.69
B/B_1	16	57.1	1.52	2.67	4.26	5.38	9.43	15.06
SPEBU Ring Study Second Round (Spring 2013)								
A/A_1	20	12.9	2.0	15.2	5.5	9.0	69.4	25.1
B/B_1	20	33.4	3.3	9.9	9.3	11.2	33.6	31.4
C/C_1	20	54.1	7.9	14.5	22.0	16.5	30.5	46.2
SPEBU Optimization Attempt (Spring 2015)								
A/A_1	2(7[b])	12.0	1.66	16.07	6.27	2.79	22.82	7.83
B/B_1	2(7[b])	60.7	2.10	3.46	5.88	4.81	7.92	13.46

[a]Calculations were made according to Methods of Analysis Statistical Analysis-4

[b]No. of operators

- Repeatability and reproducibility statistics from the ring study conducted at Oregon State University in Spring 2015 (n=7) indicate that the optimization changes worked to reduce variation and improve the SPEBU method

Potential Benefits & Recommendations

- The SPEBU method appears comparable to the BU and IAA methodology
- No need for isooctane (more environmentally friendly and reduces hazardous waste)
- Ability to be automated
- Relatively inexpensive ~$3.25-4.30 per sample; compared to IBU ~$3.30 per sample (not including waste disposal and cost of instrumentation)
- An external ring study (n ≃ 20) is needed to verify the small scale OSU ring study repeatability and reproducibility & to become an alternative ASBC MOA for beer bitterness

Contact Authors

Scott Lafontaine **Phone #:** (541)-737-8026 **E-mail:** Scott.Lafontaine@oregonstate.edu

Thomas H. Shellhammer **Phone #:** (541)-737-9308 **E-mail:** Tom.Shellhammer@oregonstate.edu

Oregon State University OSU Brewing Science

References

1. Wietstock, P. C. "SBU—A new and rapid method for determining bitterness in beer." 2012 MBAA World Brewing Congress, Analytical Session, Presentation 107.
2. ASBC Technical Committee. Sub Committee 2010 report. Determination of Bitterness Units and Iso-alpha-acid Levels in Dry-hopped Beers Using the Archived Iso-alpha-acids Spectrophotometric Method. R. Martin, *Chair*. Pages 253-254. doi:10.1094/ASBCJ-2010-0825-01