

### Objective:

- The focus of this study was to evaluate the adaptation of ASBC Method of Analysis Hops 14 to quantify residual hop acids contained in brewers' spent grain (BSG).

### Materials and Methods:

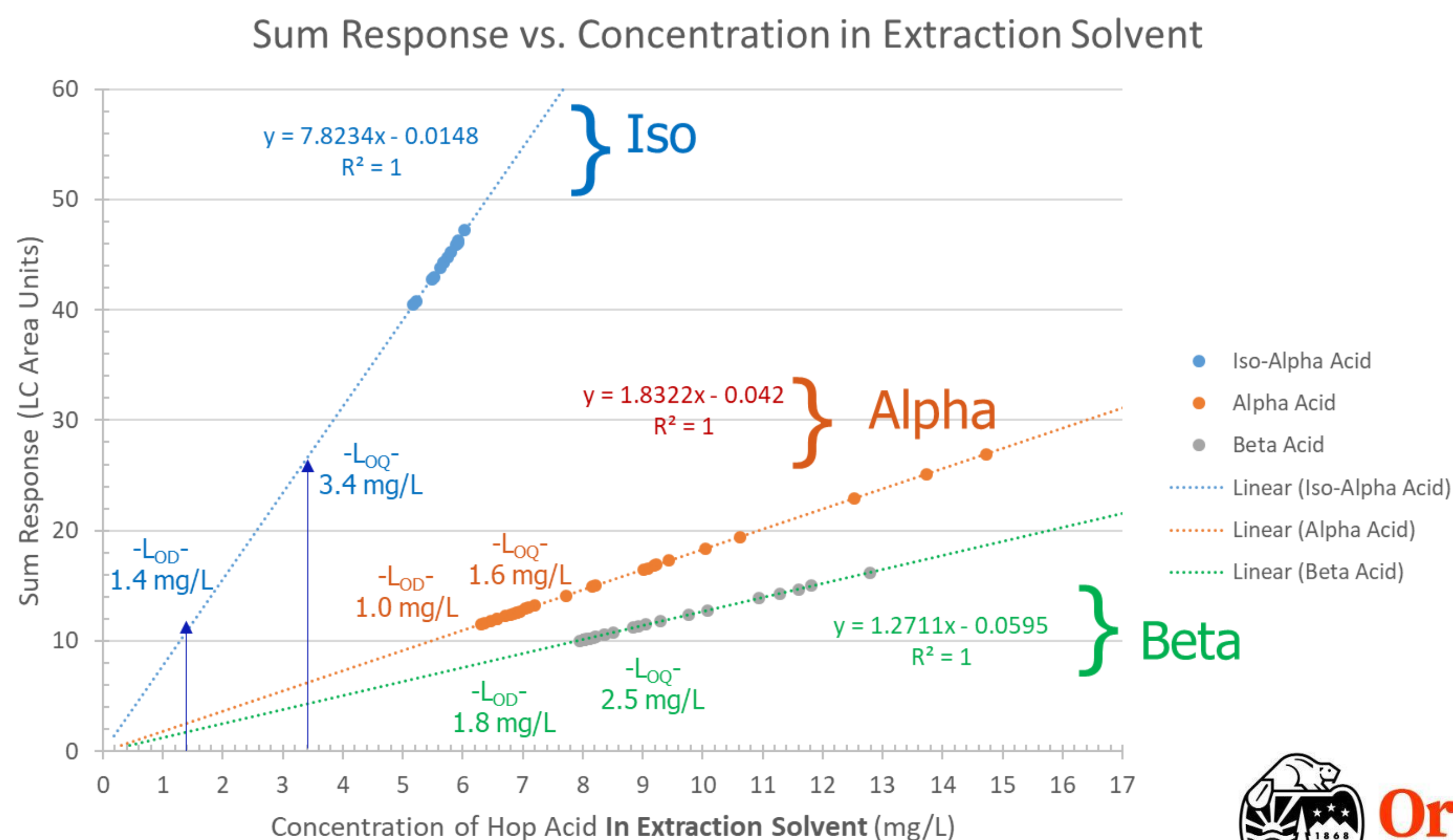
- Trials to determine the presence of iso-alpha, alpha, and beta hop acids in dried spent grain were carried out to test the feasibility of detection and quantification by HPLC analysis.
- Dose and recovery studies were carried out to evaluate the recovery rates in spent grain dosed with a mixture of purified hop acids.
- Once the method was validated for its ability to detect the hop acids under controlled conditions, an evaluation of limit of detection (LD) and limit of quantification (LQ) was carried out to better understand the limitations of the modified method Hops 14.
- Hop Acids Stock Solution Formulation:
  - Materials used:
    - 95% ethanol (EtOH)
    - Isomerized hop extract (Isohop (30% w/w), Barth Haas Group)
    - Purified alpha acids hop extract (Alpha Bio (20% w/w), Hopsteiner)
    - Purified beta acids hop extract (Beta Stab 10 (10% w/w), Barth Haas Group)
    - 100 mL volumetric flasks with stopper
    - Acidic methanol (0.5 mL H<sub>3</sub>PO<sub>4</sub> (85%) in 1 L HPLC grade methanol)
  - Build stock solution in 100 mL volumetric flask:
    - ~0.12 g Iso, ~0.18 g Alpha, ~0.36 g Beta
    - Make to the mark with 95% EtOH
  - Carry out desired dilutions of the stock solution with additional flasks
    - Make to the mark with acidic methanol (5 mL H<sub>3</sub>PO<sub>4</sub> in 1000 mL MeOH)

### Hop Acid Application and Extraction Protocol:

- 5 g of dry (~4% moisture) BSG into metal drying tin
  - Add 5.0 mL of hop acids stock solution in ethanol to the sample
  - Dry @104 C, 30 minutes to remove ethanol
  - Remove and let sample cool
  - Place entire sample in extraction jar
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- Add 5 mL of MeOH and 25 mL ethyl ether
  - Shake sample on shaker table (360 rpm, 30minutes)
  - Add 10 mL of 0.2 M HCl
  - Shake for an additional 10 minutes
  - Remove from shaker and let rest for 10 minutes
  - Filter aliquot of extract through a .45 µm PP filter directly into HPLC vial
  - Sample is ready for analysis. Run within the same day sample is taken

### Limit of Detection and Quantification Determination:

- Hop acids quantification was carried out using a modified version of ASBC Hops 14 ASBC hop acids standards – DCHA-ISO and ICE – Alpha/Beta acids
- A baseline HPLC response was determined for each of the analyte peaks in question.
- Regions for the baseline were selected based on average retention times for the individual hop acid peaks.
- Once the region was selected, a minimum and maximum height of the baseline "noise" was collected by magnification of the chromatogram.
- The standard deviation of the baseline "noise" was obtained through repeat sampling.
- This value is used in solving for LOD and LOQ instead of the actual baseline "noise" height.
- Signal-to-noise ratios (S:N) were used to calculate the Limit of Detection (LOD) and Limit of Quantification (LOQ).
  - LOD Signal-to-Noise ratio: 2.5:1
  - LOQ Signal-to-Noise ratio: 10:1



### Results:

- Recovery rates of the various hop acids ranged from 94% to 106%.
- The method yielded LD and LQ values of 1.0-1.8 mg/L in extraction solution and 1.6-3.4 mg/L in extraction solution respectively, depending on the hop acid in question.
- Detection limits in the spent grain were influenced by the spent grain:extraction solvent ratio and were 1.4, 1.0 and 1.8 mg/L for iso-alpha, alpha and beta acids, respectively.
- Further processing of the extraction solvent was deemed not necessary, and clouded the chromatogram with excess baseline signal from the grain matrix.

	mg/L In Extraction Solution		mg Hop Acid/kg Grain	
	LOD	LOQ	LOD	LOQ
ISO- Sum Response	1.4	3.4	4.7	6.8
Alpha- Sum Response	1	1.6	4.7	5.3
Beta- Sum Response	1.8	2.5	9.6	10.4

### Conclusions:

- With this adaptation of Hops 14, it is feasible to evaluate a sample of spent brewer's grain for presence of iso-alpha, alpha, and beta hop acids.

### References:

- ASBC Methods of Analysis: Available from- <http://methods.asbcnet.org/toc.aspx>
  - Method Hops 14- α-Acids and β-Acids in Hops and Hop Extracts by HPLC (International Method)
- Long, G. L.; Winefordner, J. D. Anal. Chem. 1983, 55, 712A– 724A.

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