Bitterness analysis principle in Thermo Scientific Gallery Plus Beermaster

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Introduction

Thermo Fisher Scientific has launched a new discrete Thermo Scientific analyzer, Gallery Plus Beermaster, intended for automated measurement of bitterness, SO_2, FAN and several other beer and wort parameters.

Intention of this paper is to describe the analysis principles of the beer bitterness tests in general and to evaluate the Beermaster bitterness method principle regarding to the choice of detection wavelength and elution solution. Experiments in this paper are made with water based standard solutions of iso-alpha acids.

Beer bitterness is typically analyzed by two techniques routinely: Manual/automated iso-octane extration or with HPLC. Below is a short introduction to these methods followed by results of the Beermaster spectrum analysis.

Iso-octane extraction

In the manual iso-octane extraction method, the acidified beer/wort is extracted with iso-octane by optimized shaking process. Extract is measured with manual photometer at 275 nm. Bitterness unit (BU) for beer sample is calculated by 50 x A275. BU for 1+1 diluted wort sample is calculated by 100 x A275. This is the traditional extraction method. Typically beer samples are between 5-40 BU and wort samples between 20-60 BU. The method is not specific, about 70% of absorbance is said to be iso-alpha acids. This method lacks scientific approach but is widely used due to the possibility of analysis without expensive equipment. With this method, reduced/modified iso-alpha acid extraction may be problematic to interpret because the BU often does not completely match the organoleptic study. Factor may be needed to correct the results.

Known method interference for the iso-octane method is that iso-alpha acids adsorb onto glass where the manual extraction takes place. Also the Bitterness compounds are known partly being in the beer foam and therefore complete foam dissolving by shaking is needed. Some preservatives may also interfere this method, e.g., n-heptyl-4-hydroxybenzoate, saccharin, salicylic acid, sorbic acid /sorbates. Additionally, it is known that some brewing adjuncts and coloring agents may have influence on the results (source ASBC, MEBAK, EBC). Recovery of the manual extraction varies and therefore there can be high CV between individual measurements.

Iso-octane extraction can be automated with the flow injection analyzer. Method principle and is very similar to the manual extraction. Acidified beer/wort is extracted with iso-octane and extract is measured with the photometer at 275nm. Results are expressed as BU. With this automated method, recovery is standardized with constant calibrations. CVs are typically lower compared to the manual extraction method but the instrumentation may require a lot of maintenance.

According to Christensen et al (2005) and others referred in this article, the iso-octane technique is costly, time consuming, and involves the use of undesirable organic solvents; also a high uncertainty is introduced in the manual extraction step. The absorbance at 275 nm is the sum of all species extracted from beer into iso-octane that absorbs UV light, and minor contributions from species not contributing to the bitterness, such as polyphenols can appear.

The IBU method can thus be considered as a rather crude technique, which also lacks the ability to discriminate between different iso-alpha acid species. Although all iso-alpha acids possess the same chromophore, their UV spectra are not exactly the same. Furthermore, different stereo-isomers have been shown to possess different absorptivities and absorption maxima wavelengths. Despite the limitations, the IBU method is widely used as an indicator of the bitterness in quality control.

HPLC

In the HPLC method, beer is analyzed either directly or after solid phase extraction. Peaks are measured at 270nm and concentration (mg/l) of each individual natural and reduced iso-alpha acid is reported. This is very specific method for iso-alpha acids (Iso) and reduced iso-alpha acids (Rho, Tetra, Hexa). However, depending on the system, some of the substances might co-elute so that a complete separate quantification of all individual iso-alpha acid species is not possible, instead a sum quantification must be used. Chromatograms contain also unidentified non-iso-alpha acid compounds (e.g., iso-beta acids). Sum of iso-alpha acids represents BU, but is not completely correlated to the iso-octane extraction method.

There are also method improvement studies of the official off-line HPLC method. See, e.g., the reference list for the article about “Effective On-line Sample Clean-Up and Analyte Enrichment for UHPLC Analyses”.

Automated Gallery Plus Beermaster method principle

In Gallery Plus Beermaster discrete analyzer, bitterness analysis is fully automated. A degassed sample is inserted to the analyzer. In the analyzer, bittering substances from the sample are extracted using a solid-phase extraction column. The acidified sample is aspirated to the column where the bittering substances are bound to the column. The sample matrix is then washed out of the column. Next, the analytes are released from the column by aspirating eluent, which is automatically pipetted to the measuring cuvette within the analyzer and absorbance at 275 nm is read. After elution, the column is flushed twice with eluent to clean the column completely.

The response of the Beermaster bitterness method is absorbance, but the absorbance values are not directly comparable to the manual method. If the Beermaster method needs to be compared to the iso-octane extraction method, the method must be calibrated using samples with known BU values as the calibrators.

Experimental

In this paper the Gallery Plus Beermaster bitterness measurement is compared to the manual iso octane extraction.

Samples

Calibration standards for HPLC analysis of isomerized and reduced alpha-acids were used as samples: Tetra (ICS-T2), DCHA-Iso (ICS-I3), DCHA-Rho (ICS-R2), DCHA-Hexa (ICS-H1) from Labor Veritas (Zurich, Ch.) Stock solutions of the standards were prepared according to the Table 1. Stock solution solvent was acidic methanol prepared as instructed by Labor Veritas (0.5 ml 85% H3PO4 diluted to 1000 ml with MeOH). Working solutions were prepared according to the Table 2. Solutions were made in de-mineralized water.

Table 1. Stock solutions of Iso-alpha acids

	Weighed amount of preparate g	Solvent volume ml	Iso-alpha acid content according to manufacturer %	Weighed amount of iso-alpha acids g	Iso-alpha acid concentration mg/l
Tetra	0.0044	0.005	99.4	0.00437	874.72
DCHA-Iso	0.0066	0.005	62.3	0.00411	822.36
DCHA Hexa	0.0065	0.005	65.7	0.00427	854.1
DCHA Rho	0.0066	0.005	65.3	0.00431	861.96

Table 2. Working solutions of Iso-alpha acids

	Pipetted stock solution ml	Final volume ml	Theoretical iso-alpha acid concentration mg/l	Concentration of stock solution solvent %
Tetra	1.143	50	20.00	2.3
DCHA-Iso	1.216	50	20.00	2.4
DCHA Hexa	1.171	50	20.00	2.3
DCHA Rho	1.16	50	20.00	2.3

Methods

Spectrum measurements were done with Thermo Scientific Multiskan GO spectrophotometer using a quartz cuvette at 25° C. The spectra were recorded from 200 to 350 nm at 2 nm intervals. The zero-baseline for the spectrophotometer was recorded with pure iso-octane or BC Eluent.

Manual iso-octane method was modified from the official EBC method: 2 ml sample, 0.1 ml 6 M HCl and 4 ml iso-octane were pipetted in a 20 ml erlenmayer flask. The flask was put on a horizontal shaker for 30 minutes at 250 rpm. After shaking the iso-octane was poured directly from the flask to the measuring cuvette.

The Gallery method was modified from the default Bitter AU method such that the eluent was left on the sample prep block, from where it was pipetted manually to the photometer cuvette for spectrum measurement. Eluents from four consecutive runs of the same sample were pooled for each measurement. Gallery Plus Beermaster was equipped with the BCM column (part number 986226). Sample and eluent volumes were 300 µl. Reagents used in the method were the system reagents BC Eluent (984355), BC Diluent (984354) and BC System liquid (984353).

Results and Discussion

Qualitative spectrum measurements

In the Figures 1-4 are shown the spectra of iso-octane and Beermaster extracts of the four iso-alpha acid products. The measuring wavelength 275 nm is marked in the graphs. Note that in the Figures 1-4 the spectra are normalized so that the peak maximum near 275 nm has been given the value 1.

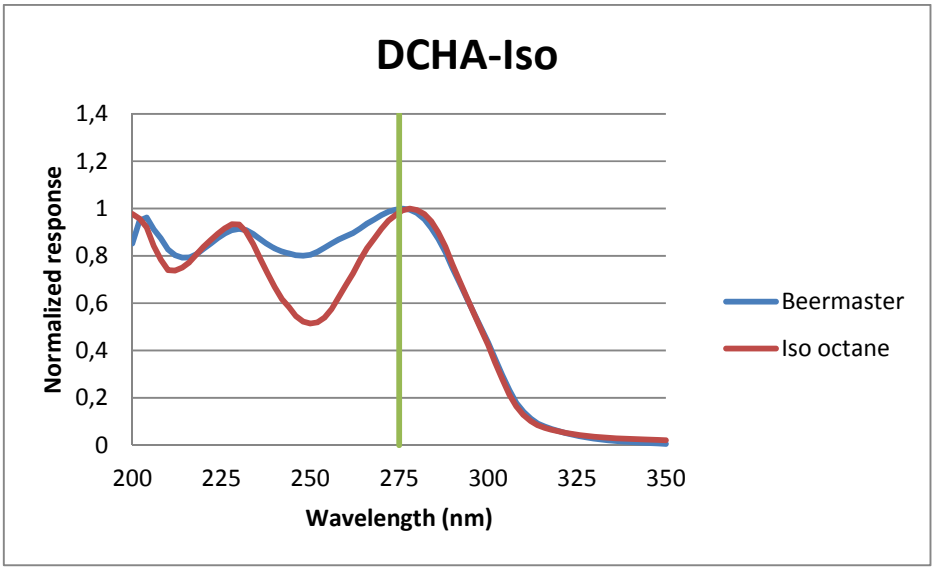

Figure 1. Water based DCHA-Iso sample absorbance spectra from iso-octane extraction method (red) and after Beermaster extraction (blue).

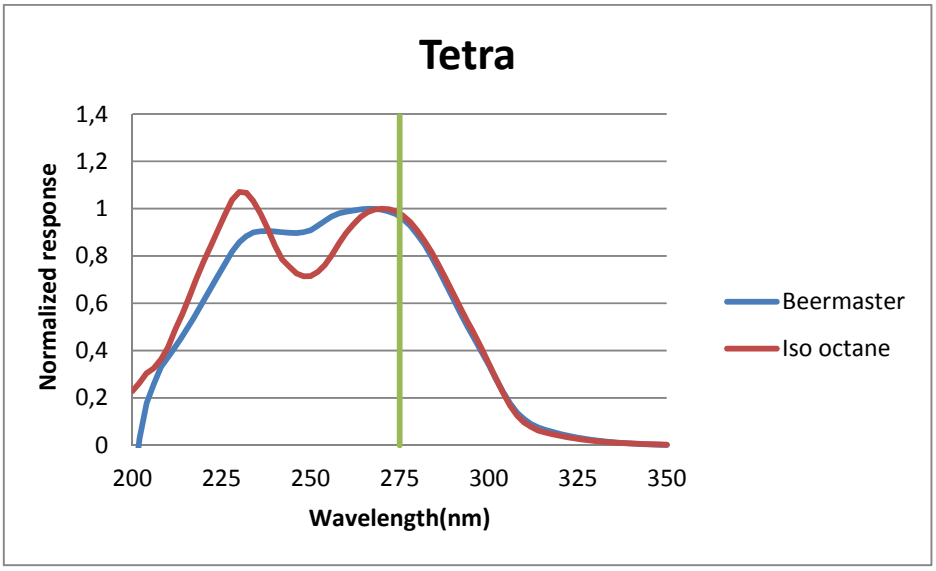

Figure 2. Water based DCHA-Tetra sample absorbance spectra from iso-octane extraction method (red) and after Beermaster extraction (blue).

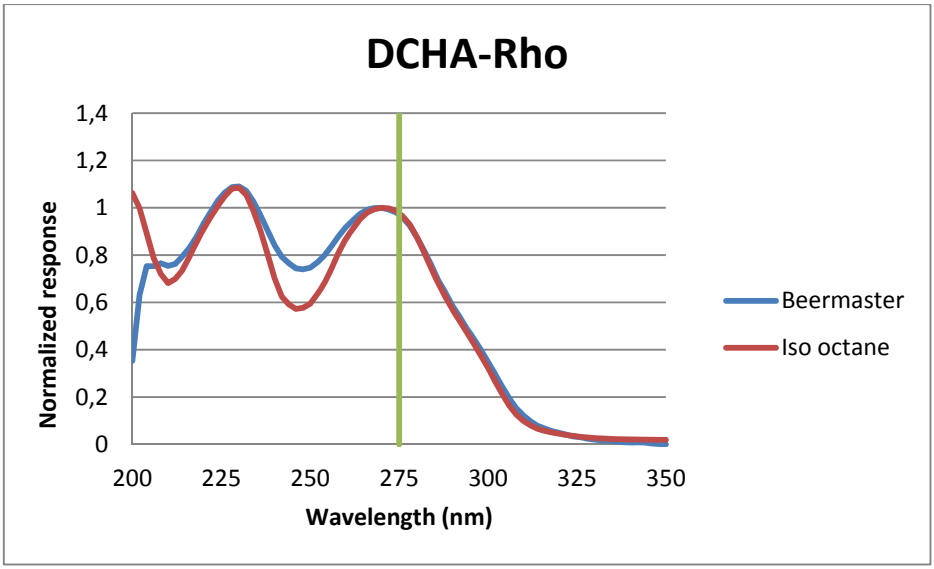

Figure 3. Water based DCHA-Rho sample absorbance spectra from iso-octane extraction method (red) and after Beermaster extraction (blue).

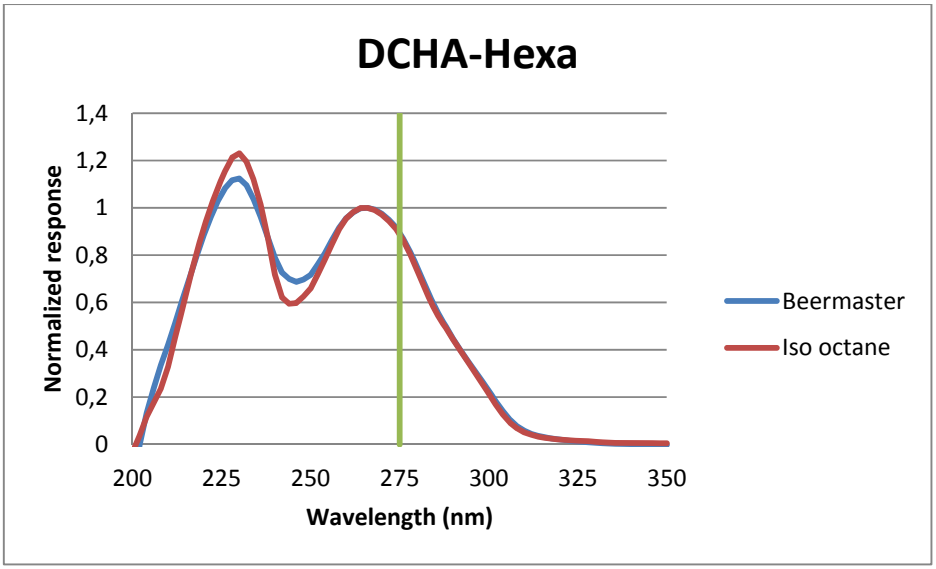

Figure 4. Water based DCHA-Hexa sample absorbance spectra from iso-octane extraction method (red) and after Beermaster extraction (blue).

As can be seen from Figures 1-4, the wavelength of the absorbance maximum near 275 nm is the same in both Beermaster BC Eluent and in the iso-octane of all four iso-alpha acids. Thus, the BC Eluent reagent is comparable to iso-octane as the background solvent in the detection of iso-alpha acids at 275 nm.

Conclusion

Gallery Plus Beermaster bitterness method is a novel method which measurement spectra are qualitatively very similar to the iso-octane extraction with all tested reduced iso-alpha acids. Refer to Technical Note 2/2012 for the quantitative comparison of these two methods.

References

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- Rapid Determination of Bitterness in Beer Using Fluorescence Spectroscopy and Chemometrics. Jakob Christensen, Anne Marie Ladefoged and Lars Nørgaard. (J. Inst. Brew. 111(1), 3–10, 2005)