Understanding bitterness measurement; interferences and contribution

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INTRODUCTION

Bitterness perceived in the flavour profile of a beer is largely constituted with iso-alpha acids which derived from hops during kettle boiling. While other components also contribute to bitterness or bitter-like attribute in different intensities, their effect is practically insignificant in comparison to iso-alpha acids. Since the last decade, many breweries have used some more sophisticated hopping strategies i.e. late hopping, double or triple hopping as well as dry hopping to the standard brewing process in order to diversify and to generate unique characteristics of their products. Consequently accurately quantifying 'bitterness in beer' becomes even more important to a modern brewery.

The traditional method for beer bitterness quantification is by manual extraction of the iso-alpha acids from beer using iso-octane, followed by UV measurement, with factors employed to estimate bitterness units or concentration (mg/L) in beer. Separation of iso alpha-acids in beer by High Pressure Liquid Chromatography has is also widely used in the brewing industry nowadays. It has the advantage of measuring the concentration of different forms of iso-alpha acids against standards with known purities.

This poster discusses the accuracy of bitterness and iso-alpha acids results at each stage of the brewing process and the contributions from malt and hop materials that impact the accuracy of the bitterness measurement by manual extraction and HPLC methods. Some industrial applications of IsoLC measurement have also been introduced.

Key words:
Iso-alpha acids, IsoLC, bitterness quantification

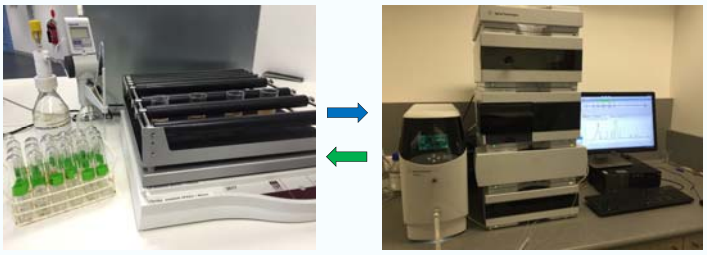
Bitterness IN BEER

Different hops derived products and their application in the modern breweries have made bitterness evaluation even more challenging. Comparison of bitterness intensity of isohumulones and reduced isohumulones[1] shown below:

Compounds	Relative Bitterness
Isohumulones (iso α-acids)	1.0
Dihydro-isohumulones (rho)	0.6 - 0.7
Tetrahydro-isohumulones (Tetra)	1.5 - 1.9

EQUIPMENT AND METHODS

Manual bitterness measurement was carried out using the IBD method[2] within 45min of arrival at the laboratory. Wort samples were filtered and bittering acids were extracted by iso-octane and consequently measured at UV 275nm. Colour was also measured at the same time. Beer samples were degassed by shaking for 10min prior to measurement.



For IsoLC sample preparation, the sample was degassed and filtered through a 0.45µm nylon filter before analyse.

An Agilent 1260 HPLC system with a DAD detector was used in this study. The system was fitted with an Agilent Poroshell120 C18 4.6x75mm, 2.7µm column. All samples were analysed at 30°C @248nm with a flow rate of 1.0ml/min[3]. This method separates most isomers within five minutes and is suitable for brewing process control.

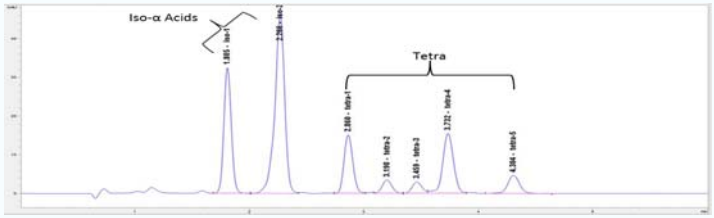


Fig 1: A typical chromatogram of iso-alpha acids and reduced iso-alpha acids (labelled Tetra). Iso-alpha acids contains iso-1 (co isomer) and iso-2 (n+ad isomers) and for reduced iso-alpha acids there are a group of five peaks, named from tetra-1 to 5 being the major isomers present in ICE-3 standard.

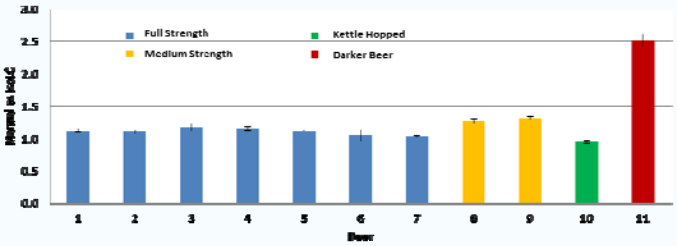
Iso-octane extraction results from unhopped worts

Unhopped worts used in the study were supplied from either the breweries or were prepared under laboratory conditions with 100% malt charge. The gravity of all worts was between 14-16 Plato.

Beer variety	Malt charge (%)	Colour (EBC)	Manual bitterness value (EBC)
Wort A	71	13.0	1.6 – 3.6
Wort B	67	14.0	1.7 – 2.5
Wort C	71	21.5	2.2 – 2.3
Wort D	100	12.4	2.8
Wort E*	100	9.2	1.0
Wort F*	100	42.3	8.7
Wort G*	100	>100	22.5

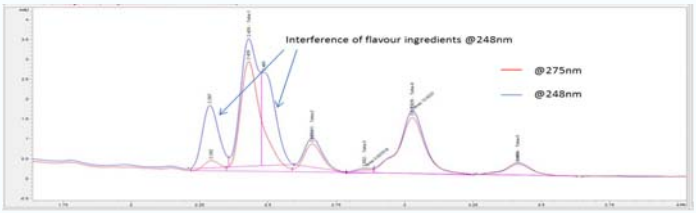
Wort E, F and G were prepared under laboratory conditions. IsoLC results confirmed there were no hop derived bitterness peaks in the un-hopped worts (not shown here). The results suggest the bitterness level in beer can be over-estimated while using the manual method by a number of factors, particularly by wort colour in this case.

Fiqure 2: Correlation between the extraction and IsoLC methods data in different type of beers.



A case study from a NPD project

It has been found that some natural flavour ingredients can interfere with IsoLC measurement and lead to an over-estimated bitterness result @248nm if peak integration is not optimal. However with optimisation of the wavelength in use to 275nm, the interferences were reduced significantly and therefore bitterness can be quantified more accurately[4].



CONCLUSION

While the factors employed by the iso-octane method for the estimation of the iso-alpha acid concentration produces a reasonable estimate of the actual measured concentration for regular beers, even unhopped wort would produce a result of up to 4mg/L. This is due to the method measuring other compounds that can be extracted and have an absorbance at 275nm. Degradation compounds of iso-alpha acids and notable contributions to the manual bitterness results from kettle hopping and from beers brewed using coloured or crystal malts can also lead to over-estimated results, whilst the beer may have a relatively low bitterness.

The outcome is that there is no simple factor to convert all iso-octane bitterness values to iso-alpha acids concentration as the factor may differ for example by malt type, hopping regime and hop quality. Beers brewed with lower malt charge and lighter in colour exhibit the least different, whilst coloured malt beers with a large quantity of hops added are expected to show the greatest difference. An understanding of these factors is important when considering both the calculation of utilisation rates and the marketing of bitterness values on products.

References

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3. EBC HPLC bitterness method, EBC method 7.8
4. A revised HPLC method for flavoured beers, CUB QA method

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