Save the Flavor – Robust Iso-α-Acids Assaying in Beer within Ten Minutes

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Key Words
Beer, Bitterness, Bitter Substances, Isohumulones, Fingerprint, Characteristic Pattern, Cis- and Trans-isomerism

Goal
Determination of the beer bitterness by measuring the contents of iso-humulones in untreated beer – quickly, including sample preparation, HPLC separation, and data review. Furthermore, the application needs to be robust and must provide reproducible results, increased lifetime of the analytical column, and provide a fingerprint or characteristic pattern of the cis- and trans-ratios.

Introduction
Isohumulones (iso-α-acids) are derived by humulones (α-acids), essential constituents of hop resins. The poorly water-soluble α-acids are isomerized to the better water-soluble iso-α-acids during wort-boiling. Iso-α-acids form approximately eighty percent of the typical bitterness of beer. Their antimicrobial effect leads to a sterile beverage, their tensioactive character stabilizes the foam, and they have a major influence on the general flavor, smell, and smoothness of beer.1 The three major iso-α-acid variants which are basically present in beer only differ in their acyl side chain and comprise iso-n-humulone, iso-cohumulone, and iso-adhumulone. Due to the stereochemistry of iso-α-acids, all of them occur as cis- and trans-isomer (Figure 1).

Each iso-α-acid variant provides different contributions to beer taste and foam stability. Recent investigations have shown that these differences are even true between both cis- and trans-isomers of the same iso-α-acid.1 Furthermore, the lifetimes of cis- and trans-isomers significantly differ from each other. Degradation products of iso-α-acids sensitively influence the important beer attributes mentioned above and the avoidance of less stable iso-α-acid variants is beneficial.2 Precise as well as comparable information about the genuine beer bitterness is only achievable by specific quantitation of bitter substances (iso-humulones) in beer. Furthermore, the fingerprint or characteristic pattern of the cis- and trans-ratios is very important information due to the reasons mentioned above. High-Performance Liquid Chromatography (HPLC) is the only analytical method that provides these results. If injected untreated, beer causes reproducibility issues and compromises its lifetime of the column as beer does not only consist of iso-humulones but also of a very complex matrix. Manual beer sample pretreatment steps, like off-line solid phase extraction (SPE) are commonly used but are very time consuming. Furthermore, traditional HPLC analyses last about half an hour.

Experiment

Equipment: System package with on-line SPE RS configuration (P/N 5200.0500) and iso-humulones starter kit for on-line SPE RS system (P/N TS-MKIT0012).

Samples: Beer (German Pilsner, purchased from a local grocery store) and certified iso-humulones standard DCHA-Iso, ICS-I3 (part of P/N TS-MKIT0012).

Conditions: Experimental data: listed in Figure 2.

Figure 1: Chemical structure of cis- and trans-iso-α-acids.

<table>
<thead>
<tr>
<th>Trans-isomer</th>
<th>Cis-isomer</th>
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<tbody>
<tr>
<td>R= CH(CH3)2</td>
<td>Iso-cohumulone</td>
</tr>
<tr>
<td>R= CH2CH(CH3)2</td>
<td>Iso-n-humulone</td>
</tr>
<tr>
<td>R= CH(CH3)CH2CH3</td>
<td>Iso-adhumulone</td>
</tr>
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</table>
UHPLC & On-Line SPE Speeds Up Analysis of Isohumulones in Beer


Useful Links
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References

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