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AppNote 5/2001

The Analysis of the Bitter and Other Flavour Compounds in Beer and Wort by Stir Bar Sorptive Extraction (SBSE) Followed by HPLC

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INTRODUCTION

Generally the analysis of the bitter principles in beer and wort is done by liquid/liquid extraction [1] or C8 SPE extraction followed by HPLC analysis [2]. Unfortunately, there is a lack of reproducibility, a high consumption of organic solvents and only the main compounds i.e. the humulones and iso-humulones, can be monitored by this methodology. Hops contain hundreds of components, however of particular interest for a brewer are the main hop α - and β -acids (Figure 1).

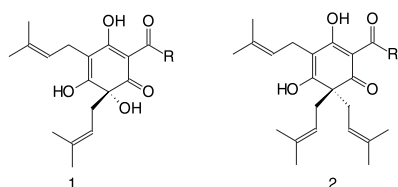


Figure 1. Hop α - and β -acids.

Table I. Hop α - and β -acids.

Group "R"	α -Acids (1)	β -Acids (2)
$\text{CH}_2\text{CH}(\text{CH}_3)_2$	Humulone (1a)	Lupulone (2a)
$\text{CH}(\text{CH}_3)_2$	Cohumulone (1b)	Colupulone (2b)
$\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$	Adhumulone (1c)	Adlupulone (2c)

These compounds are the precursors of beer bitterness. During wort boiling a thermal isomerisation, an acyloin-type ring contraction, converts the α -acids into iso- α -acids. Each α -acid gives rise to two epimeric iso- α -acids (example of humulone 1a shown in Figure 2).

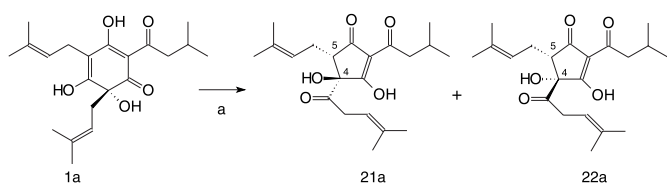


Figure 2. Rearrangement of humulone (1a) to trans-isohumulone (21a) and cis-isohumulone (22a) during the wort boiling.

Consequently, 6 major iso- α -acids are present in beer resulting from the conversion of the 3 major α -acids, humulone, cohumulone and adhumulone, respectively.

There is a need, however, for a method with which other flavour compounds derived from hops can be monitored during the production process (in wort) and in the final product beer. Additionally the sample preparation should be optimized for time and costs. A new sample preparation technique namely Stir Bar Sorptive Extraction (SBSE) [2] was evaluated for the enrichment of hop-derived solutes in wort and beer. SBSE offers an effective, easy-handling and low-cost opportunity for the isolation of organic solutes from aqueous matrices. A small stir bar, coated with polydimethylsiloxane is placed directly in the sample and stirred for minutes. After sampling, the stir bar is placed in a thermal desorption unit for subsequent CGC separation under mild conditions. Several hop-derived solutes can be elucidated and identified in this way. Alternatively, the stir bar can be desorbed by a liquid followed by HPLC analysis. This allows analysis of some of the thermostable hop derived solutes. Both approaches

are complementary in nature and give a quite complete profile of the hoppy flavour in beer. The SBSE approach has also been compared to the classical SPE-HPLC technique.

EXPERIMENTAL

Reagents. All chemicals were purchased from Fluka (Neu-Ulm, Germany) in the highest purity available, except for the following substances: Methanol and acetonitrile were LiChroSolv gradient grade from Merck (Darmstadt, Germany). Crystalline dicyclohexylamine (DCHA) salts of trans-iso- α -acids were purchased from Versuchsstation Schweizerischer Brauereien (VSB, Zürich, Switzerland). The DCHA salts were calculated to contain 66.5 %w hop acids. Extracts of iso- α -acids and the α - and β -acids are from Hopstabil GmbH (Wolnzach, Germany).

HPLC Conditions. The HPLC system consisted of a HP 1090 with a photodiode array detector. The detector was set to measure absorbance at 275 nm (iso- α -acids) and 314 nm (α -acids and β -acids). The column from Macherey Nagel (ET 250/3 Nucleosil 100-5 C18 Ab, Düren, Germany) was kept at 40°C. Injection volume was 20 μL . For the complete separation the mixture A was acetonitrile:methanol: citric acid buffer) 17:25:57 (v/v/v) and mixture B was acetonitrile: citric acid buffer 55:45 (v/v). The citric acid buffer was adjusted to pH 7 with 50% NaOH solution and filtered separately before combination with the organic solvents. The mobile phase gradient is given in Table II.

Table II. Mobile phase program for the HPLC method.

Time [min]	A) [%]	B) [%]
0	100	0
5	98	2
14	95	4
32	90	10
50	65	35
60	10	90
62	10	90
63	100	0
66	100	0

Sample preparation for GC and HPLC Analysis. The isooctane extraction was done by the method described in [1], the octyl solid phase extraction was described in [2].

For the stir bar sorptive extraction a 10 mm stir bar was added to 0.5 mL sample diluted with 0.5 mL deionized water and 0.05 mL phosphoric acid (85%w). The samples and standard solutions were stirred for 15 min. Stir bars were removed with forceps, rinsed briefly in distilled water.

For HPLC analysis the stir bar was eluted with 1ml of a mixture of acetonitrile: water: (50:50)(v/v). 20µl eluate was injected in the HPLC.

RESULTS AND DISCUSSION

The isooctane extraction has frequently been used to isolate beer bitter substances. However the samples of this standard preparation for photometry and HPLC methods [1] had a low amount of matrix, the procedure has higher costs with respect to time, manpower and organic solution waste. The alternative solid phase extraction method with a C8 SPE cartridge and an eluate mixture of acetonitrile :methanol :water :phosphoric acid is a rapid and easy-handling technique, but there are still some reproducibility problems and there are more matrix effects compared to the liquid/liquid extraction method. Very pure chromatograms with excellent peak shapes and resolutions were obtained using the new rapid SBSE technique. Figure 3 shows chromatograms from a beer sample using the different methods.

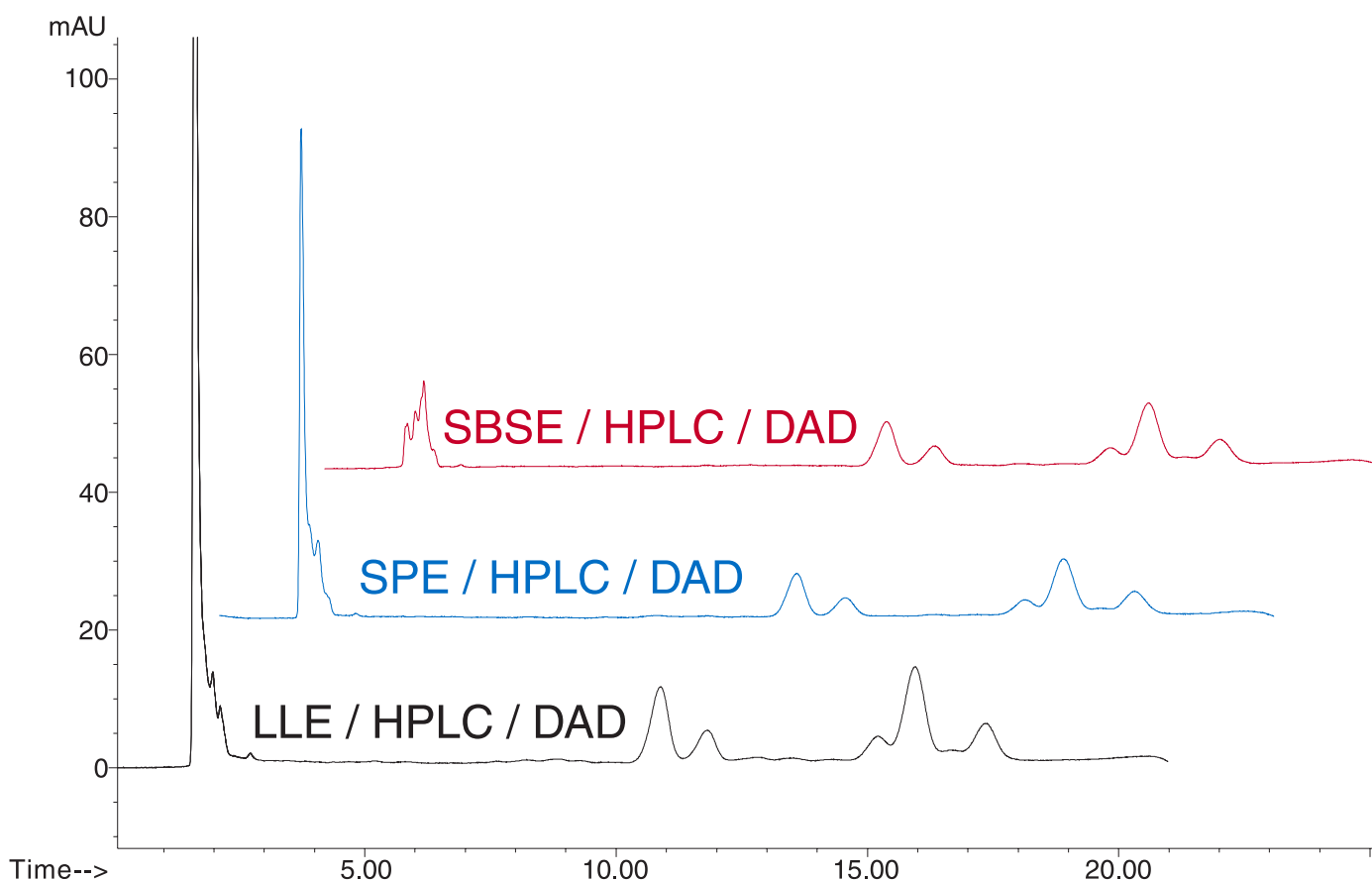


Figure 3. Comparative chromatograms for sample preparation with different techniques.

A chromatogram of an injected 30 ppm iso-humulone standard is presented (Fig. 4). Calibration functions were recorded in the linear range from 10 to 100 ppm total iso-humulone (Fig.5). The RSD (n=4) for the standards ranged from 1% to 3%.

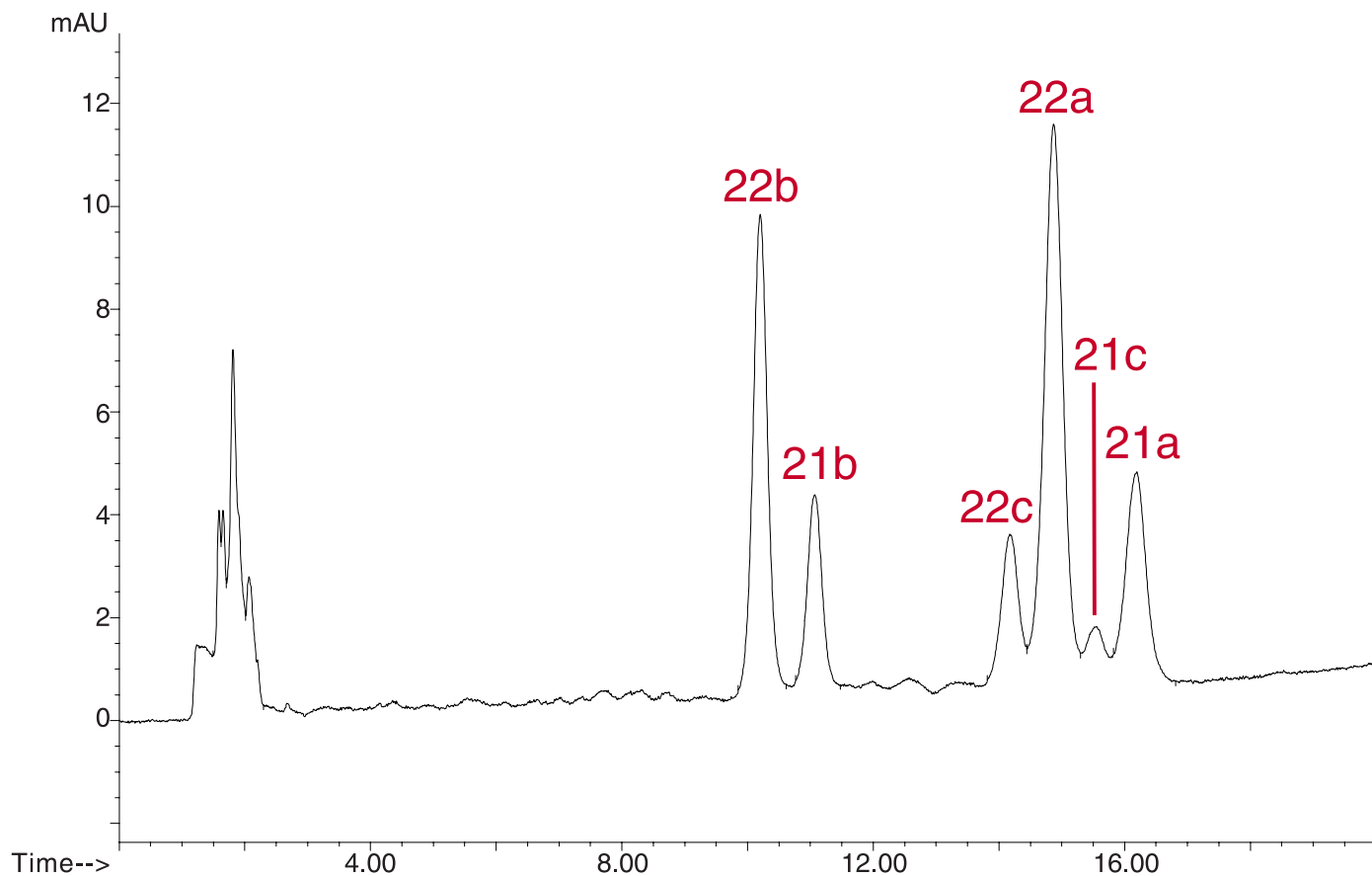


Figure 4. Chromatograms of an iso-humulone standard with 30ppm total, Peaks in following order: cis-isocohumulone (22b); trans-isocohumulone (21b); cis-isoadhumulone (22c); cis-isohumulone (22a); trans-isoadhumulone (21c); trans-isohumulone (21a).

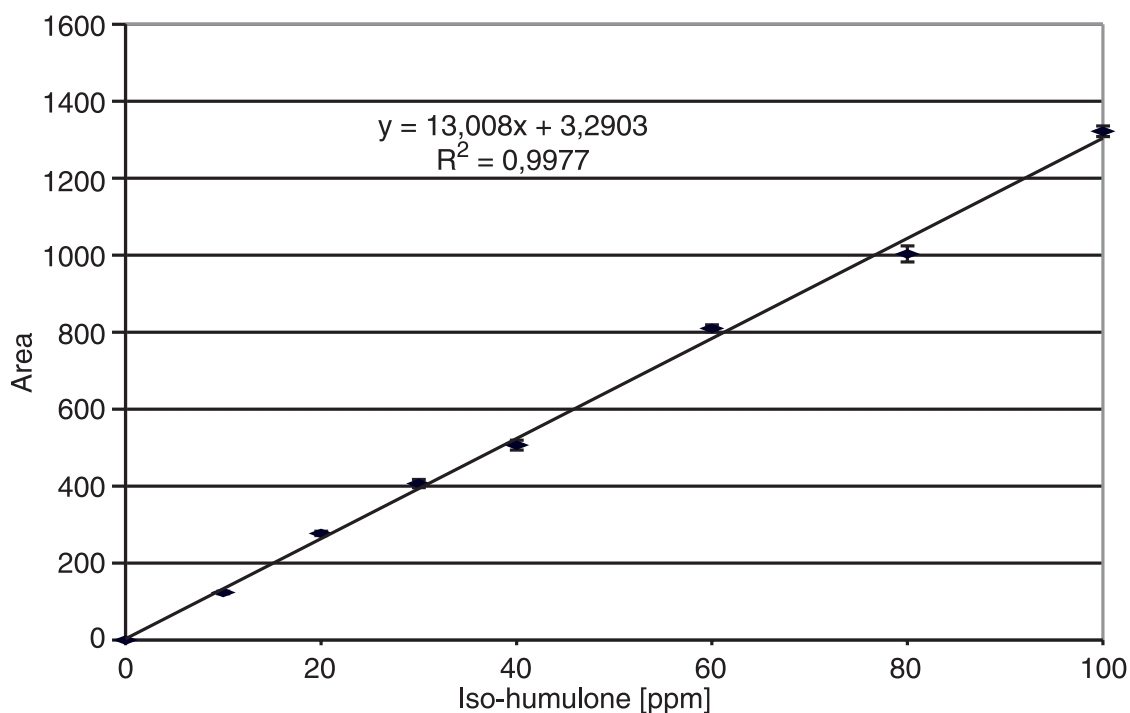


Figure 5. Calibration curve for the total iso-humulone amount using the SBSE technique.

Samples taken during the brewing process were analyzed with the Twister. The following chromatograms show the results from different steps during wort boiling (Fig. 6-8). The significant increase of the α -acid and iso- α -acid amounts can be observed in the HPLC chromatograms.

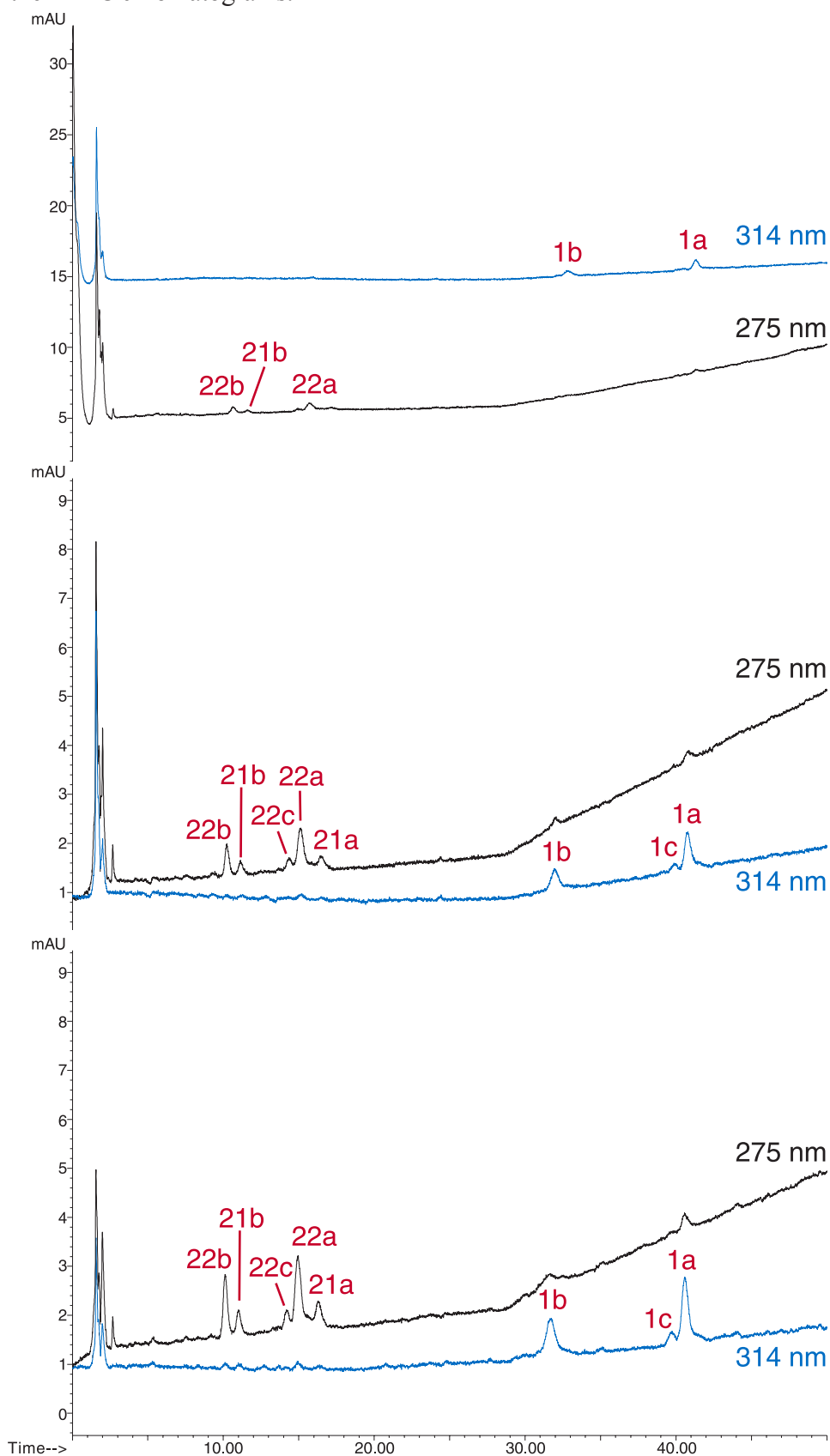


Figure 6-8. Chromatograms of the increasing amounts of iso-humulone and α -humulones during wort boiling. Peaks in following order: cis-isocohumulone (22b); trans-isocohumulone (21b); cis-isoadhumulone (22c); cis-isohumulone (22a); trans-isohumulone (21a); cohumulone (1b); adhumulone (1c); humulone (1a).

Several beers and hop extracts have been analyzed successfully using Twister when compared to existing methods such as SPE sample preparation with following UV/vis detection, and the established Continuous Flow Analyzer (CFA) method [4] based on an automated liquid/liquid extraction and UV/vis detection (Fig. 9). The method was applied to investigations of samples with complex matrices which can be found especially in wort samples. The CFA method, however, exhibits satisfactory agreement with the other methods only for the beer sample. The wort samples shows higher results caused by interference from the matrix.

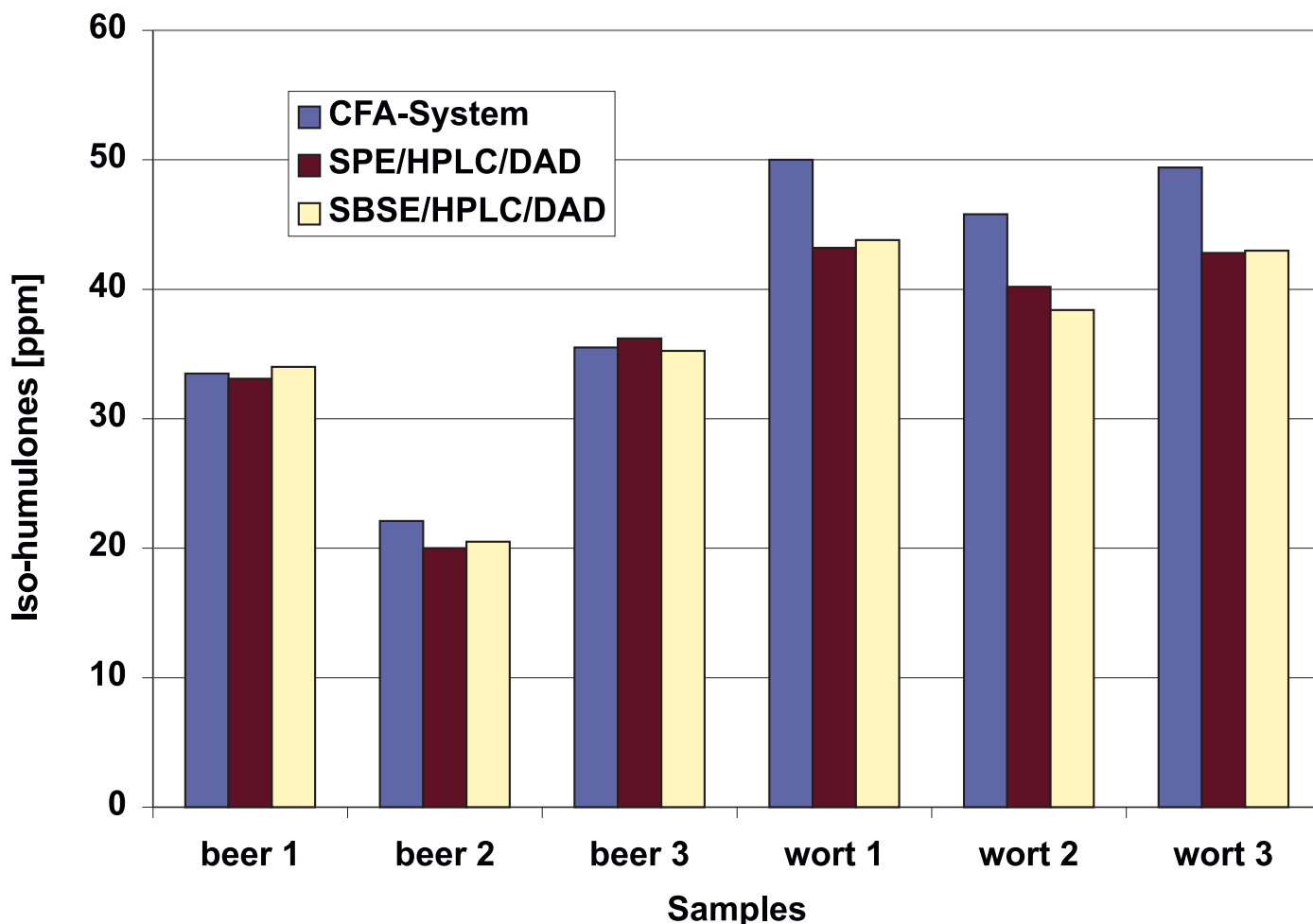


Figure 9. Investigations of beer and wort samples using different techniques.

CONCLUSIONS

Stir bar sorptive extraction is a very good alternative to the classical iso-octane extraction or the SPE sample preparation for bitterness analysis in beer and hop products. In comparison with the other techniques, precision is improved and sample preparation costs and time are reduced. SBSE without any doubt has increased the options available to brewers to control the flavor and taste of beer.

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