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ARTICLE

Quantitative Sensomics Profiling of Hop-Derived Bitter Compounds Throughout a Full-Scale Beer Manufacturing Process

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Although the complex taste profile of beer is well accepted to be reflected by the molecular blueprint of its sensometabolites, the knowledge available on the process-induced transformation of hop-derived phytochemicals into key sensometabolites during beer manufacturing is far from comprehensive. The objective of the present investigation was, therefore, to develop and apply a suitable HPLC-MS/MS method for the simultaneous and comprehensive quantitative monitoring of a total of 69 hop-derived sensometabolites in selected intermediary products throughout a full-scale beer manufacturing process. After data normalization, the individual sensometabolites were arranged into different clusters by means of agglomerative hierarchical analysis and visualized using a sensomics heatmap to verify the structure-specific reaction routes proposed for their formation during the beer brewing process.

KEYWORDS: Beer; hops; α-acids; β-acids; xanthohumol; iso-α-acids; hulupones; sensomics; sensometabolites; sensometabolome

INTRODUCTION

For many centuries the cones (female flowers), pellets, or extracts of hop (*Humulus lupulus* L.) are used as essential ingredients in beer manufacturing to impart the attractive aroma as well as the typical bitter taste to the final beverage. It is well accepted in the scientific community that the authentic flavor signature of food and beverages such as beer is reflected by the molecular blueprint of its sensory active, low-molecular weight compounds, coined sensometabolites (*1*, *2*). Much progress has been made in recent years in the field of sensomics to systematically identify, catalog, and quantify the sensory active key metabolites that are present in raw materials and/or produced upon food processing and do reflect the sensory phenotype of that product (*2*, *3*).

During beer manufacturing, several hop components are extracted and/or transformed upon wort boiling to give the sensometabolites **1**−**36** (**Figure 1**) contributing to the typical bitter taste profile of freshly brewed beer as well as the compounds **37**−**57** (**Figure 2**) recently identified in aged beers (*4*−*8*). Among the group of flavonoids, the prenylated chalcone xanthohumol (**1**) is long known to give the bitter isoxanthohumol (**2**) upon cyclization (**Figure 3**) (*9*). In addition, the three α-acids cohumulone (**3**), humulone (**4**), and adhumulone (**5**) isomerize upon wort boiling into the six iso-α-acids *trans*-isocohumulone (**6**), *trans*-isohumulone (**7**), *trans*-isoadhumulone (**8**), *cis*-isocohumulone (**9**), *cis*-isohumulone (**10**), and *cis*-isoadhumulone (**11**) (**Figure 3**), respectively, which have been reported as the major contributors to the bitter taste of beer (*8*−*10*). Besides the iso-α-acids, bitter tasting co-, n-, and ad-humulinones **12**−**14** (**Figure 1**) are reported to be formed from the corresponding α-acids upon oxidation (**Figure 3**) (*11*).

Another major class of hop phytochemicals are the β-acids colupulone (**15**), lupulone (**16**), and adlupulone (**17**) (**Figure 1**). Upon wort boiling, these β-acids are transformed into the bitter tasting co-, n-, and ad-hulupones **18**−**20** (**Figure 3**) (*12*) as well as a series of recently identified tricyclic degradation products, named nortricyclo-co/n/ad-lupone (**21**−**23**), dehydrotricyclo-co/n/ad-lupone epimers (**24a**/**24b**−**26a**/**26b**), tricyclo-co/n/ad-lupone epimers (**27a**/**27b**−**29a**/**29b**), hydroxytricyclo-co/n/ad-lupone epimers (**30a**/**30b**−**32a**/**32b**), and hydroperoxytricyclo-co/n/ad-lupone epimers (**33a**/**33b**−**35a**/**35b**) (**Figure 1**) (*4*, *5*). The hulupones **18**−**20** are further degraded to give hulupinic acid (**36**) as their common degradation product (**Figure 3**) (*13*, *5*).

Very recently, the *trans*-iso-α-acids **6**−**8** (**Figure 1**) were found to be not stable during storage of beer, but are further degraded by means of a proton-catalyzed cyclization reaction to give the co-, n-, and ad-congeners of lingering, harsh bitter tasting, tricyclic degradation products such as the tricyclohumol congeners **37**−**39** (**Figure 2**, **3**) (*7*). Whereas the formation of these tricyclohumols **37**−**39** from the iso-α-acids **6**−**8** were confirmed to be *trans*-specific (*14*), the humulinic acids **40**−**45** (**Figure 2**, **3**), the alloisohumulonehydroxides **46**−**51**, and the corresponding peroxides **52**−**57** were found as oxidation products of both, the *cis*- and *trans*-iso-α-acids **6**−**11** (**Figure 3**), in particular, when beer samples were accessible to air oxygen (*15*).

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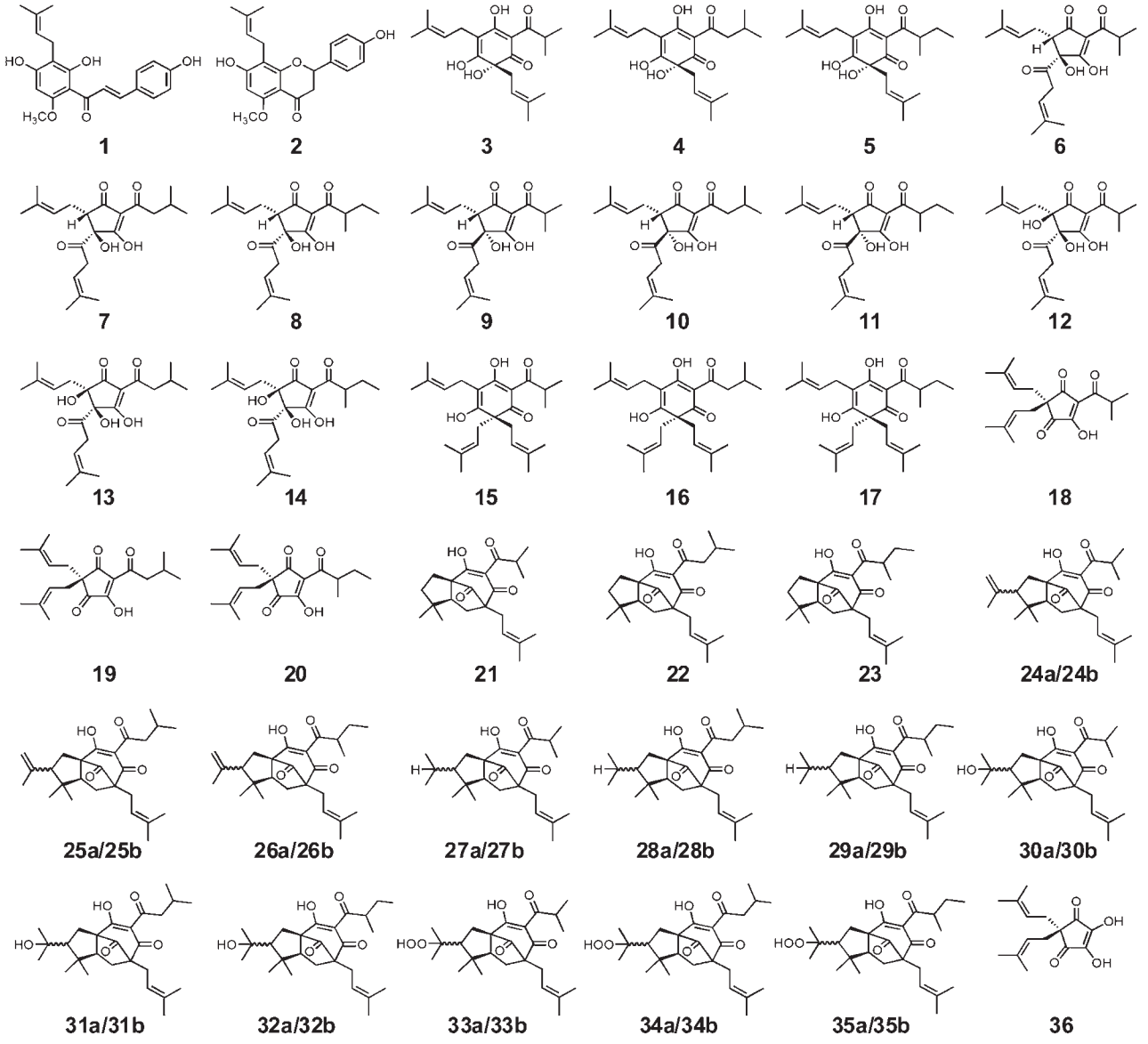

Figure 1. Chemical structures of bitter sensometabolites identified in hops and freshly brewed beer: xanthohumol (**1**), isoxanthohumol (**2**), cohumulone (**3**), humulone (**4**), adhumulone (**5**), *trans*-isocohumulone (**6**), *trans*-isohumulone (**7**), *trans*-isoadhumulone (**8**), *cis*-isocohumulone (**9**), *cis*-isohumulone (**10**), *cis*-isoadhumulone (**11**), cohumulinone (**12**), humulinone (**13**), adhumulinone (**14**), colupulone (**15**), lupulone (**16**), adlupulone (**17**), cohulupone (**18**), hulupone (**19**), adhulupone (**20**), nortricyclocolupone (**21**), nortricyclolupone (**22**), nortricycloadlupone (**23**), dehydrotricyclocolupone epimers (**24a/24b**), dehydrotricyclolupone epimers (**25a/25b**), dehydrotricycloadlupone epimers (**26a/26b**), tricyclocolupone epimers (**27a/27b**), tricyclolupone epimers (**28a/28b**), tricycloadlupone epimers (**29a/29b**), hydroxytricyclocolupone epimers (**30a/30b**), hydroxytricyclolupone epimers (**31a/31b**), hydroxytricycloadlupone epimers (**32a/32b**), hydroperoxytricyclocolupone (**33a/33b**), hydroperoxytricyclolupone epimers (**34a/34b**), hydroperoxytricycloadlupone epimers (**35a/35b**), and hulupinic acid (**36**).

As hops are rather costly ingredients and the iso-α-acids **6**−**11** are considered to be the major contributors to the bitter taste of beer, the rate of the so-called "hop utilization", that means the extraction yield of α-acids from the hop material as well as the degree of α-acid isomerization during wort boiling, is a crucial factor in evaluating the efficiency of the hopping dosage during wort boiling (*16*−*18*). Any losses of these valuable bitter compounds throughout the brewing process occurring during wort boiling, fermentation, storage, and filtration, respectively, need to be minimized as much as possible. Whereas some literature data are available on the fate of xanthohumol (**1**) and isoxanthohumol (**2**) during beer manufacturing (*19*−*21*), detailed quantitative information on the fate of α- and β-acids as well as the evolution of their transformation products throughout an entire industrial brewing trial is still far from comprehensive.

Since RP-HPLC analysis with UV detection (*22*, *23*) do not enable the quantitation of all the bitter compounds **1**−**57** in beer samples, the objective of the present investigation was to develop and apply a suitable HPLC-MS/MS method for the simultaneous and comprehensive quantitative monitoring of the evolution and/or degradation of these hop-derived sensometabolites in selected intermediary products throughout an industrial, full-scale beer manufacturing process.

MATERIALS AND METHODS

Chemicals and Materials. The following chemicals were obtained commercially: formic acid (Merck, Darmstadt, Germany); hydrochloric acid, sodium hydroxide (Riedel-de-Haen, Seelze, Germany); solvents were of HPLC grade (Merck, Darmstadt, Germany). Deionized water used for chromatography was purified by means of a Milli-Q Gradient A10 system

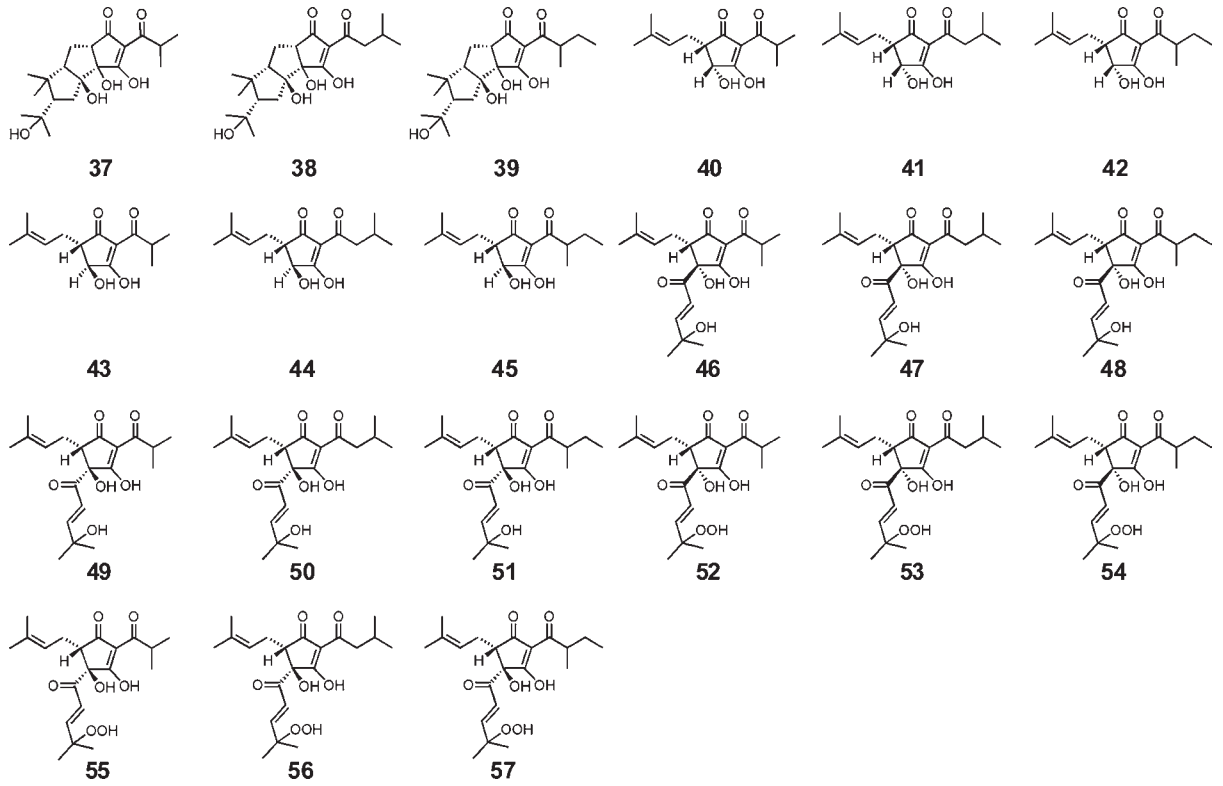

Figure 2. Chemical structures of bitter sensometabolites identified in aged beer: tricyclocohumol (**37**), tricyclohumol (**38**), tricycloadhumol (**39**), *cis*-cohumulinic acid (**40**), *cis*-humulinic acid (**41**), *cis*-adhumulinic acid (**42**), *trans*-cohumulinic acid (**43**), *trans*-humulinic acid (**44**), *trans*-adhumulinic acid (**45**), *cis*-alloisocohumulonehydroxide (**46**), *cis*-alloisohumulonehydroxide (**47**), *cis*-alloisoadhumulonehydroxide (**48**), *trans*-alloisocohumulonehydroxide (**49**), *trans*-alloisohumulonehydroxide (**50**), *trans*-alloisoadhumulonehydroxide (**51**), *cis*-alloisocohumulonehydroperoxide (**52**), *cis*-alloisohumulonehydroperoxide (**53**), *cis*-alloisoadhumulonehydroperoxide (**54**), *trans*-alloisocohumulonehydroperoxide (**55**), *trans*-alloisohumulonehydroperoxide (**56**), and *trans*-alloisoadhumulonehydroperoxide (**57**).

(Millipore, Billerica, MA). An ethanolic hop extract (year 2009) made from Hallertauer Taurus (Hopsteiner, Germany), an iso-α-acid extract (30% iso-α-acids, year 2009), a crude xanthohumol extract (year 2009), as well as a purified reference sample of cohumulinone **12** (**Figure 1**) were provided by the Hallertauer Hopfenveredelungsgesellschaft (Mainburg, Germany). All intermediary samples as well as beers collected from an industrial, full-scale Pilsner-type beer production process (**Figure 4**) were provided by the Bitburger Braugruppe GmbH (Bitburg, Germany). The type 90 hop pellets used for beer brewing consisted of two aroma hop varieties (50% Perle, 50% Tradition), whereas the ethanolic hop extract used was made from two bitter hop varieties (60% Magnum, 40% Taurus).

Preparation of Reference Compounds. Following the protocols reported recently (*7*), xanthohumol (**1**) was purified from a commercial xanthohumol extract by means of RP-HPLC, isoxanthohumol (**2**) was prepared from **1** by alkali-catalyzed cyclization, the α-acids **3**-**5** as well as the *β*-acids **15**−**17** were isolated from an ethanolic hop extract by means of RP-HPLC, and the individual *trans*- and *cis*-iso-α-acids (**6**−**11**) were isolated from a commercially available iso-α-acid extract by dicyclohexylamine precipitation, followed by HPLC purification. Cohulupone (**18**) was synthesized from colupulone (**15**) by thermal treatment in the presence of platinum/charcoal (10% Pt) under alkaline conditions (*5*). Hulupinic acid (**36**) was prepared by heating purified cohulupone (**18**) in ethanol (*5*). The cocongeners of the tricyclic compounds **21**, **24a**/**24b**, **27a**/**27b**, **30a**/**30b**, and **33a**/**33b** were prepared from colupulone (**15**) upon laboratory wort-boiling conditions and, then, purified by means of RP-HPLC (*5*). Tricyclocohumol (**37**) was obtained from *trans*-isocohumulone (**6**) by acid-catalyzed cyclization (*7*) *cis*-cohumulinic acid (**40**) was isolated by means of RP-HPLC from an aqueous solution of *cis*-isocohumulone (**9**) heated under alkaline conditions (*24*), and *cis*-alloisocohumulonehydroxide (**46**) and *cis*-alloisocohumulonehydroperoxide (**52**) were synthesized by oxidation of *cis*-isocohumulone (**9**) in ethyl acetate solution in the presence of oxygen (*15*). After confirming their structural identity as well as purity (>98%) by means of HPLC/UV, HPLC/MS, and ^{1}H NMR spectroscopy, the individual bitter compounds were used as external standards for the HPLC-MS/MS analysis of the sensometabolites in beer as well as intermediary samples of the beer production line.

Sample Preparation. Wort samples and beer samples I−VII (**Figure 4**) were degassed by ultrasonification, diluted 1:10 with methanol/water (50/50, v/v) and, after membrane filtration (0.45 μm, Sartorius, Göttingen, Germany), were directly injected into the HPLC-MC/MS system. Samples of the collected diatomite material (1.0 g), the polyvinylpyrrolidone (PVP) material (1.0 g), trub (1.0 g), yeast samples I−IV (1.0 g), the ethanolic hop extract (0.1 g), and hop pellets (1.0 g), respectively, were intimately homogenized with methanol (3 × 30 mL) containing 1% formic acid, filtered, and, then, diluted 1:10 (for diatomite and PVP), 1:100 (for trub and yeast samples I−IV), or 1:1000 (for hop extract and hop pellets) with methanol/water (50/50, v/v) prior to HPLC-MS/MS analysis. Samples of the sheet filter material (**Figure 4**) were frozen with liquid nitrogen, ground in an analytical mill (IKA Werke GmbH & Co. KG, Staufen, Germany), aliquots (2.0 g) of the powder were extracted with methanol (50 mL) containing 1% formic acid, filtered, diluted 1:100 with methanol/water (50/50, v/v) and, then, injected into the HPLC-MS/MS system.

High Performance Liquid Chromatography/Mass Spectrometry (HPLC-MS/MS). The Agilent 1200 Series HPLC-system, consisting of a pump, a degasser, and an autosampler (Agilent, Waldbronn, Germany), was connected to an API 4000 Q-TRAP mass spectrometer (AB Sciex Instruments, Darmstadt, Germany) which was equipped with an electrospray ionization (ESI) source and operated in the negative ionization mode. The ion spray voltage was set to −4500 V and the declustering potential and the MS/MS parameters were optimized for each substance to induce fragmentation of the pseudo molecular ion $[M - H]^-$ to the corresponding target product ions after collision-induced dissociation (**Figure 5**). The dwell time for each mass transition was 44 ms. The declustering potential (DP), the cell exit potential (CXP), and the collision energy (CE) were optimized to the data given in **Table 1**. Nitrogen was used as the collision gas (4×10^{-5} Torr). Quantitative analysis was performed by

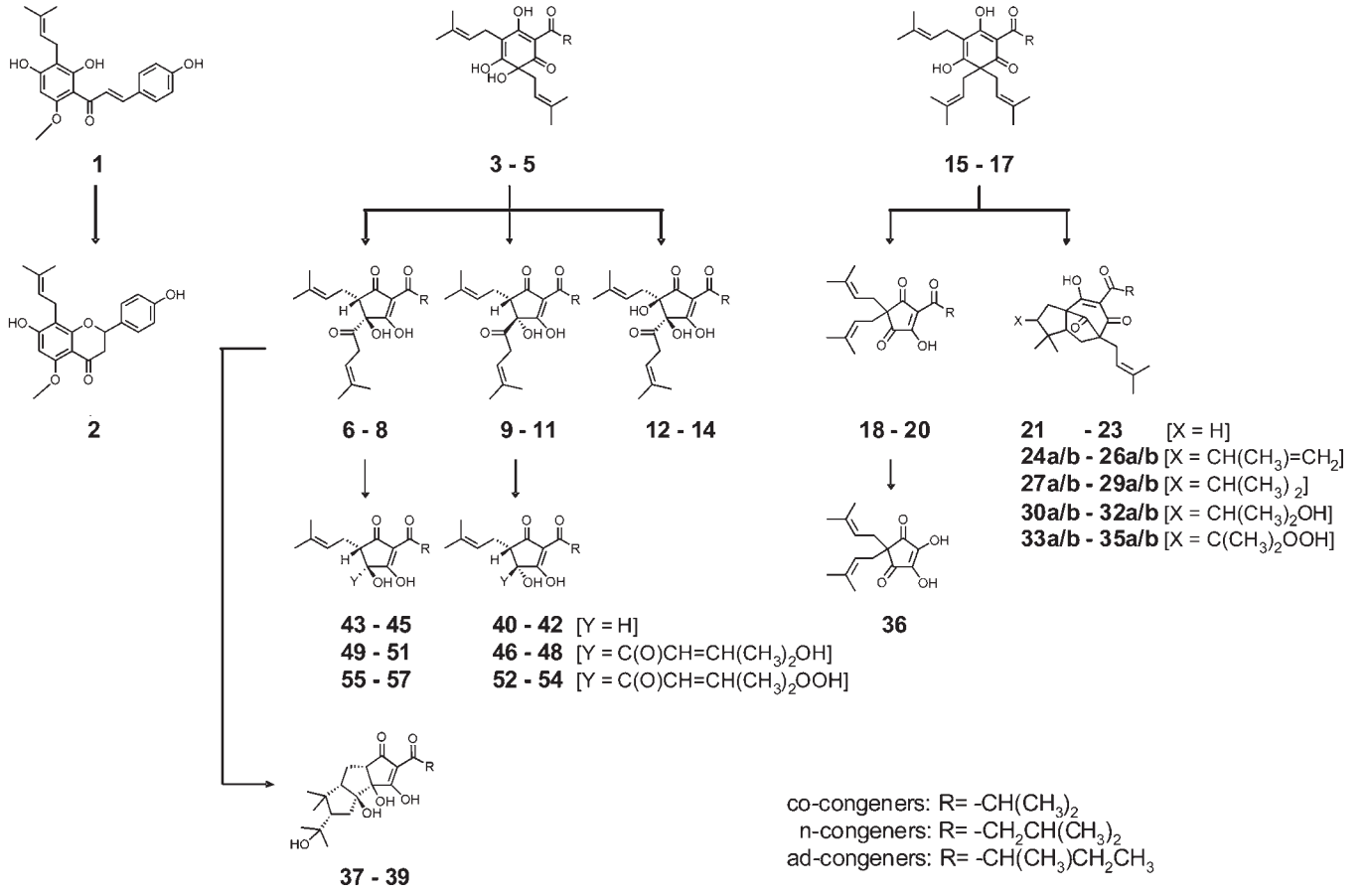

Figure 3. Reaction routes for the transformation of the hop-derived bitter compounds xanthohumol (**1**), α-acids (**3**−**5**), and β-acids (**15**−**17**) upon beer manufacturing leading to the formation of isoxanthohumol (**2**), *trans*- and *cis*-iso-α-acids (**6**−**11**), humulinones (**12**−**14**), hulupones (**18**−**20**), tricyclic β-acid cyclization products (**21**−**35**), hulupinic acid (**36**), tricyclohumols (**37**−**39**), *cis*- and *trans*-humulinic acids (**40**−**45**), *cis*- and *trans*-alloisohumulonehydroxides (**46**−**51**), and the corresponding -hydroperoxides (**52**−**57**).

means of the multiple reaction monitoring (MRM) mode using the fragmentation parameters optimized prior to analysis. Data processing and integration was performed by using the Analyst software version 1.4.2 (AB Sciex Instruments, Darmstadt, Germany). Chromatography was performed on a 150 × 2.0 mm, 5 μm, Synergi 4u Hydro-RP column (Phenomenex, Aschaffenburg, Germany) using acetonitrile containing 0.1% formic acid as solvent A and aqueous formic acid (0.1% in water) as solvent B. Using a flow rate of 250 μL/min, chromatography was done by increasing solvent A from 20 to 60% within 20 min, then to 70% in 15 min, to 92% during 28 min, and, finally, to 100% within 2 min.

Quantitative Analysis of Bitter Sensometabolites. To enable the quantitation of compounds **1**−**57**, 6-point external calibration curves were recorded for xanthohumol (**1**), isoxanthohumol (**2**), cohumulone (**3**), *trans*-isocuhumulone (**6**), cohumulinone (**12**), colupulone (**15**), cohulupone (**18**), nortricyclocolupone (**21**), hulupinic acid (**36**), tricyclocohumol (**37**), *cis*-cohumulinic acid (**40**), *cis*-alloisocohumulonehydroxide (**46**), and *cis*-alloisocohumulonehydroperoxide (**52**), respectively. To obtain the best fit for all calibration curves, second-order polynomial equations were calculated. To avoid negative or exaggerated estimates at the low end of the concentration ranges, the functions were forced through zero, thus leading to correlation coefficients of >0.99 for all the reference compounds. The iso-α-acids (**6**−**11**) were quantitated using the calibration curve of their isomer **6**, the n- and ad-congeners of α-acids (**4**, **5**), β-acids (**16**, **17**), humulinones (**13**, **14**), hulupones (**19**, **20**), and tricyclohumols (**38**, **39**) were analyzed on the basis of the calibration curve of the corresponding cocongener (**3**, **15**, **12**, **18**, **37**). The tricyclic β-acid degradation products (**21**−**35b**) were analyzed using nortricyclocolupone (**21**) as the external standard. Quantitative analysis of *cis*- and *trans*-humulinic acids (**40**−**45**) was performed by using the calibration function of *cis*-cohumulinic acid (**40**), and *cis*- and *trans*-alloisohumulonehydroxides (**46**−**51**) and -hydroperoxides (**52**−**57**) were analyzed on the basis of the calibration curve of the *cis* cocongeners **46** and **52**, respectively.

Multivariate Analysis. Data analysis was performed within the programming and visualization environment R (version 2.10.0) (*25*). The sensomics heatmap (**Figure 6**) was calculated using the heatmap.2 function of R based on the raw concentration data (**Tables 2** and **3**) normalized to the wort content of each sensometabolite. The dendrogram was constructed by means of an agglomerative average linkage algorithm (*26*), whereas the distance between two clusters is defined as the average of distances between all pairs of objects and each pair is made up of one object from each group.

RESULTS AND DISCUSSION

To quantitatively monitor the evolution of hop-derived bitter compounds **1**−**57** and to visualize generation, transformation, degradation, and/or adsorption of these sensometabolites in the most important intermediary steps of a Pilsner-type beer production line, samples from the wort, from six intermediate beer samples (beers I−VI) taken at different stages of the brewing process, the final beer (sample VII), as well as the byproduct trub, yeast (samples I−IV), and filter materials (diatomite, PVP, sheet filter) were collected from an industrial, full-scale beer manufacturing process (**Figure 4**). Besides barley, yeast, and water, two different hop products, namely an ethanolic hop extract (30.2 kg) as well as hop pellets (14.7 kg), were added prior to wort boiling. To monitor the hop-derived sensometabolites throughout the entire brewing process, first, their selective and sensitive detection by means of mass spectrometry needed to be optimized.

HPLC-MS/MS Analysis of Sensometabolites 1−**57.** Aimed at detecting the individual sensometabolites with high selectivity using the multiple reaction monitoring (MRM) mode, sample

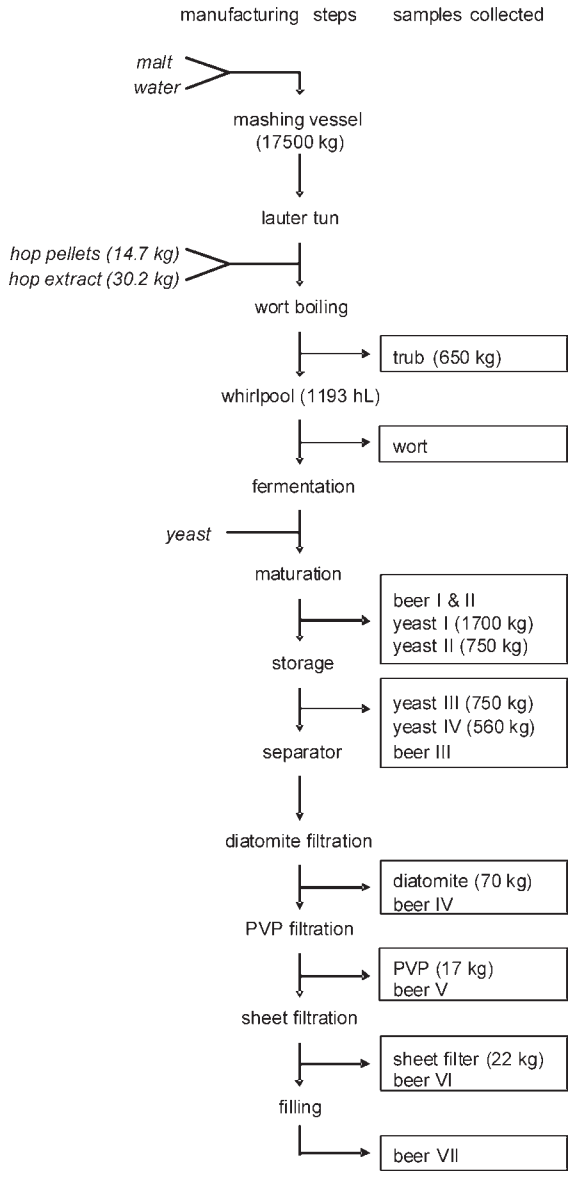

Figure 4. Scheme showing the industrial production line of a Pilsner-type beer and samples collected for analysis. Beers I and II were collected after the first and second yeast separation, beer III was collected prior to the separator, beer IV was collected after separation of the diatomite, beer V was collected after addition of polyvinylpyrrolidone (PVP), beer VI was collected after the sheet filtration, and beer VII was collected after the filling and represents a commercial beer sample.

solutions of the individual analytes were repeatedly injected into the tandem mass spectrometer to determine the pseudomolecular ion ($[M - H]^-$) as well as the daughter ions in full scan mode in the range from 100 to 500 amu. Upon flow injection of the reference substances, the instrument settings were optimized between −125 and −45 for the declustering potential, between −72 to −22 for the collision energy, and between −23 and −5 for the cell exit potential (**Table 1**), thus enabling the maximization of the product ion intensity. To enable the quantitation of the sensometabolites, 6-point external calibration curves were determined by means of HPLC-MS/MS revealing correlation coefficients of >0.99 for all reference compounds.

To determine the utilization of the hop-derived bitter molecules during the brewing process, wort and beer samples (I−VII) were diluted with methanol/water and, after filtration, the target compounds **1**−**57** (**Figure 3**) were directly analyzed by means of HPLC-MS/MS operating in the multiple reaction monitoring mode (MRM) with negative electrospray ionization. As shown for example for the analysis of a wort sample (**Figure 5**), a total number of 48 bitter compounds were detectable in wort. In addition, samples of hop pellets, hop extracts, yeast, trub, and filter materials were homogenized, extracted with acidified methanol and, after filtration and dilution, analyzed for the sensometabolites by means of HPLC-MS/MS.

Concentrations of Sensometabolites in Hop Extract and Pellets. To determine the utilization of the hop-derived sensometabolites during beer manufacturing, first, the bitter compounds **1**−**57** were analyzed in the ethanolic hop extract as well as the hop pellets used in the brewing process (**Table 2**). High concentrations of xanthohumol (**1**), the α-acids **3**−**5**, as well as the β-acids **15**−**17** were detectable in both hop products but, as to be expected, the amounts of any isomerization and/or degradation products such as, for example, isoxanthohumol (**2**), iso-α-acids (**6**−**11**), or hulupones (**18**−**20**) in these samples were below the detection limit of the HPLC-MS/MS method used. The hop extract, made from the two bitter hop varieties Magnum and Taurus, was found to be rich in α-acids and contained 19.92% humulone (**4**), 10.66% cohumulone (**3**), and 6.36% adhumulone (**5**), respectively (**Table 2**). As expected, the hop pellets, consisting of the two aroma hop varieties Perle and Tradition, contained significantly lower amounts of bitter-tasting α- and β-acids as well as xanthohumol, for example, the ethanol extract contained 9- and 3-fold higher levels of cohumulone (**3**) and xanthohumol (**1**) when compared to the hop pellets (**Table 2**). Considering the amounts of ethanol extract and pellets used for the entire brewing process, a total amount of 1635.7 g xanthohumol (**1**), 6575.0 g α-acids (**3**−**5**), and 3878.7 g β-acids (**15**−**17**) were added to the gyle, which is defined as the amount of wort used at any one time, to produce 1193 hL finished wort (**Table 2**). For the later calculation of the yields of the corresponding sensometabolites in the gyle, these amounts of α- and β-acids as well as xanthohumol were defined as 100%.

Concentrations of Sensometabolites in Wort and Beer Samples. HPLC-MS/MS analysis revealed the presence of 48 and 44 out of the 69 sensometabolites in wort and beer samples I−VII, respectively (**Table 3**). The bitter compounds **21**−**29a**/**b** and **33a**/**b**−**35a**/**b** were not detectable in any wort or beer sample collected and, therefore, are not listed in **Table 3**. The bitter tasting prenylflavonoids xanthohumol (**1**) and isoxanthohumol (**2**) were found in the final wort in concentrations of 105.9 and 341.9 g/gyle, thus indicating that a total of 6.5 and 20.9% of the starting amount of xanthohumol was extracted into the wort and/or has been isomerized to give isoxanthohumol (**2**). In comparison, the concentrations of these prenylflavonoids in the beer samples I−VII were found to be more than 10 times lower and were in the range between 0.7 and 21.8 g/gyle for xanthohumol (**1**) and between 45.7 and 284.0 g/gyle for isoxanthohumol (**2**), respectively (**Table 3**). The final beer VII contained 4.2 and 195.5 g/gyle xanthohumol (**1**) and isoxanthohumol (**2**), respectively, thus corresponding to an utilization rate of only 0.3 and 12.0% for **1** and **2**. This low utilization rate confirms earlier reports (*19*) and might be explained by the insufficient extraction of **1** from the hop material and/or might be due to further chemical degradation into unknown reaction products.

Quantitative analysis of the α-acids (**3**−**5**) revealed rather high concentrations in the wort but only low levels in beer, for example, the high level of 918.4 g/gyle humulone (**4**) decreased to 52.0 g/gyle in the intermediary beer sample I collected after the fermentation (**Table 3**). The concentrations of the α-acids cohumulone (**3**), humulone (**4**), and adhumulone (**5**) in the production step "wort → beer I" dropped by factors of 8, 17, and 25,

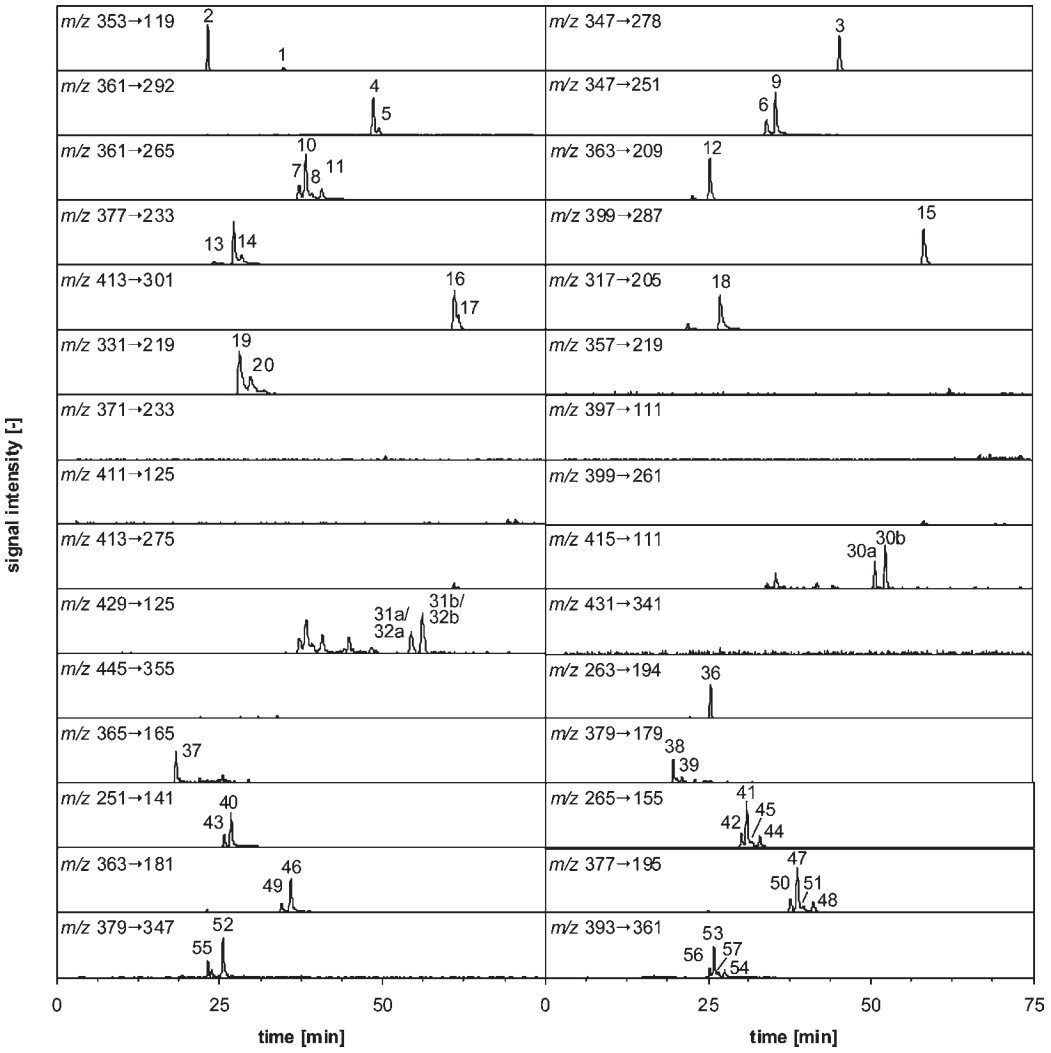

Figure 5. HPLC-MS/MS analysis of the sensometabolites in a wort sample. Signal intensity of each mass transition is normalized. Peak numbering refers to the chemical structures given in **Figures 1** and **2**.

respectively, thus reflecting the decreasing water solubility of α-acids in order of increasing hydrophobicity upon cooling beer sample I. In addition, the decrease of the pH value from 5.4 in the wort to 4.3 (**Table 3**) after fermentation might impact the solubility of the α-acids (*18*, *27*, *28*).

Also the concentrations of the iso-α-acids **6**−**11** were found to be somewhat higher in the wort when compared to the beer samples (**Table 3**). Interestingly, the isomerization rate seem to depend on the α-acid congener, for example, cohumulone (**3**) exhibited a significantly higher isomerization rate than humulone (**4**), and the utilization rate was observed to vary from 16.5 and 18.5% for *trans*-isohumulone (**7**) to 48.3 and 48.8% for *cis*-isocohumulone (**9**) in beer samples I and VII, respectively (**Table 3**). This seems to be well in line with the lower pK_s-value and the higher polarity of the cocongeners when compared to the less water-soluble n- and ad-isomers (*29*). Comparison of the total amount of iso-α-acids (**6**−**11**) in the beer samples I−VII revealed only a slight increase, for example, the major *cis*-isohumulone (**10**) increased from 1051.0 to 1154.5 g/gyle (**Table 3**).

About 10% of the starting amounts of the α-acids (**3**−**5**) added with the hop dosage were found to be oxidized to the corresponding humulinones **12**−**14** in the wort (**Table 3**), which show an increased water solubility when compared to their precursors (*17*). Comparison of the concentrations of **12**−**14** found in the wort and the beer samples I−VII indicated a slight decrease of these humulinones. The final beer sample VII contained 128.2, 208.1, and 87.5 g/gyle cohumulinone (**12**), humulinone (**13**), and adhumulinone (**14**), thus corresponding to a final yield of 6.0−7.4% (**Table 3**).

Compared to the α-acids, the concentration of *β*-acids (**15**−**17**) in the wort was found to be rather low, for example, only 25.2 g/gyle were found in the wort for colupulone (**15**) (**Table 3**). This is well in line with the limited water solubility of these *β*-acids counteracting their extractability from the hop material during wort boiling and favoring their precipitation upon cooling of the final wort (*29*, *30*). Although degradation reactions of *β*-acids (**15**−**17**) during an authentic brewing process are not described in literature, about 20% of the starting amount of *β*-acids added with the hops was found to be oxidized to give 445.9 g cohulupone (**18**), 247.3 g hulupone (**19**), and 126.1 g adhulupone (**20**) per gyle in the final wort (**Table 3**). However, the initial concentration of these hulupones in wort decreased to a lower level of 51.2−147.5 g/gyle in the finished beer VII, corresponding to a utilization rate of 4.8−10.4% (**Table 3**). Comparatively low concentrations were found for hulupinic acid (**36**), for example, only 6.8 and 4.8 g/gyle were present in the wort and beer sample VII (**Table 3**). Interestingly, the decreasing concentrations of hulupones during beer processing were not accompanied by an accelerated formation of hulupinic acid (**36**). These findings give first evidence that in contradiction to literature data (*13*), the degradation of hulupones does not lead exclusively to the formation of hulupinic

acid (**36**), and further unknown pathways contribute to the decrease of hulupones (**18**−**20**) in beer.

Besides the various oxidation products, the tricyclic degradation products **21**-**35a/b** were recently identified as β-acid transformation products in model wort boiling experiments (*4*, *5*). As the HPLC-MS/MS screening in the Pilsner-type beer allowed only the detection of the transformation products **30a**/**30b**, **31a**/**31b**, and **32a**/**32b**, the quantitative analysis was focused on these sensometabolites. Whereas the concentrations of the two epimers of the cocongener **30a**/**30b** were found to be 78.9 and 139.6 g/gyle, an appropriate separation of the epimers of the tricyclic n- and ad-congeners **31a**/**32a** and **31b**/**32b** could not be successfully achieved (**Figure 5**). Therefore, the total amount of **31a**+**32a** (31.6 g/gyle) and **31b**+**32b** (60.1 g/gyle) was determined in the wort. The concentration of the two coepimers **30a**/**30b** were found to decrease by about 50% from the wort to the final beer VII and, most likely due to the low solubility of the n- and ad-congeners, neither **31a/b**, nor **32a/b** were detectable in any of the Pilsner-type beer samples I−VII (**Table 3**).

Quantitative analysis of the tricyclic *trans*-iso-α-acid transformation products **37**−**39** and the oxidation products **40**−**57** revealed significant amounts of these molecules already in the freshly prepared wort (**Table 3**). At this stage already about 4.1% of the α-acids (**3**-**5**) added with the hop dosage seem to be degraded to the oxidation products **40**−**57** (**Table 3**). Among the oxidation products present in wort, *cis*-alloisohumulonehydroxide (**47**), *trans*-alloisohumulonehydroxide (**50**), and *cis*-alloisohumulonehydroperoxide (**53**) were found in the highest concentrations of 19.6, 10.7, and 10.5 g/gyle, respectively (**Table 3**). In comparison, the beer samples I−VII contained more than 50% less of these oxidation products. On the other hand, the tricyclic *trans*-iso-α-acid transformation products **37**−**39** were not detectable in the

Table 1. Optimized Mass Spectrometric Parameters for the Quantitative Analysis of Hop-Derived Sensometabolites **1**−**57.**

compound[a]	mass transition	mass loss [amu]	DP [V][b]	CE [V][c]	CXP [V][d]
1, **2**	353 → 119	234	−95	−42	−7
3	347 → 278	69	−65	−28	−17
4, **5**	361 → 292	69	−65	−28	−23
6, **9**	347 → 251	96	−90	−22	−11
7, **8**, **10**, **11**	361 → 265	96	−80	−26	−11
12	363 → 209	154	−50	−22	−7
13, **14**	377 → 223	154	−50	−22	−7
15	399 → 287	112	−80	−38	−15
16, **17**	413 → 301	112	−105	−40	−7
18	317 → 205	123	−55	−40	−13
19, **20**	331 → 219	123	−75	−36	−13
21	357 → 219	138	−45	−40	−21
22, **23**	371 → 233	138	−45	−40	−13
24a, **24b**	397 → 111	286	−120	−64	−23
25a, **25b**, **26a**, **26b**	411 → 125	286	−120	−64	−15
27a, **27b**	399 → 261	138	−120	−44	−13
28a, **28b**, **29a**, **29b**	413 → 275	138	−120	−44	−15
30a, **30b**	415 → 111	304	−125	−72	−5
31a, **31b**, **32a**, **32b**	429 → 125	304	−125	−72	−21
33a, **33b**	431 → 341	90	−50	−34	−15
34a, **34b**, **35a**, **35b**	445 → 355	90	−50	−34	−23
36	263 → 194	69	−50	−22	−11
37	365 → 165	200	−105	−48	−9
38, **39**	379 → 179	200	−105	−48	−9
40, **43**	251 → 141	110	−75	−28	−7
41, **42**, **44**, **45**	265 → 155	110	−75	−28	−7
46, **49**	363 → 181	182	−80	−42	−13
47, **48**, **50**, **51**	377 → 195	182	−80	−42	−13
52, **55**	379 → 347	32	−85	−26	−9
53, **54**, **56**, **57**	393 → 361	32	−85	−26	−9

[a] Chemical structures of the individual compounds are given in **Figures 1** and **2**. [b] Declustering potential. [c] Collision energy. [d] Cell exit potential.

Table 2. Concentrations (Standard Deviation) of Selected Hop-Derived Bitter Compounds in Hop Samples and Total Hop Dosage Used for the Entire Gyle

	concentration [g/100 g] in		
compound[a]	ethanolic hop extract[b]	hop pellets[c]	total dosage [g/gyle][d]
xanthohumol (**1**)	6.45 (±0.08)	2.28 (±0.02)	1635.7
cohumulone (**3**)	10.66 (±0.02)	1.20 (±0.07)	1929.2
humulone (**4**)	19.92 (±0.16)	1.78 (±0.07)	3465.6
adhumulone (**5**)	6.36 (±0.23)	0.81 (±0.04)	1180.3
colupulone (**15**)	8.49 (±0.13)	1.75 (±0.06)	1778.3
lupulone (**16**)	7.82 (±0.09)	1.52 (±0.05)	1608.1
adlupulone (**17**)	2.32 (±0.02)	0.50 (±0.06)	492.2

[a] Chemical structures of the individual compounds are given in **Figures 1** and **2**. [b] Ethanol extract used was made from the bitter hop varieties Magnum and Taurus. [c] Pellets type 90 were made from the aroma hop varieties Perle and Tradition. [d] Total dosage of bitter compounds originating from ethanol extract (30.2 kg) and hop pellets (14.7 kg) to a gyle (1193 hL wort).

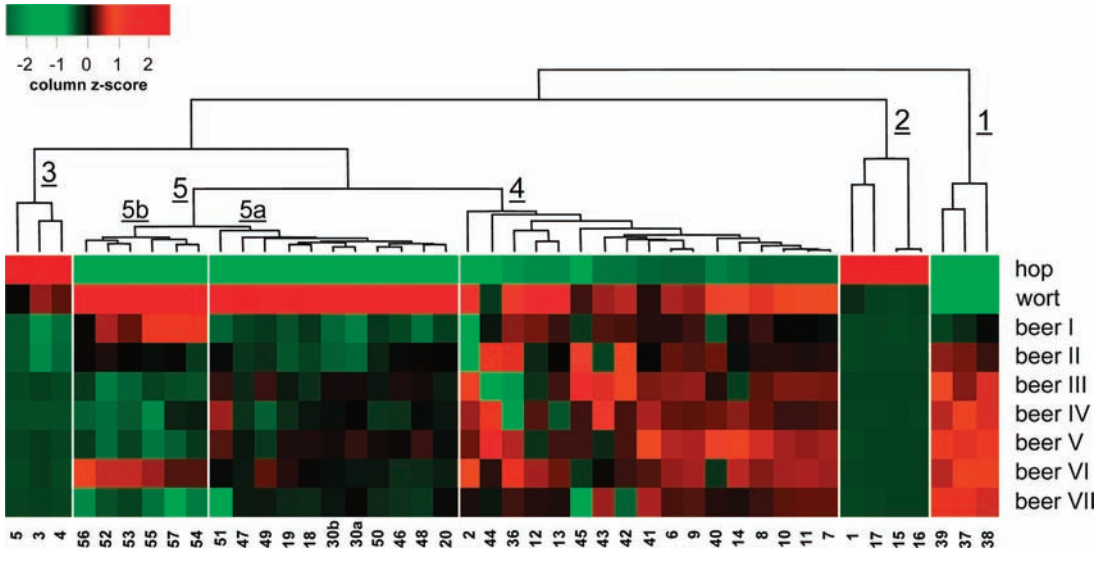

Figure 6. Sensomics heatmap calculated from the quantitative data (**Table 3**) of each bitter compound normalized to its content in wort. The dendrogram is based on an agglomerative linkage algorithm (*26*). Chemical structures of the individual compounds are given in **Figures 1** and **2**. Sample numbering refers to **Figure 4**.

Table 3. Concentrations and Utilization of Hop-Derived Bitter Compounds in Wort and Beer Samples I- VII

bitter compound[a]	concentration [g/gyle] (utilization [%])[b]							
	wort	beer I	beer II	beer III	beer IV	beer V	beer VI	beer VII
xanthohumol (**1**)	105.9 (6.5)	1.8 (0.1)	0.7 (<0.1)	21.8 (1.3)	14.7 (0.90)	8.5 (0.5)	18.7 (1.1)	4.2 (0.3)
isoxanthohumol (**2**)	341.9 (20.9)	45.7 (2.8)	59.7 (3.6)	274.8 (16.8)	234.9 (14.4)	219.3 (13.4)	284.0 (17.4)	195.5 (12.0)
cohumulone (**3**)	710.4 (36.8)	89.5 (4.6)	100.2 (5.2)	291.0 (15.1)	256.2 (13.3)	304.8 (15.8)	293.9 (15.2)	284.4 (14.7)
humulone (**4**)	918.4 (26.5)	52.0 (1.5)	58.5 (1.7)	227.7 (6.6)	199.8 (5.8)	247.5 (7.1)	221.8 (6.4)	237.8 (6.9)
adhumulone (**5**)	188.6 (16.0)	7.7 (0.7)	8.7 (0.7)	35.4 (3.0)	30.9 (2.6)	41.5 (3.5)	34.5 (2.9)	39.2 (3.3)
trans-isocohumulone (**6**)	593.5 (30.8)	528.8 (27.4)	557.7 (28.9)	578.5 (30.0)	559.7 (29.0)	600.4 (31.1)	584.8 (30.3)	548.7 (28.4)
trans-isohumulone (**7**)	768.2 (22.2)	568.6 (16.4)	609.0 (17.6)	651.8 (18.8)	631.7 (18.2)	679.9 (19.6)	678.4 (19.6)	642.2 (18.5)
trans-isoadhumulone (**8**)	564.6 (47.8)	485.9 (41.2)	475.6 (40.3)	501.4 (42.5)	505.3 (42.8)	555.7 (47.1)	517.7 (43.9)	487.8 (41.3)
cis-isocohumulone (**9**)	999.9 (51.8)	931.5 (48.3)	946.3 (49.1)	995.3 (51.6)	949.1 (49.2)	1020.7 (52.9)	1008.9 (52.3)	941.7 (48.8)
cis-isohumulone (**10**)	1365.0 (39.4)	1051.0 (30.3)	1091.9 (31.5)	1183.5 (34.1)	1117.0 (32.2)	1222.4 (35.3)	1227.2 (35.4)	1154.5 (33.3)
cis-isoadhumulone (**11**)	583.8 (49.5)	436.8 (37.0)	455.9 (38.6)	497.6 (42.2)	470.9 (39.9)	510.5 (43.3)	519.0 (44.0)	486.2 (41.2)
cohumulinone (**12**)	213.4 (11.1)	139.5 (7.2)	113.1 (5.9)	108.3 (5.6)	134.9 (7.0)	108.0 (5.6)	146.8 (7.6)	128.2 (6.6)
humulinone (**13**)	350.6 (10.1)	215.7 (6.2)	202.2 (5.8)	218.0 (6.3)	162.1 (4.7)	219.2 (6.3)	228.7 (6.6)	208.1 (6.0)
adhumulinone (**14**)	118.1 (10.0)	87.1 (7.4)	82.4 (7.0)	73.2 (6.2)	97.6 (8.3)	111.2 (9.4)	101.7 (8.6)	87.5 (7.4)
colupulone (**15**)	25.2 (1.4)	13.0 (0.7)	14.8 (0.83)	32.9 (1.8)	28.2 (1.6)	36.0 (2.0)	28.0 (1.6)	32.3 (1.8)
lupulone (**16**)	22.5 (1.4)	14.9 (0.9)	15.5 (1.0)	25.8 (1.6)	22.3 (1.4)	28.5 (1.8)	24.4 (1.5)	24.9 (1.5)
adlupulone (**17**)	16.8 (3.4)	14.1 (2.9)	13.6 (2.8)	17.4 (3.5)	15.8 (3.2)	18.1 (3.7)	18.0 (3.7)	16.2 (3.3)
cohulupone (**18**)	445.9 (25.1)	136.1 (7.7)	127.9 (7.2)	142.2 (8.0)	158.2 (8.9)	178.9 (10.1)	172.1 (9.7)	147.5 (8.3)
hulupone (**19**)	247.3 (15.4)	60.0 (3.7)	58.7 (3.6)	80.9 (5.0)	74.0 (4.6)	93.0 (5.8)	94.8 (5.9)	76.5 (4.8)
adhulupone (**20**)	126.1 (25.6)	41.2 (8.4)	52.2 (10.6)	48.5 (9.8)	49.8 (10.1)	50.4 (10.2)	48.1 (9.8)	51.2 (10.4)
hydroxytricyclocolupone 1 (**30a**)	78.9 (4.4)	16.6 (0.9)	18.9 (1.1)	31.1 (1.7)	29.8 (1.7)	33.1 (1.9)	28.3 (1.6)	30.5 (1.7)
hydroxytricyclocolupone 2 (**30b**)	139.6 (7.9)	31.9 (1.8)	33.6 (1.9)	53.5 (3.0)	48.8 (2.7)	53.3 (3.0)	49.0 (2.8)	48.2 (2.7)
hydroxytricyclolupone epimer 1 (**31a**) hydroxytricycloadlupone epimer 1 (**32a**)	31.6 (1.5)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
hydroxytricyclolupone epimer 2 (**31b**) hydroxytricycloadlupone epimer 2 (**32b**)	60.1 (1.8)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
hulupinic acid (**36**)	6.8 (0.2)	5.2 (0.1)	5.7 (0.2)	2.9 (0.1)	2.8 (0.1)	5.4 (0.1)	5.8 (0.2)	4.8 (0.1)
tricyclocohumol (**37**)	n.d.	74.4 (3.9)	98.8 (5.1)	101.6 (5.3)	121.6 (6.3)	114.4 (5.9)	121.3 (6.3)	116.0 (6.0)
tricyclohumol (**38**)	n.d.	142.5 (4.1)	159.3 (4.6)	195.7 (5.6)	195.5 (5.6)	208.1 (6.0)	208.4 (6.0)	192.5 (5.6)
tricycloadhumol (**39**)	n.d.	41.3 (3.5)	64.8 (5.5)	79.0 (6.7)	68.8 (5.8)	75.7 (6.4)	68.3 (5.8)	73.9 (6.3)
cis-cohumulinic acid (**40**)	1.9 (0.1)	1.1 (0.1)	1.5 (0.1)	1.4 (0.1)	1.5 (0.1)	1.9 (0.1)	1.2 (0.1)	1.4 (0.07)
cis-humulinic acid (**41**)	2.4 (0.1)	2.4 (0.1)	2.3 (0.1)	2.6 (0.1)	2.7 (0.1)	3.0 (0.1)	2.5 (0.1)	2.7 (0.08)
cis-adhumulinic acid (**42**)	1.1 (0.1)	1.0 (0.1)	1.2 (0.1)	1.2 (0.1)	1.0 (0.08)	1.0 (0.1)	1.0 (0.1)	0.8 (0.6)
trans-cohumulinic acid (**43**)	0.6 (<0.1)	0.2 (<0.1)	0.2 (<0.1)	0.3 (0.1)	0.3 (<0.1)	0.2 (<0.1)	0.2 (<0.1)	0.3 (<0.1)
trans-humulinic acid (**44**)	0.2 (<0.1)	0.3 (<0.1)	0.3 (<0.1)	0.2 (<0.1)	0.3 (<0.1)	0.4 (<0.1)	0.3 (<0.1)	0.3 (<0.1)
trans-adhumulinic acid (**45**)	0.1 (<0.1)	0.1 (<0.1)	0.2 (<0.1)	0.2 (<0.1)	0.1 (<0.1)	0.1 (<0.1)	0.1 (<0.1)	0.1 (<0.1)
cis-alloisocohumulonehydroxide (**46**)	9.6 (0.5)	2.8 (0.2)	3.6 (0.2)	3.9 (0.2)	3.1 (0.2)	3.8 (0.2)	3.2 (0.2)	3.1 (0.2)
cis-alloisohumulonehydroxide (**47**)	19.6 (0.6)	4.7 (0.1)	5.2 (0.2)	5.7 (0.2)	5.3 (0.2)	6.2 (0.2)	6.0 (0.2)	6.0 (0.2)
cis-alloisoadhumulonehydroxide (**48**)	7.5 (0.6)	2.0 (0.2)	3.1 (0.3)	3.2 (0.3)	3.0 (0.3)	3.4 (0.3)	2.7 (0.2)	2.5 (0.2)
trans-alloisocohumulonehydroxide (**49**	4.1 (0.2)	1.3 (0.1)	1.4 (0.1)	1.8 (0.1)	1.1 (0.1)	1.3 (0.1)	1.9 (0.1)	1.5 (0.1)
trans-alloisohumulonehydroxide (**50**)	10.7 (0.3)	3.3 (0.1)	3.7 (0.1)	3.9 (0.1)	3.4 (0.1)	4.3 (0.1)	3.9 (0.1)	3.6 (0.1)
trans-alloisoadhumulonehydroxide (**51**)	3.8 (0.3)	0.9 (0.1)	1.4 (0.1)	1.6 (0.2)	1.9 (0.2)	1.7 (0.1)	1.4 (0.1)	0.2 (<0.1)
cis-alloisocohumulonehydroperoxide (**52**)	5.7 (0.3)	2.9 (0.2)	2.3 (0.1)	1.2 (0.1)	1.3 (0.1)	1.3 (0.1)	3.0 (0.2)	1.5 (0.1)
cis-alloisohumulonehydroperoxide (**53**)	10.5 (0.3)	4.9 (0.1)	4.1 (0.1)	2.7 (0.1)	2.8 (0.1)	3.1 (0.1)	5.6 (0.2)	3.1 (0.1)
cis-alloisoadhumulonehydroperoxide (**54**)	4.0 (0.3)	2.4 (0.2)	1.3 (0.1)	1.2 (0.1)	1.5 (0.1)	1.3 (0.1)	1.9 (0.2)	1.0 (0.1)
trans-alloisocohumulonehydroperoxide (**55**)	2.4 (0.1)	1.4 (0.1)	0.9 (0.1)	0.7 (<0.1)	0.5 (<0.1)	0.5 (<0.1)	1.2 (0.1)	0.5 (<0.1)
trans-alloisohumulonehydroperoxide (**56**)	4.3 (0.1)	1.7 (0.1)	1.7 (0.1)	1.3 (<0.1)	1.1 (<0.1)	1.3 (0.4)	2.6 (0.1)	0.9 (<0.1)
trans-alloisoadhumulonehydroperoxide (**57**)	2.0 (0.2)	1.2 (0.1)	0.8 (0.1)	0.6 (0.1)	0.7 (<0.1)	0.6 (0.1)	0.9 (0.1)	0.3 (<0.1)
pH value	5.4	4.3	4.3	4.3	4.3	4.3	4.3	4.3

[a] Chemical structures of the individual compounds are given in **Figure 1** and **2**. [b] Sample numbering refers to **Figure 4**. n.d. not detectable.

freshly prepared wort, but were rapidly formed in increasing concentrations in the order of beer I to beer VII, for example, tricyclocohumol (**37**) increased from 74.4 to 116.0 in beer VII (**Table 3**). This is well in line with our recent findings that these tricyclohumols are formed from *trans*-iso-α-acids upon beer storage with decreasing pH value and increasing time (*14*).

Concentrations of Sensometabolites in Trub and Yeast. To gain a more detailed insight into the hop components present in the trub as well as those being absorbed to the yeast during fermentation, a sample of trub collected after the wort boiling as well as the yeast samples I−IV collected after fermentation (**Figure 4**) were extracted and analyzed for xanthohumol (**1**), α-acids (**3**−**5**), and *β*-acids (**15**−**17**) by means of HPLC-MS/MS.

Consistent with the limited water solubility, high amounts of *β*-acids (**15**−**17**) were found in the trub, for example, 68.9, 76.9, and 83.8% of the starting amount of colupulone (**15**), lupulone (**16**), and adlupulone (**17**) present in the hop dosage were recovered in this byproduct (**Table 4**). This is well in line with earlier findings that a vast amount of *β*-acids remains in the trub (*29*). Even for xanthohumol (**1**) a significant amount of 15.9% of the hop dosage was found in the trub, whereas only trace amounts of about 1.0−3.8% of the more polar α-acids **3**−**5** were determined (**Table 4**).

Analysis of the yeast samples revealed that a total amount of 10.5% of the added xanthohumol (**1**) is adsorbed by the yeast material, thus confirming previous reports (*18*). In contrast, only

Table 4. Concentration and Yield of Selected Hop-Derived Bitter Compounds adsorbed at Trub and Yeast Material

	concentration [g/gyle][b] (yield [%][c])					
compound[a]	trub	yeast I	yeast II	yeast III	yeast IV	sum yeast
xanthohumol (**1**)	260.2 (15.9)	48.5 (3.0)	38.1 (2.3)	44.1 (2.7)	40.6 (2.5)	171.3 (10.5)
cohumulone (**3**)	73.6 (3.8)	11.9 (0.6)	10.9 (0.6)	21.7 (1.1)	4.9 (0.3)	49.4 (2.6)
humulone (**4**)	36.1 (1.0)	20.4 (0.6)	25.9 (0.7)	40.6 (1.2)	8.8 (0.3)	95.7 (2.8)
adhumulone (**5**)	13.1 (1.1)	5.9 (0.5)	3.9 (0.3)	6.8 (0.6)	1.5 (0.1)	18.1 (1.5)
colupulone (**15**)	1224.8 (68.9)	8.7 (0.5)	8.8 (0.5)	9.7 (0.5)	3.4 (0.2)	30.6 (1.7)
lupulone (**16**)	1237.3 (76.9)	6.2 (0.4)	6.5 (0.4)	7.0 (0.4)	2.3 (0.1)	22.0 (1.4)
adlupulone (**17**)	412.4 (83.8)	1.8 (0.4)	2.1 (0.4)	2.5 (0.5)	1.0 (0.2)	7.4 (1.5)

[a] Chemical structures of the individual compounds are given in **Figure 1** and **2**. [b] Sample numbering refers to **Figure 4**. [c] Total amount of 1193 hL wort and beer, 650 kg trub, 1700 kg yeast I, 750 kg yeast II, 750 kg yeast III, and 560 kg yeast IV were obtained per gyle.

1.4−2.8% of the α-acids (**3**−**5**) and β-acids (**15**−**17**) added with the hop dosages were detected in these yeast samples (**Table 4**). Although irreversible adsorptions of hop bitter acids to yeast cells are assumed to be a sink for bitter compounds during beer manufacturing (*29*, *31*), these data indicate that the loss of α- and β-acids by yeast absorption is comparatively low.

Concentrations of Sensometabolites Adsorbed at Filter Materials. To study possible losses of bitter compounds during the final filtration steps and during the stabilization with polyvinylpyrrolidone (PVP), samples of diatomite, PVP, and the sheet filter were collected, extracted with methanol, and, then, analyzed for xanthohumol (**1**), α-acids (**3**−**5**), and β-acids (**15**−**17**) by means of HPLC-MS/MS. Only trace amounts of these bitter compounds were detectable in the different filter materials (**Table 4**), thus fitting well with the finding that the concentrations of these bitter compounds in the beer samples I−VII remain almost unaltered (**Table 3**). In conclusion, there seem to be only a marginal effect of filtration and stabilization on the levels of these sensometabolites in the final beer (**Table 5**).

Hierarchical Cluster Analysis and Sensomics Heatmap. To examine the multivariate distances between the individual sensometabolites throughout the brewing process, the concentrations determined for each compound in the hop dosage, the wort, as well as the beer samples I−VII (**Tables 2** and **3**) were normalized to their concentrations found in wort and a hierarchical cluster analysis was performed on the basis of these normalized data. The results were visualized in a sensomics heatmap that was combined with hierarchical agglomerative clustering of the sensometabolites **1**−**57** (**Figure 6**). The cluster analysis quantifies the degree of similarity between the sensometabolites by calculating the distance between all possible pairs of molecules. The two most similar sensometabolites were then grouped together and the distance measure recalculated. This iterative process was continued until all sensometabolites were members of a single cluster. This resulting hierarchical clustering is visually displayed as a dendrogram (**Figure 6**). The closer the sensometabolites are to each other in the dendrogram, the smaller the differences in their concentration patterns throughout the entire brewing process. The hierarchical analysis arranged the sensometabolites into three small clusters (labeled 1 - 3 in **Figure 6**) and two large clusters (labeled 4 and 5 in **Figure 6**).

Cluster 1 consisted of the three tricyclohumols **37**−**39**, whereas the β-acids **15**−**17** as well as xanthohumol (**1**) were well separated in cluster 2 and the α-acids (**3**−**5**) were grouped in cluster 3 (**Figure 6**). The tricyclohumols **37**−**39** (cluster 1) were absent in the hop dosage as well as in the freshly prepared wort, but were increasingly generated in the order of beer I to beer VII. In total, about 5.6−6.3% of the parent α-acids were found to be transformed into **37**−**39** (**Table 3**). This transformation occurs via the *trans*-iso-α-acids as the key intermediates as outlined in **Figure 3** (*14*).

Table 5. Concentration and Yield of Selected Hop-Derived Bitter Compounds adsorbed at Filter Materials

	concentration [mg/gyle][b] (yield [%])		
compound[a]	diatomite	PVP	sheet filter
xanthohumol (**1**)	n.d.	0.01 (<0.01)	0.05 (<0.01)
cohumulone (**3**)	n.d.	n.d.	0.52 (0.03)
humulone (**4**)	n.d.	n.d.	1.02 (0.03)
adhumulone (**5**)	n.d.	n.d.	0.23 (0.02)
colupulone (**15**)	0.17 (0.01)	0.09 (<0.01)	8.31 (0.47)
lupulone (**16**)	0.31 (0.02)	0.11 (<0.01)	7.15 (0.44)
adlupulone (**17**)	0.30 (0.06)	0.12 (<0.01)	5.26 (1.1)

[a] Chemical structures of the individual compounds are given in **Figure 1** and **2**. [b] Sample numbering refers to **Figure 4**. A total amount of 1193 hL wort and beer, 70 kg diatomite, 17 kg PVP and 22 kg sheet filter material were obtained per gyle. n. d. not detectable.

In contrast, the β-acids **15**−**17** (**Figure 1**) as well as xanthohumol (**1**), grouped in cluster 2, show a dramatic drop in their concentrations from the hop dosage over the wort to the beer samples (**Figure 6**). This is most likely due to the limited extraction of the β-acids from the hop material, for example, a majority of 68.9 - 83.8% of these sensometabolites is removed after the wort boiling with the trub (**Table 4**). A comparatively low utilization rate was found for xanthohumol (**1**), for example, only 6.5% of the amount present in the hop dosage was detected in the wort and 20.9% was found as its isomerization product isoxanthohumol (**2**).

The levels of α-acids (**3**−**5** in **Figure 1**) in cluster 3 were higher in the wort when compared to the other hop-derived phytochemicals (clusters 1 and 2) and, after running through a minimum in beer samples I and II, increased again slightly up to the final beer sample VII (**Figure 6**). On the basis of the quantitative data (**Table 3**), it can be concluded that, depending on the congener, between 3.3 and 14.7% of the starting dosage of α-acids (**3**−**5**) remain unchanged in the final beer VII.

The large cluster 4 (**Figure 6**) consisted of isoxanthohumol (**2**), the iso-α-acids (**6**−**11**), the humulinones (**12**−**14**), hulupinic acid (**36**), and *cis*- and *trans*-humulinic acids (**40**−**45**). This cluster analysis clearly demonstrate a rather similar evolution pattern of these compounds throughout the entire brewing process, thus being well in line with the proposed reaction routes for the formation of isoxanthohumol (**2**) from **1**, the iso-α-acids (**6**−**11**), humulinones (**12**−**14**), and *cis*- and *trans*-humulinic acids (**40**−**45**) from the α-acids (**3**−**5**), and hulupinic acid (**36**) from the β-acids (**15**−**17**) (**Figure 3**). An amount of 77.2, 51.8, and 82.5% of the starting dosage of α-acids (**3**−**5**) were isomerized to the corresponding iso-α-acids (**6-11**) in the final beer VII (**Table 3**), which is in the range to be expected for a Pilsner-type beer (*32*, *33*). In addition, 6.6−7.4% of the parent α-acids were found to be transformed into humulinones (**12**−**14**) and humulinic acids (**40**−**45**). Interestingly, the formation of these oxidation products from the

parent iso-α-acids seems to take place already during the wort boiling process.

In comparison, the large cluster 5 consisted of hulupones (**18**−**20**), the hydroxytricyclocolupone epimers **30a/b**, *cis*- and *trans*-alloisohumulonehydroxides (**46**−**51**) separating in subcluster 5a, and *cis*- and *trans*-alloisohumulonehydroperoxides (**52**−**57**) separating in subcluster 5b. Interestingly, all the sensometabolites grouping in cluster 5 were recently found to be formed upon autoxidative degradation of α-acids (*15*), namely **46**−**57**, and *β*-acids (*4*,*5*), namely **18**−**20** and **30a/b** (**Figure 1**). Depending on the congener, 15.4−25.6% of the *β*-acids were oxidatively transformed into the bitter tasting hulupones **18**−**20** already during wort-boiling, thereafter the concentrations declined to 4.8−10.4% in the beer sample VII (**Table 3**). The same behavior was observed for the hydroxytricyclocolupone epimers **30a/b** and *cis*- and *trans*-alloisohumulonehydroxides (**46**−**51**) as visualized by the cluster analysis (**Figure 6**).

In conclusion, quantitative profiling of the sensometabolites contributing to the bitter taste of beer revealed a comprehensive insight into the transformation of hop-derived phytochemicals and the process-induced evolution of sensometabolites throughout a full-scale beer manufacturing process. Depending on their chemical structure, the individual sensometabolites were arranged into different clusters by means of hierarchical analysis and sensomics heatmapping and confirmed the reaction routes proposed for their formation during the entire beer brewing process. The sensomics data obtained during this study offers the scientific basis for a knowledge-based optimization of the beer bitter taste by technological means.

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