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Influence of Different Hop Products on the *cis/trans* Ratio of Iso-α-Acids in Beer and Changes in Key Aroma and Bitter Taste Molecules During Beer Ageing

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ABSTRACT

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Isomerization of α-acids from hops can result in different ratios of *cis*- to *trans*-iso-α-acids. In contrast to oxidative degradation processes, the conversion of iso-α-acids to tri- and tetracyclic compounds during beer ageing is only possible in the *trans* form. Therefore, it might be an advantage to use pre-isomerized hop products in order to maximize the *cis* form in beer. For this study, beers conventionally hopped with regular pellets were compared with beers produced with isomerized pellets and isomerized extracts. In addition, inline pre-isomerization was investigated as another alternative. The four variants were produced in a pilot brewery. Bottled beers were stored at 5 and 28°C. Analysis of fresh and aged samples after 4, 8, and 12 months was focused on bitter compounds (HPLC-MS/MS) and on volatile aroma compounds (GC-MS/MS). Beers produced with isomerized hop products showed higher ratios of *cis*- to *trans*-iso-α-acids, and their amounts of degradation products of *trans*-iso-α-acids were accordingly lower after ageing. However, all of the four types of beers hopped in different ways showed very similar profiles of volatile aroma compounds even during ageing. Moreover, sensory evaluation of aged beers gave no preference for the variations with pre-isomerized hop products.

Keywords: Beer ageing, *cis*/*trans* ratio, Degradation products of iso-α-acids, Hop products, Pre-isomerization

RESUMEN

La isomerización de los α-ácidos del lúpulo puede resultar en diferentes proporciones de iso-α-ácidos *cis* y *trans*. A diferencia de los procesos de degradación oxidativas, la conversión de iso-α-ácidos a compuestos tri- y tetracíclicos durante el envejecimiento de la cerveza es únicamente posible en la forma *trans*, por lo que podría ser ventajoso utilizar lúpulos preisomerizados para maximizar la forma *cis* en la cerveza. En este estudio se compararon cervezas con pellets de lúpulo convencionales con cervezas elaboradas con pellets isomerizados y extracto isomerizado. También se investigó la preisomerización en línea como otra alternativa. Los cuatro variantes fueron elaborados en una planta piloto. La cerveza embotellada se guardó a 5°C y a 28°C. El análisis de las cervezas frescas y guardadas a los 4, 8 y 12 meses se concentró en los compuestos amargos (HPLC-MS/MS) y en los compuestos volátiles aromáticos. Las cervezas producidas con productos de lúpulo isomerizado mostraron mayores proporciones de *cis* a *trans* y la cantidad de productos de degradación de los iso-α-ácidos *trans* fueron consecuentemente más bajos después del envejecimiento. Sin embargo, las cuatro cervezas con lupulados diferentes mostraron perfiles muy similares de los compuestos volátiles aromáticos, inclusive después del envejecimiento. Es más, la evaluación sensorial de las cervezas envejecidas no mostró ninguna preferencia para las cervezas con los productos preisomerizados.

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Palabras claves: Envejecimiento de cerveza, Preisomerización, Productos de degradación de iso-α-ácidos, Productos de lúpulo, Relación *cis:trans*

Different hop products such as pellets and ethanol or carbon dioxide extracts are used as key ingredients in beer manufacturing to impart the typical bitter taste as well as the attractive aroma to the final beverage.

Isomerization processes during wort boiling are of major importance for bitter taste development in beer products. The *cis*/*trans*-iso-α-acids have been identified as the main contributors to the bitter taste of beer and are generated upon a rearrangement reaction of their hop-derived precursors, the α-acids cohumulone (1a), humulone (1b), and adhumulone (1c) (Fig. 1) (5,15,18). Isomerization of α-acids can result in different ratios of *cis*- to *trans*-iso-α-acids. Wort boiling in the brewery usually gives a ratio of ~2.5:1. However, it can be considerably higher when the α-acids are pre-isomerized using suitable catalysts.

De Cooman et al (4) already described that particularly *trans*-iso-α-acids (3a-c) are prone to degradation. Intelmann et al (11,14) revealed an acid-catalytic decomposition pathway for the *trans* form of the iso-α-acids (acids 2 and 3) to tri- and tetracyclic degradation compounds (compounds 4–8) during beer storage. The formation of degradation products of iso-α-acids under oxidative storage conditions is independent of their *cis*/*trans* configuration and was described by Intelmann et al (13). An overview of possible degradation products of *trans*- and *cis*-iso-α-acids is given in Figure 1. With regard to the findings about the degradation compounds during storage of beer, it might be beneficial to use pre-isomerized hop products in order to maximize the *cis* form in beer and, therefore, the bitter taste stability of the beverage.

In addition to the analysis of the degradation products of *trans*- and *cis*-iso-α-acids, the evaluation of beer flavor over time in storage is usually based on the determination of analytical parameters such as *trans*-2-nonenal, strecker aldehydes, β-damascenone, or the *trans*/*cis* ratio of the bitter acids (1,2,16,19). Additionally, sensory tests have revealed that the bitterness intensity of fresh beer declines with increasing age of the beverage and a more harsh lingering bitterness is produced (14).

With the help of beers exclusively bittered with *cis*- and *trans*-iso-α-acids, De Clippeleer et al (3) observed that the *trans*-specific degradation of iso-α-acids could not be linked to the formation of staling aldehydes during beer ageing. The malt quality and the brewing process itself are probably the most important factors regarding the flavor instability of beer.

To analyze the possibilities of enhancing α-acid utilization during the brewing process, Hertel and Dillenburger examined the important parameters regarding the behavior of bitter-acids among the different manufacturing steps (7–9) and demonstrated the benefits of a hop yield enhancer (10). The procedure for inline pre-isomerization using a hop yield enhancer was as follows. Partial wort quantity was taken and exposed to higher temperature

in a special isomerization vessel together with hop product added. In addition, a specific, continuous extraction using a special extraction unit was developed. This procedure can significantly increase yield of bitter substances in brewing (10). However, Schmidt et al (17) showed that there was no impact of inline pre-isomerized hop products on analytical and sensory markers for beer ageing compared with conventional hopping.

To investigate whether the bitter taste stability improves when isomerized hop products are used, this study compared beers conventionally hopped with regular pellets to beers produced with isomerized pellets and isomerized extracts. The commercial products pellets type 90 (P90), isomerized pellets (IPE), and isomerized kettle extract (IKE) used in the brewing trials are presented in Figure 2. In addition, inline pre-isomerization (IPI) was investigated as another alternative. Evaluations of fresh as well as aged beer samples after 4, 8, and 12 months were targeted on bitter components (e.g., *cis/trans*-iso-α-acids, allo-isohumulones, allo-isohumulonhydroperoxides, allo-isohumulonhydroxides, tricyclohumols, tricyclohumenes, and tetracyclohumols) by means of HPLC-DAD or HPLC-MS/MS and on volatile aroma compounds (Fig. 3) using the GC-MS/MS analysis. Sensory analyses of fresh and aged beer samples complemented the study.

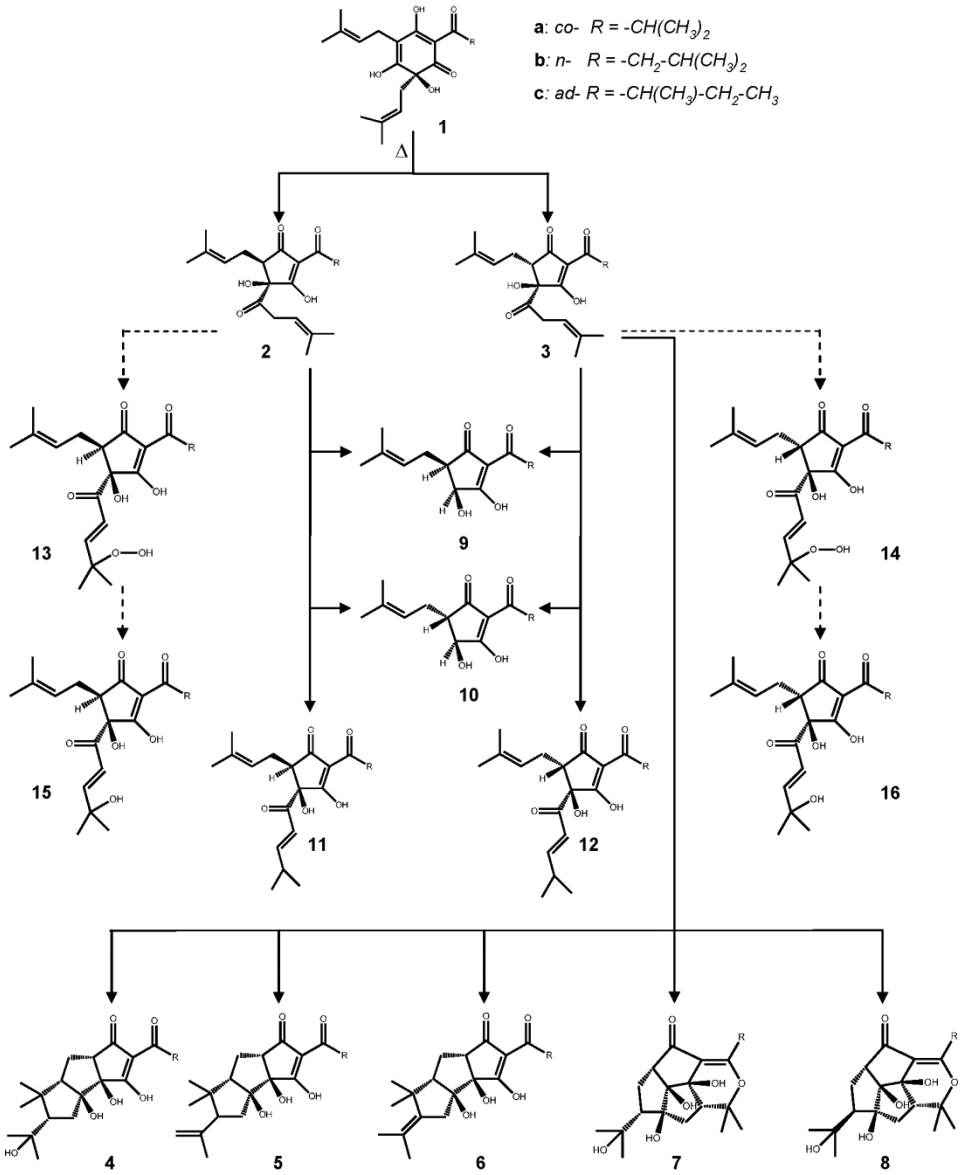

Fig. 1. Structures of the α-acids cohumulone (1a), humulone (1b), and adhumulone (1c) and selected transformation products *cis*-isocohumulone (2a), *cis*-isohumulone (2b), *cis*-isoadhumulone (2c), *trans*-isocohumulone (3a), *trans*-isohumulone (3b), *trans*-isoadhumulone (3c), tricyclocohumol (4a), tricyclohumol (4b), tricycloadhumol (4c), tricyclocohumene (5a), tricyclohumene (5b), tricycloadhumene (5c), isotricyclocohumene (6a), isotricyclohumene (6b), isotricycloadhumene (6c), tetracyclocohumol (7a), tetracyclohumol (7b), tetracycloadhumol (7c), epitetracyclocohumol (8a), epitetracyclohumol (8b), epitetracycloadhumol (8c), *cis*-cohumulinic acid (9a), *cis*-humulinic acid (9b), *cis*-adhumulinic acid (9c), *trans*-cohumulinic acid (10a), *trans*-humulinic acid (10b), *trans*-adhumulinic acid (10c), *cis*-alloisocohumulone (11a), *cis*-alloisohumulone (11b), *cis*-alloisoadhumulone (11c), *trans*-alloisocohumulone (12a), *trans*-alloisohumulone (12b), *trans*-alloisoadhumulone (12c), hydroperoxy-*cis*-alloisocohumulone (13a), hydroperoxy-*cis*-alloisohumulone (13b), hydroperoxy-*cis*-alloisoadhumulone (13c), hydroperoxy-*trans*-alloisocohumulone (14a), hydroperoxy-*trans*-alloisohumulone (14b), hydroperoxy-*trans*-alloisoadhumulone (14c), hydroxy-*cis*-alloisocohumulone (15a), hydroxy-*cis*-alloisohumulone (15b), hydroxy-*cis*-alloisoadhumulone (15c), hydroxy-*trans*-alloisocohumulone (16a), hydroxy-*trans*-alloisohumulone (16b), hydroxy-*trans*-alloisoadhumulone (16c).

EXPERIMENTAL

Chemicals and Materials

The following chemicals were obtained commercially: methanol and acetonitrile (BDH Prolabo, VWR International, Darmstadt, Germany); methanol (LiChrosolv), ammonium acetate, ortho-phosphoric acid (85%), citric acid monohydrate, disodium hydrogen phosphate dehydrate, and formic acid (Merck KGaA, Darmstadt, Germany).

Linalool (97%) and eugenol (99%) (Merck KGaA); 3-methylbutanal (97%), 2-methylbutanal (95%), ethyl propionate (99%), 1,1-diethoxyethane (99%), ethyl butanoate (99%), methional (96%), ethyl hexanoate (≥99%), phenylacetaldehyde (≥90%), 2-methoxyphenol (98%), nonanal (95%), ethyl octanoate (≥99%), 2′-aminoacetophenone (98%), β-damascenone (≥90%), pentanal (97%), methyl pentanoate (99%), and hexanol (≥99%) (Sigma-Aldrich, St. Louis); and 2-methoxy-4-vinylphenol (97%) and vanillin (99%) (Alfa Aesar, Ward Hill, MA) were also obtained commercially.

Deionized water was prepared by Synergy UV and Elix UV water purification systems (Millipore, Billerica, MA).

Quantitation of iso-α-acids was done by external calibration using dicyclohexylamine salts of trans-iso-α-acids for HPLC analysis of isomerized α-acids (ICS-I3; Labor Veritas AG, Zürich). Quantitation of α- and β-acids was carried out with the help of external calibration using International Calibration Extract (ICE-3; Labor Veritas AG).

All standards used for LC-MS/MS analyses were provided by the Chair of Food Chemistry and Molecular Sensory Science (Technische Universität München, Freising, Germany).

Brewing Procedure

The trials were carried out in a 20-hL pilot plant at Bitburger brewery. All of the four different variations were produced in duplicate. All results are given as mean values. A standard two-mash decoction procedure was used to produce Pilsener type beer from 300 kg of barley malt. All hop products were added at the beginning of wort boiling. For hop pellets, the dosage was 1,380 g per 20 hL, corresponding to α at 8.7 g/hL. In the case of isomerized hop products, the α dosages were reduced accordingly in order to achieve 30 bitter units (BU) in each beer. For IPI, 274 g of hop extract and 310 g of hop pellets were heated for 20 min at constant pressure (1.2 bar) and temperature (120°C) in a 10-L pressure vessel in water before adding to the wort. The schematic diagram of the pressure vessel used for hop yield enhancing is shown in Figure 4.

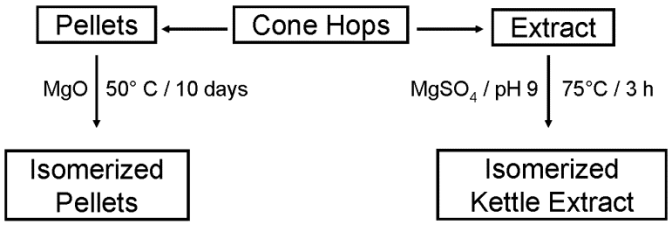

Fig. 2. Commercial hop products used in the brewing trials and their preparation conditions.

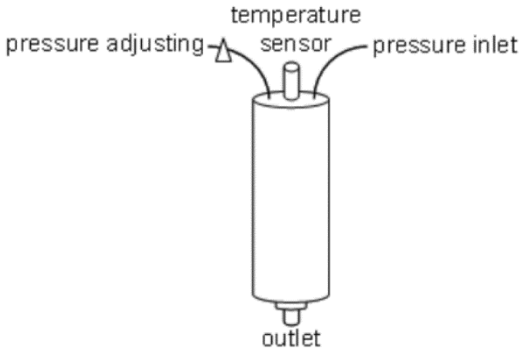

Fig. 4. Schematic diagram of pressure vessel used for inline pre-isomerization.

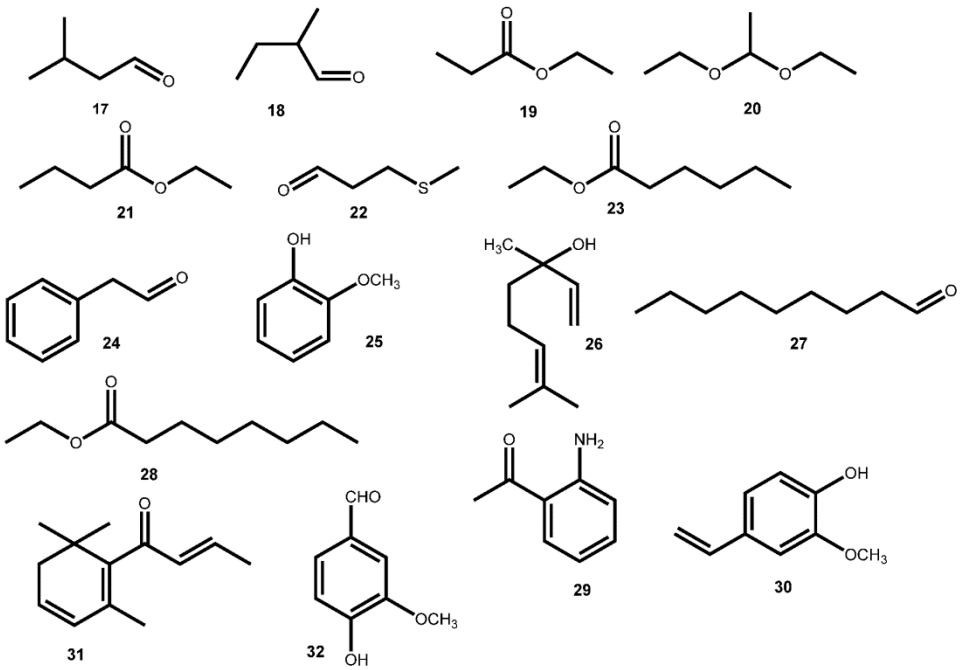

Fig. 3. Chemical structures of aroma compounds 3-methylbutanal (17), 2-methylbutanal (18), ethyl propionate (19), 1,1,-diethoxyethane (20), ethyl butanoate (21), methional (22), ethyl hexanoate (23), phenylacetaldehyde (24), 2-methoxyphenol (25), linalool (26), nonanal (27), ethyl octanoate (28), 2′-aminoacetophenone (29), 2-methoxy-4-vinylphenol (30), β-damascenone (31), and vanillin (32).

Wort was boiled for 75 min at 100°C and fermented at 10.5°C. Solely, kieselguhr was used for the filtration. The total amount of O_2 in the finished products, filled in brown glass bottles, was total O_2 at <0.05 mg/L. The wort and beer samples were examined fresh as well as after 4, 8 ,and 12 months of storage at 5°C as well as at 28°C.

Analysis of Hop Products

The α- and β-acids in hop pellets were extracted by a mixture of diethyl ether/methanol and hydrochloric acid solution. In the ether phase, dissolved substances were separated by reversed-phase HPLC and measured at 314 nm according to Analytica-EBC Method 7.7 (6). Hop extracts, including isomerized extracts, were dissolved in methanol and were separated via reversed-phase HPLC using gradient elution according to Analytica-EBC Method 7.8 (6). The separated α- and β-acids were measured at 314 nm and the iso-α-acids at 270 nm. Furthermore, the iso-α-, α-, and β-acids were extracted by a mixture of diethyl ether/methanol and hydrochloric acid solution from milled isomerized hop pellets and then also separated via reversed-phase HPLC using gradient elution according to Analytica-EBC Method 7.11 (6).

Wort and Beer Samples: Solid-Phase Extraction HPLC-DAD

For analysis of iso-α-acids and α-acids, wort and beer samples were cleaned-up via reversed-phase solid-phase extraction (SPE) (Strata C18-E; Phenomenex, Aschaffenburg, Germany) using Gilson GX-271 ASPEC with Dual 406 Syringe Pump (TRILUTION LH; Gilson, Inc., Middleton, WI). Prior to analysis, fresh and stored beer samples were degassed and filtered, while wort samples were filtered after centrifugation. A defined volume of the wort and beer samples (200 mL) was treated with ortho-phosphoric acid (85%; 400 µL). After conditioning of the SPE columns with a water/methanol mixture (50/50; v/v, acidified with 200 µL of ortho-phosphoric acid [85%]; 5 mL), 20 mL of the acidified beer and wort samples were given to the SPE columns. The treated columns were washed with acidified water (200 µL of ortho-phosphoric acid [85%] per 100 mL water; 5 mL) and eluted with methanol/water (90/10; v/v with 200 µL of ortho-phosphoric acid [85%]; 10 mL). The resulting samples were analyzed by means of HPLC-DAD with the following conditions.

The BIO-TEK Kontron Instruments HPLC system—consisting of a pump, a degasser, an autosampler, a thermostated column oven, and a diode array detector (BIO-TEK Kontron Instruments, Neufahrn, Germany)—was used for the quantitative analysis of iso-α-acids (2a-c and 3a-c), and α-acids (1a-c). For chromatography, an analytical 250-by-4.6-mm, 3-µm Gemini RP18 column (Phenomenex) equipped with a guard column of the same type was used as the stationary phase at 40°C, and citric acid/phosphate buffer (42.02 g of citric acid monohydrate was dissolved in 2 L of deionized water and mixed with 17.8 g of $Na_2HPO_4 \cdot 2H_2O$ per 500 mL of water) as solvent A and acetonitrile/water (90/10; v/v) as solvent B. Monitoring the effluent flow (0.7 mL/min) at 270 nm as well as at 314 nm, chromatography was executed by increasing the amount of solvent B from 50 to 60% within 18 min and from 60 to 100% within 5 min and, thereafter, maintaining for 100% solvent B for additional 17 min.

TABLE I
Specific Mass Transitions (*m/z* Q1→Q3) and Optimized Parameters for LC-MS/MS Analysis of Degradation Compounds 4a, 4b, 5a, and 5b Using Electrospray Ionization in the Negative Mode

Compound[a]	*m/z* Q1→Q3	DP (V)[b]	CE (V)[c]	CXP (V)[d]
4a	365→165	–81	–52	–10
4b	379→179	–105	–47	–10
5a	347→165	–110	–38	–10
5b	361→179	–95	–50	–10

[a] Numbering of compounds and chemical structures refers to Figure 1.
[b] Declustering potential.
[c] Collision energy.
[d] Cell exit potential.

BU

The bitter substances were extracted from the acidified sample (wort and beer) with iso-octane and the concentration in the extract was measured by spectrophotometry according to Analytica-EBC Method 9.8 (6).

Beer samples: HPLC-MS

Analysis of compounds 4a, 4b, 5a, 5b. The Shimadzu Corporation HPLC system—consisting of a binary pump, a degasser, an autosampler, and a thermostated column oven (Shimadzu Corporation, Kyoto, Japan)—was coupled with API 4000 Q-TRAP mass spectrometer (AB SCIEX, Darmstadt, Germany) equipped with the electrospray ionization (ESI) source running in the negative ion mode. Samples were introduced by HPLC with a solvent flow of 200 µL/min requiring the use of the turbo gas at a temperature of 450°C. The ion spray voltage was set to –4,500 V, and the declustering potential (DP) 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. The dwell time for each mass transition was 80 msec. The collision energy (CE), declustering potential (DP), and cell exit potential (CXP) were set as given in Table I. Nitrogen was used as the collision gas. To enhance selectivity, the quantitation was done using the multiple reaction monitoring (MRM) mode of the instrument with the fragmentation parameters optimized prior to analysis. The specific mass transitions of compounds 4–5 are summarized in Table I Data processing and integration was performed by using Analyst software (version 1.5; AB SCIEX). LC separation was performed using a 50-by-2.0-mm internal diameter Chromolith Fast Gradient RP-18 endcapped column (Merck KGaA) at 40°C. For elution of the compounds, 10 m*M* ammonium acetate as solvent A and methanol as solvent B was applied. Chromatography was performed by increasing solvent B from 30 to 50% within 20 min, then to 60% within 20 min, then 90% within 5 min; then it was kept constant for another 5 min. The tricyclic degradation products (4a, 4b, 5a, and 5b) were examined in degassed beer samples without the need of any clean-up procedures using the external calibration. The amounts of 4b, 5a, and 5b were calculated as tricyclohumol (4a) in milligrams per liter in each beer sample.

TABLE II
Specific Mass Transitions (*m/z* Q1→Q3) and Optimized Parameters for LC-MS/MS Analysis of Further Degradation Compounds (6a-b, 7a-b, 11a-b, 12a-b, 13a-b, 14a-b, 15a-b, and 16a-b)

Compound[a]	*m/z* Q1→Q3	DP (V)[b]	CE (V)[c]	CXP (V)[d]
6a	347→165	–60	–52	–7
6b	361→179	–60	–52	–7
7a	365→193	–120	–46	–11
7b	379→207	–120	–46	–11
11a, 12a	347→181	–70	–45	–6
11b, 12b	361→195	–70	–45	–6
13a, 14a	379→347	–85	–26	–9
13b, 14b	393→361	–85	–26	–9
15a, 16a	363→181	–80	–42	–13
15b, 16b	377→195	–80	–42	–13

[a] Numbering of compounds and chemical structures refers to Figure 1.
[b] Declustering potential.
[c] Collision energy.
[d] Cell exit potential.

Analysis of compounds 6a-b, 7a-b, 11a-b, 12a-b, 13a-b, 14a-b, 15a-b, and 16a-b. An Agilent 1100 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) equipped with the ESI source. For MS performed in the negative ion mode, the compound-specific DP, CXP, and CE were optimized prior analysis for each substance (Table II; 6a-b, 7a-b, 11a-b, 12a-b, 13a-b, 14a-b, 15a-b, and 16a-b). The specific mass transitions of the compounds 6–7 and 11–16 are also given in Table II.

Chromatography was performed using a 150-by-2.0 mm, 4-µm Synergi 4u Hydro-RP column (Phenomenex), 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 performed by increasing solvent A from 20 to 60% within 20 min, then to 70% within 15 min, to 92% within 28 min, and finally to 100% within 2 min. Data processing and integration were performed by using Analyst software (version 1.4.2; AB SCIEX). The quantitative analysis was carried out using the ECHO technique following the protocol reported by Intelmann et al (12,13). The beer samples were degassed and then directly used for quantitation.

Beer samples: GC-MS

Analysis of 3-methylbutanal (compound 17), 2-methylbutanal (compound 18), ethyl propionate (compound 19), 1,1,-diethoxyethane (compound 20), ethyl butanoate (compound 21), methional (compound 22), ethyl hexanoate (compound 23), phenylacetaldehyde (compound 24), 2-methoxyphenol (compound 25), linalool (compound 26), nonanal (compound 27), ethyl octanoate (compound 28), 2′-aminoacetophenone (compound 29), 2-methoxy-4-vinylphenol (compound 30), β-damascenone (compound 31), and vanillin (compound 32) was done by GC coupled with MS detection. Internal standards (pentanal, methyl pentanoate, hexanol, and eugenol) were added to the samples. Decarbonated beer (100 mL) was extracted twice with diethyl ether (300 mL) and dried with disodium sulfate for 15 min. The diethyl ether phase was evaporated to 2.5 mL of sample volume. After that, a 2.5-µL sample solution was injected. Gas chromatographic conditions were as follows (GC 7890A; Agilent Technologies, Waldbronn, Germany). Liquid injection of the extracted volatiles was done by a cool injection system (KAS 4; Gerstel, Mülheim, Germany). Helium was used as a carrier gas at a constant flow of 0.7 mL/min. Separation of the injected compounds was performed on a VF-WAXms capillary column (60 m by 0.25 mm, i.d. 0.25 µm) (factor4; Varian, Darmstadt, Germany). The oven program was as follows: 7 min at 40°C, followed by a temperature increase of 10°C/min up to 120°C (5-min isotherm), then at 10°C/min up to the final temperature of 240°C (8-min isotherm). For selective determination and precise quantitation, an MS detector (7000 GC/MS triple Quad; Agilent Technologies) operating in the electron ionization mode (70eV) was used. The ion source temperature was set at 230°C and the electron multiplier voltage (EMV) was 1,430 ± 200 V (ΔEMV). Analyses were performed by operating in the MS/MS mode (Table III). The identity of the compounds was confirmed using retention times of standards.

TABLE III
Specific Mass Transitions and Collision Energy (CE) Values for GC-MS/MS Analysis of Aroma Compounds 17–32

Compound[a]	Mass Transition	CE (V)[b]
17	86→58	0
18	58→57	0
19	102→74	0
20	103→75	0
21	88→71	0
22	70→48	0
23	88→61	0
24	120→91	15
25	124→109	10
26	121→93	0
27	114→81	0
28	88→61	0
29	92→65	0
30	150→135	10
31	190→121	0
32	152→151	15

[a] Numbering of compounds and chemical structures refers to Figure 3.
[b] Collision energy.

Sensory Analysis

Beer samples were analyzed by 12 trained assessors in separate sensory cabins using a triangle test followed by profile analysis where the panelists had to judge bitterness intensity, bitterness aftertaste, and beer ageing on a scale from 0 (not detectable) up to 9 (strong perception) in comparison with a fresh Pilsener type beer. The profile analyses were carried out when differences to the reference beer (fresh Pilsener type beer) were detectable. Beer samples were always presented in brown glasses. Data evaluation was executed with help of FIZZ software (Biosystemes, Couternon, France).

RESULTS AND DISCUSSION

To elucidate the impact of different hop products on the *cis*/*trans* ratio of iso-α-acids in beer and to monitor changes in key aroma and bitter taste compounds during beer storage, four selected hop products were compared with the help of sensory evaluations as well as by means of LC and GC.

Analysis of Hop Products

All hop products used were from the same variety, Hallertau Magnum (HHM, crop 2009). The amounts of α-acids, iso-α-acids, and β-acids determined in hop products tested are shown in Table IV. The amounts of the described parameters were determined using HPLC-DAD. The P90 had 12.6% of α-acids and 6.9% of β-acids. The IPE contain, as expected, a higher content of iso-α-acids (11.9%), only a negligible amount of α-acids (0.3%), and a comparable content of β-acids (6.6%).

TABLE IV
Amounts of α-Acids, Iso-α-Acids, and β-Acids in Four Different Hop Products

Hop products	α-Acids (%)	Iso-α-Acids (%)	β-Acids (%)
Pellets type 90 (P90)	12.6	-	6.9
Ethanol-extract (IPI)[a]	41.2	1.1	23.2
Isomerized pellets (IPE)	0.3	11.9	6.6
Isomerized kettle extract (IKE)	1.0	44.2	23.4

[a] Inline pre-isomerization (IPI) together with P90.

TABLE V
α Dosage and Resulting Bitter Units (BU) in Fresh Beer

Hop Products[a]	α-Acids/Iso-α-Acids (g/hL)	BU (EBC 9.8)
Pellets Type 90 (P90)	8.7/8.7	32/32
Ethanol-extract (IPI)[b]	7.7/7.7	33/28
Isomerized pellets	6.1/6.1	33/33
Isomerized kettle extract (IKE)	6.0/6.0	29/32

[a] Different variations were produced in duplicate.
[b] Inline pre-isomerization (IPI) together with P90.

For the brewing procedure, the dosages of the different hop products were calculated in order to achieve identical BU in the final beers. The measured BU were in a range between 28 and 33 IBU (Table V). General beer parameters such as alcohol, pH, or CO_2 (*data not shown*) demonstrated no significant differences between the variations tested.

Behavior of *cis*- and *trans*-Iso-α-Acids During Ageing of Beer

The analytical results of *cis*- and *trans*-iso-α-acids are presented in Tables VI–IX for each hop product.

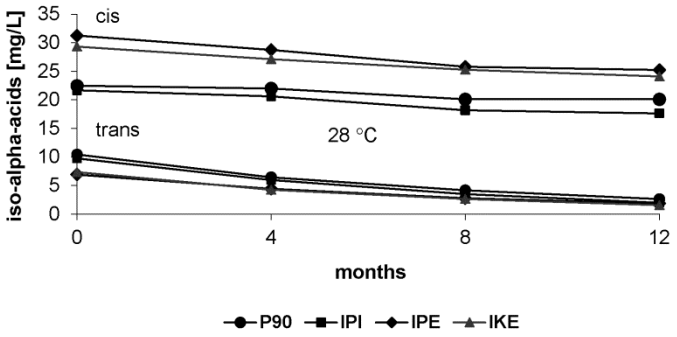

Fig. 5. Behavior of *cis*- and *trans*-iso-α-acids in beer samples prepared with different hop products during storage for 12 months at 28°C. P90 = Pellets Type 90, IPI = inline pre-isomerization (ethanol extract with P90), IPE = isomerized pellets, IKE = isomerized kettle extract.

Both the addition of regular pellets (P90) at the beginning of wort boiling and IPI in the brewery resulted in almost the same ratio of *cis*- to *trans*-iso-α-acids, with a value of ~2.2:1 (Tables VI and IX), whereas the isomerized hop products resulted in a considerably higher ratio (~4:1, Tables VII and VIII). To compare the change of the ratio of *cis*- to *trans*-iso-α-acids during storage, the values determined were normalized to their amounts in the fresh beverage. The difference of the *cis*/*trans* ratio during storage was dependent only on the storage conditions time and temperature; the hop variations showed no influence. The total amount of *cis*-isomers decreased only slightly compared with *trans*-isomers. As expected, *trans*-iso-α-acids demonstrated a higher decrease at higher temperature (Tables VI–IX). The behavior of *cis*- and *trans*-iso-α-acids in different beer samples during storage at 28°C for over one year is presented in Figure 5. The total amount of *trans*-iso-α-acids in beer samples which were prepared, for instance, with IPE decreased from 6.9 mg/L in the fresh beverage to 1.9 mg/L in the stored beer sample.

Concentrations of Degradation Compounds of Iso-α-Acids in Beer

The results of tricyclic degradation products of *trans*-iso-α-acids such as tricyclohumols (4a-b) and tricyclohumenes (5a-b) as analytical markers of beer ageing are listed in Tables VI–IX. The amounts of isotricyclohumenes (6a-b) and tetracyclohumols (7a-b) as well as of further known degradation products of *cis*- and *trans*-iso-α-acids (compounds 11–16) are summarized in Table X.

TABLE VI
Quantitation Results (± Standard Deviation; *n* = 2) of Taste and Aroma Compounds (1–5 and 17–32) in Wort and Beer Samples, Fresh and Stored, for 4, 8, and 12 Months at 5 and 28°C for Pellets Type 90

	Concentration in[a]							
			4 months		8 months		12 months	
Number[b]	Wort	Beer (Fresh)	5°C	28°C	5°C	28°C	5°C	28°C
1a-c	12.5 (0.7)	0.8 (0.4)	0.6 (0.3)	0.3 (0.1)	0.4 (0.2)	0.2 (0.1)	0.4 (0.1)	0.2 (0.1)
2a	8.7 (0.3)	7.8 (0.1)	8.1 (0.3)	7.6 (0.4)	7.3 (0)	7.1 (0.1)	6.9 (0.4)	7.1 (0)
2b	15.0 (0.7)	11.5 (0.2)	12.0 (0.6)	11.1 (0.5)	10.5 (0)	10.0 (0.2)	10.2 (0.6)	10.0 (0.1)
2c	4.1 (0.2)	3.2 (0.02)	3.4 (0.1)	3.3 (0.1)	3.0 (0.1)	3.1 (0.1)	2.8 (0.1)	3.1 (0.2)
Σ 2a-c	27.9	22.5	23.5	22.0	20.8	20.2	19.9	20.2
3a	4.1 (0.1)	3.6 (0.02)	3.4 (0.1)	2.1 (0.1)	2.6 (0.1)	1.3 (0.1)	2.4 (0.2)	1.3 (0.02)
3b	7.3 (0.3)	5.3 (0.04)	5.0 (0.2)	3.4 (0.2)	3.9 (0.1)	2.3 (0.2)	3.2 (0.2)	2.3 (0.1)
3c	2.0 (0.1)	1.5 (0)	1.5 (0.1)	0.9 (0.1)	1.0 (0.04)	0.5 (0.02)	0.9 (0.1)	0.5 (0.04)
Σ 3a-c	13.4	10.4	9.8	6.4	7.5	4.2	6.5	4.2
2a-c/3a-c[c]	2.1	2.2	2.4	3.4	2.8	4.9	3.1	4.9
4a	0.05 (0.01)	0.1 (0)	0.5 (0.1)	1.6 (0.04)	1.1 (0.2)	2.3 (0.03)	1.3 (0)	2.3 (0.05)
4b[d]	0.08 (0.01)	0.4 (0.03)	1.6 (0.2)	4.5 (0.3)	3.0 (0.6)	5.3 (0.04)	2.8 (0.04)	5.3 (0.1)
5a[d]	0.06 (0.04)	0.06 (0.01)	0.1 (0.01)	0.3 (0)	0.4 (0.1)	0.6 (0.02)	0.3 (0.01)	0.6 (0)
5b[d]	0.09 (0.01)	0.5 (0.05)	0.8 (0.02)	1.1 (0.04)	1.8 (0.2)	2.1 (0.05)	1.4 (0.02)	2.1 (0.05)
17	n.a.	7.4 (1.8)	36.8 (5.5)	35.0 (0.8)	28.3 (3.0)	22.6 (1.3)	20.2 (0.6)	20.7 (1.7)
18	n.a.	2.5 (0.7)	22.6 (9.3)	20.1 (0.2)	15.5 (0.5)	12.7 (2.0)	14.3 (0.2)	13.2 (1.0)
19	n.a.	67.3 (7.2)	69.2 (9.1)	64.6 (1.4)	74.0 (1.8)	44.9 (1.2)	62.0 (6.8)	47.4 (3.0)
20	n.a.	20.9 (3.8)	22.3 (2.5)	21.2 (6.0)	18.8 (2.3)	13.6 (2.0)	25.2 (4.4)	24.0 (2.3)
21	n.a.	84.4 (1.8)	81.9 (6.0)	73.5 (0.8)	90.9 (2.0)	54.7 (0.1)	76.5 (10.0)	66.1 (5.6)
22	n.a.	5.4 (0)	4.7 (0.6)	5.4 (0.5)	4.8 (0.05)	4.5 (0.1)	5.0 (0.5)	3.9 (0.3)
23	n.a.	1,96.5 (4.6)	119.3 (10.3)	103.2 (2.4)	146.8 (9.0)	84.2 (3.5)	88.5 (7.2)	69.8 (1.0)
24	n.a.	4.7 (1.3)	20.1 (2.2)	22.2 (1.4)	25.1 (0.2)	24.3 (1.5)	27.5 (11.2)	21.4 (0.6)
25	n.a.	12.5 (3.6)	113.7 (0.8)	113.5 (0.3)	90.3 (1.3)	90.6 (2.2)	107.0 (0.9)	99.8 (4.8)
26	n.a.	3.9 (1.1)	5.3 (0.6)	6.7 (0.8)	4.3 (0.8)	5.6 (0.7)	3.3 (0.7)	5.4 (0.3)
27	n.a.	n.d.	3.8 (0.7)	5.0 (1.1)	31.5 (6.2)	35.0 (26.1)	8.8 (1.0)	5.9 (1.9)
28	n.a.	202.1 (2.3)	144.3 (2.5)	135.8 (5.5)	139.1 (0.3)	119.6 (0.7)	201.9 (6.5)	167.7 (0)
29	n.a.	5.5 (0.2)	13.3 (0.8)	14.5 (2.6)	9.8 (0.8)	9.8 (2.6)	4.9 (0.1)	4.7 (0.1)
30	n.a.	176.7 (26.6)	1,438.6 (109)	1,386.2 (0.3)	1,266.2 (40.6)	1,273.6 (90.4)	1,322.6 (55.9)	1,226.5 (130.8)
31	n.a.	0.7 (0.1)	4.3 (0.3)	3.9 (0.1)	3.7 (0.2)	3.0 (0.2)	3.3 (0.8)	2.0 (0.1)
32	n.a.	7.6 (0.2)	22.5 (1.8)	32.8 (1.6)	26.0 (0.3)	35.5 (2.1)	27.1 (3.2)	47.1 (1.0)

[a] Results are given in milligrams per liter for 1–5 and in micrograms per liter for 17–32; n.a. = not analyzed and n.d. = not detectable.
[b] Numbering of compounds and chemical structures refer to Figures 1 and 3.
[c] The *cis*/*trans* ratio.
[d] Calculated as milligrams of tricyclocohumol (4a) per liter.

The quantitative analysis of compounds 6–7 and 11–16 was carried out only in beer samples stored for 12 months at 5 and 28°C for one brewing trial (Table X).

Due to the low amounts of *trans*-iso-α-acids in fresh beers, the concentrations of 4a-b and 5a-b were lower in samples with isomerized hop products (IPE and IKE) compared with beverages hopped with P90. The storage behavior of beer samples with IPI (Table IX) showed no significant difference compared with samples with P90 (Table VI), confirming the results of Schmidt et al (17) that there was no impact of inline pre-isomerized hop products on analytical markers for beer ageing (products 4a-b) compared with conventional hopping. The total amount of the tricyclic degradation products 4a and 4b in fresh beer samples varied between 0.37 mg/L in beer with IPE and 0.65 mg/L in beverages with IPI. During the ageing period of 12 months, the highest increase was observable for the beer samples with P90 (4.15–7.47 mg/L), comparable with the amounts of samples prepared with the IPI (3.93–7.52 mg/L) for both storage temperatures (Fig. 6). Beverages stored at higher temperature (28°C) resulted in higher amounts of the degradation products, with the exception of *cis*- and *trans*-hydroperoxy- as well as hydroxy-alloisohumulones (compounds 13–16). Moreover, the formation of compounds 13–16 was independent of the hop variations used in the brewing trial (Table X). *Cis*- and *trans*-alloisohumulones (compounds 11–12) were not detectable in the stored beverages (Table X). The described results regarding the degradation products of *trans*-iso-α-acids 4–7 and of *cis*- and *trans*-iso-α-acids 13–16 confirm the formation pathway of these substances, demonstrated in Figure 1, which was already demonstrated by Intelmann et al (11–14). However, whereas the decline of *trans*-iso-α-acids more or less corresponded to the total amount of their tri- and tetracyclic degradation products, the loss of *cis*-iso-α-acids was by far not compensated quantitatively. Not all of their degradation products seem to be identified.

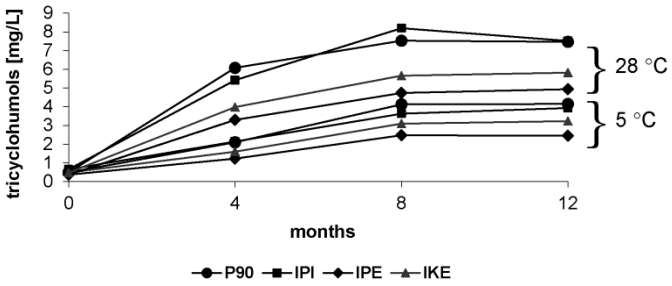

Fig. 6. Comparison of analytical markers tricyclocohumol (4a) and tricyclohumol (4b) in fresh beer samples as well as after storage for 4, 8, and 12 months at 5 and 28°C prepared with different hop products (P90 = Pellets 90, IPI = inline pre-isomerization, ethanol extract with Pellets 90, IPE = isomerized pellets, IKE = isomerized kettle extract).

TABLE VII
Quantitation Results (± Standard Deviation; *n* = 2) of Taste and Aroma Compounds (1–5 and 17–32) in Wort and Beer Samples, Fresh and Stored, for 4, 8, and 12 Months at 5 and 28°C for Isomerized Pellets

	Concentration in[a]							
			4 months		8 months		12 months	
Number[b]	Wort	Beer (Fresh)	5°C	28°C	5°C	28°C	5°C	28°C
1a-c	0.3 (0)	0.2 (0.1)	0.2 (0.1)	0.1 (0.04)	0.1 (0.04)	0.0 (0)	0.1 (0.1)	0.0 (0)
2a	12.8 (0.2)	11.3 (0)	11.1 (0)	10.4 (0.9)	10.5 (0.2)	9.3 (0.7)	9.9 (0.1)	9.2 (0.1)
2b	23.5 (0.1)	16.4 (0.2)	16.2 (0.04)	15.2 (1.5)	15.1 (0.4)	13.6 (1.2)	14.6 (0.1)	13.4 (0.2)
2c	5.0 (0.02)	3.5 (0.04)	3.5 (0.01)	3.2 (0.4)	3.1 (0.1)	2.9 (0.3)	2.9 (0.04)	2.7 (0.1)
Σ 2a-c	41.2	31.3	30.7	28.8	28.7	25.8	27.4	25.2
3a	2.6 (0.03)	2.3 (0)	2.1 (0)	1.5 (0.1)	1.7 (0.1)	0.9 (0.1)	1.6 (0.1)	0.7 (0.1)
3b	5.7 (0.03)	3.9 (0)	3.5 (0.04	2.5 (0.2)	2.8 (0.2)	1.6 (0.1)	2.4 (0.04)	1.0 (0.1)
3c	1.0 (0.07)	0.7 (0.02)	0.7 (0)	0.5 (0.1)	0.5 (0.04)	0.3 (0.04)	0.5 (0)	0.2 (0)
Σ 3a-c	9.3	6.9	6.3	4.4	4.9	2.8	4.6	1.9
2a-c/3a-c[c]	4.4	4.5	4.9	6.5	5.9	9.3	6.0	13.7
4a	0.03 (0)	0.09 (0)	0.3 (0.02)	0.7 (0.04)	0.6 (0.06)	1.3 (0.2)	0.7 (0.03)	1.5 (0.1)
4b[d]	0.06 (0)	0.3 (0.02)	1.0 (0.1)	2.6 (0.1)	1.9 (0.1)	3.5 (0.4)	1.8 (0.1)	3.4 (0.2)
5a[d]	0.07 (0)	0.02 (0)	0.05 (0.01)	0.1 (0)	0.2 (0.01)	0.4 (0.1)	0.1 (0.01)	0.3 (0.02)
5b[d]	0.06 (0)	0.08 (0)	0.1 (0)	0.3 (0.01)	0.5 (0.01)	1.5 (0.7)	0.3 (0)	0.6 (0.04)
17	n.a.	8.4 (3.0)	33.6 (6.1)	35.9 (3.7)	21.3 (2.6)	21.9 (0.3)	20.7 (0.6)	22.3 (1.2)
18	n.a.	2.6 (0.5)	20.0 (9.8)	21.3 (3.7)	10.0 (0.9)	10.6 (0.4)	13.9 (0.4)	14.9 (0.7)
19	n.a.	67.3 (1.0)	67.8 (0.4)	62.0 (0.2)	44.8 (0.6)	41.1 (1.3)	61.4 (10.3)	50.4 (0.1)
20	n.a.	21.0 (0.1)	20.9 (3.8)	17.3 (1.7)	36.3 (0)	24.6 (0.2)	28.0 (4.1)	21.0 (1.5)
21	n.a.	83.6 (1.3)	76.6 (3.7)	70.6 (0.4)	54.8 (0.6)	51.3 (0.4)	74.6 (8.3)	61.5 (1.5)
22	n.a.	6.0 (0.4)	4.7 (0.6)	6.3 (0.5)	4.0 (0.2)	4.7 (0.2)	4.8 (0.7)	4.1 (0.4)
23	n.a.	203.1 (3.0)	104.5 (2.7)	99.5 (1.7)	94.2 (0.5)	82.4 (0.9)	87.5 (7.8)	62.3 (0.7)
24	n.a.	6.2 (2.3)	20.1 (1.7)	25.3 (1.2)	19.6 (0.4)	20.6 (0.2)	33.0 (10.2)	18.0 (0.1)
25	n.a.	22.0 (10.2)	114.1 (4.1)	116.9 (1.6)	80.8 (2.4)	88.9 (0.9)	109.6 (0.4)	133.0 (24.8)
26	n.a.	2.8 (0.1)	5.3 (0)	7.1 (0.1)	3.6 (0.1)	5.9 (0.2)	3.7 (0.5)	6.1 (0.5)
27	n.a.	n.d.	3.7 (0.7)	5.7 (2.1)	13.3 (4.3)	23.4 (3.4)	8.7 (3.0)	3.9 (0.2)
28	n.a.	209.4 (4.7)	139.5 (5.1)	127.2 (2.5)	132.1 (3.3)	112.0 (1.7)	183.2 (0.7)	155.8 (0)
29	n.a.	6.2 (0.4)	12.9 (0.3)	15.9 (2.7)	10.5 (1.5)	10.8 (2.5)	5.7 (0.5)	6.2 (0.3)
30	n.a.	239.1 (53.2)	1,439.4 (83.5)	1,495.6 (38.6)	1,253.9 (95.3)	1,303.4 (49.8)	1,352.3 (31.5)	1,277.8 (76.2)
31	n.a.	0.8 (0.3)	4.2 (0.5)	4.8 (0.4)	3.1 (0.1)	3.2 (0.2)	3.4 (0.3)	2.5 (0)
32	n.a.	8.4 (0.1)	20.6 (2.3)	35.0 (0.1)	18.0 (0.2)	32.7 (1.1)	24.5 (3.7)	49.8 (4.9)

[a] Results are given in milligrams per liter for 1–5 and in micrograms per liter for 17–32; n.a. = not analyzed and n.d. = not detectable.
[b] Numbering of compounds and chemical structures refer to Figures 1 and 3.
[c] The *cis*/*trans* ratio.
[d] Calculated as milligrams of tricyclocohumol (4a) per liter.

GC-MS/MS Analysis of Volatile Compounds in Beer

In addition to analysis of key hop bitter taste compounds described above, the quantitation of 15 key aroma components was performed by means of GC-MS/MS. The results received from beer samples, fresh and after storage of 4, 8, and 12 months at two different temperatures, are given in Tables VI–IX.

A maximum increase of the strecker aldehydes was observable during the first four months of storage. Further storage of beers for 8 and finally for 12 months leads to a decline in the concentrations of the staling aldehydes. The results are independent of the hop product used. In the case of 3-methylbutanal (compound 17; Fig. 7), higher amounts were observable at higher storage temperature but there was no significant difference between the type of the hop product. Among all aroma compounds, the highest concentrations were found for 2-methoxy-4-vinylphenol (compound 30) between 1,322.5 µg/L (4 months, 28°C; Table VIII) in beverages with IKE and 1,495.6 µg/L (4 months, 28°C; Table VII) in beer samples with IPE. However, the formation of the aroma components (compounds 17–32) during storage was not influenced by the hop product tested. These findings are in agreement with the work of De Clippeleer et al (3). They pointed out that the formation of staling aldehydes is not linked with the *trans*-specific degradation of iso-α-acids.

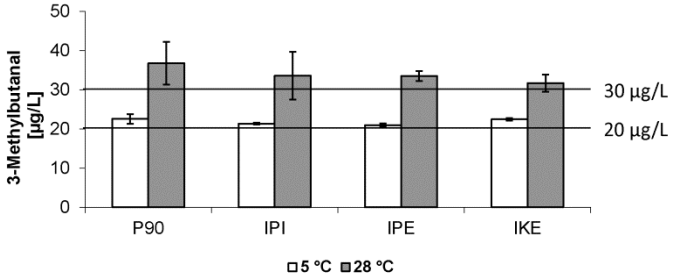

Fig. 7. Concentrations of 3-methylbutanal (compound 17) in beer with different hop products stored for four months at 5 and 28°C. Error bars demonstrate the standard deviation. (P90 = Pellets 90, IPI = inline pre-isomerization, ethanol extract with Pellets 90, IPE = isomerized pellets, IKE = isomerized kettle extract).

Sensory Evaluations of Fresh and Stored Beer Samples

Supplementary to the evaluations by means of HPLC and GC, the beer samples were analyzed by a trained sensory panel comparing the attributes bitterness intensity, bitterness aftertaste, and beer ageing of fresh beers of the four variants as well as stored beers for two and four months at 28°C with those of a reference beer which was always a fresh Pilsener type beer. Results of the sensory evaluations are shown in Figure 8.

On a scale from 0 (not detectable) to 9 (strong perception), the tasters ranged the bitterness intensity in fresh beer samples between 5.8 in samples with P90 and 6.0 in beverages hopped with IPE (Fig. 8). The decline of bitterness intensity as described in literature (4,11) was not confirmed in our study for beers stored for two and four months at 28°C.

TABLE VIII
Quantitation Results (± Standard Deviation; $n = 2$) of Taste and Aroma Compounds (1–5 and 17–32) in Wort and Beer Samples, Fresh and Stored, for 4, 8, and 12 Months at 5 and 28°C for Isomerized Kettle Extract

	Concentration in[a]							
			4 months		8 months		12 months	
Number[b]	Wort	Beer (Fresh)	5°C	28°C	5°C	28°C	5°C	28°C
1a-c	0.3 (0)	0.2 (0.05)	0.1 (0)	0.1 (0)	0.0 (0)	0.0 (0)	0.1 (0.05)	0.0 (0)
2a	12.4 (0.2)	10.7 (0.2)	10.7 (0.4)	9.9 (0.3)	10.0 (0.02)	9.3 (0.2)	9.5 (0.4)	8.9 (0)
2b	22.4 (0.5)	15.2 (0.9)	15.3 (1.2)	14.1 (1.1)	14.0 (0.4)	13.1 (0.1)	13.6 (0.9)	12.7 (0.5)
2c	5.0 (0.2)	3.4 (0.2)	3.4 (0.2)	3.1 (0.2)	3.0 (0.1)	2.9 (0)	2.8 (0.2)	2.5 (0.1)
Σ 2a-c	39.8	29.3	29.4	27.1	26.9	25.3	25.9	24.1
3a	2.9 (0)	2.5 (0.1)	2.3 (0.1)	1.5 (0.04)	1.8 (0.1)	1.0 (0.1)	1.5 (0.03)	0.6 (0.1)
3b	6.2 (0.1)	4.0 (0.3)	3.5 (0.3)	2.4 (0.1)	2.8 (0.04)	1.4 (0.2)	2.4 (0.2)	0.9 (0.02)
3c	1.2 (0.1)	0.8 (0.1)	0.7 (0)	0.5 (0)	0.5 (0.03)	0.3 (0.1)	0.5 (0.03)	0.1 (0)
Σ 3a-c	10.3	7.3	6.6	4.4	5.1	2.6	4.3	1.6
2a-c/3a-c[c]	3.9	4.0	4.5	6.2	5.3	9.7	6.0	15.6
4a	0.05 (0)	0.1 (0.02)	0.4 (0.02)	0.9 (0.1)	0.8 (0.2)	1.5 (0.3)	1.0 (0.2)	1.7 (0.1)
4b[d]	0.1 (0)	0.4 (0.1)	1.2 (0.1)	3.1 (0.6)	2.3 (0.02)	4.2 (0.6)	2.2 (0.3)	4.2 (0.5)
5a[d]	0.07 (0)	0.05 (0.02)	0.1 (0)	0.2 (0.02)	0.4 (0.02)	0.5 (0.1)	0.2 (0.02)	0.4 (0.03)
5b[d]	0.2 (0)	0.1 (0.03)	0.2 (0.02)	0.4 (0.04)	0.9 (0)	1.2 (0.2)	0.5 (0.1)	0.8 (0.1)
17	n.a.	7.2 (2.0)	33.5 (1.3)	37.4 (0.9)	21.2 (1.0)	21.0 (0.4)	17.4 (0)	23.3 (0.1)
18	n.a.	2.3 (0.3)	21.5 (5.1)	22.1 (0.2)	9.9 (0.2)	9.7 (0.2)	13.4 (0.6)	16.6 (0.1)
19	n.a.	66.3 (7.8)	57.4 (2.5)	58.6 (1.8)	43.2 (0.5)	39.3 (1.7)	59.4 (10.7)	51.9 (1.6)
20	n.a.	17.5 (3.9)	18.4 (0.9)	18.3 (2.3)	37.9 (4.0)	23.8 (1.7)	21.0 (5.4)	22.3 (3.5)
21	n.a.	76.2 (1.0)	68.8 (3.5)	65.4 (2.8)	48.7 (0.3)	48.0 (1.7)	68.2 (7.3)	57.6 (1.6)
22	n.a.	5.5 (0.2)	4.7 (0.7)	5.9 (0.8)	3.5 (0.3)	4.4 (0.6)	5.7 (0.1)	3.3 (0.2)
23	n.a.	192.5 (15.1)	103.2 (2.9)	97.4 (3.9)	89.7 (1.1)	76.2 (1.3)	81.6 (1.1)	61.1 (1.5)
24	n.a.	5.1 (0.9)	19.6 (3.7)	24.3 (2.2)	17.4 (0.2)	20.6 (1.8)	37.2 (6.6)	22.4 (4.7)
25	n.a.	10.8 (3.5)	111.5 (2.4)	112.5 (2.1)	75.0 (1.5)	86.2 (2.3)	108.6 (2.4)	102.4 (1.5)
26	n.a.	2.2 (0.2)	3.9 (0.3)	4.9 (0.2)	2.7 (0.2)	4.2 (0.1)	2.8 (0.4)	3.6 (0.2)
27	n.a.	n.d.	4.4 (1.2)	6.8 (1.3)	13.0 (3.5)	10.2 (1.1)	7.7 (3.8)	3.7 (0)
28	n.a.	199.9 (23.0)	126.1 (12.7)	115.1 (4.5)	124.9 (2.0)	100.5 (1.6)	179.0 (5.4)	139.4 (3.7)
29	n.a.	5.8 (0.9)	12.4 (1.8)	11.6 (0.9)	8.7 (0.4)	9.7 (1.6)	6.1 (0.6)	7.5 (1.6)
30	n.a.	157.8 (31.9)	1,319.2 (51.4)	1,322.5 (2.9)	1,128.7 (29.8)	1,210.8 (8.7)	1,252.4 (27.5)	1,159.1 (22.7)
31	n.a.	1.1 (0.4)	3.9 (0.6)	4.2 (0.3)	3.4 (0.2)	3.2 (0.4)	4.1 (0.2)	3.4 (1.0)
32	n.a.	7.9 (0.8)	18.5 (5.6)	32.6 (1.9)	16.7 (0.6)	31.1 (1.6)	27.8 (3.5)	58.6 (3.7)

[a] Results are given in milligrams per liter for 1–5 and in micrograms per liter for 17–32; n.a. = not analyzed and n.d. = not detectable.
[b] Numbering of compounds and chemical structures refer to Figures 1 and 3.
[c] The *cis/trans* ratio.
[d] Calculated as milligrams of tricyclocohumol (4a) per liter.

The results for bitterness aftertaste, also shown in Figure 8, demonstrated a slightly higher amount for the fresh beers with IPI compared with the other three beers. During the storage time, bitterness aftertaste was slightly increased for beer samples with IPE and remained unchanged in beers with IPI (Fig. 8).

Ageing flavor does not play a role in the fresh beer samples prepared with the different types of hop products. The ageing behavior showed the same pattern for two and four months of storage. The ageing flavor was evaluated with the highest amount in beverages with IPE, followed by beers with P90. Lower amounts of ageing were detected for beers prepared with IKE and beers with IPI (Fig. 8) but the differences were not significant.

In this study, the sensory evaluation of aged beers gave no significant preference for the variations higher in *cis*/*trans* ratios of iso-α-acids. The ageing flavor as well as bitterness intensity or quality was not influenced by the type of hop product.

CONCLUSIONS

The described experiments confirmed that specific transformation products of *trans*-iso-α-acids are suitable markers for beer ageing. The use of IPI showed no influence on the *cis*/*trans* ratio and, thus, on the stability of iso-α-acids as compared with conventional hopping. The strecker aldehydes and other aroma compounds are not influenced by the type of the hop product. It could be shown that the *cis*/*trans* ratio of iso-α-acids in the beverage can be raised with isomerized hop pellets or isomerized hop extract but higher *cis*/*trans* ratio in a fresh beer sample does not

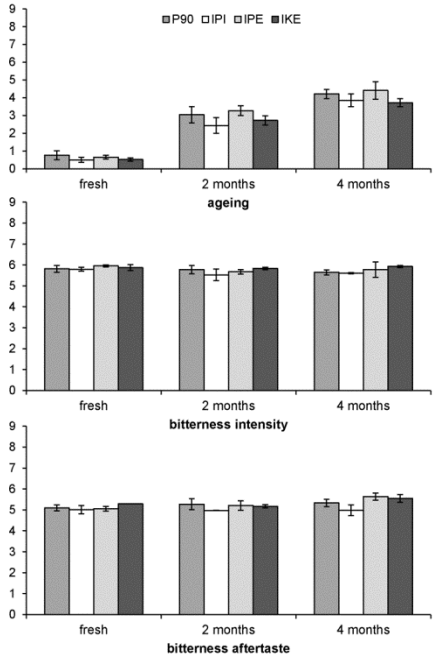

Fig. 8. Sensory evaluations of fresh and stored (two and four months, 28°C) beer samples for the sensory parameters ageing, bitterness intensity, and bitterness aftertaste (P90 = Pellets 90, IPI = inline pre-isomerization, ethanol extract with Pellets 90, IPE = isomerized pellets, IKE = isomerized kettle extract).

TABLE IX
Quantitation Results (± Standard Deviation; *n* = 2) of Taste and Aroma Compounds (1–5 and 17–32) in Wort and Beer Samples, Fresh and Stored, for 4, 8, and 12 Months at 5 and 28°C for Ethanol Extract[a]

	Concentration in[b]							
			4 months		8 months		12 months	
Number[c]	Wort	Beer (Fresh)	5°C	28°C	5°C	28°C	5°C	28°C
1a-c	8.0 (0.1)	0.5 (0.05)	0.4 (0)	0.2 (0)	0.2 (0)	0.1 (0.05)	0.3 (0)	0.1 (0.05)
2a	8.6 (0.1)	7.5 (0.4)	7.7 (0.2)	7.1 (0.2)	7.1 (0.2)	6.4 (0.4)	6.5 (0)	6.2 (0.1)
2b	15.3 (0.3)	11.2 (0.9)	11.4 (0.8)	10.7 (0.7)	10.1 (0.8)	9.3 (0.9)	9.8 (0.5)	9.4 (0.5)
2c	3.9 (0.1)	2.9 (0.2)	3.0 (0.2)	2.9 (0.2)	2.7 (0.2)	2.6 (0.3)	1.9 (0.1)	2.0 (0.04)
Σ 2a-c	27.7	21.7	22.1	20.7	19.9	18.2	18.2	17.6
3a	3.8 (0.04)	3.3 (0.2)	3.0 (0.1)	2.0 (0.1)	2.4 (0.1)	1.1 (0.05)	2.3 (0)	0.7 (0.03)
3b	7.4 (0.1)	5.1 (0.6)	4.7 (0.4)	3.2 (0.3)	3.9 (0.3)	1.9 (0.2)	3.0 (0.2)	1.0 (0.05)
3c	1.8 (0.04)	1.3 (0.1)	1.2 (0.1)	0.9 (0.1)	0.9 (0.1)	0.5 (0.1)	0.8 (0)	0.3 (0)
Σ 3a-c	13.0	9.8	9.0	6.0	7.2	3.5	6.0	2.0
2a-c/3a-c[d]	2.1	2.2	2.5	3.5	2.8	5.2	3.0	8.9
4a	0.07 (0)	0.2 (0.02)	0.5 (0.02)	1.3 (0.1)	1.0 (0)	2.4 (0.1)	1.3 (0)	2.3 (0.1)
4b[e]	0.1 (0)	0.5 (0.04)	1.7 (0.1)	4.2 (0.2)	2.6 (0.1)	5.8 (0.5)	2.7 (0.1)	5.2 (0.5)
5a[e]	0.06 (0.04)	0.1 (0)	0.2 (0)	0.3 (0)	0.4 (0)	0.8 (0)	0.3 (0.02)	0.5 (0.02)
5b[e]	0.05 (0)	0.6 (0)	0.8 (0)	1.0 (0.04)	2.0 (0.03)	2.5 (0.1)	1.5 (0.1)	1.4 (0.1)
17	n.a.	8.4 (3.2)	31.7 (2.2)	36.3 (1.7)	18.3 (2.6)	22.5 (0.4)	18.0 (0.4)	21.3 (1.1)
18	n.a.	2.7 (0.8)	17.0 (4.4)	19.7 (1.0)	9.2 (0.4)	12.6 (1.8)	14.0 (0)	15.3 (1.3)
19	n.a.	70.6 (2.7)	59.0 (1.9)	61.1 (0.3)	43.2 (1.5)	46.3 (1.1)	58.4 (11.3)	49.0 (0.8)
20	n.a.	21.8 (2.7)	20.0 (0.2)	19.9 (1.4)	28.5 (5.1)	14.5 (1.2)	26.5 (5.9)	22.8 (4.1)
21	n.a.	86.3 (2.9)	72.3 (1.7)	70.7 (1.4)	56.5 (1.4)	54.5 (1.1)	74.1 (8.7)	61.2 (0.6)
22	n.a.	5.8 (0.3)	4.8 (0.5)	6.6 (0.4)	3.4 (0.2)	4.8 (0.1)	5.2 (0.8)	5.2 (2.2)
23	n.a.	191.5 (5.1)	105.0 (1.7)	103.9 (7.0)	95.2 (8.4)	81.4 (3.5)	89.4 (1.6)	63.6 (2.7)
24	n.a.	5.1 (2.0)	20.1 (2.2)	27.9 (1.3)	17.8 (1.0)	22.8 (0.1)	32.4 (3.9)	19.8 (5.2)
25	n.a.	11.0 (4.0)	109.4 (2.2)	121.5 (2.5)	85.5 (0.2)	93.4 (0.2)	109.1 (3.6)	160.5 (31.1)
26	n.a.	2.8 (0.3)	4.9 (0.2)	6.1 (0.3)	3.6 (0.3)	4.9 (0.3)	3.6 (0.1)	3.3 (0.1)
27	n.a.	n.d.	4.3 (0)	9.5 (0.5)	15.2 (5.1)	37.7 (29.0)	8.7 (0.5)	4.2 (0.1)
28	n.a.	186.6 (17.6)	137.7 (7.8)	125.6 (8.9)	133.6 (1.2)	107.9 (2.4)	194.0 (17.0)	156.6 (0.7)
29	n.a.	4.8 (0.5)	13.1 (0.6)	12.9 (0.1)	7.9 (0.7)	9.4 (0.2)	5.0 (0.6)	4.7 (0.3)
30	n.a.	148.6 (37.1)	1,276.7 (38.6)	1,337.8 (35.1)	1,145.5 (7.4)	1,168.0 (4.3)	1,190.4 (9.6)	1,127.2 (24.6)
31	n.a.	0.6 (0.2)	4.2 (0.3)	4.6 (0)	3.4 (0)	3.2 (0.3)	4.3 (0.5)	2.9 (1.2)
32	n.a.	6.7 (0.3)	19.9 (2.8)	38.0 (1.1)	15.2 (0.5)	34.3 (1.0)	26.0 (0.2)	50.9 (1.2)

[a] Inline pre-isomerization together with Pellets Type 90.
[b] Results are given in milligrams per liter for 1–5 and in micrograms per liter for 17–32; n.a. = not analyzed and n.d. = not detectable.
[c] Numbering of compounds and chemical structures refer to Figures 1 and 3.
[d] The *cis*/*trans* ratio.
[e] Calculated as milligrams of tricyclocohumol (4a) per liter.

TABLE X
Quantitation Results of Taste Compounds (6–7 and 11–16) in Beer Samples Stored for 12 months at 5 and 28°C

	Concentration in[a]							
	P90		IPE		IKE		IPI	
Number[b]	5°C	28°C	5°C	28°C	5°C	28°C	5°C	28°C
6a	0.09	0.28	0.06	0.16	0.06	0.20	0.07	0.27
6b	0.13	0.3	0.09	0.23	0.11	0.19	0.14	0.32
7a	0.43	0.74	0.26	0.58	0.29	0.58	0.38	0.69
7b	0.83	1.37	0.57	1.11	0.64	1.05	0.85	1.34
Σ 11a-12a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ 11b-12b	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ 13a-14a	0.03	0.02	0.03	0.02	0.02	0.02	0.03	0.02
Σ 13b-14b	0.04	0.02	0.05	0.03	0.04	0.02	0.02	0.02
Σ 15a-16a	0.19	0.22	0.15	0.18	0.13	0.16	0.10	0.13
Σ 15b-16b	0.44	0.61	0.34	0.49	0.31	0.33	0.24	0.36

[a] P90 = Pellets Type 90; IPE = isomerized pellets; IKE = isomerized kettle extract; and IPI = inline pre-isomerization, ethanol extract with pellets. Results are given in milligrams per liter for one brewing trial; n.d. = not detectable.
[b] Numbering of compounds and chemical structures refer to Figure 1.

result in improved flavor and bitter taste stability. The impact of unknown degradation processes of *cis*-iso-α-acids to the taste stability of aged beverages needs further investigation in the future.

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