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Changes in bitterness as beer ages naturally

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Abstract

In order to ascertain changes in bitterness as beer aged naturally, time–intensity (TI) measurements were made by a professional sensory panel using a squeezer transducer system. TI parameters were fitted by variance components models using Restricted Maximum Likelihood (REML). It was shown that bitterness decreased with age, although this age-induced difference was smaller than the original differences among the eight fresh (commercial lager) beer types which spanned about 10 European Bitterness Units (EBU). Intensity-related parameters obtained from the TI curves were more important than time-related parameters for differentiating samples. Perceived bitterness could not be explained entirely by concentrations of five iso-α-acid peaks as measured by High Performance Liquid Chromatography (HPLC). Analytical and sensory data were correlated using Partial Least Squares (PLS2) and by fitting iso-α-acid peak areas in the REML models.

Keywords: Naturally-aged beer; Bitterness; Time–intensity; Iso-α-acids

1. Introduction

The shelf-life of beer is largely determined by its colloidal stability, although flavor changes may develop prior to any haze formation. Off-flavors due to beer staling are as varied as the beer flavors themselves. Bitterness, however, is one aspect of flavor common to all beers, albeit to different degrees. Bitterness in beer arises when the α-acids in hops are isomerized during boiling with wort (Hough, Briggs, Stevens, & Young, 1982). The formation of iso-α-acids or isohumulones has been widely reported (Keukeleire, de Vindevogel, Szücs, & Sandra, 1992; McMurrough & Byrne, 1992).

It has been claimed that beer bitterness decreases with age while sweetness increases, thus shifting the balance towards a sweeter flavor for all beer types (Dalgliesh, 1977). According to Pangborn, Lewis, and Tanno, (1977), this decrease in bitterness is accompanied by a change in its nature: fresh-bitterness is related to analytically-determined isohumulone concentrations expressed in bitter units whereas aged-bitterness is not. These authors reported no effect of storage temperature on sensory bitterness whereas bitter units decreased as a function of both time and temperature of beer storage. (For a discussion of bitter units see McMurrough & Byrne, 1992, pp. 601–602).

Isohumulones can be subject to oxidation during beer aging. Kaneda, Kano, Koshino, and Ohya-Nishiguchi, (1992) showed that the isohumulone content of beer decreased during forced aging for 10 days at 37°C. This decrease was explained in light of free radical reactions studied earlier by the same authors in connection with beer staling: oxidative degradation of isohumulones was accelerated by iron ions, hydrogen peroxide and combinations thereof. In the paper by Pangborn et al., (1977) bitter units were shown to decrease more when beer was stored under an oxygen headspace than when carbon dioxide was used. Sensory bitterness, on the other hand, was unaffected by an oxygen headspace, but a bitter aftertaste developed when beer bottles packed under carbon dioxide were stored.

Walters, Heasman, and Hughes, (1997) measured a 71% decrease in total iso-α-acids (from 15.3 to 4.5 mg/L) when the beer they studied was stored 156 days at 40°C. The same beer stored 220 days at 0 or 25°C showed no loss of total iso-α-acids. A concentration of iso-α-acids 20 mg/L or less is usually considered as not bitter whereas very bitter beers could contain upwards of 40 mg/L (Crombecq, 1995). Collin, Derdelinckx, and Dufour, (1994) prepared lager beer without hops and then added diastereoisomeric mixtures of either

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isohumulone/isoadhumulone or isocohumulone to give beers ranging from 0 to 40 EBU (European Bitterness Units). Preferred bitterness as indicated by their panel was in the range 17.5–25 EBU.

Time–intensity measurements are made in the hope of discovering temporal aspects of flavor perception that would be lost in such point-measurements as profiling by traditional scaling. Cepicka, Strejcek, and Pokorny, (1992) used a category scale to make measurements of beer bitterness at several defined moments in time: when a sample was taken into the mouth, when it was swallowed, and every 10 s thereafter for a period of 90 s. Their sample set consisted of 44 types of beer (13–25 mg/L isocompounds) ranging from pale (various original gravities) to dark, including low alcoholic beers. Although their principal component analysis indicated that the 30–50 s region after swallowing was more important for an impression of total bitterness than the initial evaluations, they were not able to distinguish any distinct separation of beer types except between those fermented to very low or very high residual sugar content.

Pangborn, Lewis, and Yamashita, (1983) compared a chart-recorder TI method to category scaling and found similar concentration-dependent bitterness for solutions of iso-α-acids in water and Natural Light lager beer. Hughes and Simpson, (1994) evaluated the bitterness of iso-α-acids separated by preparative HPLC. Their computerized-TI measurements showed no differences among these acids when they were evaluated in sodium phosphate buffer pH 4.15 containing 0.05% (v/v) ethanol.

The current work was undertaken to study age-related changes in iso-α-acids for eight different types of beer subjected to natural aging. The samples were commercial lagers covering a range in bitterness of about 9–19 EBU based on previous measurements (King, 1995). The changes in iso-α-acids were correlated to sensory bitterness as measured by the time–intensity technique. A major goal of the data processing was to put age-related changes in perspective to other sources of bitterness variation measured.

2. Materials and methods

2.1. Samples

Eight different types of beer (coded A–H followed by a number related to sample age) were obtained locally and evaluated in 22 sessions over a period of 5 months as given by the scheme shown in Table 1. The following samples were of the same batch of beer according to labeling: A4 and A5, B4 and B5, C2 and C3. This means that for all intents and purposes aging effects are confounded with batch effects. Beer was naturally aged in a dark basement storeroom at $19 \pm 2°C$.

For the time–intensity tests, 15 ml of beer was served in plastic cups coded with 3-digit numbers. At the time of evaluation, the temperature of this volume of beer was $13 \pm 2°C$.

2.2. Sensory measurements

A paid, professional panel consisting of 16 women, 41–59 years old, was used in these experiments. They all had at least 2 years of experience in making time–intensity evaluations of beer bitterness and, on the average, 10 years of service on the Quest Sensory Research Panel. Time–intensity measurements were made by using a special transducer system: a squeezable handgrip that transmits hand force via a strain gauge to an amplifier. The device, the method for using it and panel training in this technique have been described previously (King & Moreau, 1996). For experiments discussed in this paper, panelists were instructed to empty the contents of the cup in their mouths, click the squeezer on and begin evaluating bitterness, then count to five before swallowing and simultaneously pressing the computer space bar. Panelists pressed the space bar again whenever they swallowed during the 121 s of measurement.

A neutral beer was served at the beginning of each session as a palate cleanser. Crackers and water were consumed between measurements. Panelists made no more than four evaluations per session and no less than two repetitions of each sample (most panelists made all three repetitions) according to the scheme shown in Table 1. Sessions 1–3, 4–6 and 7–9 spanned 7 days each. Sessions 10–12 were carried out within 6 days. Sessions 13–22 required 26 days for completion.

2.3. Instrumental measurements

Iso-α-acids in all beer samples were analyzed by HPLC (Hewlett–Packard 1090 M) using a diode array detector (270 nm, bandwidth 4 nm) with a Hypersil ODS 5 column 100×2.1 mm id. An isocratic solvent system phosphoric acid–acetonitrile–magnesium chloride–water (20:500:6:500 w/w) was employed at a flow rate of 1 ml min^{-1}.

Five peaks were measured in triplicate for the following components, in order of elution: *trans*-isocohumulone, *cis*-isocohumulone, *trans*-isohumulone, *cis*-isohumulone + *trans*-isoadhumulone, *cis*-isoadhumulone.

2.4. Data processing

Time–intensity data were collected per sample/session/panelist as a matrix of squeezer intensity units by 1102 squeezer sampling time indices. (Squeezer software records 9.1 measurements per s as determined by the internal computer clock.) The raw data were filtered in order to remove digitation noise while keeping all other

Table 1
Experimental design for TI evaluation of eight beer types (A–H)

Sample Code	Age[a]	Session number																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	16	17	18	19	20	21	22
A1	−4	1	1	1																		
A2	−2				1	1	1															
A3	2	1	1	1																		
A4	5				1	1	1															
A5	7	1	1	1																		
A6	11	1	1	1																		
B1	−5							1	1	1												
B2	−4				1	1	1															
B3	−1							1	1	1												
B4	2				1	1	1															
B5	3							1	1	1												
B6	7							1	1	1												
C1	−4										1	1	1									
C2	−3													1	1	1						
C3	−1										1	1	1									
C4	4													1	1	1						
D1	−4														1		1	1				
D2	3														1		1	1				
E1	−5													1					1			1
E2	3													1					1			1
F1	−6																1		1	1		
F2	1																1		1	1		
G1	−3																	1			1	1
G2	4																	1			1	1
H1	−4															1				1	1	
H2	3															1				1	1	

[a] Difference between evaluation date and best before date in months.

features intact (see Appendix A). Six parameters were extracted from the filtered data via proprietary software in S-PLUS (1995 Windows version 3.3, release 1: Mathsoft Inc. Seattle, WA) as shown in Fig. 1 and defined thereunder. In order to stabilize the variance, logarithmic transformations of the parameters (except T1/2) were used in all calculations.

All variables were considered to be random in the REML models used to calculate variance percentages. Negative variance components were dropped. The variances calculated were converted to percentages by choosing an appropriate multiplication factor so that the sum of components was equal to 100%. Genstat 5 (1996 PC/Windows 95, release 3.2: Lawes Agricultural Trust, IACR Rothamsted, UK) was used to perform all the statistical analyses.

The REML model for a given time-intensity parameter comprised the sum of terms for the following effects: beer + age(beer) + panelist + panelist * beer + age(panelist * beer) + session(panelist) + order(panelist) + error where age is nested in beer, session is nested in panelist, etc. The order(panelist) effect was shown to be negligible, and this term was therefore dropped for all the calculations discussed in this paper. The error terms were calculated for the full 6-term model:

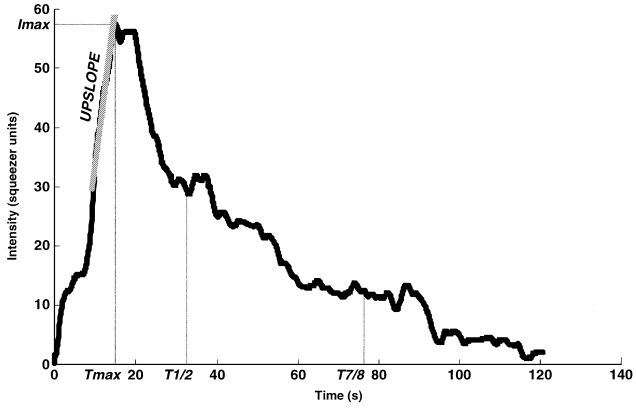

Fig. 1. A time–intensity curve showing filtered data and parameters used in calculations: AREA, area under filtered curve calculated from baseline zero; T1/2, point on time axis from which a vertical line would separate the area under the filtered curve into two equal portions; T7/8, point on time axis from which a vertical line would separate the area under the filtered curve into two portions such that 7/8 of the total area is to the left and 1/8 to the right; Tmax, average time at which intensity of filtered curve attains a maximum peak/plateau; Imax, maximum intensity of filtered curve at peak/plateau; UPSLOPE, slope of line drawn along last 10 points of filtered curve before attaining 97.5% of the maximum peak/plateau value.

$$\text{TI parameter} = \text{panelist} + \text{beer} + \text{age (beer)} + \text{panelist} * \text{beer} + \text{age (panelist} * \text{beer)} + \text{session (panelist)} + \text{error} \quad (1)$$

A simpler REML model was used to analyze HPLC data because samples were measured on fewer days, thus confounding a potential 'session' effect with an age(beer) effect. The model for a given log peak area was thus:

$$\text{log peak area} = \text{beer} + \text{age (beer)} + \text{error} \quad (2)$$

Principal Component Analysis (PCA) was carried out on the correlation matrix (26 beers, six time–intensity parameters) for sensory data and on the covariance matrix (26 beers, five HPLC log peak areas) for the chemical data. Mean values for these and other analyses were determined from one-way ANOVA (chemical data) and from the REML analyses (sensory data) using model (3) where beer was a fixed effect and panelist, panelist * beer were random effects.

$$\text{TI parameter} = \text{panelist} + \text{beer} + \text{panelist} * \text{beer} + \text{error} \quad (3)$$

Correlation of HPLC measurements with time–intensity data was carried out in different ways: two procedures were based on variance component models, and a third procedure made use of PLS2.

In the first procedure mean log HPLC peak areas were treated as a fixed covariate term despite the fact that the peak areas are measurements subject to error. Both panelist and session(panelist) were considered as random effects. The model, taking only one peak area at a time, becomes:

$$\text{TI parameter} = \text{log peak area} + \text{panelist} + \text{session (panelist)} + \text{error} \quad (4)$$

In an attempt to incorporate the contribution from more iso-α-acids simultaneously, a second variance component procedure [referred to as model (4a)] employed loadings from one, two or three PCA components of the HPLC data instead of log peak area in model (4). This model was further expanded by including terms for beer and panelist * beer in order to put more emphasis on aging effects.

The third procedure made use of a PLS2 model in which the Y-block consisted of the sensory data (26 beers, six time–intensity parameters) and the X-block comprised the chemical data (26 beers, five HPLC peak areas) with mean values obtained as described for PCA.

3. Results and discussion

3.1. Sensory data

The choice of parameters extracted from time–intensity curves is somewhat arbitrary, and the nature of these curves implies correlation of many commonly-used parameters. With the exception of Tmax, which was significantly correlated (5% level) only with AREA and T1/2, all other parameters used in this paper were highly correlated with each other as can be seen in Table 2. A PCA of the sensory data (Fig. 2) explained 89.5% of the variance in two dimensions. The less bitter beer types B and F are clearly separated from the other beer types along the first dimension whereas batch/age differences are more associated with the second dimension.

Table 3 indicates that most of the variation in the TI parameters can be attributed to panelists. This is typical for time–intensity data (Pangborn et al., 1983). Model (1) is shown to fit the data very well for parameters such as AREA and Imax which have only a small percent of unexplained variance (error). The percentage error is very large for the other four parameters.

Table 2
Pearson correlation coefficients between TI parameters

TI parameter	Log AREA	Log Imax	T1/2	Log T7/8	Log Tmax	Log UPSLOPE
Log AREA	1	0.95*	0.87*	0.93*	0.42*	0.51*
Log Imax	0.95*	1	0.71*	0.83*	0.29	0.56*
T1/2	0.87*	0.71*	1	0.94*	0.47*	0.46*
Log T7/8	0.93*	0.83*	0.94*	1	0.34	0.57*
Log Tmax	0.42*	0.29	0.47*	0.34	1	−0.22
Log UPSLOPE	0.51*	0.56*	0.46*	0.57*	−0.22	1

* Values significant at $p < 0.05$ ($n = 26$).

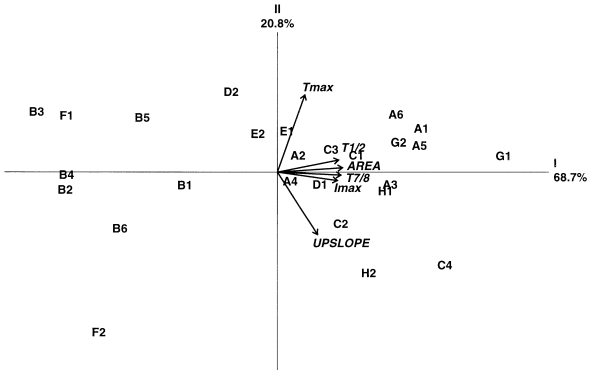

Fig. 2. First two dimensions from PCA of TI parameters.

Table 3
REML Variance components (%) for TI peaks using model (1)

Model terms	Log AREA	Log Imax	T1/2	Log T7/8	Log Tmax	Log UPSLOPE
Panelist	85.5	85.8	32.3	48.1	41.1	62.4
Beer	3.4	1.8	5.3	4.5	0.4	0.2
Age (beer)	0.3	0.2	0.0	0.0	0.6	0.5
Panelist*beer	1.7	1.1	5.9	2.9	2.7	1.8
Age (panelist*beer)	0.1	0.2	0.0	1.8	0.0	0.0
Session(panelist)	1.6	1.4	5.6	2.7	2.6	0.0
Error	7.4	9.6	51.0	40.0	52.6	35.1

AREA can be considered as an expression of total perceived bitterness, an integrated effect including both temporal and concentration effects. As such, AREA is subject to panelist variation resulting from scaling differences, not conceptual differences: it is the parameter which best separates beer types (Wald statistic = 84.9 with 7df, $p < 0.001$; note also that panelist * beer variation is smaller than variation for beer). The eight beer types can be ranked in bitterness (from least to most bitter) as follows: F, B, D, E, C, A, H, G.

The same ranking of beer bitterness is obtained with parameter Imax (Wald statistic = 59.2 with 7df, $p < 0.001$), as might be expected given the high correlation of Imax with AREA.

Table 3 indicates that the differences in bitterness among beer types based on AREA and Imax are greater than the change in bitterness attributable to batch/aging. Scatter plots of the data for these parameters showed irregularities for beer types A, B, C. Trends (based on only two age/batch measurements) for the other beer types showed decreases (types H, G, D), virtually no change (type E) or a slight increase (type F) in AREA and Imax as samples aged.

The parameters T1/2 and T7/8 were also able to separate beer types well according to the pattern previously given (Wald statistics with 7df were 50.7 and 65.2, respectively; $p < 0.001$ in both cases). Both parameters explained a larger percent variance for beer type than was the case with either AREA or Imax, although model (1) fit neither of these time parameters well (51% respectively 40% error). On the basis of T1/2 the beer types were split into two distinct groups ($p = 0.05$) with the least bitter beer types (F and B) achieving half of the area under the curve more than 3 s faster than the other beer types. For the point at which 7/8 of the area under the curve was reached, beer types B and F were 11 s faster than the other types.

Table 3 indicates that the batch/age effect was not present for either T1/2 or for T7/8. If bitterness were to shift with age from an initial sensation to a lingering, after-palate sensation, T1/2 and T7/8 would increase with age. A scatter plot of the data showed irregularities for beer types A and B but increases (in $s\ month^{-1}$) for type C (T1/2 = 0.13, T7/8 = 0.25), type F (T1/2 = 0.04, T7/8 = 0.23) and type H (T1/2 = 0.07, T7/8 = 0.23). For beer type G T1/2 increased by 0.13 s/m but T7/8 decreased by 0.30 s/m. There was virtually no change for beer type E (T1/2 decreased by 0.05 s/m; T7/8 increased by 0.017 s/m). For beer type D both parameters decreased (T1/2 = 0.10, T7/8 = 0.55).

Tmax and UPSLOPE made only slight distinction among the beer types: the Wald statistics with 7df were 10.6 ($p < 0.20$) and 8.5 ($p < 0.30$), respectively. At the 5% level, beers D, G and A reached their maximum bitterness 2 s later than beer F, the least bitter beer.

Tmax and UPSLOPE were the only parameters showing larger percent variance for batch/age than for beer type, although the differences are small. These two parameters loaded heavily on the second PCA dimension shown in Fig. 2. The importance of an age effect for Tmax can be attributed to the large decrease (0.4 s/m) shown for beer F. Tmax decreased moderately for beer types G and H (0.15 respectively 0.13 s/m) and decreased slightly for beer type E (0.05 s/m). Beer D was once again the exception to the general trend: Tmax increased 0.14 s/m. The changes for beer types A, B, C were too irregular to merit further discussion.

The parameter UPSLOPE showed a significant difference at the 5% level only between beers C and B: the slope was greater for the more bitter beer C, as could be expected. The age effect for this parameter was such that the older beer rose faster to its maximum bitterness intensity for types F and H but slower for types D, E and G. Once again the patterns for beers A, B, C were irregular.

3.2. HPLC data

The first three dimensions of the PCA on log peak areas accounted for more than 99% of the explained variance: I = 89.9%, II = 5.5%, III = 3.9%. The biplot in Fig. 3 depicts dimension I as being dependent on beer type whereas dimension II is age-related.

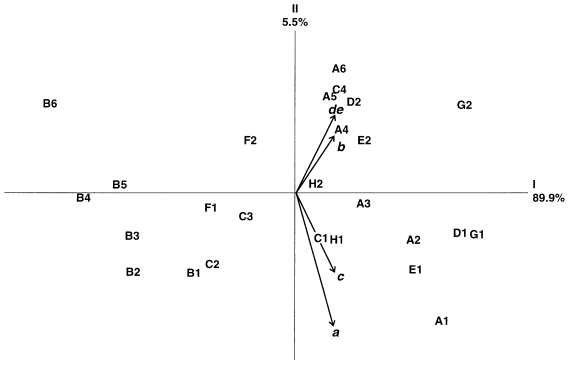

Fig. 3. First two dimensions from PCA of log area for HPLC peaks: (a) *trans*-isocohumulone, (b) *cis*-isocohumulone, (c) *trans*-isohumulone, (d) *cis*-isohumulone + *trans*-isoadhumulone, (e) *cis*-isoadhumulone.

Table 4 shows that the main source of variation in the HPLC data is the beer type, which is to be expected given the difference in hops and hopping procedures encountered in brewing. The small error variation given in Table 4 indicates that model (2) could describe these data adequately. The *cis* :*trans* ratio depends on the α-acid isomerization conditions (Verzele & de Keukeleire, 1991). The *cis*-isomers are always present to a larger extent (e.g. 80:20 or 68:32), thus it is not surprising that these peaks have a higher percent variance in Table 4. [According to Walters et al. (1997), the peak eluted fourth, *cis*-isohumulone + *trans*-isoadhumulone is approximately 90% *cis*-isohumulone.] Hop variety, on the other hand, is reflected in the ratio of isocohumulones to isohumulones (Nickerson & Williams, 1986). Isohumulone/isoadhumulone bitterness was favored above isocohumulone bitterness according to Collin et al. (1994) because of its mildness.

Data from the HPLC analyses are plotted in Figs. 4–8 where concentration is in arbitrary integration units and age is given as the difference in months between evaluation date and 'best before' date indicated on the bottle. (Positive age values mean that the sample was 'old' whereas negative age values mean that it had not yet reached its 'best before' date.) From these figures and the variance components given in Table 4 it is clear that only the peaks for *trans*-isocohumulone and for *trans*-isohumulone show aging effects, namely a decrease in concentration with time.

As indicated earlier, beer age was confounded with beer batch in these experiments. If one assumes no age effect for *cis*-isocohumulone, *cis*-isohumulone + *trans*-isoadhumulone and *cis*-isoadhumulone, then the 18.1, 8.1 and 9.1% variance indicated for these three peaks in Table 4 can be attributed to beer batch. An average of these three values, namely 11.8%, could be used to determine a batch effect for the other two HPLC peaks. The age effect for *trans*-isocohumulone would then be estimated as $63.6\% - (0.118 \times 35.8\%) = 59.4\%$. Similarly, the estimated age effect for *trans*-isohumulone would be 23.1%.

The results shown in Figs. 4–8 are in keeping with those reported by Walters et al. (1997). The *cis*-isomers for the beer they used showed no decline when beer was stored at 0 or 25°C and only a slight decrease when beer was kept for 156 days at 40°C. The *trans*-isomers, on the other hand, decreased rapidly when stored at 40°C,

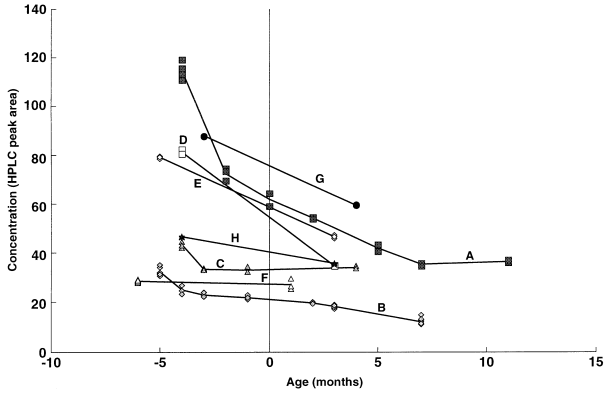

Fig. 4. Concentration of *trans*-isocohumulone (HPLC peak area) as a function of age relative to 'best before' date for eight beer types.

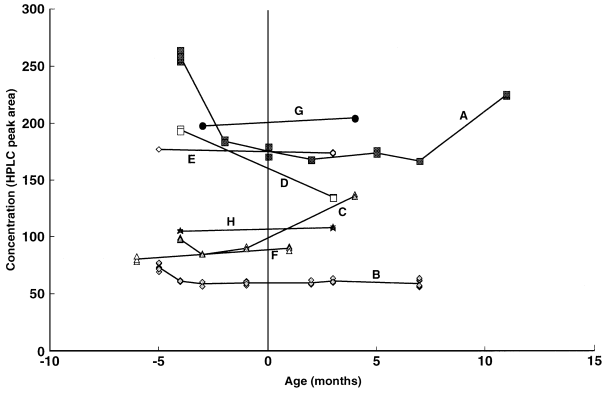

Fig. 5. Concentration of *cis*-isocohumulone (HPLC peak area) as a function of age relative to 'best before' date for eight beer types.

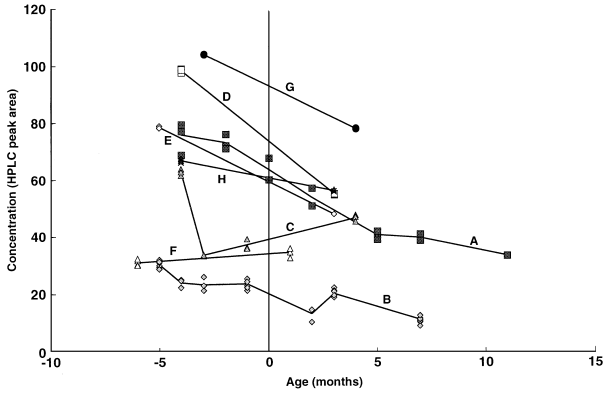

Fig. 6. Concentration of *trans*-isohumulone (HPLC peak area) as a function of age relative to 'best before' date for eight beer types.

Table 4
REML Variance components (%) for log area of HPLC peaks using model (2)

Model terms	*Trans*-isocohumulone	*Cis*-isocohumulone	*Trans*-isohumulone	*Cis*-isohumulone + *trans*-isoadhumulone	*Cis*-isoadhumulone
Beer	35.8	81.6	66.0	89.9	88.2
Age (beer)	63.6	18.1	30.9	8.1	9.1
Error	0.6	0.2	3.1	2.0	2.7

decreased slightly at 25°C, and showed no change when stored at 0°C. From an initial concentration of about 2.5 mg/L, *trans*-isohumulone leveled off at about 1.75 mg/L after 120 days (30% decrease) whereas *trans*-isocohumulone declined from about 1.6 to 1.0 mg/L after 156 days (37.5% decrease).

It is difficult to compare data for the eight beers evaluated in the current study with that of Walters et al. (1997) concerning percent decrease after 5 months storage for *trans*-isomers for several reasons. Firstly, the exact bottling dates for these samples were not available. A commonly-quoted shelflife for European lagers is 6 months, which means that only beer type F was evaluated within a month of bottling. Moreover, different batches of each beer type were evaluated in the current study. Secondly, Walters et al. (1997) had standards to calibrate their HPLC data and could therefore determine that the initial concentration of *trans*-isohumulone was higher than the initial concentration of *trans*-isocohumulone. By comparing HPLC peak areas only, as in the current study, it was shown that some of the beer types (C, D, G, H) had more *trans*-isohumulone initially, other types (A, B) had more *trans*-isocohumulone, while for types E and F both *trans*-isomers were present to about the same extent.

Some indication of decrease in the *trans*-isomers with age can be made for these eight beer types by estimating the difference in peak areas between the youngest sample available for each beer type and the corresponding (interpolated) values at time zero, the 'best before' date. Table 5 shows that, with the exception of beer types C and E, there was a larger decrease in *trans*-isocohumulone independent of the relative initial concentrations of *trans*-isomers.

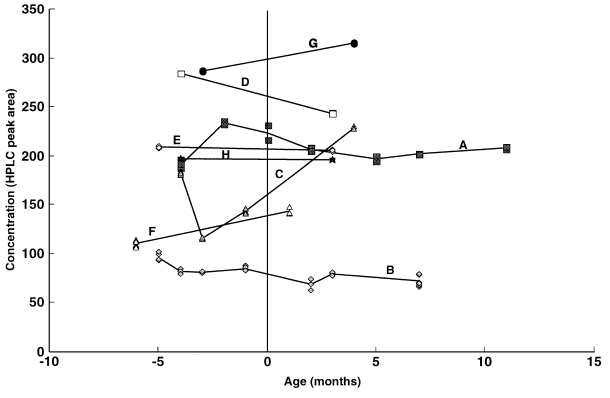

Fig. 7. Concentration of *cis*-isohumulone + *trans*-isoadhumulone (HPLC peak area) as a function of age relative to 'best before' date for eight beer types.

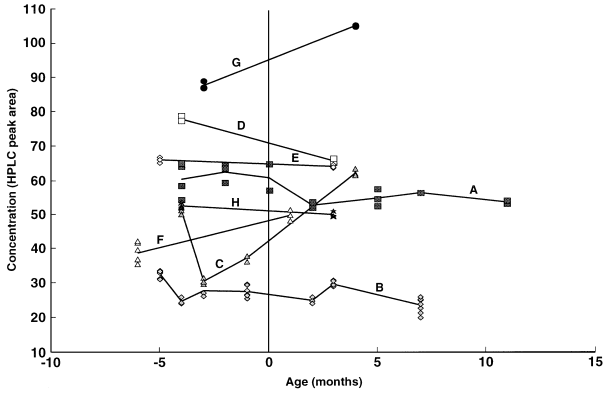

Fig. 8. Concentration of *cis*-isoadhumulone (HPLC peak area) as a function of age relative to 'best before' date for eight beer types.

3.3. Correlation of sensory and HPLC data

Table 6 shows that the five HPLC peak areas are all highly correlated (critical value of $r_{26,0.05} = 0.39$). For this reason the first approach using variance components was model (4) where only one of the five log peak areas was introduced at a time. Table 7 indicates that the Wald statistic was significant for all combinations of HPLC peaks with time–intensity parameters except *trans*-isocohumulone with UPSLOPE (critical value for 5% level and 1df = 3.84).

It was shown that limiting model (4a) to PC-I is acceptable for all parameters. Model (4a) offers no advantage above model (4) when there is a judicious choice of the HPLC peak. Likewise, expanding the model to include beer and beer-interaction effects only reduced the Wald statistics further.

Table 5
Percentage change in *trans*-isomer peak areas between youngest sample available and interpolated area at 'best before' date

Beer type	*Trans*-isocohumulone	*Trans*-isohumulone
A	−46	−17
B	−36	−33
C	−23	−38
D	−32	−26
E	−25	−25
F	−3	+10
G	−14	−12
H	−13	−10

Table 6
Pearson correlation coefficients between log areas of HPLC peaks

Peak	a	b	c	d	e
a	1	0.85*	0.92*	0.80*	0.81*
b	0.85*	1	0.79*	0.90*	0.89*
c	0.92*	0.79*	1	0.90*	0.89*
d	0.80*	0.90*	0.90*	1	0.97*
e	0.81*	0.89*	0.89*	0.97*	1

a, *Trans*-isocohumulone; b, *cis*-isocohumulone; c, *Trans*-isohumulone; d, *cis*-isohumulone + *trans*-isoadhumulone; e, *cis*-isoadhumulone.
* Values significant at $p < 0.05$ ($n = 26$).

Table 7
Wald statistic and estimate for effect log areas of HPLC peaks using model (4)

Compound	Log AREA	Log Imax	T1/2	Log T7/8	Log Tmax	Log UPSLOPE
Trans-isocohumulone	130.2	47.1	43.0	42.6	8.4	2.9
	0.2741	0.1573	6.298	0.0898	0.0798	0.1042
Cis-isocohumulone	163.6	64.5	57.2	60.8	10.3	3.9
	0.3563	0.2113	8.251	0.1199	0.0989	0.1329
Trans-isohumulone	152.7	62.2	47.8	48.1	6.0	5.3
	0.2776	0.1680	6.187	0.0885	0.0627	0.1285
Cis-isohumulone +	176.7	77.6	59.4	65.4	6.0	7.8
trans-isoadhumulone	0.3794	0.2376	8.666	0.1282	0.0784	0.1929
Cis-isoadhumulone	141.8	60.4	49.7	50.8	6.1	5.0
	0.3890	0.2386	9.016	0.1292	0.0896	0.1757

Multicollinearity is not a problem with the PLS2 technique. Table 8 indicates that one dimension is sufficient to explain the Y-block sensory data, although only three parameters (AREA, T1/2, T7/8) are explained for more than 50%. It is clear that with larger standard errors of the mean values used, lower percent explained variation can be expected: both the sensory and the HPLC data treated by PLS2 were subject to measurement errors.

In order to investigate the quality of the sensory data a simulation study was carried out based on the idea that maximum expected values of explained variation for each time–intensity parameter would be obtained in the situation where these data were used to predict the same parameters from another hypothetical panel having the same 'noise' level in time–intensity tests. Normal distributions were obtained with means determined per parameter and product from the variance components models and standard errors determined over all products from the same variance components models. One thousand simulation data sets were constructed by random selection from these normal distributions. These data sets were then regressed against the original data giving a distribution of percent explained variance per time–intensity parameter. The mode for percentage explained variance was determined from each distribution and shown to be significantly higher than the percents given in Table 8, namely: AREA 93%, Imax 87%, T1/2 80%, T7/8 85%, Tmax 62%, UPSLOPE 65%. Therefore, low percent explained variation in Table 8 cannot be excused on the basis of poor sensory measurements; the HPLC data were insufficient.

Table 8
PLS2 explained variance % of Y variables

Dimension	Log AREA	Log Imax	T1/2	Log T7/8	Log Tmax	Log UPSLOPE
1	58.0	44.6	63.0	56.7	19.9	12.5
2	0.8	3.4	0.7	1.9	3.3	6.8
3	4.9	3.8	4.9	7.9	3.3	1.0
4	3.9	4.2	1.2	2.2	0.4	4.7

Bitterness in beer can be caused by components other than the iso-α-acids included in the PLS2 model. Tetrahydroisohumulones or ρ-isohumulones are often used as bittering agents instead of isohumulones to avoid oxidation reactions that lead to stale flavor development. These components were identified in several of the beer types examined for this paper, but incomplete analyses prohibited their inclusion in the models. This fact clarifies to some extent the low percentages of explained sensory variation given in Table 8.

The X-block HPLC data, on the other hand, can be fully described by two dimensions in the PLS2 analysis (Table 9). The fact that *cis*-isohumulone explains the most sensory bitterness and *trans*-isocohumulone the least could be related to the findings of Hughes and Simpson (1996) who showed that *cis*-isohumulone was the most bitter hop acid of the four iso-α-acids they studied while *trans*-isocohumulone was the least bitter. The sensory differences were not attributed to differences in temporal bitterness because TI curves for the four iso-α-acids were not different (Hughes & Simpson, 1994). In earlier work Verzele, Jansen, and Ferdinandus (1970) claimed a similar bitterness in quality and intensity for *cis* and *trans* iso-α-acids, although this was refuted by Verhagen (1994) who showed a greater bitterness for *cis*-isomers. Rigby (1972) reported that low cohumulone hop varieties were believed to impart a smooth, pleasant hop bitterness. He found little difference in bitterness between beer containing isocohumulone versus isohumulone when consumed in small sips, but a sharper bitterness was indicated for isocohumulone when larger drafts were consumed. These differences (as well as the presence of lingering bitter aftertaste) were explained in terms of the re-establishment of oral pH (6.5–7.5) which causes dissociation of iso-α-acids.

Fig. 9 visualizes the relationships among these 11 variables and corroborates the information given for model (4) in Table 7. The lack of large age effects in the sensory data means that the TI parameters are best predicted from HPLC peaks that likewise showed no age effect: *cis*-isocohumulone, *cis*-isohumulone + *trans*-isoadhumulone and *cis*-isoadhumulone.

Table 9
PLS2 explained variance % of X variables (log area of HPLC peaks)

Dimension	*Trans*-isocohumulone	*Cis*-isocohumulone	*Trans*-isohumulone	*Cis*-isohumulone + *Trans*-isoadhumulone	*Cis*-isoadhumulone
1	85.2	87.5	90.3	93.5	92.7
2	12.9	0.4	0.1	6.3	3.0
3	0.1	8.5	3.8	0.1	1.4
4	1.6	3.5	5.6	0.0	3.0

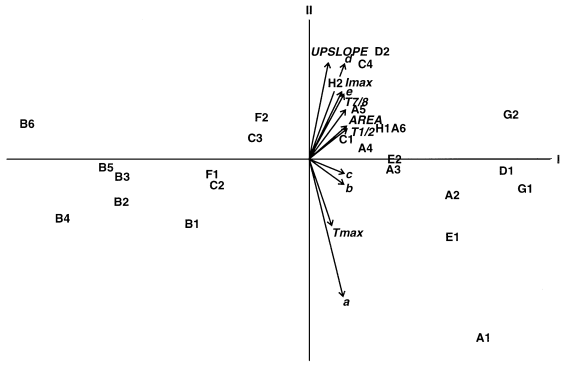

Fig. 9. First two dimensions from PLS2: scores for 26 beers, Y-loadings for six TI variables and X-loadings for five HPLC variables labeled a–e as in Fig. 3.

In conclusion it can be stated that both the sensory and the analytical data showed sample separation based on beer type and, to a lesser extent, batch/age. Sensory bitterness generally decreased with age as shown by lower maximum intensity and less area under the TI curve. As a natural consequence of this decrease, some beer types reached maximum intensity much earlier and at a faster rate when they were aged. For some beer types the TI curves shifted to a longer-lasting bitterness with age.

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4. Appendix

A symmetrical filter with no time shift and having the general form given by Eq. (A1) was used where y_t is the filtered value at sample time y_t.

$$y_i = \sum_{n=-1}^{1} h_n x_{t+n} \quad \text{(A1)}$$

Table 10
Weights for Eq. (A3)

n	h_n
0	0.112275173
1	0.107956897
2	0.095961686
3	0.078825671
4	0.059798784
4	0.041859149
6	0.027005903
7	0.016034755
8	0.008746230
9	0.004373115
10	0.001999138
11	0.000832974
12	0.000315179

This filter replaces each intensity for sample time x_t by a weighted sum of observed sample times where h_n is the weight for an observation n sample time units from x_t.

$$y_t = 0.25x_{t-1} + 0.5x_t + 0.25x_{t+1} \quad \text{(A2)}$$

Eq. (A2) was repeated 25 times, which is equivalent to writing Eq. (A3) and using the 13 weights given in Table 10.

$$Y_t = h_0 x_t + \sum_{n=1}^{12} h_n (x_{t+n} + x_{t-n}) \quad \text{(A3)}$$

References

Cepicka, J., Strejcek, F., & Pokorny, J. (1992). Development of the sensory bitterness during the tasting of beer. *Monatsschrift für Brauwissenschaft, 10*, 329–331.

Collin, S., Derdelinckx, G., & Dufour, J.-P. (1994). Relationships between the chemical composition and sensory evaluation of lager beers. *Food Quality and Preference, 5*, 145–149.

Crombecq, P. (1995). *Bier Jaarboek 1995–1996*, Kosmos Z & K: Utrecht/Antwerpen.

Dalgliesh, C. E. (1977). Flavour stability. *Proc. Congr. Eur. Brew. Conv., 9*, 623–659.

Hough, J. S., Briggs, D. E., Stevens, R., & Young, T. W. (1982). *Malting and brewing science*, (Vol. 2, 2nd ed). London: Chapman and Hall, p. 389.

Hughes, P. S., & Simpson, W. J. (1994). Sensory impact of hop-derived compounds. *Monograph-European Brewing Convention (1994), 22*, 128–140.

Hughes, P. S., & Simpson, W. J. (1996). Bitterness of congeners and stereoisomers of hop-derived bitter acids found in beer. *Journal of the American Society of Brewing Chemists, 54* (4), 234–237.

Kaneda, H., Kano, Y., Koshino, S., & Ohya-Nishiguchi, H. (1992). Behavior and role of iron ions in beer deterioration. *Journal of Agricultural and Food Chemistry, 40*, 2102–2107.

Keukeleire, D.de., Vindevogel, J., Szücs, R., & Sandra, P. (1992). The history and analytical chemistry of beer bitter acids. *Trends in Analytical Chemistry, 11* (8), 275–280.

King, B. M. (1995). The use of sensory analysis to describe and classify different types of beer. Proceedings of the Fifth Scientific and Technical Convention, Institute of Brewing Central and Southern (pp. 9–21). African Section, Victoria Falls, Zimbabwe, 5–9 March, 1995.

King, B. M., & Moreau, N. (1996). A comparison of bitter perception in high-alcohol, low-alcohol and alcohol-free beer. *J Inst Brew, 102*, 419–425.

McMurrough, I., & Byrne, J. R. (1992). HPLC analysis of bittering substances, phenolic compounds, and various compounds of alcoholic bevarages. In L. M. L. Nollet (Ed.), *Food analysis by HPLC*. New York: Marcel Dekker, (Chapter 16).

Nickerson, G. B., & Williams, P. A. (1986). Varietal differences in the proportion of cohumulone, adhumulone and humulone in hops. *Journal of the American Society of Brewing Chemists, 44*, 91–94.

Pangborn, R. M., Lewis, M. L., & Tanno, L. A. S. (1977). Sensory quantification of bitterness and flavour of beer during storage. *Journal of the Institute of Brewing, 83*, 244–250.

Pangborn, R. M., Lewis, M. L., & Yamashita, J. F. (1983). Comparison of time-intensity with category scaling of bitterness of iso-α-acids in model systems and in beer. *Journal of the Institute of Brewing, 89*, 349–355.

Rigby, F. L. (1972). A theory on the hop flavor of beer. *Proceedings of the American Society of Brewing Chemists. 30*, 46–50.

Verhagen, L. C. (1994). Beer flavour. In J. R. Piggott, & A. Paterson (Eds.), *Understanding natural flavors*, (pp. 211–227). Blackie Academic & Professional, London.

Verzele M., & de Keukeleire, D. (1991). *Chemistry and analysis of hop and beer bitter acids*. (P. 105). London: Elsevier.

Verzele, M., Jansen, H. E., & Ferdinandus, A. (1970). Organoleptic trials with hop bitter substances. *Journal of the Institute of Brewing, 76*, 25–28.

Walters, M. T., Heasman, A. P., & Hughes, P. S. (1997). Comparison of (+)-catechin and ferulic acid as natural antioxidants and their impact on beer flavor stability. Part 2: extended storage trials. *Journal American Society of Brewing Chemists, 55* (3), 91–98.