

Comparative study of the stability of iso- α -acids, dihydroiso- α -acids and tetrahydroiso- α -acids during beer ageing[†]

Luc De Cooman^{1*}, Guido Aerts¹, An Witters¹, Marjan De Ridder¹, Annick Boeykens¹,
Koen Goiris¹, and Denis De Keukeleire²

¹ KaHo St.-Lieven, Laboratory of Enzyme and Brewing Technology, Gebroeders Desmetstraat 1, B-9000 Ghent, Belgium; ² University Ghent, Faculty of Pharmaceutical Sciences, Laboratory of Pharmacognosy and Phytochemistry, Harelbekestraat 72, B-9000 Ghent, Belgium

[†] Summary of a presentation at "Trends in de brouwerij" 20.04.2001 at KaHo St.-Lieven, Gent, Belgium and EBC, 28th Congress, 2001, Budapest, Hungary

ABSTRACT

Reduced iso- α -acids are believed to exhibit a higher resistance to oxidation in the beer matrix, as compared with iso- α -acids. However, evidence for this assumption is not available. Our study on the stability of the different bitter acids by HPLC profiling of selected ageing beers, showed that *trans*-iso- α -acids are more prone to oxidation than their *cis* counterparts. Tetrahydroiso- α -acids are extremely resistant to oxidative deterioration, whilst the 'light-stable' dihydroiso- α -acids are not at all stable on storage. Tetrahydroiso- α -acids are the most stable bitter acids towards oxidation, followed by *cis*-iso- α -acids, dihydroiso- α -acids, and *trans*-iso- α -acids. For 'normal' beers, bitterness consistency can be improved by the use of isomerised hop extracts, or by the partial substitution of iso- α -acids for tetrahydroiso- α -acids. For beers packaged in colourless bottles, dihydroiso- α -acids are to be used, but oxidative deterioration of these compounds will occur anyhow. The potential of the hexahydroiso- α -acids should be re-evaluated.

Keywords: beer bitterness, beer ageing, iso- α -acids, dihydroiso- α -acids, tetrahydroiso- α -acids

Cerevisia 26(3), 2001

INTRODUCTION

Iso- α -acids, derived from the hop α -acids, are primary flavour constituents of beer (7). Beer contains six major iso- α -acids, i.e. the *trans*- and *cis*-isomers of isochumulone, isohumulone, and isoadhumulone (for the structures, see Fig. 1). Together with some minor iso- α -acids, these compounds are largely responsible for the characteristic bitter beer taste (15, 18). One of the most striking sensory changes in ageing beer is a decrease in bitterness intensity and quality, often occurring from the onset of the ageing process (2). Such undesirable changes are associated with oxidative transformations on beer storage (1, 12, 19). In this respect, isohumulones are indeed prone to oxidative decomposition during the brewing process and on storage of finished beer (3, 18). Iso- α -acids deterioration in ageing beer has been reported in several studies and is believed to be caused by free-radical reactions (4, 10, 11, 13, 14, 17, 20). Moreover, iso- α -acids degradation is often quoted as a possible pathway

to the generation of ageing off-flavours, including staling carbonyls (2, 4, 5, 18, 19). Recent investigations demonstrated that *trans*-iso- α -acids are significantly less stable in the beer matrix than *cis*-iso- α -acids (4, 9, 14, 20). In a previous study, we emphasised the marked instability of *trans*-iso- α -acids in ageing beer and hence, their adverse impact on bitterness consistency (4). It was clearly shown that the ratio of the sum of the concentrations of *trans*-isochumulone (1a) and *trans*-isohumulone (1b) to the sum of the concentrations of *cis*-isochumulone (2a) and *cis*-isohumulone (2b), defined as the 'trans-cis ratio', proves to be a reliable parameter to evaluate bitterness deterioration in ageing beers (4). In contrast to iso- α -acids, tetrahydroiso- α -acids appeared to be remarkably stable on beer storage (4). These earlier observations prompted us to conduct a more comprehensive study on the stability of both conventional and reduced iso- α -acids, including dihydro- and tetrahydroiso- α -acids. Nowadays, dihydro- and tetrahydroiso- α -acids are used in the preparation of light-stable beers to circumvent the formation of 3-methyl-2-butene-1-thiol by photosensitised degradation of iso- α -acids (3, 8) (for the structures of the reduced iso- α -acids, see Fig. 1). Tetrahydroiso- α -acids are also frequently applied in 'normal' beers to enhance foam stability and bitterness consistency (3). Besides their light-proofing properties, it is accepted that reduced iso- α -acids also exhibit a higher resistance to oxidative decomposition in the beer matrix in comparison with conventional iso- α -acids (6, 14). However, conclusive evidence for this assumption is not available in literature. We therefore examined the stability of the different bitter acid types in pilot beers brewed at our institute, and in commercial beers with a known bitter acid profile.

MATERIALS AND METHODS

Preparation of pilot beers

Three pilsener beers were prepared at our pilot scale brewery (2 hl) from one and the same brew. By post-fermentation addition of iso- α -acids, dihydroiso- α -acids, and tetrahydroiso- α -acids, respectively, to green beer derived from the same fermentation vessel, a similar matrix for these pilot beers is obtained. Beer preparation was carried out as follows. Grist: pilsener malt (34 kg) and maize flakes (6 kg); brewing water: reverse osmosis (1.7 hl) with addition of Ca²⁺ (40 ppm); brewing scheme: 36°C (15 min), 49°C (30 min), 63°C (20 min), 72°C (30 min),

78°C (120 min, including wort filtration with lauter tun); wort boiling: 75 min; wort clarification: whirlpool; original gravity: 12.2°P; pitching rate: 10^7 cells/ml; fermentation temp. and duration: 12°C, 8 days; hopping: at transfer to cask with either iso- α -acids (3.13 g/hl; presumed utilisation: 80%), dihydroiso- α -acids (4.20 g/hl; utilisation: 85%), or tetrahydroiso- α -acids (2.26 g/hl; utilisation: 65%); lagering: in cask (10 days at 2°C); beer filtration: kieselguhr/cellulose sheets (1 μ m); packaging: manual filling (Servinox) in brown glass bottles (25 cl).

Beer samples

Two commercial beers, indicated as 'A' (pellet hopping) and 'E' (hopped with reduced isomerised extracts), and three pilot beers, indicated as 'B', 'C', and 'D', were

studied. Next to fresh beers, the following samples were analysed:

- Beers 'A', 'B', 'C', 'D', 'E': samples aged at 60°C for 1, 3, 5, 7, and 10 days.
- Beer 'A': samples kept in the dark at room temperature for 1 to 6, 10 and 12 months.
- Beer 'E': samples kept in the dark at room temperature for 1 to 6, 9 and 12 months.

Extraction of bitter acids from beer and HPLC analysis of (reduced) iso- α -acids

The bitter acids were extracted from the beers and subsequently analysed by high-performance liquid chromatography (HPLC) as described earlier (4).

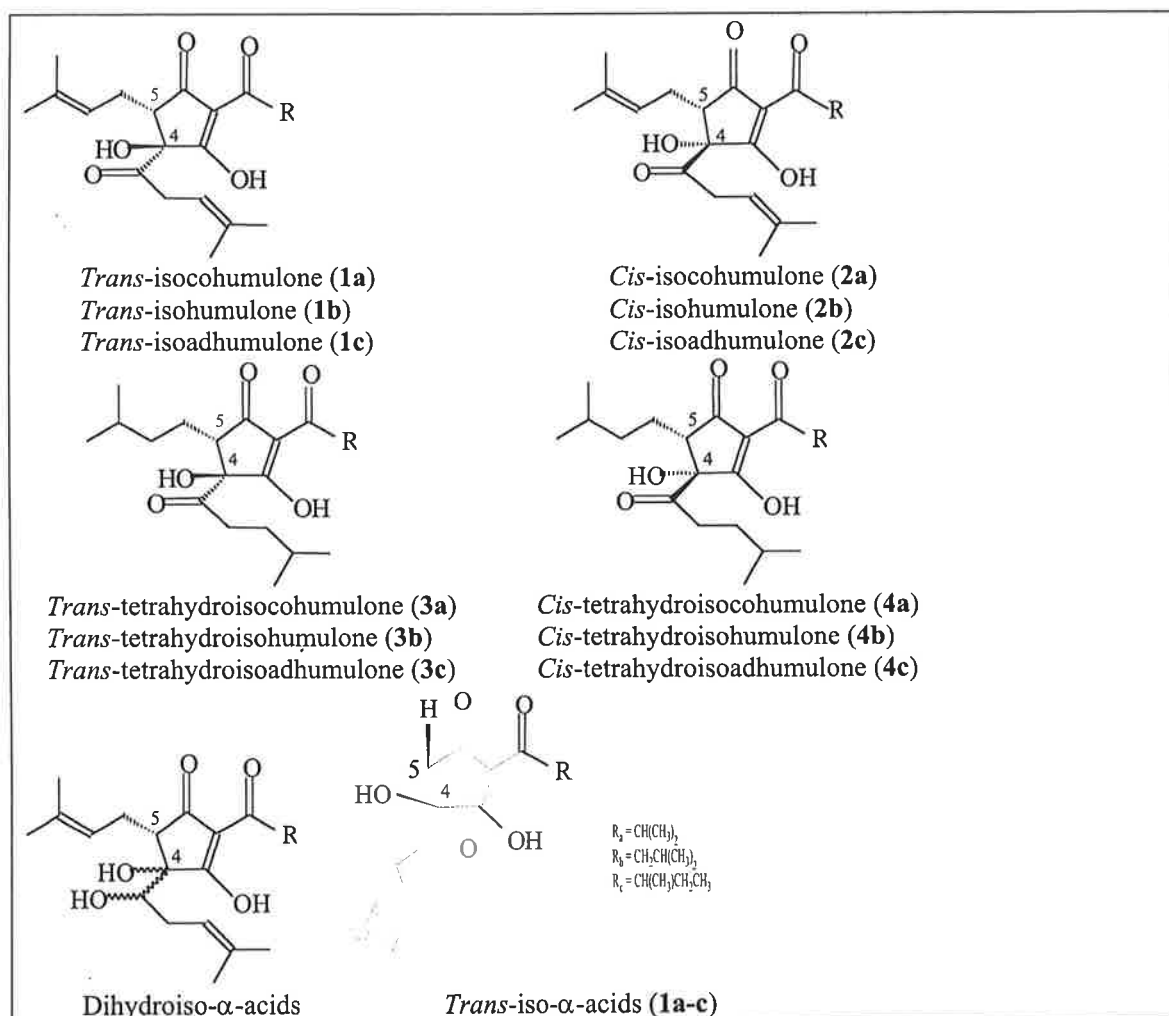


Figure 1: Structures of iso- α -acids, tetra- and dihydroiso- α -acids.

RESULTS AND DISCUSSION

Evaluation of the stability of iso- α -acids in a commercial pilsener beer

A kettle hopped commercial pilsener, indicated as beer 'A', was studied as a function of both spontaneous and forced ageing. Figure 2A shows the iso- α -acids profile of the fresh beer. The applied HPLC method allows for the full separation of the 6 major iso- α -acids, i.e. *trans*-isocohumulone (1a), *cis*-isocohumulone (2a), *trans*-isohumulone (1b), *cis*-isohumulone (2b), *trans*-isoadhumulone (1c), and *cis*-isoadhumulone (2c). The HPLC

analysis of a beer sample that had been stored for one year in the dark at room temperature, is represented in figure 2B. Comparison between the analyses in figure 2 points to the differential behaviour of *trans*- and *cis*-iso- α -acids on beer storage. *Trans*-isomers are obviously deteriorated at a much faster rate than *cis*-isomers. In fact, the small peaks of *trans*-iso- α -acids in figure 2B (1a, 1b, 1c) suggest a high sensitivity of these compounds towards oxidative degradation in the beer matrix. The contents of iso- α -acids in samples of beer 'A' that were aged spontaneously for 1 to 12 months are summarised in table 1.

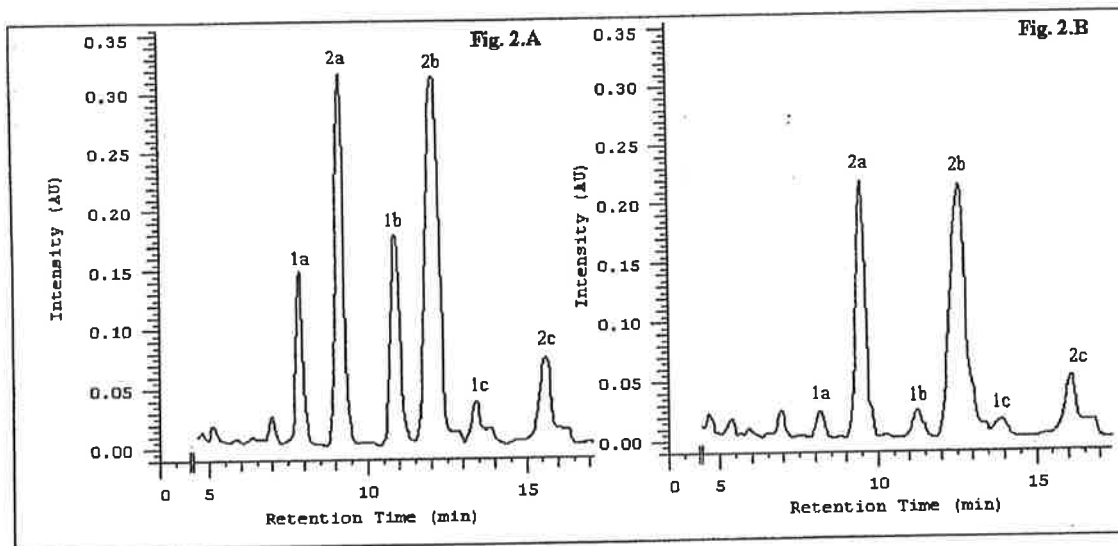


Figure 2: HPLC analysis of the iso- α -acids in a commercial pilsener beer 'A'; A: fresh sample, B: sample after spontaneous ageing for 12 months.

Table 1: Iso- α -acids (ppm) in beer 'A' as a function of spontaneous ageing.

Compound	Ageing time (months)								
	0 ^a	1 ^b	2 ^b	3 ^b	4 ^b	5 ^b	6 ^b	10 ^b	12 ^b
Trans-ich	2.2	1.9	1.7	1.5	1.4	1.2	1.0	0.6	0.5
Cis-ich	5.1	4.9	4.9	4.9	4.8	4.7	4.6	4.6	4.3
Trans-inh	3.5	2.9	2.6	2.4	2.2	1.8	1.5	0.9	0.7
Cis-inh	9.1	8.8	8.8	8.8	8.5	8.2	8.1	7.9	7.4
Trans-iah	1.2	1.1	1.0	1.0	0.8	0.8	0.8	0.6	0.6
Cis-iah	2.4	2.4	2.2	2.3	2.3	2.2	2.1	2.0	2.0
Total (ppm)	23.5	22.0	21.2	20.9	20.0	18.9	18.1	16.6	15.4
Total (%)	100	94	90	89	85	80	77	71	66
T/C ratio (%)	40	35	31	29	27	23	20	16	10

^a = fresh sample

^b = samples stored in the dark at room temperature

After one year, total bitterness is reduced by 34%. The T/C ratio amounts to 40% for the fresh sample but only 10% for the one year-old beer, reflecting the differential behaviour of *trans*- and *cis*-iso- α -acids. After one year of spontaneous ageing, about 75% of *trans*-isomers are lost versus 18% of *cis*-components. A shelf half-life for *trans*-iso- α -acids of less than one year was also noticed previously in a commercial top-fermented beer subjected to spontaneous ageing (4). As a consequence, even after relatively short storage times of only one or two months, distinct decreases in the levels of *trans*-iso- α -acids can be measured (see also table 1). Similar findings as to the differential behaviour of *trans*- and *cis*-iso- α -acids, were obtained by forced ageing of beer 'A' at 60°C (see table 2). The marked instability of *trans*-iso- α -acids was explained earlier in terms of the stereochemical arrangement of the isohexenoyl and the prenyl side chains at C(4) and C(5), respectively (4). As shown in figure 1, only in *trans*-iso- α -acids, the proximity of the unsaturated sites provides a pool of high electron density, allowing ready initiation of radical-type oxidation reactions.

Evaluation of the stability of conventional and reduced iso- α -acids in pilot beers

Three pilot beers with a similar beer matrix were prepared as described in 'Materials and Methods'. After primary fermentation, either iso- α -acids (beer 'B'), dihydroiso- α -

acids (beer 'C'), or tetrahydroiso- α -acids (beer 'D'), were added. The contents of iso- α -acids on forced ageing of Beer 'B' are summarised in table 3. In the case of our pilot beer, a relatively low T/C ratio (28%) is measured in the fresh sample, which is typical when using pre-isomerised hop extract (9). Nevertheless, the results obtained on the pilot beer 'B' (table 3) largely parallel those collected for the commercial beer 'A' (table 2), i.e. clear reductions in total bitterness and especially in the T/C ratio are observed on forced ageing.

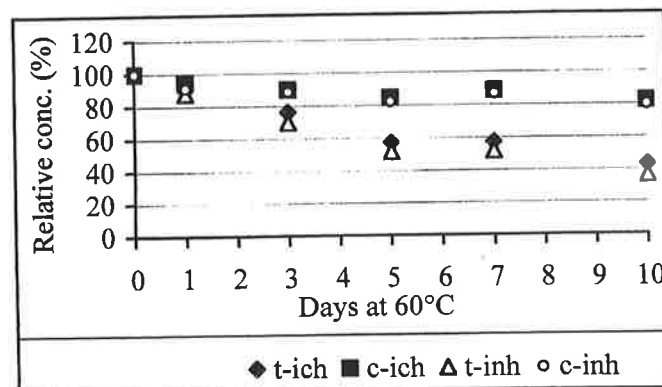


Figure 3: Iso- α -acids (%) in beer 'B' as a function of ageing at 60°C.

Table 2: Iso- α -acids (ppm) in beer 'A' as a function of forced ageing.

Compound	Ageing time (days)					
	0 ^a	1 ^b	3 ^b	5 ^b	7 ^b	10 ^b
Trans-ich	2.2	1.7	1.2	1.0	0.7	0.5
Cis-ich	5.1	4.7	4.5	4.5	4.1	4.0
Trans-inh	3.5	2.6	1.7	1.4	0.9	0.7
Cis-inh	9.1	8.3	8.1	8.1	7.2	6.9
Trans-iah	1.2	1.1	0.9	0.9	0.8	0.8
Cis-iah	2.4	2.1	2.1	2.1	1.9	1.7
Total (ppm)	23.5	20.5	18.5	18.0	15.6	14.6
Total (%)	100	87	79	77	66	62
T/C ratio (%)	40	33	23	19	14	11

^a = fresh sample

^b = samples aged in a thermostated room at 60°C

Table 4: Dihydroiso- α -acids (ppm) in beer 'C' as a function of forced ageing.

Compound	Ageing time (days)					
	0 ^a	1 ^b	3 ^b	5 ^b	7 ^b	10 ^b
Dihydro-ich(a)	6.6	6.1	5.9	5.3	5.1	4.5
Dihydro-ich(b)	10.2	9.4	9.1	8.4	7.8	7.0
Dihydro-inh(a)	5.1	4.6	4.4	4.0	3.8	3.3
Dihydro-inh(b)/ dihydro-iah(a)	11.4	10.2	10.0	9.0	8.8	7.7
Dihydro-iah(b)	3.1	2.8	2.8	2.6	2.5	2.3
Total (ppm)	36.4	33.1	32.2	29.3	28.0	24.8
Total (%)	100	91	88	81	77	68

^a = fresh sample

^b = samples aged in a thermostated room at 60°C

Figure 3 shows the relative concentration (the content of each iso- α -acid in the fresh sample was equalised to 100%) of the major iso- α -acids, i.e. *trans*-isocohumulone (t-ich), *cis*-isocohumulone (c-ich), *trans*-isohumulone (t-inh), and *cis*-isohumulone (c-inh), as a function of forced ageing time. As opposed to the differential behaviour of *trans*- and *cis*-iso- α -acids, co- and normal homologues degrade at a similar rate. When comparing the results in table 3 with those in table 2, it is further interesting to notice that bitterness was more stable in the pilot beer 'B' than in the commercial beer 'A'. Closer examination of the quantitative data reveals that this is mainly due to a greater stability of the *trans*-iso- α -acids in the pilot beer. Obviously, the rate of decline of the T/C ratio varies from one beer to another and such interesting differences are easily detected by high-resolution HPLC (4).

Totally new results were obtained on pilot beer 'C' containing only dihydroiso- α -acids. The quantitative data are represented in table 4. Because the identity of the dihydroiso- α -acids is not completely solved, the compounds are tentatively indicated as dihydroisocohumulone (a), dihydroisocohumulone (b), dihydroisohumulone (a), dihydroisohumulone (b), dihydroisoadhumulone (a) (Dihydroisoadhumulone is not resolved from dihydroisohumulone. Hence, these peaks were quantified together.), and dihydroisoadhumulone (b), analogous to the identification of iso- α -acids and tetrahydroiso- α -acids. At first sight, the dihydroiso- α -acids concentration in the fresh sample seems to be rather high (36.4 ppm), but according to Benitez et al. (3) such a level corresponds to a sensory bitterness, equivalent to about 25 ppm of iso- α -acids. Clear reductions in the

Table 3: Iso- α -acids (ppm) in beer 'B' as a function of forced ageing.

Compound	Ageing time (days)					
	0 ^a	1 ^b	3 ^b	5 ^b	7 ^b	10 ^b
Trans-ich	2.1	1.9	1.6	1.2	1.2	0.9
Cis-ich	7.9	7.5	7.1	6.7	7.0	6.5
Trans-inh	3.3	2.9	2.3	1.7	1.7	1.2
Cis-inh	11.4	10.4	10.1	9.4	9.9	9.1
Trans-iah	0.8	0.7	0.7	0.6	0.6	0.5
Cis-iah	2.8	2.6	2.6	2.4	2.4	2.3
Total (ppm)	28.3	26.0	24.4	22.0	22.8	20.5
Total (%)	100	92	86	78	81	72
T/C ratio (%)	28	27	23	18	17	13

^a = fresh sample

^b = samples aged in a thermostated room at 60°C

content of all dihydroiso- α -acid components are detected on forced ageing, resulting in a relative total bitterness of only 68% after 10 days at 60°C. The changes in the levels of the individual components are visualised in figure 4. All dihydroiso- α -acids show a similar degradation rate, in contrast to iso- α -acids. A plausible explanation for this finding is that the dihydroiso- α -acids of the applied hop extract would possess the same configuration, i.e. the *cis*-configuration as claimed by Ting & Goldstein (16). According to this view, when comparing the data obtained on the beers 'B' and 'C' (see tables 3 and 4), dihydroiso- α -acids would be more sensitive to deterioration in beer than iso- α -acids. Anyhow, as apparent from the figures 3 and 4, the currently available dihydroiso- α -acids show a faster degradation than *cis*-iso- α -acids.

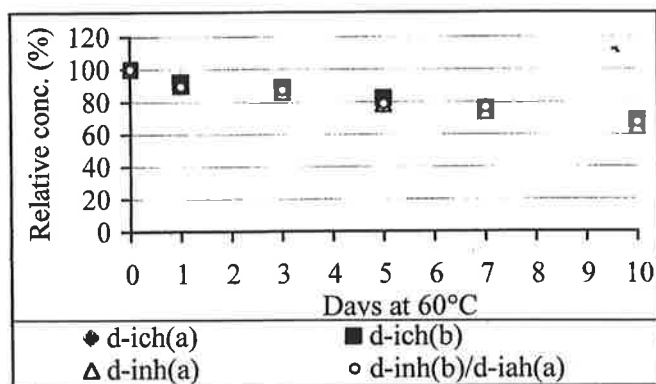


Figure 4: Dihydroiso- α -acids (%) in beer 'C' as a function of ageing at 60°C.

In tetrahydroiso- α -acids, the double bonds in the side chains at C(4) and C(5) are reduced (see Fig. 1). On account of this modification, it has been predicted that tetrahydroiso- α -acids should behave very stable towards oxidation in finished beer (3, 18). This view is confirmed by the results obtained on pilot beer 'D' (see table 5 and Fig. 5). Only a relatively small reduction in the level of tetra-components (approx. 10%) was detected on forced ageing. In our previous paper, a marked stability of tetrahydroiso- α -acids was also noticed in a commercial pilsener, subjected to prolonged spontaneous ageing (4). By comparing the data collected for the pilot beers, we can conclude that tetrahydroiso- α -acids are the most stable bitter acids towards oxidative deterioration, followed by *cis*-iso- α -acids, dihydroiso- α -acids, and *trans*-iso- α -acids.

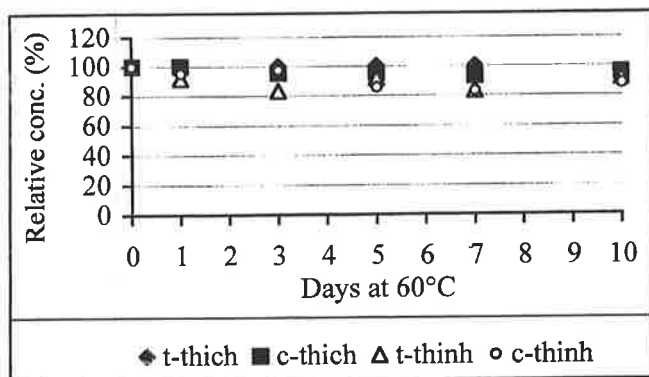


Figure 5: Tetrahydroiso- α -acids (%) in beer 'D' as a function of ageing at 60°C.

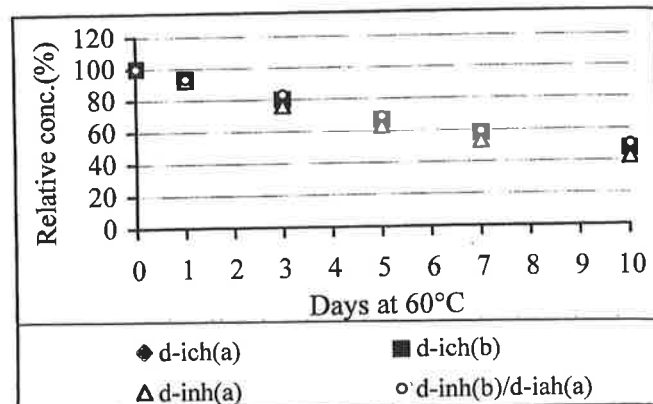


Figure 6: Dihydroiso- α -acids (%) in beer 'E' as a function of ageing at 60°C.

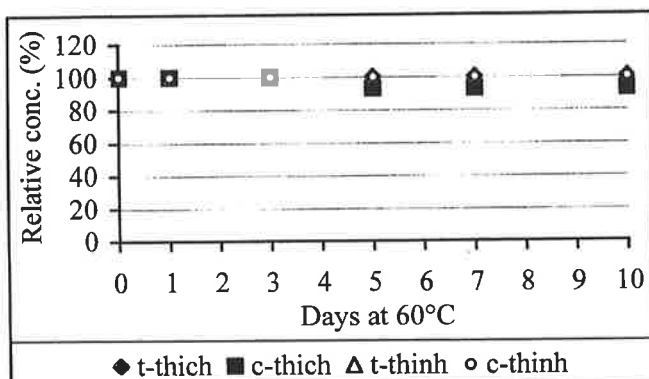


Figure 7: Tetrahydroiso- α -acids (%) in beer 'E' as a function of ageing at 60°C.

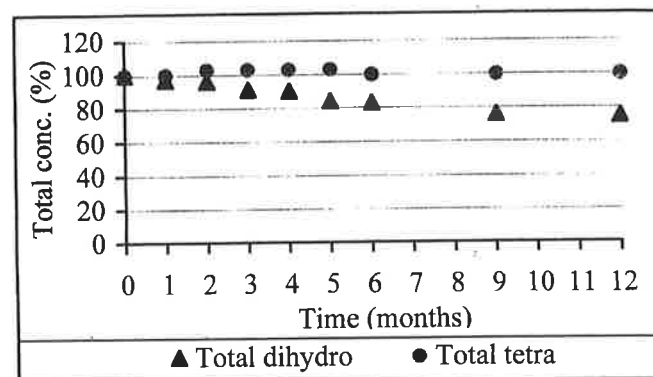


Figure 8: Total dihydro- and tetrahydroiso- α -acids (%) in beer 'E' as a function of spontaneous ageing.

Table 5: Tetrahydroiso- α -acids (ppm) in beer 'D' as a function of forced ageing.

Compound	Ageing time (days)					
	0 ^a	1 ^b	3 ^b	5 ^b	7 ^b	10 ^b
Trans-thich	1.4	1.4	1.4	1.4	1.4	1.3
Cis-thich	4.4	4.4	4.2	4.2	4.1	4.2
Trans-thinh	1.2	1.1	1.0	1.1	1.0	1.1
Cis-thinh	4.2	4.0	4.1	3.6	3.5	3.7
Trans-thiah	0.3	0.3	0.3	0.3	0.2	0.3
Cis-thiah	1.1	1.0	1.0	1.0	0.9	1.0
Total (ppm)	12.6	12.2	12.0	11.6	11.1	11.6
Total (%)	100	97	95	92	88	92

^a = fresh sample

^b = samples aged in a thermostated room at 60°C

Evaluation of the stability of reduced iso- α -acids in a commercial beer

The study of the pilot beer 'C' revealed instability of dihydroiso- α -acids on forced ageing. To determine whether these observations would also hold in other beers, a commercial light-stable beer 'E' was investigated. As required to light stability, beer 'E' is completely bittered with reduced iso- α -acids, the fresh beer containing both dihydroiso- α -acids (20 ppm) and tetrahydroiso- α -acids (3 ppm). In our study on beer 'E', samples of the same brew were subjected to forced or spontaneous ageing at various time intervals. The results are summarised in the figures 6 to 8. In conformity with our findings obtained on beer 'C', a relatively strong and similar reduction in all dihydro-components is observed on forced ageing of beer 'E' (Fig.

6). When comparing the results in the figures 6 and 4, it can be noticed that the dihydroiso- α -acids behaved more stable in the pilot beer 'C' than in the commercial beer 'E'.

Figure 7 again demonstrates the stability of the tetrahydroiso- α -acids. As further shown in figure 8, similar results on the dihydro- and tetrahydroiso- α -acids were obtained on prolonged spontaneous ageing of beer 'E'.

CONCLUSIONS

Trans-iso- α -acids are more prone to oxidation in ageing beer than *cis*-iso- α -acids, and the corresponding *trans/cis* iso- α -acids ratio is useful to monitor and evaluate bitterness deterioration. In contrast with conventional iso- α -acids, tetrahydroiso- α -acids are extremely resistant to oxidative decomposition. Surprisingly, the light-stable dihydroiso- α -acids are not at all stable on storage. Thus, the generalised view that reduced iso- α -acids exhibit enhanced resistance to oxidative deterioration in the beer matrix is only true for tetrahydroiso- α -acids. The decomposition kinetics of the three bitter acid types in the pilot beers, demonstrated that the most stable bitter principles towards oxidation in beer are the tetrahydroiso- α -acids, followed by *cis*-iso- α -acids, dihydroiso- α -acids, and *trans*-iso- α -acids. For beers stored in dark-coloured glass bottles, bitterness consistency can be substantially improved by the use of pre-isomerised hop products with a high proportion of *cis*-iso- α -acids, and/or by the partial

substitution of iso- α -acids for tetrahydroiso- α -acids. Replacement of iso- α -acids by dihydroiso- α -acids in 'normal' beers is not recommended, considering the susceptibility of the dihydro-components to oxidation. For beers packaged in colourless glass bottles, dihydroiso- α -acids should be used as the main bittering principles because of their inherent light-stability. However, special attention must be paid to the issue of oxidative flavour deterioration in order to slow down, among other things, dihydroiso- α -acids decay. It also seems worthwhile to reconsider the potential benefits of the hexahydroiso- α -acids.

Acknowledgements

The authors thank the Flemish Institute for the Promotion of Scientific-Technological Research in Industry (IWT, Brussels, Belgium) for financial support (HOBUE IWT-Fund, project numbers 970117 and 990070).

REFERENCES

1. Araki, S., Kimura, T., Shimizu, C., Furusho, S., Takashio, M. & Shinotsuka, K., *Journal of the American Society of Brewing Chemists*, 1999, 57, 34-37.
2. Bamforth, C.W., *Brauwelt International*, 1999, 2, 98-110.
3. Benitez, J.L., Forster, A., De Keukeleire, D., Moir, M., Sharpe, F.R., Verhagen, L. C. & Westwood, K. T., *Hops and Hop Products - EBC Manual of Good Practice*, Nürnberg: Fachverlag Hans Carl, 1997.
4. De Cooman, L., Aerts, G., Overmeire, H. & De Keukeleire, D., *Journal of the Institute of Brewing*, 2000, 106, 169-178.
5. Hashimoto, N. & Kuroiwa, Y., *Proceedings of the American Society of Brewing Chemists*, 1975, 33, 104-111.
6. Held, R., *Technical Quarterly of the Master Brewers Association of the Americas*, 1998, 35, 133-140.
7. Hough, J.S., Briggs, D.E., Stevens, R. & Young, T.W., *Malting and Brewing Science*, Vol. 2, *Hopped Wort and Beer*, Second Edition, London: Chapman and Hall, 1982, Chapter 23.
8. Hughes, P.S., *Cerevisia*, 1999, 24 (2), 21-25.
9. Hughes, P.S., Menneer, I.D., Walters, M.T. & Marinova, G., *Proceedings of the 26th Congress of the European Brewery Convention*, Maastricht, 1997, 231-238.
10. Kaneda, H., Kano, Y., Koshino, S. & Ohya-Nishiguchi, H., *Journal of Agricultural and Food Chemistry*, 1992, 40, 2102-2107.
11. Kaneda, H., Kano, Y., Osawa, T., Kawakishi, S. & Kamada, K., *Journal of the American Society of Brewing Chemists*, 1989, 47, 49-53.
12. Kaneda, H., Kobayashi, N., Furusho, S., Sahara, H. & Koshino, S., *Technical Quarterly of the Master Brewers Association of the Americas*, 1995, 32, 90-94.
13. Kaneda, H., Takashio, M., Tamaki, T. & Osawa, T., *Journal of the Institute of Brewing*, 1997, 103, 21-23.
14. King, B.M. & Duineveld, C.A.A., *Food Quality and Preference*, 1999, 10, 315-324.
15. Moir, M., *Journal of the American Society of Brewing Chemists*, 2000, 58, 131-146.
16. Ting, P.L. & Goldstein, H., *Journal of the American Society of Brewing Chemists*, 1996, 54, 103-109.
17. Uchida, M. & Ono, M., *Journal of the American Society of Brewing Chemists*, 1996, 54, 198-204.
18. Verzele, M. & De Keukeleire, D., *Chemistry and Analysis of Hop and Beer Bitter Acids*, Amsterdam, Elsevier, 1991.
19. Wackerbauer, K. & Hardt, R., *Brauwelt International*, 1997, 4, 320-327.
20. Walters, M.T., Heasman, A.P. & Hughes, P.S., *Journal of the American Society of Brewing Chemists*, 1997, 55, 91-98.