

# Flavour Instability of Pale Lager Beers: Determination of Analytical Markers in Relation to Sensory Ageing

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## ABSTRACT

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Flavour changes of six Belgian pale lager beers were studied in order to estimate the importance of different parameters and reactions in relation to the ageing process. An attempt was made to link analytical data with sensory evaluation using multivariate statistical analysis. Partial least squares regression techniques (PLSR) were employed on the analytical and sensory data. As apparent from the PLSR model, significant indicators of lager beer ageing are aldehyde markers (especially total aldehydes, furfural, hexanal, 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal), cold and permanent haze, and beer colour. Conversely, compounds or parameters that load negatively in the PLSR model for beer ageing are *trans*-isohumulones, *cis*-isohumulones, total bitterness, the T/C-ratio, polyphenolic markers (especially proanthocyanidins), the flavanoid content, and, to a lesser extent, the TB-index and reducing power (TRAP). The integrated analytical-sensorial methodology is proposed as a useful tool for evaluation of the flavour instability of pale lager beers.

**Key words:** analytical markers, beer ageing, flavour instability, partial least squares regression.

## INTRODUCTION

Every brewer aims at a pleasant and consistent beer flavour by selection of high-quality raw materials and application of a controlled production process. Nevertheless, flavour instability upon storage remains one of the most important quality problems of beer<sup>51,52</sup>. Flavour instability is relevant to both ales and lagers, but especially pale lager beers seem to be very sensitive to flavour deterioration<sup>3,18</sup>.

Due to the complexity of both malt and beer production and considering the intricate composition of the beer matrix, a multitude of parameters may have an effect on

the flavour stability of the finished product<sup>7</sup>. Typically, a decline in beer bitterness (both in quality and intensity), the development of sweet taste and the appearance of cardboard flavour are connected to flavour instability<sup>14,44</sup>. In particular, formation of volatile aldehyde compounds is recognised as one of the major causes of flavour deterioration upon storage. The development of these typical ageing flavours during beer storage has been linked to oxidation of higher alcohols, oxidative degradation of hop-derived bitter acids, Strecker degradation of amino acids, oxidation of unsaturated fatty acids, and aldol condensations<sup>7,8,52,55</sup>. Most research in relation to beer flavour instability and the accompanying generation of volatile aldehydes, has been focused on the cardboard-flavoured *trans*-2-nonenal and on its formation by lipid oxidation, because of the extremely low flavour threshold value of this compound<sup>34,37,39,60</sup>. However, more recently, it has been stated repeatedly that other flavours related to beer ageing, *e.g.* Strecker aldehydes<sup>45,46</sup> and  $\beta$ -damascenone ('cooked apple' or honey-like)<sup>13,22,26</sup>, may be equally or even more important for the overall unpleasant sensory perception of aged beer. Moreover, besides formation of staling volatile aldehyde compounds, a lot of other unwanted reactions *e.g.* oxidation of beer bitter acids<sup>5,17-19,31</sup> and oxidation and polymerisation of malt and hop polyphenols<sup>41</sup>, also occur during beer storage.

Clearly, the chemistry behind the ageing process is extremely complex, as many constituents<sup>1,8,10,12,20,25,34,35,52,56-58</sup> derived from the raw materials or formed during the production process, may contribute in one way or another to beer flavour stability (*e.g.* anti-oxidants such as polyphenols) or flavour instability (*e.g.* Strecker aldehydes, intermediates of lipid oxidation). The central question now arises which of these constituents are of greatest interest in relation to beer flavour instability, in other words which compounds are to be regarded as true markers for flavour instability.

The aim of this study is comprehensive characterisation of the sensory and chemical ageing properties of commercial pale lager beers in order to determine key marker constituents for flavour instability and, finally, to establish an integrated methodology allowing adequate determination of the sensitivity of pale lagers towards flavour deterioration. Due to large variations in the raw materials used and the applied production processes, it can be expected that the ageing behaviour will differ

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**Table I.** Brewing information of the six commercial pale lager beers.

	Brew A	Brew B	Brew C	Brew D	Brew E	Brew F
Mashing-in temperature (°C)	52	54	54	45	40	53
pH-correction at mashing-in	No correction	Lactic acid	Phosphoric acid	Lactic acid	No correction	Lactic acid
pH at mashing-in	5.60	5.40	5.65	5.43	5.80	5.33
Increased reducing power in brewing liquor	–	–	–	+	–	–
Use of adjuncts	+	+	+	+	+	+
Wort filtration	LT*	LT*	TBF**	LT*	LT*	LT*
Hopping	Pellets + CO <sub>2</sub> -extract	Pellets	Pellets + CO <sub>2</sub> -extract + tetrahydro-iso- $\alpha$ -acids	Pellets + CO <sub>2</sub> -extract	Pellets	Pellets
Wort boiling time (min)	90	90	70	90	90	90
Anti-oxidants post-fermentation	+	–	+	+	–	+
Colloidal stabilisation						
Protein-side	+	–	+	+	+	+
Polyphenol-side	–	–	+	–	–	–

\* LT = lauter tun.

\*\* TBF = thin bed filter.

greatly between different pale lager beers. Therefore, partial least squares regression analysis (PLSR)<sup>23,38,49</sup> was applied, in order to reveal possible relationships between chemical data and sensory evaluation of beer ageing and to gain improved understanding of the ageing process of different commercial pale lager beers.

## MATERIALS AND METHODS

### Beer samples and ageing conditions

Six fresh commercial lager beers, obtained from different Belgian breweries and delivered immediately after bottling, were stored at 0°C to preserve freshness. All beers were bottled with total oxygen levels below 0.1 mg/L (for more information on the preparation of these beers, see Table I). For evaluation of flavour stability, samples were aged at 22°C during 9 months (spontaneous ageing) or at 30°C for 60 days (forced ageing).

### Sensory evaluation of beer ageing

Sensory evaluation of flavour deterioration upon ageing of the six pale lager beers was performed by our trained panel (12 panellists). Panellists were asked to give overall-ageing-scores (OAS), after tasting fresh and aged beer samples, respectively, without disclosing the identity of the samples (OAS: 0: fresh; 2: very weakly aged; 4: weakly aged; 6: clearly aged; 8: strongly aged, undrinkable).

### Determination of lipoxygenase activity

Extraction and measurements of potential lipoxygenase activity in malt were carried out using our in-house procedure<sup>15</sup>.

### Gas chromatographic analysis of trihydroxy fatty acids

Determination of trihydroxy fatty acids in malt, wort, and beer samples was based on the published procedures of Möller-Hergt *et al.*<sup>43</sup> and Wackerbauer and Meyna<sup>57</sup>. Equipment: GC-FID (Thermo Quest CE Trace 2000 (Interscience Benelux)) equipped with an AS 2000 auto-sampler (Interscience Benelux), a cyano-phenyl-methyl deactivated retention gap (2.5 m × 0.53 mm i.d., Varian,

The Netherlands), and a fused silica analytical capillary column (CP-Sil 5 CB LOW BLEED/MS; 50 m × 0.25 mm i.d., 0.25  $\mu$ m film thickness, Varian, The Netherlands). Data processing was performed by Chromcard software 1.0.7.

### Standard analyses

The alcohol content of beer was measured by the Anton Paar Alcoyser.

Standard beer analyses were carried out according to EBC-methods: FAN (free amino nitrogen): 9.10 (for FAN determination of wort: 8.10); total polyphenols: 9.11; flavanoid content: 9.12; foam stability, using the NIBEM-T Meter (Haffmans): 9.42.

Cold haze: analysis of turbidity of beer kept for a minimum of 24 h at 0°C (Haffmans VOS ROTA 90 Turbidity meter, 90° light scatter).

Permanent haze: analysis of turbidity of beer kept for 24 h at 20°C (Haffmans VOS ROTA 90 Turbidity meter, 90° light scatter).

Beer colour: IOB method: 9.1.

Determination of proanthocyanidins: method according to Bate-Smith<sup>9</sup>.

Soluble protein: Bio-Rad protein assay, according to Bradford<sup>11</sup>.

TB-index (thiobarbituric acid) in malt, wort and beer: method according to Thalacker and Bößendörfer<sup>50</sup>.

TRAP (total reactive antioxidant potential) in wort and beer: method according to Araki *et al.*<sup>4</sup>.

### GC-MS determination of aldehydes

Volatile aldehydes in beer were determined according to Vesely *et al.*<sup>54</sup>, using headspace-solid phase micro-extraction (HS-SPME) with on-fibre PFBOA (*o*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine) derivatisation and capillary gas chromatography/mass spectrometry (CGC/MS) (Dual Stage Quadrupole (DSQ<sup>TM</sup> II) GC/MS system (Interscience Benelux)). The DSQ<sup>TM</sup> II was coupled to a Thermo Trace GC Ultra (Interscience Benelux) equipped with a CTC-PAL autosampler, a split/splitless injector with a narrow glass inlet liner (0.5 ml volume), and a RTX-1 fused-silica capillary column (40 m × 0.18 mm i.d., 0.2  $\mu$ m film thickness, Restek, Interscience Benelux).

**Table II.** Lipoxygenase activity, trihydroxy fatty acid content and TB-index of the malts used for the commercial brews A-F.

Malt	LOX (U/g d.m.)	THOE (mg/kg)	TBI*
A	125.0	17.8	12.3
B	148.8	20.7	14.6
C	121.3	18.7	15.7
D	112.6	14.7	9.8
E	143.1	21.0	11.5
F	80.2	18.9	14.0

\* TB-index for malt: index for 10.0 g malt.

Data processing was performed by the XCalibur™ data system (Thermo Electron Corporation).

### HPLC determination of beer bitterness

Extraction of the bitter acids from beer was carried out using our in-house procedure<sup>18</sup>. HPLC analysis of iso- $\alpha$ -acids was performed on a Hitachi liquid chromatograph (Merck, Darmstadt, Germany) which consists of a programmable HPLC pump (L-7100) with a quaternary low pressure gradient system, a diode array detector (L-7450A), an interface module (D-7000), a solvent degasser (L-7612), an autosampler (L-7200), a Compaq Deskpro 2000 (Merck D-7000 Rev. 2.1 HPLC system manager software), and an Alltima 5  $\mu$ m C18 column (150 mm  $\times$  4.6 mm i.d.; Alltech Associates, Deerfield, USA). Chromatographic conditions were as described by De Cooman *et al.*<sup>18</sup>. For quantification of iso- $\alpha$ -acids, a dicyclohexylamine-iso- $\alpha$ -acids ICS-II complex (66.5% (w/w) iso- $\alpha$ -acids) (Labor Veritas, Zürich, Switzerland) was used as an external standard. Besides quantitative determination of the levels of individual iso- $\alpha$ -acids, the T/C-ratio was calculated as follows:

$$T/C (\%) = \frac{[trans - \text{isocohumulone}] + [trans - \text{isohumulone}]}{[cis - \text{isocohumulone}] + [cis - \text{isohumulone}]} \times 100\%$$

### HPLC profiling of polyphenols

Polyphenolic extract from beer was prepared as described by Goiris *et al.*<sup>27</sup>. HPLC analysis of polyphenols was performed on a Hitachi Lachrom system (Merck, Darmstadt, Germany), consisting of a L-7100 programmable pump, a L-7450A DAD detector, a L-7350 column oven, a L-7250 programmable autosampler and a D-7000 interface. Solvents were degassed in line using a Recipe DG-4000 degasser (Recipe, Munich, Germany). Separations were carried out on an Alltima 5  $\mu$ m C18 column (Alltech Associates, Deerfield, USA) of 250 mm  $\times$  4.6 mm (temperature 35°C, flow rate 0.9 mL/min). The detector was set at 280 nm to quantify flavanoids and cinnamic acids, and at 350 nm for the detection and quantification of flavonol glycosides. The mobile phases were (A) formic acid/water (1/99) and (B) acetonitrile/methanol (5/95). Gradient conditions: linear gradient from 100% A to 100% B in 120 min; reverse gradient in 15 min; 100% A for 2 min.

### GC-MS profiling of esters and higher alcohols

Extraction of volatile esters and higher alcohols from beer was performed by headspace-solid phase micro-extraction (HS-SPME) for 30 min at 40°C using a 65  $\mu$ m PDMS-DVB fiber coating. Components were separated

**Table III.** FAN, TBI, reducing power, and trihydroxy fatty acid content of worts of the commercial brews A-F (BB: wort before boiling; PW: pitching wort).

Brew		FAN (mg/L)	TBI*	TRAP (mM ascorbic acid eq.)	THOE (mg/L)
A	BB	151.8	25.8		9.2
	PW	155.8	54.2	1.524	7.5
B	BB	183.0	25.1		10.6
	PW	157.0	49.2	1.524	9.6
C	BB	119.3	23.7		9.9
	PW	144.7	50.2	1.364	7.2
D	BB	232.0	40.8		9.2
	PW	203.0	58.6	1.902	7.8
E	BB	125.0	27.7		9.8
	PW	150.0	47.9	1.204	10.0
F	BB	168.0	30.2		7.7
	PW	201.0	48.8	1.454	5.7

\* TB-index for wort: index for 100.0 mL wort.

and detected by capillary gas chromatography/mass spectrometry (CGC/MS) (Dual Stage Quadrupole (DSQ™) GC/MS system (Interscience Benelux) operating in the electron impact mode). The DSQ™ was coupled to a ThermoFinnigan Trace GC (Interscience Benelux) equipped with a CTC-PAL autosampler, a split/splitless injector with a narrow glass inlet liner (0.5 mL volume), and a RTX-1 fused-silica capillary column (40 m  $\times$  0.18 mm i.d., 0.2  $\mu$ m film thickness, Restek, Interscience Benelux). Helium was the carrier gas at a flow rate of 0.8 mL/min. The inlet temperature was 230°C and the injection occurred in the split mode (split ratio 1/12). The oven temperature was held at 40°C for 3 min, then raised to 200°C at 6°C/min, followed by an increase to 250°C (at 15°C/min) and finally held at 250°C for 3 min. Processing of the chromatographic data was performed by the Xcalibur™ data system (Thermo Electron Corporation). For each component, a calibration curve was made in order to quantify the component in beer.

### Statistical methods

Analytical and sensorial data were analysed by a multivariate data analysis software package (The Unscrambler®; CAMO, Oslo, Norway) in order to determine relevant parameters for beer flavour instability. Partial least squares regression analysis (PLSR) was employed to develop a model between the chemical data (X matrix) and the sensorial overall-ageing-score (Y matrix).

## RESULTS AND DISCUSSION

### Evaluation of malt

The malts used for the preparation of the six commercial lager beers, were evaluated for parameters considered to be significant in terms of their influence on the flavour stability of beer<sup>28</sup>. These potentially relevant quality parameters of malt are lipoxygenase activity, trihydroxy fatty acid content, and TB-index (see Table II).

In previous work<sup>6,15,16,29,36</sup> the importance of lipoxygenase (LOX) activity in malt for beer flavour stability was emphasized. Clearly, the malt used in brewery F contains the lowest residual LOX activity. The content of trihydroxy fatty acids (THOE) is lowest in malt D. This malt also shows the lowest TB-index (TBI), an indicator of

**Table IV.** Potential contribution of malt to the TB-index and trihydroxy fatty acid content of wort at the beginning of wort boiling of the brews A-F.

Brew	TBI (potential contribution of malt)	Increase in TBI during mashing and wort filtration	THOE (potential contribution of malt (mg/L))	Increase in THOE during mashing and wort filtration (mg/L)
A	15.6	10.2	2.3	6.9
B	19.4	5.7	2.8	7.8
C	20.9	2.8	2.5	7.4
D	15.7	25.1	2.4	6.8
E	13.9	13.8	2.5	7.3
F	18.0	12.2	2.4	5.3

heat load during malting. All TBI values of the malts are lower than 20, this is recommended for pale malts<sup>50</sup>.

### Evaluation of wort samples

During brewing, wort samples were taken at the beginning of wort boiling and after wort cooling (pitching wort). The free amino nitrogen content (FAN), the TB-index, the reducing power (TRAP) and the concentration of trihydroxy fatty acids were measured (see Table III).

The free amino nitrogen content is rather low in the wort samples of brewery C and brewery E. High levels of FAN in the pitching wort as found in the brews D and F may result in high residual FAN in the final beer, which has been shown to be negative in terms of flavour stability<sup>21</sup>. As a result of the heat load during wort boiling, a clear increase in TB-index is observed for every brew. The pitching wort of brewery D clearly has a higher reducing power (TRAP), which can be explained by an increase of the reducing power of the brewing water (see also Table I). Except for brew E, there is a clear decline in the levels of trihydroxy fatty acids during wort boiling and hot wort clarification, possibly attributed to thermal degradation, association with cold break compounds or decomposition to lipid oxidation off-flavours<sup>24,47</sup>.

The potential contribution of the malt to the TB-index and the trihydroxy fatty acid content of the respective wort at the beginning of boiling is given in Table IV. These potential contributions were calculated on the basis of the analytical value of the malt, the amount of malt used, and the volume of the brew at the beginning of wort boiling. The increases during mashing and wort filtration as indicated in Table IV represent the difference between the measured value of the wort at the beginning of wort boiling and the potential contribution of the corresponding malt. The smallest increase in TB-index during mashing and wort filtration is noticed for brewery C due to the shortest lautering time (88 min) using a thin bed filter. On the other hand, brew D shows the highest increase in TB-index due to the very long lautering time (240 min).

Except for brewery F, the increase in trihydroxy fatty acids during mashing and wort filtration is always relatively high, *i.e.* approx. 7–8 mg/L. Brew F shows a smaller increase in trihydroxy fatty acids (approx. 5 mg/L). This may be on account of a combination of a lower pH at mashing-in (pH 5.33, see Table I) and a lower residual LOX activity introduced by the malt at mashing-in (see Table II)<sup>1,35,53</sup>.

**Table V.** Mean overall-ageing-scores of the fresh, forced aged and spontaneously aged beers.

Beer	Fresh	60 days at 30°C	9 months at 22°C
A	0.2	4.5	4.8
B	0.4	4.2	6.7
C	0.1	3.1	4.1
D	0.2	3.2	4.1
E	0.1	2.5	4.3
F	0.3	4.2	6.2

Fresh: fresh beer sample; 60 days at 30°C: beer sample aged for 60 days at 30°C; 9 months at 22°C: beer sample aged for 9 months at 22°C.

### Sensory evaluation of pale lager beers

Sensory evaluation of the six fresh pale lager beers, forced aged beer samples (60 days at 30°C), and spontaneously aged beer samples (9 months at 22°C) was carried out by our trained taste panel. Panellists were asked to give an overall-ageing-score, without knowing the identity of the beers. The results of all sensory assessments are summarised in Table V. Higher overall-ageing-scores were obtained for the beers aged during 9 months at 22°C. Clearly, after 60 days of ageing at 30°C, the beers C, D, and E showed the lowest overall-ageing-scores. This is still reflected upon ageing for 9 months at 22°C. Based on the overall-ageing-scores, the beers C and D are more flavour stable than the other beers during spontaneous ageing, whereas the beers B and F appear to be the most sensitive towards flavour deterioration.

### Standard analyses of pale lager beers

Table VI shows the results of the standard analyses on the fresh beers, the forced aged beers (60 days at 30°C), and the spontaneously aged beers (9 months at 22°C), respectively. The six commercial pale lager beers have an alcohol content between 4.4 and 5.5% (v/v), whereas the pH varies between 4.15 and 4.51. Large differences in free amino nitrogen content (FAN) are noted between the fresh pale lager beers. In particular, beer D shows a high FAN level, reflecting the high FAN level of the corresponding pitching wort (see Table III). Based on comparison of the FAN levels of the pitching wort (see Table III) and the final beer (see Table VI), the consumption of free amino nitrogen by yeast can differ greatly. A high concentration of soluble proteins implies good foam stability (see fresh beer B). The fresh beers A, B and F show a high value of cold haze. The beer colour and the TB-index are rather similar for the six fresh pale lager beers, although beer C shows a somewhat lower TB-index. In agreement with literature data<sup>32,48</sup>, a clear relationship is noticed between the polyphenol content and the reducing power of the beers. Beer D has the highest total polyphenol content and the highest reducing power (TRAP). Also the pitching wort of this brew showed the highest reducing power (see Table III). Beer C, on the other hand, has a low polyphenol content (see total polyphenols, flavanoid content and proanthocyanidin content) and a corresponding low reducing power. When comparing the results obtained on the reducing power in Table III and Table VI, it can be seen that the reducing power of the pitching wort is determinative for the reducing power of the finished beer. The concentrations of trihydroxy fatty acids are quite similar in the fresh beers A-D. The brews E and F having the highest

**Table VI.** Standard analyses of fresh and aged beer samples of the brews A-F.

		Sample	Beer A	Beer B	Beer C	Beer D	Beer E	Beer F	
Alcohol	% (v/v)	Fresh	5.1	5.5	4.7	5.2	4.9	4.4	
pH		Fresh	4.15	4.29	4.15	4.51	4.46	4.38	
FAN	mg/L	Fresh	89.2	99.8	52.2	137.8	87.6	71.6	
		60d 30°C	91.4	98.0	52.4	137.8	89.2	71.2	
		9m 22°C	94.3	97.6	53.1	137.8	88.8	73.7	
Soluble proteins	mg/L	Fresh	312	400	317	237	216	226	
		60d 30°C	261	391	309	235	199	189	
		9m 22°C	241	387	314	233	178	195	
Foam stability (NIBEM)	s	Fresh	239	326	292	218	232	291	
		60d 30°C	179	300	258	216	245	270	
		9m 22°C	212	322	273	230	238	280	
Cold haze	(EBC)	Fresh	1.2	1.0	0.3	0.6	0.5	1.8	
		60d 30°C	9.8	5.7	1.2	1.5	2.8	2.1	
		9m 22°C	46.8	57.3	5.9	8.2	14.7	15.7	
Permanent haze	(EBC)	Fresh	0.4	0.4	0.1	0.6	0.5	0.8	
		60d 30°C	1.2	1.6	0.7	1.2	1.0	1.1	
		9m 22°C	3.1	15.9	1.4	1.4	1.9	4.7	
Beer colour	(IOB)	Fresh	6.9	7.1	7.2	7.1	7.1	6.7	
		60d 30°C	7.3	8.0	7.4	9.5	7.6	7.1	
		9m 22°C	7.6	10.8	8.2	8.9	8.3	8.5	
Total polyphenols	mg/L	Fresh	171.4	178.8	96.4	234.1	140.2	157.4	
		60d 30°C	173.8	171.8	95.9	236.9	136.9	154.6	
		9m 22°C	161.9	175.4	96.4	227.6	135.2	156.2	
Flavanoids	mg/L	Fresh	34.0	35.3	20.4	34.5	28.1	33.7	
		60d 30°C	31.0	32.0	19.4	31.2	25.5	29.3	
		9m 22°C	26.6	28.5	18.1	29.8	22.7	27.5	
Pro-antho- cyanidins	mg/L	Fresh	27.1	28.6	16.3	25.1	17.5	22.1	
		60d 30°C	28.0	30.3	15.7	23.6	19.5	25.6	
		9m 22°C	28.9	32.2	16.2	29.1	18.5	20.3	
TBI	index for 100.0 mL beer	Fresh	39.4	36.5	33.2	39.6	36.4	38.0	
		60d 30°C	36.3	32.2	31.0	33.2	34.0	34.1	
		9m 22°C	36.3	32.6	31.9	32.9	32.4	35.2	
TRAP	mM	Fresh	1.325	1.378	1.018	1.759	1.261	1.305	
		ascorbic acid eq.	60d 30°C	1.257	1.232	0.895	1.582	1.091	1.184
		9m 22°C	1.106	1.106	0.856	1.414	0.959	0.957	
Trihydroxy fatty acids	mg/L	Fresh	9.0	9.3	8.9	9.3	14.4	7.4	
		60d 30°C	9.0	9.4	8.3	8.4	11.6	7.0	
		9m 22°C	8.6	7.8	5.6	6.3	8.7	5.7	

Fresh: fresh beer sample; 60d 30°C: beer sample aged for 60 days at 30°C; 9m 22°C: beer sample aged for 9 months at 22°C.

and lowest levels of trihydroxy fatty acids in the pitching worts, respectively (see Table III), also showed the highest and lowest levels of trihydroxy fatty acids in the corresponding beers (see Table VI).

During the studied period of beer ageing, the free amino nitrogen content does not alter significantly. The concentration of soluble proteins decreases during ageing and with that a concomitant decline in foam stability is noticed. Reduction in foam stability is especially pronounced upon forced ageing of the beers (60 days at 30°C). An increase in cold and permanent haze formation is noticed during beer ageing, especially the beers A, B and F show a high increase in haze formation upon ageing. Beer colour increases upon beer ageing for 60 days at 30°C and even more during 9 months of ageing at 22°C, especially in beer B, which also showed the highest overall-ageing-score after spontaneous ageing (see Table V). Furthermore, for all beers, a small decrease in the TB-index is noticed during ageing. In all beers, the flavanoid content decreases on ageing (upon both forced and spontaneous ageing). The evolution of total polyphenols and proanthocyanidins during ageing is less pronounced, although in most cases a decrease in total polyphenols and an increase in proanthocyanidins is observed. In accordance with a decline in flavanoids and total polyphenols,

the reducing power of the beers lowers during beer ageing, especially during spontaneous ageing for 9 months. Similarly, a decrease is found in the content of trihydroxy fatty acids, particularly after 9 months of ageing at 22°C. This may be attributed to degradation of trihydroxy fatty acids to off-flavour aldehydes<sup>35,43</sup>.

### Evaluation of aldehyde profiles

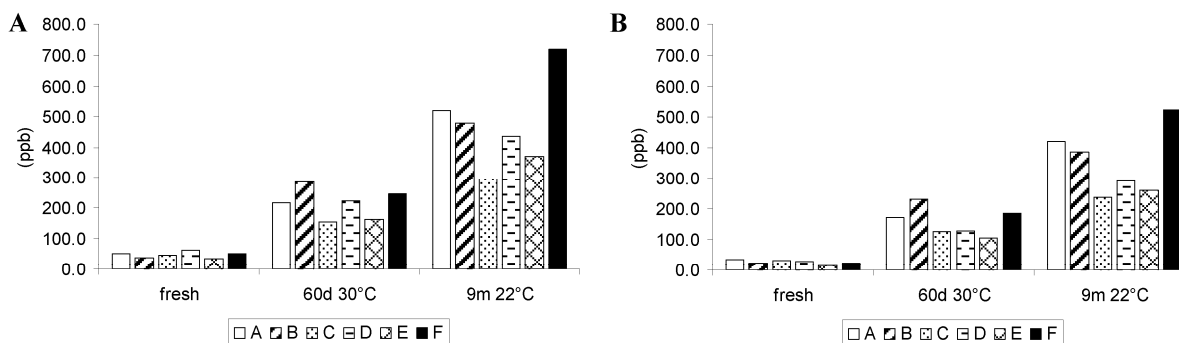
Quantitative GC-MS profiling of aldehyde markers was performed on the fresh pale lager beers and on forced aged (60 days at 30°C) and spontaneously aged (9 months at 22°C) samples (see Table VII). The investigated aldehyde markers can be classified into Strecker degradation aldehydes (2-methylpropanal, 2- and 3-methylbutanal, methional, benzaldehyde and phenylacetaldehyde), aldehydes formed during Maillard reactions (furfural) and lipid oxidation aldehydes (hexanal and *trans*-2-nonenal). In Fig. 1, the increases in concentration upon beer ageing of total aldehyde markers and furfural, are depicted.

The fresh pale lager beers B and E have a relatively low content of aldehyde markers. During ageing of beer B, a large increase in the concentration of aldehyde markers takes place. This is in accordance with sensory evaluation (see Table V), where beer B was given a high overall-ageing-score after 60 days at 30°C (4.2) as well as after 9

**Table VII.** Concentrations of aldehyde markers ( $\mu\text{g/L}$ ) in fresh, forced aged and spontaneously aged beer samples of the brews A-F.

Beer	Sample	2-Methylpropanal	2-Methylbutanal	3-Methylbutanal	Methional	Benzaldehyde	Phenylacetaldehyde	Furfural	Hexanal	t-2-Nonenal	Sum aldehyde markers
A	Fresh	6.9	1.5	2.9	1.2	1.2	3.5	34.0	0.4	0.03	51.7
	60d 30°C	25.5	3.5	6.5	2.7	1.5	6.7	170.8	0.9	0.03	218.1
	9m 22°C	59.0	7.0	14.0	5.5	1.9	11.8	419.7	1.6	0.04	520.4
B	Fresh	5.0	1.3	2.7	1.1	1.1	3.2	21.7	0.3	0.02	36.5
	60d 30°C	28.9	3.6	6.8	2.6	1.6	8.6	232.5	0.9	0.05	285.5
	9m 22°C	49.8	6.9	15.1	4.7	2.4	12.4	386.4	1.8	0.04	479.5
C	Fresh	3.8	1.8	3.5	1.1	1.6	3.1	31.2	0.2	0.02	46.4
	60d 30°C	9.5	3.1	5.6	2.0	1.9	5.2	125.8	0.5	0.03	153.5
	9m 22°C	22.5	5.8	11.8	5.6	2.4	8.2	237.2	1.3	0.05	294.8
D	Fresh	11.2	1.5	5.5	5.3	3.2	6.2	29.9	0.5	0.04	63.3
	60d 30°C	35.9	3.1	11.8	17.8	3.3	20.2	129.6	1.1	0.03	222.8
	9m 22°C	52.4	6.3	20.9	29.2	4.4	31.2	290.4	1.9	0.03	436.7
E	Fresh	5.8	1.5	3.1	1.5	1.7	4.7	14.7	0.3	0.02	33.3
	60d 30°C	32.9	2.9	7.3	3.4	2.5	8.6	105.6	0.8	0.02	163.9
	9m 22°C	61.0	5.1	16.7	7.5	3.3	15.4	259.7	2.2	0.04	371.1
F	Fresh	10.2	1.8	5.1	1.8	1.4	7.1	22.8	0.4	0.03	50.7
	60d 30°C	38.2	3.0	7.3	3.8	1.7	8.6	184.7	0.6	0.03	247.9
	9m 22°C	146.8	6.0	15.9	7.2	3.0	14.7	524.1	1.3	0.05	719.1

Fresh: fresh beer sample; 60d 30°C: beer sample aged for 60 days at 30°C; 9m 22°C: beer sample aged for 9 months at 22°C.



**Fig. 1.** Evolution of levels of aldehyde markers as a function of beer ageing during 60 days at 30°C or 9 months at 22°C, A: sum of all aldehyde markers, B: furfural.

months at 22°C (6.7). On the other hand, during ageing of beer E the increase in the content of aldehyde markers is less pronounced. This can also be related with the low overall-ageing-score (2.5 after 60 days at 30°C and 4.3 after 9 months at 22°C). Beer C shows the lowest content of aldehyde markers after ageing. This beer was also given a low overall-ageing-score after forced ageing (3.1), as well as after spontaneous ageing (4.1). Beer F shows a very large increase in aldehyde markers, especially after ageing for 9 months at 22°C, which corresponds well with the overall-ageing-score after spontaneous ageing (6.2). As illustrated in Fig. 1, the increase in furfural, not necessarily an off-flavour but an indicator of flavour deterioration<sup>40</sup>, has the largest impact on the increase of the sum of the aldehyde markers during ageing. The beers with the highest overall-ageing-scores (beers A, B and F) present the highest increase in the concentration of furfural. Also the Strecker degradation aldehydes increase significantly during beer ageing, whereas the lipid oxidation aldehydes

show a slight increase on a quantitative basis, but a significant increase on a relative basis, in particular for hexanal.

### Evaluation of bitterness profiles

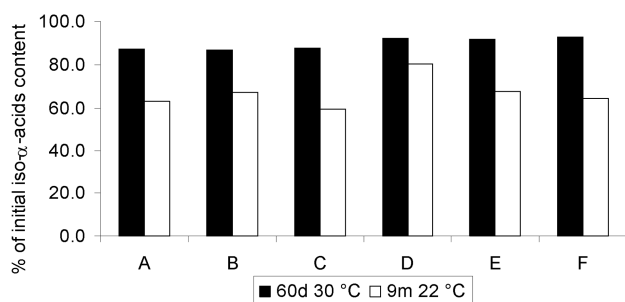
Bitterness profiles of fresh and aged beers (60 days at 30°C and 9 months at 22°C, respectively) were analysed by HPLC. Table VIII shows the levels of individual *trans*- and *cis*-iso- $\alpha$ -acids in the fresh and aged beers, respectively. The total concentrations of iso- $\alpha$ -acids after ageing, relative to the initial concentrations in the fresh beers, are presented in Fig. 2. As reported in several studies<sup>8,18,30,31,33,59</sup>, significant degradation of iso- $\alpha$ -acids occurs during beer ageing, due to oxidative deterioration. After ageing for 60 days at 30°C, the remaining relative concentration of iso- $\alpha$ -acids in the beers is comparable. Conversely, spontaneous ageing of the beers (9 months at 22°C) points to significant differences in the extent of iso- $\alpha$ -acids deterioration between the different beers. In beer

**Table VIII.** Concentrations of individual iso- $\alpha$ -acids (mg/L) in fresh, forced aged and spontaneously aged beer samples of the brews A-F.

Beer	Sample	t-ich	c-ich	t-inh	c-inh	t-iah	c-iah	Sum iso- $\alpha$ -acids	T/C-ratio
A	Fresh	2.1	5.0	2.4	7.3	0.9	2.3	20.0	0.37
	60d 30°C	1.4	4.8	1.6	6.8	0.7	2.1	17.4	0.26
	9m 22°C	0.6	4.2	0.6	5.4	0.3	1.6	12.7	0.12
B	Fresh	2.4	5.5	3.3	9.6	1.0	2.5	24.3	0.37
	60d 30°C	1.6	5.2	2.3	8.8	0.8	2.4	21.1	0.28
	9m 22°C	0.8	4.7	1.0	7.6	0.4	1.8	16.3	0.15
C	Fresh	0.9	1.8	1.7	4.0	0.5	1.0	9.9	0.44
	60d 30°C	0.6	1.7	1.1	3.9	0.4	1.0	8.7	0.30
	9m 22°C	0.2	1.5	0.3	2.9	0.2	0.7	5.9	0.12
D	Fresh	1.7	3.5	2.2	6.1	0.7	1.7	15.8	0.40
	60d 30°C	1.3	3.5	1.6	5.9	0.7	1.7	14.6	0.31
	9m 22°C	0.8	3.4	1.1	5.5	0.4	1.5	12.7	0.21
E	Fresh	1.6	3.3	1.9	5.1	0.7	1.5	14.1	0.42
	60d 30°C	1.3	3.2	1.5	4.9	0.6	1.4	12.9	0.34
	9m 22°C	0.6	2.7	0.8	3.9	0.3	1.1	9.5	0.22
F	Fresh	1.7	3.9	2.6	7.7	0.8	2.0	18.8	0.37
	60d 30°C	1.3	3.9	1.9	7.7	0.7	1.9	17.4	0.27
	9m 22°C	0.6	3.4	0.8	5.6	0.3	1.4	12.1	0.16

Fresh: fresh beer sample; 60d 30°C: beer sample aged for 60 days at 30°C; 9m 22°C: beer sample aged for 9 months at 22°C.

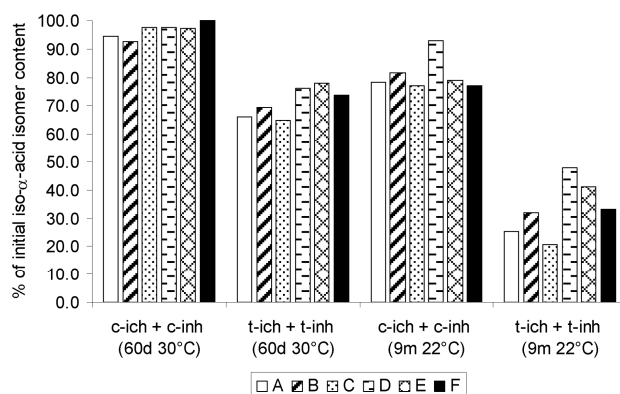
Compound identification: t-ich: *trans*-isochumulone; c-ich: *cis*-isochumulone; t-inh: *trans*-isohumulone; c-inh: *cis*-isohumulone; t-iah: *trans*-isoadhumulone; c-iah: *cis*-isoadhumulone.



**Fig. 2.** Relative concentrations of total iso- $\alpha$ -acids (%) in the commercial pale lager beers as a function of beer ageing during 60 days at 30°C or 9 months at 22°C.

D, the relative concentration of iso- $\alpha$ -acids is approx. 80% of the initial iso- $\alpha$ -acid content, whereas for the other beers, less than 70% of the initial iso- $\alpha$ -acids level remains after spontaneous ageing. The relatively high stability of iso- $\alpha$ -acids in beer D is in accordance with the results obtained by sensory evaluation. The overall ageing-score for beer D after 9 months at 22°C appears to be low (4.1; see Table V). Also beer C showed a low overall ageing-score after spontaneous ageing (4.1), although this aged beer contains less than 60% of the initial iso- $\alpha$ -acid content. This may be explained by the low absolute concentration of iso- $\alpha$ -acids in the fresh beer (approx. 10 ppm) and consequently, the rather small decrease in absolute levels (approx. 4 ppm) upon spontaneous ageing. Furthermore, beer C is also bittered with tetrahydro-iso- $\alpha$ -acids (see Table I), known to be very resistant towards oxidative degradation<sup>19,31</sup>.

The relative concentrations of *trans*- and *cis*-iso- $\alpha$ -acids upon beer ageing are presented in Fig. 3. *Cis*-iso- $\alpha$ -acids are clearly more stable than *trans*-iso- $\alpha$ -acids, which is in accordance with literature<sup>5,18</sup>. After 9 months of ageing at 22°C, the lowest degradation of *trans*-iso- $\alpha$ -acids is observed in beer D. Also, partial stabilisation of the *cis*-iso- $\alpha$ -acids is noticed in this beer where the reducing



**Fig. 3.** Relative concentrations of iso- $\alpha$ -acid stereo-isomers (%) in the commercial pale lager beers as a function of beer ageing during 60 days at 30°C or 9 months at 22°C. Compound identification: t-ich: *trans*-isochumulone; c-ich: *cis*-isochumulone; t-inh: *trans*-isohumulone; c-inh: *cis*-isohumulone.

power was increased at mashing-in (see Table I). A comparable stabilising effect on *cis*-iso- $\alpha$ -acids has also been found previously<sup>1,2,27</sup>, where gallotannins or hop polyphenols were added to the brewing and sparging liquor in order to increase the reducing power from the onset of brewing.

### Evaluation of polyphenol profiles

The content of selected polyphenolic markers in fresh and aged beers was determined by quantitative HPLC analysis. These results are reported in Table IX. The fresh beers C and E contain a relatively low amount of polyphenolic markers, in accordance with the results obtained by standard analysis of polyphenols and with the low reducing power of these fresh beers (see Table VI). During ageing, the total concentration of the polyphenolic markers decreases, especially after spontaneous ageing for 9 months at 22°C. The lowest decrease in polyphenolic markers is observed in beer E. In particular the flavanoids

**Table IX.** Concentrations of polyphenolic markers (mg/L) in fresh, forced aged and spontaneously aged beer samples of the brews A-F.

	Sample	Beer A	Beer B	Beer C	Beer D	Beer E	Beer F
Prodelphinidin trimer	Fresh	0.5	0.6	0.3	0.3	0.2	0.5
	60d 30°C	0.3	0.4	0.1	0.2	0.2	0.3
	9m 22°C	0.2	0.4	0.2	0.3	0.2	0.2
Prodelphinidin B3	Fresh	3.4	3.8	1.7	2.9	1.0	3.4
	60d 30°C	2.1	2.2	0.9	2.0	0.8	1.9
	9m 22°C	0.9	1.9	0.7	1.9	0.9	1.5
Procyanidin trimer	Fresh	1.5	1.6	0.9	1.2	0.6	1.4
	60d 30°C	1.0	1.1	0.7	0.8	0.6	0.9
	9m 22°C	0.8	1.0	0.6	1.0	0.6	0.5
Procyanidin B3	Fresh	5.3	5.7	2.5	5.2	1.6	4.3
	60d 30°C	4.1	4.5	1.6	3.8	1.1	4.0
	9m 22°C	2.3	2.1	0.7	1.8	0.9	1.7
(+) -Catechin	Fresh	5.0	5.3	1.9	5.2	3.6	5.0
	60d 30°C	4.5	4.8	1.8	4.8	3.2	4.3
	9m 22°C	3.8	4.2	1.6	4.0	2.6	3.6
(-) -Epicatechin	Fresh	0.8	0.8	0.7	2.7	0.6	0.6
	60d 30°C	0.7	0.8	0.7	2.6	0.6	0.7
	9m 22°C	0.6	0.7	0.5	0.6	0.6	0.6
p-Coumaric acid	Fresh	1.0	1.2	0.8	1.7	1.2	1.0
	60d 30°C	1.0	1.2	0.9	1.7	1.2	1.0
	9m 22°C	0.9	1.2	0.8	1.5	1.2	1.0
Ferulic acid	Fresh	2.0	1.8	1.6	2.6	2.4	1.8
	60d 30°C	1.8	1.7	1.6	2.5	2.8	1.7
	9m 22°C	1.9	1.9	1.9	2.5	2.7	1.9
Rutin	Fresh	1.5	2.1	0.3	1.6	1.1	1.4
	60d 30°C	1.4	2.1	0.4	1.7	1.1	1.4
	9m 22°C	1.5	2.2	0.5	1.9	1.3	1.6
Quercetin derivative	Fresh	0.3	0.5	0.2	0.4	0.3	0.3
	60d 30°C	0.2	0.3	0.2	0.3	0.2	0.2
	9m 22°C	0.2	0.2	0.2	0.2	0.2	0.2
Kaempferol glucoside	Fresh	1.0	1.2	0.2	1.0	0.7	1.0
	60d 30°C	0.9	1.3	0.3	1.0	0.8	1.0
	9m 22°C	0.9	1.3	0.3	1.4	0.8	1.2
Kaempferol derivative	Fresh	0.3	0.5	0.2	0.4	0.4	0.4
	60d 30°C	0.2	0.3	0.2	0.2	0.2	0.2
	9m 22°C	0.2	0.2	0.1	0.1	0.1	0.0
Isoxanthohumol	Fresh	0.7	1.0	0.1	0.4	0.6	0.6
	60d 30°C	0.8	1.0	0.1	0.4	0.6	0.6
	9m 22°C	0.7	1.0	0.1	0.4	0.6	0.5
Sum polyphenol markers	Fresh	23.3	25.9	11.2	25.6	14.4	21.7
	60d 30°C	19.1	21.7	9.5	22.1	13.5	18.2
	9m 22°C	14.9	18.3	8.1	17.6	12.7	14.4

Fresh: fresh beer sample; 60d 30°C: beer sample aged for 60 days at 30°C; 9m 22°C: beer sample aged for 9 months at 22°C.

prodelphinidin trimer, prodelphinidin B3, procyanidin trimer, procyanidin B3, (+)-catechin and (-)-epicatechin decrease as a function of beer ageing, which is also in agreement with the previously shown spectrophotometric data on the total flavanoid content (see Table VI). Flavanoids are notorious for their extreme sensitivity towards oxidative degradation<sup>42</sup>. On the other hand, cinnamic acid derivatives, flavonol glycosides, and prenylated hop flavonoids appear to be much more stable during beer ageing.

### Evaluation of profiles of esters and higher alcohols

Quantitative GC-MS profiling of esters and higher alcohols was performed on the fresh pale lager beers and on forced aged and spontaneously aged samples (see Table X). The fresh beers D and E contain the highest concentration of aroma markers, especially due to relatively high levels of 3-methylbutan-1-ol, ethyl acetate, isoamyl acetate, and phenylethyl acetate. Beer A has clearly the

lowest total level of aroma markers. The levels in higher alcohols, *i.e.* 3- and 2-methylbutan-1-ol, hardly change upon beer ageing, whereas, the concentrations of the esters (ethyl acetate, isoamyl acetate, ethyl hexanoate, ethyl octanoate, phenylethyl acetate and ethyl decanoate) decrease significantly in most beers, especially upon spontaneous ageing.

Based on all previous results, a short lautering time associated with a smaller increase in TB-index seems to be positive in view of flavour stability of beer (see beer C). Although brew D shows a high FAN content and a high increase in TB-index during brewing, this beer is the most stable in terms of flavour deterioration, which may be attributed to an increased reducing power from the onset of the brewing process till the finished beer. Even though brew F was mashed-in at a lower pH (pH 5.33), combined with a low lipoxygenase activity in the malt and a relatively small increase in trihydroxy fatty acids during brewing, this beer shows a high overall-ageing-score due to a very large increase in aldehyde markers. A high in-

**Table X.** Concentrations of esters and higher alcohols in fresh, forced aged and spontaneously aged beer samples of the brews A-F.

Beer	Sample	3-Methylbutan-1-ol (mg/L)	2-Methylbutan-1-ol (mg/L)	Ethyl acetate (mg/L)	Isoamyl acetate (µg/L)	Ethyl hexanoate (µg/L)	Ethyl octanoate (µg/L)	Phenylethyl acetate (µg/L)	Ethyl decanoate (µg/L)	Sum aroma markers (µg/L)
A	Fresh	32.2	9.0	8.8	392.8	143.6	159.9	92.1	19.4	50,868
	60d 30°C	34.0	8.6	8.5	356.7	131.8	164.7	80.5	20.8	51,867
	9m 22°C	32.5	8.9	8.5	227.4	118.8	118.9	74.0	13.5	50,400
B	Fresh	44.2	8.9	17.7	1154.4	120.1	120.5	222.5	11.9	72,389
	60d 30°C	42.7	8.8	16.8	1024.6	107.6	132.1	214.6	12.0	69,784
	9m 22°C	45.6	8.9	16.5	835.3	78.8	88.8	186.7	9.6	72,272
C	Fresh	44.0	11.4	11.5	1037.8	181.0	53.0	293.1	8.4	68,439
	60d 30°C	44.6	11.4	10.9	922.9	154.3	38.0	268.2	6.6	68,255
	9m 22°C	43.9	11.4	10.0	707.0	100.1	26.6	239.1	9.5	66,354
D	Fresh	55.4	12.5	25.3	2601.5	114.8	43.5	512.6	9.3	96,390
	60d 30°C	55.8	12.2	24.4	2317.3	61.8	24.9	485.2	9.9	95,261
	9m 22°C	54.1	11.9	21.5	1865.9	37.0	21.4	431.6	9.9	89,916
E	Fresh	50.3	13.7	24.6	1674.7	163.3	189.1	406.6	15.8	91,074
	60d 30°C	51.3	14.6	24.2	1544.9	155.6	184.6	387.7	14.4	92,390
	9m 22°C	48.3	13.2	21.6	1141.1	122.9	158.4	310.0	16.3	84,896
F	Fresh	51.8	12.9	14.3	1452.3	93.9	131.9	314.7	17.6	81,006
	60d 30°C	52.6	12.6	12.6	1370.4	90.7	117.3	302.4	12.1	79,667
	9m 22°C	53.6	13.2	13.3	1203.7	91.5	131.0	267.7	16.6	81,841

Fresh: fresh beer sample; 60d 30°C: beer sample aged for 60 days at 30°C; 9m 22°C: beer sample aged for 9 months at 22°C.

**Table XI.** Analytical parameters and sensory evaluation (OAS) used for PLSR.

Code	Analytical parameter	Code	Analytical parameter	Code	Analytical parameter
t-ich	<i>trans</i> -Isocohumulone	PF11	Kaempferol glucoside	ALD7	Furfural
c-ich	<i>cis</i> -Isocohumulone	PF12	Kaempferol derivative	ALD8	Hexanal
t-inh	<i>trans</i> -Isohumulone	PF13	Isoxanthohumol	ALD9	<i>trans</i> -2-Nonenal
c-inh	<i>cis</i> -Isohumulone	PF	Sum polyphenol markers	ALD	Sum aldehyde markers
t-iah	<i>trans</i> -Isoadhumulone	ARO1	3-Methylbutan-1-ol	FLAV	Flavanoid content
c-iah	<i>cis</i> -Isoadhumulone	ARO2	2-Methylbutan-1-ol	TRAP	Reducing power
ISO	Total iso- $\alpha$ -acid content	ARO3	Ethyl acetate	TBI	TB-index
T/C	T/C-ratio	ARO4	Isoamyl acetate	THOE	Trihydroxy fatty acids
PF1	Prodelphinidin trimer	ARO5	Ethyl hexanoate	CH	Cold haze
PF2	Prodelphinidin B3	ARO6	Ethyl octanoate	PH	Permanent haze
PF3	Procyanidin trimer	ARO7	Phenylethyl acetate	PROANT	Proanthocyanidins
PF4	Procyanidin B3	ARO8	Ethyl decanoate	TPF	Total polyphenols
PF5	(+)-Catechin	ALD1	2-Methylpropanal	BC	Beer colour
PF6	(-)-Epicatechin	ALD2	2-Methylbutanal	FAN	Free amino nitrogen
PF7	p-Coumaric acid	ALD3	3-Methylbutanal	SOL_P	Soluble proteins
PF8	Ferulic acid	ALD4	Methional	FOAM	Foam stability
PF9	Rutin	ALD5	Benzaldehyde	OAS	Overall-ageing-score
PF10	Quercetin derivative	ALD6	Phenylacetaldehyde		

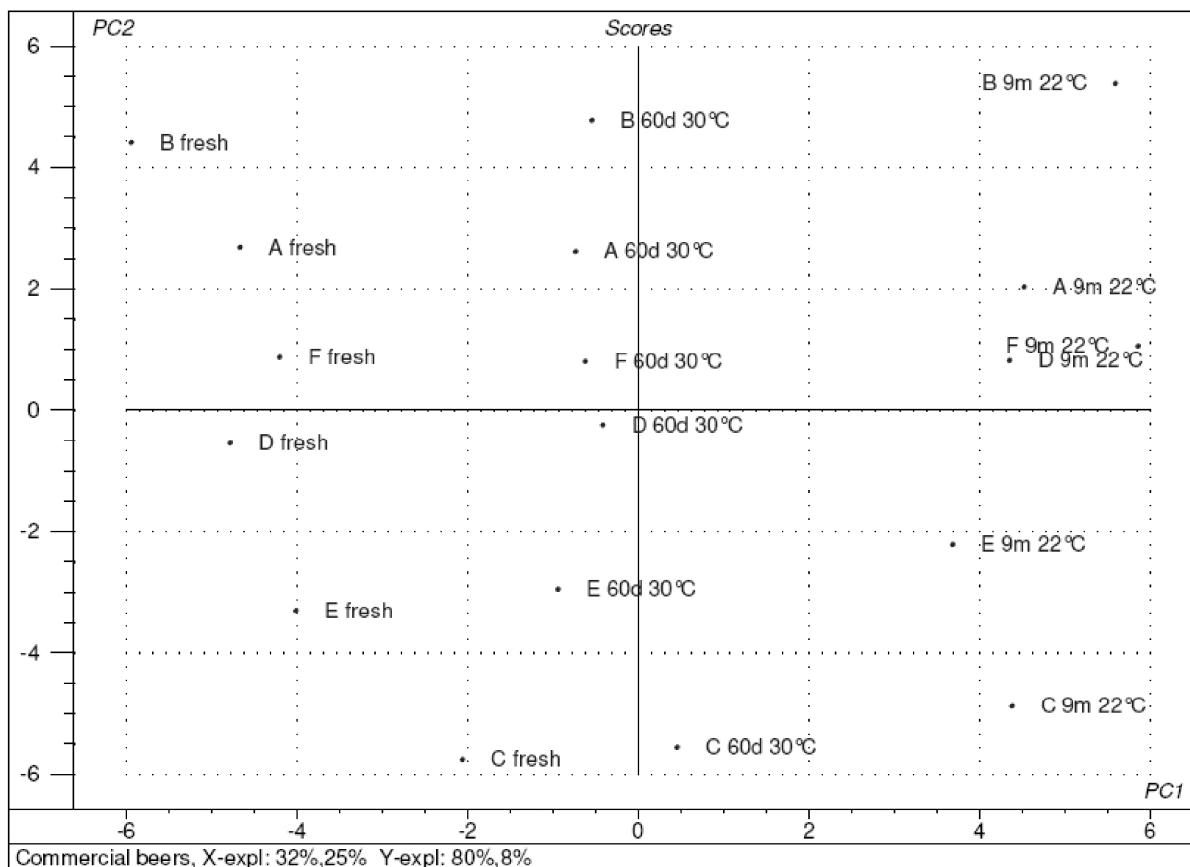
crease in aldehyde markers (especially furfural) during beer ageing is clearly correlated with flavour instability (see beers A, B and F). Conversely, relatively low levels of aldehydes after beer ageing are connected with prolonged flavour stability (see beers C and E).

### Multivariate data analysis

To determine the most relevant parameters in relation to flavour instability, multivariate data analysis was performed on the analytical data and on the sensory evaluation of the fresh, forced aged and spontaneously aged beer samples of the six pale lagers. Multivariate partial least squares regression techniques (PLSR) were employed to develop a model between the analytical data, represented

in the X matrix, and the overall-ageing-score (OAS), represented in the Y matrix<sup>23,49</sup>. All used parameters with their corresponding code are summarised in Table XI.

Fig. 4 shows the score plot of the PLSR analysis of the fresh, forced aged and spontaneously aged beer samples based on the data of the analytical parameters (X matrix) compared to the OAS (Y matrix). The fresh, forced aged, and spontaneously aged beer samples are clearly divided into three groups. The beer samples on the left side of the score plot represent the fresh samples. The more the samples move to the right side of the score plot, the more aged they are. It is obvious that upon storage for 9 months at 22°C the beers are more aged than after 60 days of storage at 30°C. The correlation loadings plot of the PLSR



**Fig. 4.** Score plot of PLSR analysis of fresh, forced aged (60 days at 30°C) and spontaneously aged (9 months at 22°C) beer samples based on analytical data compared with OAS (overall-ageing-score).

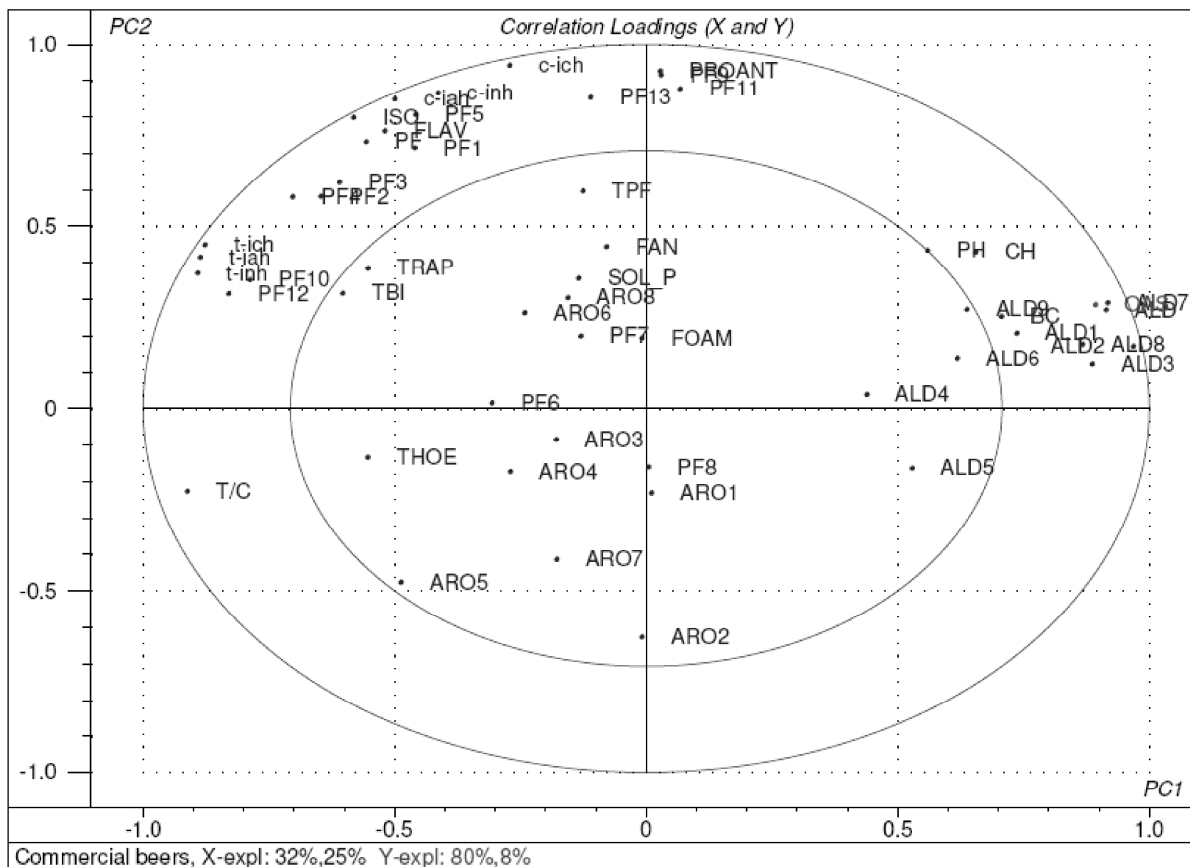
analysis is shown in Fig. 5. Ellipses represent a correlation coefficient of the analytical data with the OAS of 50% and 100%. A decrease in the level of the analytical parameters on the left side (the fresh beer side) of the correlation loadings plot (decrease in total bitterness, *cis*-iso- $\alpha$ -acids and especially *trans*-iso- $\alpha$ -acids, the T/C-ratio, some polyphenolic markers (especially proanthocyanidins and the flavonoids PF10 (quercetin derivative) and PF12 (kaempferol derivative)), flavanoid content, and to a lesser extent TB-index and reducing power (TRAP)) is correlated with an increase in overall-ageing-score. Clearly, these parameters situated on the fresh beer side of the correlation loadings plot represent substances known for their high sensitivity towards oxidative degradation. Conversely, an increase in the level of the analytical parameters on the right side (aged beer side) of the correlation loadings plot is correlated with an increased overall-ageing-score. This is true for several aldehyde markers (especially total aldehyde content, furfural, hexanal and the Strecker degradation aldehydes 2-methylpropanal, 2-methylbutanal and 3-methylbutanal), beer colour, and cold and permanent haze.

By means of the correlation loadings plot (Fig. 5), the different positions of the beers in the score plot (Fig. 4) can be explained. For instance, the fresh beer C is positioned at the bottom of the score plot because of its high level in ethyl hexanoate (ARO5). The fresh beer E also contains a high concentration of ethyl hexanoate (ARO5)

and the highest content of trihydroxy fatty acids (THOE). On the other hand, the fresh beer B is situated at the top of the score plot because this beer shows the highest concentration of *cis*- and *trans*-iso- $\alpha$ -acids (c-ich, c-inh, c-iah, t-ich, t-inh, t-iah), the highest flavanoid content (FLAV), and the highest level in polyphenolic markers, such as prodelphinidin B3 (PF1), prodelphinidin trimer (PF2), procyanidin B3 (PF3), procyanidin trimer (PF4) and (+)-catechin (PF5). Also the fresh beers A and F show a relatively high content of these polyphenolic markers. The fresh beer D is characterised by high levels in (-)-epicatechin (PF6), ferulic acid (PF8), ethyl acetate (ARO3) and isoamyl acetate (ARO4). Upon ageing, all beers show a higher colour (BC), more haze (PH; CH), a higher level of aldehyde markers, and a significant overall-ageing-score (OAS), especially after spontaneous ageing. The highest OAS is given for the spontaneously aged beers B and F, which are situated very near to the right side (aged beer side) of the score plot.

## CONCLUSIONS

In this paper, six commercial pale lager beers from different Belgian breweries were evaluated in relation to flavour stability. Notwithstanding significant differences in the composition of the raw materials (malt, hops, brewing liquor), brewing technology and parameters, and processing aids, several different analytical parameters rele-



**Fig. 5.** Correlation loadings plot of PLSR analysis of fresh, forced aged (60 days at 30°C) and spontaneously aged (9 months at 22°C) beer samples based on analytical data compared with OAS (overall-ageing-score).

vant for adequate evaluation of beer flavour stability have been identified on the basis of multivariate data analysis. The most relevant parameters comprise *trans*-isohumulones, *cis*-isohumulones, total bitterness, the T/C-ratio, aldehyde markers (especially furfural, hexanal, 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal), polyphenolic markers (especially proanthocyanidins), flavanoid content, cold and permanent haze formation, and beer colour. Clearly, compounds known to be very sensitive towards oxidative deterioration (in particular *trans*-isoo- $\alpha$ -acids and proanthocyanidins) are correlated with sensory beer ageing, as well as an increase in aldehydes (especially furfural and the Strecker degradation aldehydes 2-methylpropanal, 2-methylbutanal and 3-methylbutanal).

In conclusion, a reliable integrated analytical-sensorial methodology for determination of the flavour stability of pale lager beers has been established. This methodology will be applied for the evaluation of the quality of raw materials and for optimisation of brewing technology and process parameters in view of prolonged flavour stability of pale lager beers.

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