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Detailed Multivariate Modeling of Beer Staling in Commercial Pale Lagers

It is generally recognized that flavour quality and flavour(in)stability cannot be grasped by one parameter, since the multitude of flavour chemicals involved. To identify beer chemistry changes during staling in commercial pilsner beer, an integrated analytical-sensorial methodology using multivariate statistical analysis was applied on samples subjected to ageing at 30 °C. Application of this technique to model the taste(in)stability in an objective way, offers the opportunity to more thoroughly investigate the influence of raw materials, brewing methods and applied technologies on flavour stability. The models obtained showed differences in aging behaviour of six commercial pale lager beers. Furthermore, detailed multivariate analysis allowed us to identify chemical compounds related to beer staling and facilitates a better understanding of beer flavour (in)stability by pinpointing the impact of process parameters and applied technologies.

Descriptors: Beer ageing, flavour stability, pale lager, multivariate statistical analysis

1 Introduction

Currently, one of the main quality problems of beer is the change of its chemical composition during storage. Many different flavours may (dis)appear resulting from many chemical reactions, which alters the sensory properties. Each of these reactions may contribute with lesser or greater extent to the decline in overall flavour quality/stability and "drinkability". As previously mentioned by *Malfliet* et al. 2008, most of these changes are caused by oxidation: oxidation of higher alcohols [4], oxidative degradation of hop-derived bitter acids [6,7, 21,22, 35, 36, 38, 39, 41, 42, 47, 64], Strecker degradation of amino acids [5, 32], oxidation of unsaturated fatty acids [18, 24, 25] and aldol condensations [34]. The current knowledge about the (bio)chemical origin of various ageing flavours and the possible reaction mechanisms responsible for their formation have been further reviewed by *Vanderhaegen* et al. [63].

Both acceleration and deceleration of the various flavour changes, that appear in time in packaged beer depend on storage conditions [9, 28, 33], pH-value [31, 33, 46], temperature [29, 45, 52], oxygen and free radical content as well as on the exposure to light. In particular, the storage temperature of beer is very important since the rate of chemical reactions largely depends on temperature. The reaction rate increase for a certain temperature increase depends on the reaction activation energy. This activation energy differs with the reaction type, which means that the rates of different reactions do not equally increase with increasing temperature. Consequently, beer storage at different temperature will lead to different relative amounts of staling compounds, in other words, lead to different ageing profiles [63]. Conversely, beer kept at low temperature (0–4 °C) fail to show signs of oxidation even after several months of storage [11]. Furthermore, finishing and packaging conditions can influence the amount of oxygen in the bottle. Therefore, since oxygen can start a large range of chemical reactions, higher oxygen levels can increase the rate of beer staling significantly [29, 62]. Moreover, due to the complexity of both malt and beer production as well as the intricate composition of the beer matrix, a multitude of parameters may have an effect on the flavour stability of the finished product [52]. In addition, different beer types do not only age at a different rate but often in a different way. *Bamforth* [13] summarizes the issue of flavour instability as follows: "Perhaps the greatest technical challenge facing the brewer remains the achievement of flavour stability." In practice, the outcome of scientific investigation resulted in the design and implementation of certain anti-oxidative beer production systems [1, 2, 49, 65]. Nevertheless, flavour stability is still a serious problem for every brewery, especially when their beers are exported all over the globe.

The aim of the current work was to evaluate the sensory and analytical changes of six commercial pale lager beers in relation to their flavour instability. To identify beer chemistry changes during staling in commercial pilsner beer, an integrated analytical-sensorial methodology using multivariate statistical analysis was applied on fresh samples and samples subjected to ageing at 30 °C for 30, 60, 90 and 120 days. Additionally, the influence of the quality of the raw materials (malt and hops) was investigated in relation to beer flavour instability.

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Tables and figures see Appendix

2 Materials and methods

2.1 Beer samples

Six fresh commercial pale lager beers, obtained from different Belgian breweries delivered immediately after bottling, were stored at 0 °C to preserve freshness. All breweries delivered their

beers three times, each delivery representing a completely distinct batch of production.

2.2 Ageing of beer samples

All the bottled beer samples were aged in the dark under forced conditions at 30 °C in a thermostatically controlled room for 30, 60, 90 and 120 days, respectively.

2.3 Standard analysis of fresh and aged beers

Standard beer analyses were performed according to EBC-methods [26], IOB-methods [37] and in-house procedures:

- Total Polyphenols: EBC-method: 9.11;
- FAN (Free Amino Nitrogen): EBC-method: 9.10;
- Flavanoid content: EBC-method: 9.12;
- TB-index (thiobarbituric acid) in malt and beer: method according to *Thalacker* and *Böβendörfer* [60];
- Foam stability, using the NIBEM-T-meter: EBC-method: 9.42;
- Soluble Protein: Bio-Rad Protein Assay, according to *Bradford* [17];
- Beer colour: IOB method: 9.1;
- Dissolved oxygen content (Intap 4000 portable DO-meter; Mettler Toledo, Zaventem, Belgium);
- pH.

Alcohol, original extract, real extract, and attenuation were determined using an Anton Paar Alcolyser with a DMA 5000 density meter. The reducing power of beer was determined by the TRAP reducing activity test according to *Araki* et al. [5] and of malt according to *Woffenden* et al. [69].

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

Permanent haze: analysis of turbidity of beer kept for a minimum of 24 h at 20 °C (VOS ROTA 90 haze meter (Haffmans, Venlo, The Netherlands)). Wort filtration rate was determined according to Malfliet et al. [51].

2.4 Evaluation of colloidal stability upon forced beer ageing

The beer samples were placed 6 days at 60 °C for forcing the beer. After cooling the forced aged beer samples for 24 h at 0 °C, the cold haze was measured using a Haffmans VOS ROTA 90 haze meter (Haffmans, Venlo, The Netherlands). After the measurement, samples were kept for 24 h at 20 °C and measured again.

2.5 UPLC determination of (reduced) iso-α-acids

UPLC separations of (reduced) iso-α-acids were performed on an Acquity UPLC (Waters, Milford, USA), consisting of a PDA detector, column heater, sample manager, binary solvent delivery system and an Acquity UPLC HSS C18 1.8µm column (2.1 i.d. × 150 mm; Waters, USA). Data reprocessing was done using Empower 2 software.

Chromatographic conditions were: eluent A: milli-Q water adjusted to pH 2.80 with H_3PO_4 (85 %, Merck, Darmstadt, Germany); eluent B: HPLC-grade CH_3CN (Novasol, Belgium). Elution: isocratic using 52 % (v/v) solvent B and 48 % (v/v) solvent A. Analysis time: 12 min. Flow rate: 0.5 mL.min^{-1}. Column temperature: 35 °C. UV detection: 270 nm (iso-alpha-acids) and 254 nm (tetrahydro-iso-α-acids). The *trans*/*cis* iso-α-acids ratio (T/C-ratio) was based on the measured concentrations of *trans*- and *cis*-isocohumulone and *trans*- and *cis*-isohumulone.

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

2.6 UPLC determination of amino acids

The Acquity Ultra Performance LC (UPLC) separation system from Waters was used to quantify individual free amino acids in beer. The UPLC system uses relatively new technology to achieve excellent separation of over 20 amino acids in less than 10 minutes between injections. Before derivatization of amino acids in the beer samples, the proteins, were removed. Therefore, 20 µl Carrez I reagent (106 g potassium ferrocyanide trihydrate ($K_4Fe(CN)_6.3H_2O$ in 1,000 ml water) and 20 µl Carrez II reagent (220 g zinc acetate dihydrate ($Zn(CH_3COO)_2.2H_2O$) and 30 ml acetic acid is made-up to volume with mQ-water 1,000ml) was added to 1 ml of previously degassed beer. After addition sample was mixed and centrifuged (in 2 ml eppendorf microtube for 5 min.). Sample derivatization was done using the Waters AccQ•Tag Ultra Chemistry Package.

UPLC separations of amino acids were performed on an Acquity UPLC (Waters, Milford, USA), consisting of a PDA detector, column heater, sample manager, binary solvent delivery system and an AccQ•Tag™ Ultra column (2.1 i.d. × 100 mm; Waters, USA). Data reprocessing was done using Empower 2 software.

Chromatographic conditions were: AccQ•Tag Ultra Eluent A Concentrate (10 times diluted) (Waters, Milford, USA); AccQ•Tag Ultra Eluent B (Waters, Milford, USA). Elution: gradient elution according to Waters AccQ•Tag Ultra method. Analysis time: 9.5 min. Flow rate: 0.7 mL.min^{-1}. Column temperature: 60 °C.

2.7 GC determination of trihydroxy fatty acids

Determination of the hydroxy fatty acids, based on the procedures of *Möller-Hergt* et al. [56] and *Wackerbauer* and *Meyna* [68], was carried out using GC-FID (Thermo Quest CE Trace 2000 (Interscience, Benelux)) equipped with an AS 2000 autosampler (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 µm film thickness, Varian, The Netherlands). Data reprocessing was done using the Chromcard software 1.0.7.

2.8 GC-MS determination of aldehydes

Volatile aldehydes in beer were determined according to *Vesely* et al. [67], using headspace-solid phase microextraction (HS-SPME) with on-fibre PFBOA (*o*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine) derivatization and capillary gas chromatography/mass spectrometry (CGC/MS) (Dual Stage Quadrupole (DSQ™ II) GC/MS system, Interscience Benelux). The DSQ™ II was coupled to a Thermo Trace GC Ultra (Interscience Benelux) equipped with a CTC-PAL autosampler (including SPME sampling), 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 µm film thickness, Restek, Interscience Benelux). Data reprocessing was done by the XCalibur™ data system (Thermo Electron Corporation).

2.9 Sensory evaluation of fresh and aged samples

Beers were evaluated by the trained taste panel of of KAHO St.-Lieven (from 8 up to 12 panellists). For the fresh beer samples, the panellists were asked to evaluate the smell and aroma intensity, as well as bitterness quality, sweetness, sourness, fullness and astringency by giving scores from 0 to 8.

The flavour stability of the beers was evaluated after ageing (30, 60, 90 and 120 days at 30 °C). Fresh and aged samples were always presented as a series of fresh and aged samples of one brew, without disclosing the identity of the samples. The degree of staling for each beer sample was determined by giving an overall-ageing-score (OAS) based on an 8-point scale (in-house procedure) (OAS: 0: fresh, oxidized flavour not detectable; 2: very weakly aged; 4: weakly aged; 6: clearly aged; 8: strongly aged, undrinkable).

2.10 Multivariate statistical analysis

Sensory and analytical data were analyzed using a multivariate data analysis software package (The Unscrambler®; CAMO, Oslo, Norway) in order to compare flavour stability of the pale lager beers and further to determine those parameters relevant for their instability.

3 Results and discussion

3.1 Raw materials in relation to beer flavour stability

3.1.1 Evaluation of malt

The malts used for the preparation of the six commercial pale lager beers were evaluated for parameters believed to be essential in terms of their influence on beer flavour stability. For pilsner malt, an extensive set of specification or quality demands (pH, viscosity, corn size distribution, colour, FAN, total protein content) is used by the malting and brewing industry. However, additional information can be required to gain a better understanding of the malt properties and their influence on beer flavour quality and stability. The influence of the malt composition on beer quality and flavour stability was tackled earlier in several studies [16, 19, 20, 30, 65]. In this work, we further determined aldehyde content, trihydroxy fatty acid (THOE) content, filtration rate, free amino nitrogen (FAN), metal content, antioxidative activity (TRAP) and TB-Index (for the results see Table 1 and 2).

Concerning the results of free marker aldehydes in the malt samples (see Table 1), it is interesting to see the differences in their content between the malts. The total marker aldehydes level ranges from 5953 µg/kg (malt E) to 11786 µg/kg malt (malt A). In all malt samples, 3-methylbutanal was found to be present in the highest concentration, followed by 2-methylpropanal and 2-methylbutanal. Further, it was found that the malt samples from brewery A and D contain the highest aldehydes concentration and the malt from brewery E the lowest. The heat-load indicator furfural was found to be in the highest range in the malt samples A and D. The lowest furfural content was found in the malt from brewery C and brewery E. Also the TB-Index was the lowest in these two samples (see Table 2). According to *Kunze* [48], the TB-Index for pilsner malt can be at a maximum of 14. Only malt from brewery F has a TB-Index higher than the allowed maximum.

It is further interesting to observe that the pilsner malts A and D contain relatively high concentrations of furfural and Strecker aldehydes (2-methylpropanal, 2-methylbutanal, 3-methylbutanal, methional and phenylacetaldehyde), but malts A and D can be differentiated on the basis of the content in fatty acids oxidation aldehydes (hexanal and *trans*-2-nonenal) (see Table 1). Indeed, malt D is high in fatty acids oxidation aldehydes whereas malt A is low. On the other hand pilsner malt C contains relatively high concentrations of hexanal and *trans*-2-nonenal, but low concentrations of furfural and Strecker aldehydes. Furthermore, pilsner malt E contains relatively low concentrations of the three groups of marker aldehydes. All of the above mentioned observations are confirmed by PCA analysis as shown in figure 1.

With regard to the trihydroxy fatty acids content (THOE) (see Table 2), clear differences were found between the malt samples. The lowest THOE's content was found in malt B (13.3 mg THOE's/kg malt), whereas 25.7 mg THOE's/kg malt was found in malt from brewery F. Trihydroxy fatty acids are considered as indicators for the oxidation of unsaturated fatty acids, in particular during mashing. Filtration rates differ from 80 ml/hour for malt C up to 114 ml/hour for malt E. Malt E also shows the lowest FAN value which is positive from the view of beer flavour stability in that way that high FAN can lead to higher production of off-flavours (Maillard reaction, Strecker degradation) and thus lower flavour stability. Moreover, high FAN value can indicate strong malt modification.

No significant differences in the iron and copper content were found between the malt samples. The antioxidant activity varies from 210 (µM (+)-catechine eq.) for malt E up to 295 (µM (+)-catechine eq.) for malt D (see Table 2).

3.1.2 Evaluation of hops

Next to malt samples also the quality of hops has been evaluated in relation to flavour stability. Besides determination of the α- and β-acids content, the content of iron and copper in hops was

measured. Several studies indicate that free-radical reactions are responsible for beer deterioration. During the storage of beer several reactive oxygen species are produced which initiate free-radical reactions, leading to flavour staling and haze formation. Transition metal such as Fe and Cu play an important role in these radical reactions. They participate in metal catalysed reactions such as the Fenton and Haber-Weiss reactions to produce the active oxygen species [44]. In the Fenton reaction, iron(II)-ions are oxidized to iron(III) by hydrogen peroxide, forming a hydroxyl radical and a hydroxyl ion. The iron(III) eventually reacts with a further molecule of hydrogen peroxide generating two protons and a superoxide radical. These superoxide radicals react with copper(II)-ions to copper(I) and oxygen in the Haber-Weiss scheme. The copper(I)-ion that is produced is capable of splitting a hydrogen peroxide molecule into a hydroxyl ion and a hydroxyl radical. The formed radicals from both Fenton and Haber-Weiss schemes are very reactive and may give rise to radical chain reactions. According to Bamforth and *Parsons* [14], hydroxyl radicals are the most important intermediates in the formation of aged flavour compounds in beer. The use of radical scavengers could improve beer flavour stability. In a later study, Bamforth [10] further discovered that an addition of peroxides and heavy metal ions to beer led to a very rapid development of stale flavour and that peroxides catalyzed by copper ions according to the Haber-Weiss reaction gave rise to the formation of hydroxyl radicals.

Nowadays, it is generally accepted that molecular oxygen is relatively stable and needs to be activated before developing its damaging impact in bottled beer [61]. The degradation of hydrogen peroxide can be considered the last step of this activation, while heavy metals are catalyzing this degradation. Metals can also catalyze the formation of other radicals in beer without the influence of oxygen (e.g. in the formation of fatty acid radicals). Heavy metal ions are therefore of decisive importance for beer ageing [70].

As has been reported by *Aron* and *Shellhammer* [8], iron and copper from the raw materials side may have relatively little influence on the flavour stability of the final beer.

Nevertheless, we wish to emphasize that significant differences in the metal content were measured between the hops samples used for the preparation of the commercial lager beers. Iron content varies from 14.6 mg Fe/100 g hops up to 48.7 mg Fe/100 g hops (see Table 3). Copper levels vary from 6.6 mg Cu/100 g hops up to 60.8 mg Cu/100 g.

3.2 Evaluation of beer samples

3.2.1 Sensory evaluation of fresh beers

Sensory evaluation of the six fresh pale lager beers was carried out by the trained taste panel of KAHO St.-Lieven. Panellists were asked to describe the aroma, smell and the taste including bitterness intensity and quality. In all beers, fruity and hoppy aromas were found (1–2 points out of 8). Furthermore, very moderate up to moderate sulphur impressions were noticed (approx. 3 points). Bitterness of the beers was clearly noticeable (approx.6 points out of 8) with relatively high bitterness quality described sometimes as somewhat sharp and/or lingering. Moreover, all six lagers scored low for astringency and were given 3 to 4 points out of 8 for fullness. In the lager beer from brewery F and especially beer F3, some adverse aromas were found, such as diacetyl (2 points out of 8), rancidity (2 points) and a light metallic note (3 points out of 8) (data not shown).

3.2.2 Analytical evaluation fresh beers

Aiming at detailed characterization of the six fresh commercial pale lager beers, extensive analytical evaluation, including standard beer analyses (pH, FAN, soluble protein, total polyphenols, flavanoids, etc.), advanced GC (trihydroxy fatty acids, aldehydes) and UPLC profiling of amino acids and bitter acids, was performed. The results of this investigation clearly show differences between the beers. In general, the main differences were noticed for the total levels of polyphenols, flavanoids, reducing power (TRAP), total bitterness, amino acids and aldehydes profiles, and levels in THOE's. The bi-plot of PCA on the analytical data of the fresh beers (see Fig. 2), clearly points to a separation of the samples in three groups, i.e., beers from the breweries A, D, and E vs. beers from the breweries B and C, and beers from brewery F. Lager beer F differs significantly from the other lagers based on the higher levels of THOE, haze formation, high reducing power, and apparent attenuation, and a significantly higher T/C-ratio and pH. Also the FAN (free amino nitrogen) value and the amino acids content is one of the highest in this beer. Based on PCA analysis (Fig. 2), it can also be concluded that the reproducibility of the brewing of the three series of lager beer C is somewhat higher than the reproducibility of preparation of the other commercial lagers.

The results of the standard analyses performed on both fresh as well as aged beer samples are summarised in table 7. The fresh commercial pale lager beers have an average alcohol content of 5.2 (v/v; %), and the pH value varies between pH 4.19 (beers D1 and D2) and pH 4.72 for beer F. The oxygen content measured directly after beer delivery is for all the beers lower than 100 ppb with exception of lager beer A2 and E2 (116 ppb and 228 ppb, respectively). Large differences in free amino nitrogen (FAN) are further noticed between the fresh pale lager beers. In particular, high FAN values were found in beers E and F (mean value 107.6 mg/L and 124.1 mg/L, respectively) in comparison with the other lager beers. The lowest free amino nitrogen value was measured for lager beer B (58.8 mg/L). Furthermore, relatively large variations in free amino nitrogen content were found for the beers from the same brewery. On account of relatively large differences in FAN content between the three series of beers E and F, it can be concluded that the consumption of free amino nitrogen by the yeast can differ significantly during different fermentations.

A positive relation between the polyphenol content and the reducing power (TRAP) of the beers was found, which is in agreement with earlier published data [45, 52, 59]. Beer D has the lowest total polyphenol and flavanoid content, and the lowest reducing power (TRAP) in contrast to beer F with the highest polyphenol content and reducing power. Beer B shows the highest flavanoid content (mean value 42.6 mg/L) probably due to high hops addition. The content of sulfite (SO_2) ranges from 7.4 mg/L to 18.0 mg/L among

the samples. Furthermore, the concentration of soluble protein varies between 178 mg/L and 411 mg/L.

All fresh commercial lager beers were also evaluated for their content in trihydroxy fatty acids (THOE). These oxidized fatty acids can be formed during mashing-in via interaction of LOX-enzyme originating from the malt. However, mashing-in at 64 °C and pH 5.2 can reduce the formation of trihydroxy fatty acids significantly [3]. Furthermore, part of the trihydroxy fatty acids is derived from malt itself. During beer aging the trihydroxy fatty acids may be further converted into aldehydes (*trans*-2-nonenal and hexanal). The THOE levels formed during the brewing process of the commercial lagers are summarised in table 4. In the third column, the contribution to the THOE value by the brewing process is calculated. The lowest trihydroxy fatty acids content was found in lager beers A (mean value 4.74 mg/L) and B (mean value 4.85 mg/L) and the highest in beer F (approx.10 mg/L THOE). From the values in the third column (see Table 4), it is apparent that most of the trihydroxy fatty acids were formed during brewing of beer F. On the other hand the THOE contribution derived from brewing is lowest for beers from brewery A.

Amino acids can be classified according to their rate of absorption from the medium during fermentation. In complex media such as wort, brewers usually distinguish four groups of amino acids [43]. In the usual model, group A, which includes aspartate, threonine, serine, glutamate, lysine, and arginine, is reported to be immediately absorbed and almost totally consumed after a few hours of fermentation. group B, which includes valine, methionine, isoleucine, leucine, and histidine, is said not to be removed rapidly, but absorbed gradually throughout fermentation. Alanine, tyrosine, phenylalanine, tryptophane, and glycine define group C, which is characterized by a very long lag phase. These amino acids are used only when group A is totally depleted. Proline, finally, is known to be only slightly absorbed from wort under anaerobic conditions [58]. Too high levels of amino acids in the finished beer may result in excessive production of Strecker degradation aldehydes during beer ageing and thus may contribute to the staling flavour of aged beer [23]. Therefore, in this work, a detailed amino acids profile of the fresh commercial beers was determined by UPLC after sample derivatization using the Waters AccQ•Tag Ultra Chemistry Package. From figure 3, it is obvious that beers E and F contain more amino acids than the other lager beers in particular amino acids that are usually removed relatively fast (group A and group B) are still present in high amounts.

The iso-α-acids profiles of all beers (see Table 5) were analysed immediately after delivery (fresh samples) and after forced ageing in the dark at 30 °C for 30, 60, 90 and 120 days. The applied chromatographic methodology results in full separation of the six individual iso-α-acids, allowing a detailed study of iso-α-acids behaviour during beer storage. All beers were bittered with conventional hop products, but the beers from brewery A and E (beer from the third series E3) also contain small amounts of tetrahydroiso-α-acids. Total iso-α-acids content varies from 12.37 mg/L (beer A2) up to 23.30 mg/L (beer C3). Remarkable is the low T/C-ratio of the brew A3 (27.5 %) pointing to the use of pre-isomerised hop extract during preparation of this beer. Furthermore, the relatively low T/C-ratios found in beers B, C and D may indicate losses of *cis*- and especially *trans*-isomers during the brewing process or additional use of pre-isomerised hop products since a T/C-ratio between 45 % and 48 % is expected when using only conventional hop products. Relatively high T/C-ratio for beer F (48.7 % to 51.7 %) can be due to higher pH of this beer.

For the beers from the breweries A and B, relatively large differences in iso-α-acids concentrations between the three series were found. Such fluctuations in bitterness content can be the result of varying utilisation of the hop α-acids as utilisation is known to be influenced by numerous factors. The isomerisation process itself and the hop utilisation in the kettle can be influenced by temperature, vigour and length of boil, pH of the wort, specific gravity, hopping rate, type of hop product(s) used, and kettle design [15, 40, 41, 42, 54, 55, 57, 66].

Finally, quantitative GC-MS profiling of aldehyde markers was performed on all fresh beer samples. Different groups of aldehydes are present in the final beer. Some aldehydes originate from fermentation, others are reaction products of Strecker degradation of amino acids or result from oxidation of unsaturated fatty acids [63]. Straight carbon chain aldehydes have typical aldehyde odours that become more unpleasant as the chain length increases. As beer ages in commercial packages, the very potent *trans*-2-nonenal contributes to the characteristic "old beer" flavour (staling flavour) but, as mentioned above, nowadays an array of marker aldehydes is usually determined in relation to flavour instability. Malfliet et al. [52] selected nine marker aldehydes for this purpose. 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). As apparent from table 6, furfural is generally the major aldehyde marker (highest level) in the fresh beers. However, in the beer from brewery F, 2-methylpropanal is present in the highest concentration. This aldehyde is also found in the malt A in relatively high concentrations. Interpretation of the results in table 6 and the figure 4 shows some interesting observations. Large variation in aldehydes profile was found for brewery F. Furthermore, beer D3 is clearly deviating from the other beers derived from this brewery because of high furfural levels. Moreover, beer A1 is clearly different from other two beers from brewery A, mainly because of Strecker degradation aldehydes: 2-methylpropanal, 3-methylbutanal, methional, and some lesser extend to 2-methylbutanal, phenylacetaldehyde. Relatively constant aldehyde profiles and low aldehyde levels were found for fresh brew B and E.

3.2.3 Sensory evaluation of forced aged beers

The results of the sensory evaluation of the beers after ageing for 0, 30, 60, 90 and 120 days in the dark (30 °C) are shown in figure 5. Forced aged and fresh beers were evaluated by the tasting panel for comparison and determination of the extent of ageing by assessing an overall ageing score (OAS).

Stale flavours were not noticed in the fresh samples. Furthermore, a decrease in overall sensory appreciation, fullness, bitterness intensity and quality, was noticed in all tested aged beer samples. After 60 days of storage at 30 °C, differences in flavour stability

between the commercial lagers became apparent. Figure 5 demonstrates that, based on the overall-ageing score beers C and F are sensorially the most sensitive towards ageing. On the other hand beers B, D and E shows the lowest overall-ageing scores as a function of storage time. In the beers B and E less off-flavours were perceived probably due to a masking effect on ageing flavours, since large amounts of hops were introduced during brewing of these beers. Sensory evaluation of the flavour stability of beer, especially beer B and F, corresponds well with developed models of beer ageing (see further). However, the sensory data are not in full agreement with the analytical data on bitter acids degradation and formation of carbonyl markers (see further).

3.2.4 Analytical evaluation of aged beers

All lager beers were subjected to forced ageing (for 30, 60, 90 and 120 days at 30 °C in the dark) and evaluated both analytically and sensorially in respect of flavour quality and stability. From the data of the standard analyses (see Table 7), UPLC determination of bitter acids (Table 8) and GC-MS analysis of marker aldehydes (Fig. 7) on the aged lagers, the following changes can be observed upon ageing: an increase in cold haze, beer colour and aldehydes content, and a decrease in bitterness and TB-Index. The total polyphenol content changes a little bit or not as a function of forced aging time at 30 °C. In all the beers, a decline in flavanoid content was noticed. Flavanoids are generally considered as components that are very sensitive to oxidation. Within the total polyphenolic group, flavanoids will be therefore oxidize relatively faster during beer aging than the other polyphenol classes. The reducing power as determined by the TRAP assay also decreases during beer aging. Furthermore, the pH remains unchanged.

Several substances can cause haze in beer but the most frequently encountered problem is due to cross-linking of polyphenols and proteins. These components exist in equilibrium in beer and manifest themselves as a haze when the polyphenols start to polymerize. The proteins particularly involved in haze are rich in proline [12].

The colloidal stability of lager beer B (high polyphenol content, in particular flavanoids see Table 7) is significantly lower than the stability of the other beers. On the other hand, lager beer D (low polyphenol content) has higher colloidal stability in comparison to the other lagers.

Next to the colloidal stability, also bitterness stability of the 6 commercial pilsners was evaluated. The change in concentrations of iso-α-acids was measured at selected ageing intervals by UPLC. The results are summarised in table 8. Because *trans*- and *cis*-isomers are fully separated in our UPLC system, also the evolution of the individual iso-α-acids was monitored.

During ageing, a decline in iso-α-acids levels was observed in all beers (see Table 8) which is in agreement with earlier published results [11, 21, 41, 52]. However, the rate of bitterness decline was not the same for all beers. In the lager beer F (highest T/C-ratio), the relative concentration of iso-α-acids is 81 % of the initial iso-α-acids content after 120 days of ageing, whereas for the other beers, approx. 75 % up to 77 % of the initial iso-α-acids level remains after this period of forced ageing. The higher stability of iso-α-acids in beer F is probably due to the higher pH of this beer. According to *Intelmann* et al. [38], the stereospecific transformation of *trans*-iso-α-acids into degradation products is pH dependent and will occur slower at higher pH. This finding is further confirmed by our own data shown in figure 6. Nevertheless, the relatively high bitterness stability in beer F is not in accordance with sensory evaluation of overall beer ageing, since beer F is proved to be organoleptically one of the most sensitive beers towards ageing.

Furthermore, the UPLC separation patterns clearly demonstrated the differential behaviour of *trans*- and *cis*-iso-α-acids during beer ageing (see also Fig. 7). Decay of the *trans*-isomers is obvious, whereas in most cases the levels of their *cis*-counterparts show a minor decrease upon ageing. This observation agrees fully with our previous research [19, 21, 22, 41, 42]. However, the results demonstrated in figure 7 (see in particular for beer A and E) suggest that some brewers have significantly more difficulties with the oxygen content in the beer. It is apparent that beer A (first series) and beer E (series 1 and 3), lost approx. 20 % of the *cis*-isomers after 120 days at 30 °C, clearly pointing to oxygen rich conditions in these beers. On the other hand, the relatively high stability of *trans*-iso-α-acids in beer F is probably due to the higher pH of this beer.

To estimate the degree of flavour deterioration during beer ageing also the evolution of selected marker carbonyl compounds was studied. Quantitative headspace SPME GC-MS profiling was performed on the fresh (for the results see Table 6) and forced aged (30, 60, 90 at 120 days at 30 °C in the dark) beer samples. The results regarding the formation of aldehydes upon beer ageing as a function of time, are summarised in figure 8. During ageing of beer F, the lowest increase in the total concentration of aldehydes markers takes place. More detailed investigation of the data in figure 9 shows some further striking observations. Upon ageing for 120 days, the highest level in Strecker and lipid oxidation aldehydes was found for beer F in comparison with the other lagers. Conversely, the furfural level upon ageing of beer F was the lowest. Lager beers B, C and D show high amounts of total aldehydes as function of forced aging but mostly due to the furfural. Furthermore, it is interesting to observe that the amounts of Strecker and lipid oxidation aldehydes increase up to 2.5 times compared to the level in the fresh beers whereas furfural increases 10 up to 30 times of the initial value.

In figure 10 the concentration of the Strecker aldehydes measured after 120 days aging is plotted as a function of the concentration of the corresponding amino acids in the fresh beers. It can be observed that lower concentrations of amino acids in fresh beers can be positively correlated with lower concentrations of the corresponding Strecker aldehydes in the aged samples. It is further demonstrated (see Fig. 11) that the beer pH is positively correlated with the concentration of lipid oxidation and Strecker degradation aldehydes after 120 days aging at 30 °C. This is in contrast with the expectation that release of the aldehydes from their Schiff's bases during beer aging should proceed faster at a lower pH. An inverse correlation is found between the concentration of furfural after 120 days aging and the pH of the fresh beer (see Fig. 11).

This correlation is in agreement with the expectation that release of the aldehydes from their Schiff's bases during beer aging should proceed faster at a lower pH.

Multivariate data analysis is one of the most widely used methodologies for expressing the dependence of a response variable on several independent (predictor) variables [27, 50]. Therefore, multivariate data analysis was performed on the results from the analytical and sensory evaluation (overall-ageing-score) of the fresh and the forced aged beer samples from the six Belgian breweries. An integrated analytical-sensory tool for flavour stability assessment of pale lager beers and light beers was presented earlier [52, 53]. Essentially the same methodology was applied in this work. First of all, based on PCA analysis, the analytical data of the fresh and aged beer samples was compared (see Fig. 12). Parameters on the left side of the bi-plot (such as the different aldehydes markers, cold and permanent haze, beer colour) are positively correlated with beer ageing, whereas parameters on the right side of the plot are negatively correlated (for instance, the T/C-ratio, *trans*-iso-α-acids, TB-Index, flavanoid content, etc). On the right side of the plot are thus parameters found which decrease during beer ageing. In contrary, on the left side of the plot are parameters located that increase during beer ageing. Upon beer ageing, a shift of the beer samples to the left in the score-plot is noticed. Beers located most to the right side of the plot can be described as the most fresh samples, whereas samples located most to the left as most aged samples.

Furthermore, detailed inspection of figure 12 shows differences in ageing behaviour between the commercial lager beers. Lager beers from brewery F are located towards the top of the score-plot due to higher pH value, polyphenol content, TRAP, bitterness, T/C-ratio and a lower content in some of the marker aldehydes. During ageing of this beer relatively less furfural and *trans*-2-nonenal are formed/released in comparison with the other lager beers. On the other hand, more marker aldehydes such as ALD1, ALD2, ALD3, ALD4, ALD5, ALD6, ALD8 (for component identification see Fig. 2) are formed/released during ageing of beer F. Based on the analytical data, aged beer F3 can be described as the most aged beer. This is probably due to the highest increase in the levels of staling aldehydes. Also, from figure 12 it can be seen that beers from brewery B and C can be described as the most fresh samples. Moreover, it can be seen that corresponding aged samples from brewery B and C are positioned more to the right side (fresh beer side) than the other commercial lager beers. According to their location on the bi-plot, based on the analytical markers, the aged lager beers B and C are less aged than any other forced aged beer involved in this study.

Next to the PCA-analysis, Multivariate Partial Least Squares Regression (PLSR) techniques were applied on all fresh and aged beer samples in order to develop an overall model between the analytical data, represented in a matrix X, and the overall-ageing-score (OAS), represented in matrix Y. Figure 13, shows the correlation loading plot of this PLS analysis. Based on the "explained variance" for the Y-matrix (on the bottom of the figure), it can be deduced that the two principal components in the model explained together 89 % of the variance in the over-all-ageing score between all beer samples (PC1 82 %, PC2 7 %).

Based on the investigated analytical parameters, it was found that, although the extent of the flavour changes upon ageing is clearly different for the different lager beers, (see above), all basically commercial lager beers age in the same way, i.e., the same analytical parameters are either positively or negatively correlated with overall sensory ageing. Analytical parameters that loaded strongly positively in the PLSR model for beer ageing (see Fig. 13) are the sum of the aldehydes markers, and the individual aldehydes markers (especially 2-methylbutanal, 3-methylbutanal, phenylacetaldehyde, and furfural). Conversely, a decrease in the level of the analytical parameters such as total bitterness, *cis*- and *trans*-iso-α-acids appears to be correlated with an increase in the overall-ageing score.

Besides the overall PLS model of beer ageing as shown in figure 13, specific linear individual beer ageing models, based on the OAS and analytical data, were developed for each brewery (see Fig. 14). The slopes obtained from linear regression of the calculated OAS are indicative for the rate of aging of a particular beer values as a function of ageing time. Based on these multivariate data analysis models, it can be concluded that the lager beers A, C and F are the most prone to flavour change during ageing. On the contrary, beers B and E appear to be significantly less sensitive. In these last two beers, off-flavours appearing during aging are probably masked by aromas derived from the hops added during brewing. Based on the developed models as shown in figure 14, it should be now possible to predict flavour stability of beers from the breweries A-F through determination of analytical data related to flavour (in)stability.

Finally, we also looked for correlations between analytical data obtained on the raw materials and the rate of beer ageing (expressed as the slope of the increase in overall ageing score; see Fig. 15). Following this approach, it was found that, for instance, better filterability of the malt (see Fig. 15b) is positively correlated with lower slope of increase in OAS, i.e. with improved flavour stability. Moreover, an increased FAN content of the malt imparts a risk for a faster overall beer ageing and thus less flavour stable beer (see Fig. 15a). Earlier, our research group already noticed that a high residual FAN content in the beer, as well as more trihydroxy fatty acids (THOE) formation during brewing, show a negative influence on beer flavour stability [23].

4 Conclusions

In conclusion, based on PCA on analytical data, the six commercial fresh lager beers can be separated in three groups. Clearly, in comparison with the other lagers, beer F differs based on higher levels of THOE, haze, reducing power, higher pH, a higher apparent attenuation and a significantly higher T/C-ratio. Also FAN and the amino acids content is relatively high in this beer.

In general, sensory evaluation of beer ageing is not always in agreement with the analytical data on bitterness degradation and aldehydes formation/release, as the addition of large amounts of hops during brewing may exhibit a masking effect on off-flavours. Furthermore, a higher beer pH has a positive effect from the view of bitter acids deterioration and because of less formation/re-

lease of furfural. On the other hand, high amounts of amino acids (residual FAN), as well as a higher beer pH result in a higher Strecker aldehydes content upon beer ageing. A higher beer pH also results in higher amounts of lipid oxidation aldehydes in aged beer samples.

Application of an optimised integrated analytical-sensorial methodology (multivariate data analysis) for flavour stability assessment of pale lager beers revealed several interesting additional findings. Basically, the six commercial pale lager beers age in the same way i.e., the same analytical parameters are either positively or negatively correlated with overall sensory ageing, but changes in relation to these parameters occur at a different rate, depending on the ageing beer.

Considering the influence of raw materials on beer flavour quality and stability, it was found that the rate of beer ageing is positively correlated with FAN of the malt. A higher free amino nitrogen content of the malt clearly results in lower flavour stable beers. On the other hand, better malt filterability imparts prolonged beer flavour stability.

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5 References

1. Aerts, G.: Improvement of the flavour stability of beer, Chair J. De Clerck XII. Leuven, 10–13 September. 2006.
2. Aerts, G.; De Cooman, L.; De Pril, I.; De Rouck, G.; Jaskula, B.; De Buck, A.; De Pauw, C. and van Waesberghe, J.: Improved brewhouse performance and beer stability by addition of a minimal. but effective concentration of gallotannins to the brewing and sparging liquor, Proc. 29th Cong. Eur. Brew. Conv. Dublin, (2003), pp. 384-396.
3. Aerts, G. and van Waesberghe, J.: Innovative wort production in relation to 21st century wort boiling and optimised beer flavour quality and stability, Proc. 31st Cong. Eur. Brew. Conv. Venice, (2007), pp. 518-525.
4. Andersen, M. L. and Skibsted, L. H.: Electron spin resonance spin trapping identification of radicals formed during aerobic forced aging of beer, Journal of Agricultural and Food Chemistry, **46** (1998), pp. 1272-1275.
5. Araki, S.; Kimura, T.; Shimizu, C.; Furusho, S.; Takashio, M. and Shinotsuka, K.: Estimation of antioxidative activity and its relationship to beer flavor stability, Journal of the American Society of Brewing Chemists, **57** (1999), no 1, pp. 34-37.
6. Araki, S.; Takashio, M. and Shinotsuka, K.: A new stale flavour index to improve flavour stability, World Brewing Congress 2000 Poster Abstracts. Monatsschrift für Brauwissenschaft, **5/6** (2000), pp. 109-110.
7. Araki, S.; Takashio, M. and Shinotsuka, K.: A New Parameter for determination of the extent of staling in beer, Journal of the American Society of Brewing Chemists, **60** (2002), pp. 26-30.
8. Aron, P.M. and Shellhammer T.H.: A discussion of polyphenols in beer physical and flavour stability, Journal of the Institute of Brewing, **116** (2010), no. 4, pp. 369-380.
9. Back, W.; Forster, C.; Lehmann, J.; Sacher, B. and Thum, B.: New research findings on improving taste stability, BRAUWELT International, **5** (1999), pp. 394-405.
10. Bamforth, C. W.: Beer flavour stability, The Brewer, **72** (1986), pp. 48-51.
11. Bamforth, C. W.: The science and understanding of the flavour stability of beer: a critical assessment, BRAUWELT International, **2** (1999a), pp. 98-110.
12. Bamforth, C.W.: Beer haze, Journal of the American Society of Brewing Chemists, **57** (1999b), no. 3, pp. 81-90.
13. Bamforth, C. W.: A critical control point analysis for flavor stability of beer, Technical Quarterly: Master Brewers Association of the Americas, **41** (2004), no. 2, pp. 97-103.
14. Bamforth, C. W. and Parsons, R.: New procedures to improve the flavor stability of beer, Journal of the American Society of Brewing Chemists, **43** (1985), pp. 197-202.
15. Benitez, J.L.; Forster, A.; De Keukeleire, D.; Moir, M.; Sharpe, F.R.; Verhagen, L.C. and Westwood, K.T.: European Brewery Convention. Manual of Good Practice, Hops and Hop Products. Fachverlag Hans Carl, Nürnberg, Germany, 1997.
16. Boivin, P.: A review of pro- and anti-oxidant of malt. Eur. Brew. Conv. 'Flavour and Flavour Stability', Nancy, contr. 1, 2001.
17. Bradford, M.: A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Analytical Biochemistry, **72** (1976), pp. 248.
18. De Buck, A.; De Rouck, G.; Aerts ,G. and Bonte, S.: Lipoxygenase activity and beer ageing, Cerevisia, **23** (1998), pp. 25-37.
19. De Clippeleer, J.; De Rouck, G.; De Cooman, L. and Aerts, G.: Influence of the hopping technology on the storage-induced appearance of staling aldehydes in beer, Journal of the Institute of Brewing, **116** (2010a), no. 4, pp. 381-398.
20. De Clippeleer, J.; Van Opstaele, F.; Vercammen, J.; Francis, G. J.; De Cooman, L. and Aerts, G.: Real-time profiling of volatile malt aldehydes using selected ion flow tube mass spectrometry (SIFT-MS), LCGC North America, **28** (2010b), no. 5, pp. 1-9.
21. De Cooman, L.; Aerts, G. and Overmeire, H.: Alterations of the profiles of iso-α-acids during beer ageing, marked instability of t*rans*-iso-α-acids and implications for beer bitterness consistency in relation to tetrahydroiso-α-acids, Journal of the Institute of Brewing, **106** (2000), pp. 169-178.
22. De Cooman, L.; Aerts, G.; Witters, A.; De Ridder, M.; Boeykens, A.; Goiris, K. and De Keukeleire, D.: Comparative study of the stability of iso-α-acids, dihydroiso-α-acids and tetrahydroiso-α-acids during beer ageing, Proc. 28th Cong. Eur. Brew. Conv. Budapest (2001), pp. 566-575.
23. De Rouck, G.; De Clippeleer, J.; Poiz, S.; De Cock, J.; van Waesberghe, J.; De Cooman, L. and Aerts, G.: Prolonged flavour stability by production of beer with low residual FAN using active dry yeast, Proc. 31st Cong. Eur. Brew. Conv. Venice, (2007), pp. 455-467.
24. Drost, B. W.; Van Eerde, P.; Hoekstra, S. F. and Strating, J.: Fatty acids and staling of beer, Proc. Cong. Eur. Brew. Conv.(1971), pp. 451-458.
25. Clapperton, J. F.: Study on sensory analysis of staling beer, Proc. Cong. Eur. Brew. Conv. 23 (1976), pp. 1-237.

26. European Brewery Convention. Analytica-EBC. Fachverlag Hans Carl, Nürnberg: 1998.
27. Foster, R.; Samp, E.J. and Patino, H.: Multivariate modeling of sensory and chemical data to understand staling in light beer, Journal of the American Society of Brewing Chemists, **59** (2001), no. 4, pp. 201-210.
28. Fratianni. A. J.: Results of the effect of storage temperature on packaged beer flavour, Technical Quarterly: Master Brewers Association of the Americas, **38** (2001), no. 3, pp. 159-161.
29. Furusho, S.; Kobayashi, N.; Nakae, N.; Takashio, M.; Tamaki, T. and Shinotsuka, K.: A developed descriptive sensory test reveals beer flavor changes during storage, Technical Quarterly: Master Brewers Association of the Americas, **36** (1999), no. 2, pp. 163-166.
30. Gastl, M.; Spieleder, E.; Hermann, M.; Thiele, F.; Burberg, F.; Kogin, A.; Ikeda, H.; Back, W. and Narziss, L.: The influence of malt quality and malting technology on the flavour stability of beer, BrewingScience – Monatsschrift für Brauwissenschaft, **59** (2006), pp. 163-174.
31. Gijs, L.; Chevance, F.; Jerkovic, V. and Collin, S.: How low pH can intensify beta-damascenone and dimethyl trisulfide production through beer aging, Journal of Agricultural and Food Chemistry, **50** (2002), pp. 5612-5616.
32. Guedes de Pinho, P.; Silva Ferreira, A.C.: Role of Strecker aldehydes on beer flavour stability, Flavour Science: Recent Advances and Trends, **43** (2006), pp. 529-532.
33. Guyot-Declerck, C.; Francois, N.; Ritter, C.; Govaerts, B. and Collin, S.: Influence of pH and ageing on beer organoleptic properties. A sensory analysis based on AEDA data, Food Quality and Preference, **16** (2005), pp. 157-162.
34. Hashimoto, N.: Oxidation of higher alcohols by melanoidins in beer, Journal of the Institute of Brewing, **78** (1972), pp. 43-51.
35. Hofmann, T. and Intelmann, D.: The (in)stability of the beer's bitter taste – Elucidation of the mechanisms involved in *trans*-iso-alpha-acid degradation and formation of bitter off-taste compounds upon beer storage, Proc. 32nd Eur. Brew. Conv. Hamburg, Contribution L29, 2009.
36. Hughes, P.S.; Menneer, I.D.; Walters, M.T. and Marinova, G.: Differential behaviour of *cis*- and *trans*-iso-α-acids, Proc. 26th Cong. Eur. Brew. Conv. Maastricht, (1997) pp. 230-238.
37. Institute of Brewing. Method of Analysis. The Institute of Brewing. London. England. 1997.
38. Intelmann, D.; Demmer, O.; Desmer, N. and Hofmann, T.: 18O stable isotope labeling. quantitative model experiments. and molecular dynamics simulation studies on the *trans*-specific degradation of the bitter tasting iso-α-acids of beer, Journal of Agricultural and Food Chemistry, **57** (2009a), pp. 11014-11023.
39. Intelmann, D.; Kummerlöwe, G.; Haseleu, G.; Desmer, N.; Schulze, K.; Fröhlich, R.; Frank, O.; Luy, B. and Hofmann, T.: Structures of storage-induced transformation products of the beer's bitter principles revealed by sophisticated NMR spectroscopic and LC-MS techniques, Chemistry – A European Journal, **15** (2009b), pp. 13047-13058.
40. Irwin, A.J.; Murray, C.R. and Thompson, D.J.: An investigation of the relationship between hopping rate, time of boil and individual alpha acid utilization, Journal of the American Society of Brewing Chemists, 43 (1985), no. 3, pp. 145-152.
41. Jaskula, B.; Syryn, E.; Goiris, K.; De Rouck, G.; Van Opstaele, F.; De Clippeleer, J.; Aerts, G. and De Cooman, L.: Hopping technology in relation to beer bitterness consistency and flavor stability, Journal of the American Society of Brewing Chemists, **65** (2007), no. 1, pp. 38-46.
42. Jaskula, B.; Goiris, K.; Van Opstaele, F.; De Rouck, G.; Aerts, G. and De Cooman, L.: Hopping technology in relation to α-acids isomerization yield, final utilization. and stability of beer bitterness, Journal of the American Society of Brewing Chemists, 67 (2009), no. 1, pp. 44-57.
43. Jones, M. and Pierce, J.: Absorption of amino acids from wort by yeasts, Journal of the Institute of Brewing, 70 (1964), pp. 307-315.
44. Kaneda, H.; Kano, Y.; Koshino, S. and Ohya-Nishiguchi, H.: Behavior and role of iron ions in beer deterioration, Journal of Agricultural and Food Chemistry, 40 (1992), no. 11, pp. 2102-2107.
45. Kaneda, H.; Kobayashi, N.; Furusho, S.; Sahara, H. And Koshino, S.: Chemical evaluation of beer flavor stability, Technical Quarterly: Master Brewers Association of the Americas, 32 (1995), pp. 76-80, 1995.
46. Kaneda, H.; Takashio, M.; Tomaki, T. and Osawa, T.: Influence of pH on flavour staling during beer storage, Journal of the Institute of Brewing, 103 (1997), pp. 21-23.
47. King, B.M. and Duineveld, C.A.A.: Changes in bitterness as beer ages naturally, Food Quality and Preference, 10 (1999), pp. 315-324.
48. Kunze, W.: Technology Brewing and Malting, 3rd edition, VLB Berlin, Germany, 2004.
49. Lustig, S.; Bellmer, H.-G.; Eils, H.G. and Gromus, J.: Geschmacksstabilität und Sudhaustechnologie – Praktische Erfahrungen mit einem neuen Anlagenkonzept, Proc. 27th Cong. Eur. Brew. Conv. Cannes. (1999), pp. 583-592.
50. Liu, J.; Li, Q.; Dong, J.; Chen, J., and Gu, G.: Multivariate modeling of aging in bottled lager beer by principal component analysis and multiple regression methods, Journal of Agricultural and Food Chemistry, 56 (2008), pp. 7106-7112.
51. Malfliet, S.; De Cooman, L. and Aerts. G.: Application of thermostable xylanases at mashing and during germination, BrewingScience – Monatsschrift für Brauwissenschaft. November/December, **63** (2010), pp. 133-141.
52. Malfliet, S.; Van Opstaele, F.; De Clippeleer, J.; Syryn, E.; Goiris, K.; De Cooman, L. and Aerts, G.: Flavour instability of pale lager beers: Determination of analytical markers in relation to sensory ageing, Journal of the Institute of Brewing, 114 (2008), no. 2, pp. 180-192.
53. Malfliet, S.; Goiris, K.; Aerts. G. and De Cooman, L.: Analytical-sensory determination of potential flavour deficiencies of light beers, Journal of the Institute of Brewing, 115 (2009), no. 1, pp. 49-63.
54. Malowicki, M.G. and Shellhammer, T.H.: Isomerization and degradation kinetics of hop (Humulus lupulus) acids in model wort-boiling system, Journal of Agricultural and Food Chemistry, 53 (2005), no. 11, pp. 4434-4439.
55. Malowicki, M.G. and Shellhammer, T.H.: Factors affecting hop bitter acid isomerization kinetics in a model wort boiling system, Journal of the American Society of Brewing Chemists, 64 (2006), no. 1, pp. 29-32.
56. Möller-Hergt, G.; Roderfeld, H.-J. and Waterkamp, H.-J.: Changes of hydroxyl acids and other ageing relevant compounds in wort and beer depending on storage of malt and barley, Eur. Brew. Conv. 'Flavour and Flavour Stability', Nancy, contr. 7, 2001.
57. Narziss, L.: Die Brauerei, Band 2. Die Technologie der Würzebereitung, 7. Auflage. Ferdinand Enke Verlag, Stuttgart, 1992
58. Perpète, P.; Santos, S.; Bodart, E. and Collin, S.: Uptake of amino acids during beer production: The concept of a critical time value, Journal of the American Society of Brewing Chemists, 63 (2005), no. 1, pp. 23-27.
59. Syryn, E.; Malfliet, S.; Goiris, K.; Van Opstaele, F.; De Rouck, G.; De Cooman, L. and Aerts, G. An integrated analytical – sensorial methodology for adequate evaluation of the flavour stability of pilsner beer, Proc. 31st Cong. Eur. Brew. Conv. Venice, (2007), pp. 895-914.

60. Thalacker, R. and Bößendörfer, G.: Thiobarbituric acid index (TBI), Brauwelt International, **1** (2005), pp. 35-38.
61. Uchida, M. and Ono, M.: Improvement for oxidative flavor stability of beer – role of OH-radical in beer oxidation, Journal of the American Society of Brewing Chemists, **54** (1996), no. 4, pp. 198-204.
62. Vanderhaegen, B.; Neven, H.; Coghe, S.; Verstrepen, K. J.; Verachtert, H. and Derdelinckx, G.: Evolution of chemical and sensory properties during aging of top-fermented beer, Journal of Agricultural and Food Chemistry, 51 (2003), no. 23, pp. 6782-6790.
63. Vanderhaegen, B.; Neven, H.; Verachtert, H. and Derdelinckx, G.: The chemistry of beer aging – a critical review, Food Chemistry, **95** (2006), pp. 357-381.
64. Van Opstaele, F.; Goiris, K.; Syryn, E.; De Rouck, G.; Jaskula, B.; De Clippeleer, J.; Aerts, G. and De Cooman, L.: Hop: aroma and bitterness perception, Cerevisia, **31** (2006), no. 4, pp. 167-188.
65. Van Waesberghe, J.; Aerts, G. and De Cooman, L.: Flavour stability starts with malt and compensations in the brewhouse for LOX and aldehydes in malt, Eur. Brew. Conv. 'Flavour and Flavour Stability', Nancy, contr. 6, 2001.
66. Verzele, M.: Practical aspects of the isomerization of alpha-acids. Proc. 10th Eur. Brew. Conv. Congr. Stockholm, (1965), pp. 398-404.
67. Vesely, P.; Lusk, L.; Basarova, G.; Seabrooks, J. and Ryder, D.: Analysis of aldehydes in beer using solid-phase microextraction with on-fiber derivatization and gas chromatography/mass spectrometry, Journal of Agricultural and Food Chemistry, **51** (2003), pp. 6941-6944.
68. Wackerbauer, K. and Meyna, S.: The occurrence of free and triglyceride-bonded hydroxy fatty acids in barley and malt, EBC Monograph 31. EBC Symposium 'Flavour and Flavour Stability'. Nancy. France. 2001. Nürnberg: Fachverlag Hans Carl. Cont.4, 2001.
69. Woffenden, H.M.; Ames, J.M. and Chandra, S.: Relationship between antioxidant activity, color and flavor compounds of crystal malt extracts, Journal of Agricultural and Food Chemistry, **49** (2001), no. 11, pp. 5524–5530.
70. Zufall, C. and Tyrell, T.: The influence of heavy metal ions on beer flavour stability, Journal of the Institute of Brewing, **114** (2008), no. 2, pp. 134-142.

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Appendix

Table 1 Aldehyde profiling of the malt samples

Malt	A		B		C		D		E		F	
	n = 3	SD	n = 3	SD	n = 3	SD	n = 3	SD	n = 3	SD	n = 3	SD
Compound	(µg/kg)	(+/–)	(µg/kg)	(+/–)	(µg/kg)	(+/–)	(µg/kg)	(+/–)	(µg/kg)	(+/–)	(µg/kg)	(+/–)
2-methylpropanal	3480.03	203.01	1736.22	75.87	722.04	40.54	2185.26	65.91	795.65	61.64	1106.02	92.34
2-methylbutanal	2411.35	116.30	1296.92	51.31	612.63	38.39	1748.49	56.08	693.98	62.53	928.03	73.27
3-methylbutanal	3634.92	77.15	3294.65	111.74	3226.76	196.61	4215.01	75.33	3053.75	104.59	3227.66	242.96
Hexanal	561.99	50.08	1054.87	83.15	1123.17	110.71	1090.06	15.85	861.10	94.19	495.29	65.03
Furfural	409.01	7.13	181.86	53.03	128.13	19.55	651.26	80.23	89.20	8.19	211.04	8.40
Methional	523.20	30.70	523.53	14.14	171.13	13.82	633.86	7.73	191.17	12.22	327.85	43.80
Benzaldehyde	19.73	2.36	23.18	5.65	14.99	4.39	16.81	6.54	13.46	3.73	16.24	1.94
Phenylacetaldehyde	724.63	36.99	800.63	51.63	193.59	11.63	1014.52	25.03	224.98	7.35	324.00	37.84
t-2-nonenal	20.87	3.73	29.66	3.36	31.54	6.60	45.49	14.04	29.63	4.16	17.32	1.09
Total	11785.73		8559.27		6223.98		11600.76		5952.92		6653.45	

Table 2 Chemical-analytical evaluation of malt samples

Parameter / Malt	THOE (mg/kg)	Filtration rate (ml/h)	Dry matter (%)	Ash (%)	Fe ** (mg/100 g DM)	Cu ** (mg/100 g DM)	TBI*	FAN (mg/L in extract)	FAN (%; m/m)	TRAP (µM (+) – catechine eq.)
A	23.4	96	95.7	1.9	2.5	0.4	13.0	92.27	0.082	253
B	13.3	111	96.3	1.9	2.6	0.4	10.9	85.72	0.087	234
C	15.3	80	95.0	1.9	2.6	0.4	8.5	95.00	0.086	213
D	20.7	106	95.4	1.9	2.4	0.5	13.3	93.18	0.083	295
E	22.1	114	95.4	2.0	2.5	0.4	10.0	85.31	0.076	210
F	25.7	102	92.2	1.8	1.9	0.5	15.0	104.91	0.096	224

* TB-Index for malt: index for 10.0 g malt

** metals were determined using Atomic Absorption Spectrometry

Table 3 Chemical-analytical evaluation of hops and hop products

Parameter / Hops			Iso-α-acids; tetrahydroiso-α-acids (%; M/V)	Alpha-acids (%)	Beta-acids (%)	Dry matter DM (%)	As h (%)	Fe ** (mg/ 100 g DM)	Cu** (mg/ 100 g DM)
A	Hops*			5.0	5.7	92.8	7.0	27.1	60.8
	CO_2 extract (a)			39.6	34.7				
	CO_2 extract (b)			58.8	19.7				
	Tetra extract		8.7						
B	**B1**	Hops (a)		2.3	2.3	92.4	8.4	31.3	20.7
		Hops (b)		3.0	6.6	92.6	7.5	20.6	27.9
		CO_2 extract		48.3	29.9				
	B2 and B3	Hops (c)		2.9	4.8	92.2	7.8	21.8	19.6
		Hops (a)		2.3	2.3	92.4	8.4	31.3	20.7
		CO_2 extract		48.3	29.9				
C	Hops (a)			6.1	7.8	92.4	6.6	48.7	31.2
	Hops (b)			5.3	7.1	89.5	6.4	16.2	40.3
	CO_2 extract			50.9	26.5				
D	Hops			8.7	5.0	91.8	6.0	14.6	10.7
	CO_2 extract			39.2	30.7				
E	Hops (a)			8.8	4.9	93.1	6.0	18.2	6.6
	Hops (b)			15	7.2	95.6	7.9	17.4	7.5
	Hops (c)			5.9	7.6	90.7	6.2	17.8	40.8
	Hops (d)			14.7	7.0	92.6	8.2	16.3	7.0
	Hops (e)			6.9	7.6	92.6	5.8	13.7	34.4
	CO_2 extract			49.3	32.0				
F	Hops			11.2	4.2	91.3	8.3	26.0	1.4

* Hops – hop cones or hop pellets were used during beer production

** metals were determined using Atomic Absorption Spectrometry

Table 4 Trihydroxy fatty acids (THOE) content in malt, beer and calculated amount derived from the brewing process

Beer	THOE in beer (mg/L)	THOE in malt (mg/kg)	THOE derived from brewing* (mg/L)
A1	4.96	23.40	2.14
A2	4.96	23.40	2.31
A3	4.29	23.40	1.40
B1	4.41	13.05	2.75
B2	5.40	13.05	3.75
B3	4.73	13.05	3.08
C1	6.16	15.30	4.12
C2	6.92	15.30	4.90
C3	5.81	15.30	3.76
D1	6.83	21.70	4.36
D2	7.72	21.70	5.27
D3	5.38	21.70	2.90
E1	5.64	22.05	2.80
E2	7.90	22.05	5.12
E3	6.29	22.05	3.45
F1	10.88	25.65	7.57
F2	10.85	25.65	7.59
F3	9.35	25.65	6.11

* THOE derived from brewing = THOE in beer [mg/L beer] – (Malt [kg]/Pitching wort [L]) * THOE in malt[mg/kg malt]

Table 5 Bitterness profile fresh commercial lager beers from six different breweries

Beer	t-ich mg/L	c-ich mg/L	t-ih mg/L	c-ih mg/L	t-iah mg/L	c-iah mg/L	Sum iso-α-acids	T/C-ratio %	tetrahydroiso-α-acids mg/L
A1	1.86	3.71	2.57	5.48	0.60	1.44	15.67	48.2	2.38
A2	1.42	3.03	1.93	4.37	0.48	1.13	12.37	45.2	2.94
A3	1.46	5.18	1.82	6.74	0.41	1.61	17.22	27.5	2.17
B1	1.74	3.94	2.20	6.10	0.48	1.42	15.87	39.2	-
B2	2.52	5.89	2.71	7.53	0.78	2.29	21.71	39.0	-
B3	2.33	5.19	2.19	5.81	0.64	1.81	17.97	41.1	-
C1	2.26	5.05	3.37	8.96	0.80	2.11	22.56	40.2	-
C2	2.18	4.99	2.94	8.24	0.69	1.95	20.99	38.6	-
C3	2.17	5.09	3.40	9.53	0.84	2.27	23.30	38.1	-
D1	1.71	4.29	2.53	6.86	0.56	1.62	17.56	38.0	-
D2	1.82	4.59	2.54	7.09	0.58	1.70	18.31	37.3	-
D3	1.65	4.65	1.75	5.64	0.40	1.45	15.54	33.0	-
E1	1.66	3.46	1.97	4.93	0.56	1.39	13.98	43.3	-
E2	1.66	3.49	1.81	4.57	0.51	1.30	13.34	43.0	-
E3	1.60	3.24	1.73	4.15	0.49	1.16	12.37	45.1	1.50
F1	2.03	3.90	3.08	6.38	0.76	1.36	17.51	49.7	-
F2	2.10	3.88	3.07	6.11	0.80	1.58	17.54	51.7	-
F3	1.85	3.76	2.61	5.39	0.66	1.21	15.48	48.7	-

* Compound identification: t-ich: *trans*-isocohumulone; c-ich: *cis*-isocohumulone; t-ih: *trans*-isohumulone; c-ih: *cis*-isohumulone; t-iah: *trans*-isoadhumulone; c-iah: *cis*-isoadhumulone; the *trans/cis* iso-α-acids ratio (T/C-ratio) was based on the measured concentrations of *trans*- and *cis*-isocohumulone and *trans*- and *cis*-isohumulone

Table 6 **Aldehydes profile of fresh commercial lager beers from six different breweries (aldehyde levels are expressed in µg/L)**

Beer	2-methyl-propanal	2-methylbutanal	3-methylbutanal	hexanal	furfural	methional	benzaldehyde	phenylacetalde-hyde	t-2-nonenal	Sum aldehydes
A1	3.97	1.11	6.03	0.46	16.75	3.54	1.12	6.11	0.02	39.10
A2	4.31	0.48	4.38	0.33	23.38	2.84	0.66	3.82	0.02	40.21
A3	1.44	0.76	2.69	0.24	8.66	2.05	0.17	4.12	0.01	20.13
B1	2.05	0.51	1.10	0.13	12.00	0.37	0.12	1.21	0.02	17.52
B2	3.16	0.45	1.54	0.13	16.62	0.33	0.15	1.56	0.02	23.97
B3	2.65	0.40	1.22	0.20	18.14	0.77	0.51	2.19	0.01	26.09
C1	1.92	0.20	1.33	0.17	10.79	0.91	1.17	2.23	0.02	18.74
C2	2.09	0.20	1.54	0.12	10.52	0.46	1.08	1.65	0.02	17.67
C3	4.02	1.21	2.07	0.21	16.27	1.30	0.19	3.07	0.03	28.38
D1	3.40	0.20	3.80	0.10	18.70	1.00	0.30	2.40	0.01	29.91
D2	2.35	0.84	3.00	0.19	14.57	1.21	1.06	5.57	0.03	28.82
D3	5.81	0.90	2.73	0.09	65.29	2.47	0.39	5.71	0.01	83.41
E1	3.71	0.19	2.69	0.27	9.24	1.22	0.59	2.51	0.02	20.44
E2	3.08	0.11	2.38	0.15	8.31	1.31	0.29	2.61	0.02	18.26
E3	2.79	1.29	3.05	0.16	10.31	1.37	0.45	5.09	0.05	24.57
F1	8.57	0.44	3.25	0.88	8.50	2.28	1.06	2.49	0.00	27.46
F2	11.01	1.02	4.69	2.20	6.99	5.29	2.13	4.52	0.01	37.87
F3	30.99	2.47	6.38	0.73	17.32	8.67	2.27	9.64	0.05	78.51

Table 7 **See pages 132-133**

Table 8 **Mean concentration (mg/L) of iso-α-acids as a function of beer ageing**

	A		**B**		**C**		**D**		**E**		**F**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fresh	15.1	2.5	18.5	3.0	22.3	1.2	17.1	1.4	13.2	0.8	16.8	1.2
30d	14.0	2.4	16.6	2.8	20.0	0.4	15.8	1.3	12.1	0.6	16.2	1.4
60d	13.3	2.4	15.9	3.0	18.6	0.3	14.8	0.7	11.2	0.5	15.4	0.9
90d	12.4	2.2	14.3	3.1	18.0	0.7	13.6	1.2	10.4	0.8	14.2	0.9
120d	11.6	2.3	13.9	3.1	17.2	0.9	12.9	0.8	9.9	0.9	13.6	0.8

Table 7 **Standard analyses of fresh and forced aged commercial lager beer samples of the breweries A–F**

Parameters / Beer sample		A1	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	D3	E1	E2	E3	F1	F2	F3
Alcohol content (v/v %)	fresh	5.08	4.73	5.28	5.11	5.21	5.20	5.27	5.22	5.31	5.09	5.03	5.13	5.14	5.15	5.39	5.24	5.23	5.20
App. extract (g/100 g)	fresh	3.60	3.35	3.64	3.87	3.63	3.67	3.90	3.86	3.97	3.99	3.98	4.02	4.15	3.87	3.84	3.36	3.64	3.48
Real extract (g/100 g)	fresh	1.75	1.60	1.68	2.01	1.74	1.74	2.00	1.97	2.01	2.14	2.16	2.12	2.29	2.00	1.86	1.73	1.56	1.55
Original gravity (° P)	fresh	11.36	10.68	11.64	11.66	11.57	11.56	11.92	11.80	11.99	11.74	11.64	11.78	11.96	11.72	11.94	11.63	11.45	11.39
Real attenuation (%)	fresh	69.62	69.67	70.46	68.17	69.95	69.98	68.65	68.69	68.73	67.42	67.18	67.73	66.72	68.33	69.63	70.07	71.06	71.07
Apparent attenuation (%)	fresh	84.60	84.79	85.61	82.73	84.97	85.02	83.28	83.36	83.37	81.77	81.49	82.16	80.86	82.91	84.52	85.12	86.40	86.42
FAN (mg/L)	fresh	70.6	57.8	51.8	49.1	55.9	71.4	66.5	74.4	84.1	79.4	64.9	55.9	126.7	109.4	86.8	141.2	126.0	105.0
Foam stability (s)	fresh	249	285	254	219	270	204	280	335	279	268	312	254	186	273	249	211	318	191
Soluble protein (µg/ml)	fresh	330	411	350	287	282	282	296	364	236	217	270	178	189	307	244	222	300	238
THOE (mg/L)	fresh	4.96	4.96	4.29	4.41	5.40	4.73	6.16	6.92	5.81	6.83	7.72	5.38	5.64	7.90	6.29	10.88	10.85	9.35
SO_2 (mg/L)	fresh	10.9	8.4	10.5	11.0	9.2	10.6	11.2	9.4	9.1	11.1	8.4	8.0	15.4	11.7	7.4	18.0	10.4	13.5
Forced haze (0 °C) (EBC)	6d 60°C	0.27	4.16	3.47	9.88	9.27	12.94	1.93	2.69	7.12	0.70	0.75	1.99	0.36	0.67	3.54	1.56	2.54	3.53
Forced haze (20 °C) (EBC)	6d 60°C	0.22	0.72	0.44	3.00	1.70	1.69	0.67	0.70	1.18	0.34	0.35	0.60	0.28	0.38	0.58	0.60	0.77	0.60
Oxygen (ppb)	fresh	62	116	45	78	20	33	40	16	24	27	34	59	47	228	82	66	45	11
pH	fresh	4.49	4.35	4.36	4.28	4.20	4.23	4.33	4.36	4.41	4.19	4.19	4.22	4.37	4.36	4.39	4.70	4.60	4.72
	30d	4.20	4.28	4.50	4.05	4.14	4.25	4.28	4.31	4.44	4.14	4.17	4.21	4.30	4.29	4.39	4.54	4.56	4.76
	60d	4.25	4.36	4.33	4.08	4.24	4.17	4.33	4.40	4.36	4.20	4.23	4.08	4.32	4.39	4.36	4.95	4.69	4.67
	90d	4.35	4.19	4.35	4.16	4.08	4.19	4.41	4.23	4.38	4.25	4.05	4.17	4.45	4.21	4.35	4.69	4.48	4.63
	120d	4.15	4.42	4.40	4.03	4.24	4.24	4.24	4.40	4.44	4.09	4.22	4.22	4.27	4.35	4.37	4.46	4.63	4.72
TBI (index for 100 ml)	fresh	44.7	36.4	41.3	32.8	29.6	33.5	30.3	34.1	40.1	30.4	31.6	32.1	33.2	37.3	37.3	26.8	29.3	29.3
	30d	41.3	34.7	38.9	32.2	25.8	30.9	30.2	30.2	37.0	28.8	29.4	30.2	32.3	32.6	34.7	26.7	26.5	27.2
	60d	43.0	34.6	38.6	30.5	25.9	29.8	28.3	29.9	36.8	28.6	29.4	29.4	30.1	31.9	32.2	21.9	26.0	26.5
	90d	42.7	35.1	38.2	30.5	25.9	28.8	26.9	29.8	35.1	28.0	29.5	29.3	30.0	32.1	32.1	22.3	25.8	25.4
	120d	41.7	35.8	38.0	30.4	25.3	28.6	26.5	29.4	35.1	27.9	29.1	29.1	29.7	31.5	31.6	21.8	24.5	25.4
Total polyphenols (mg/L)	fresh	135.3	123.4	131.3	207.2	215.3	193.6	174.1	168.9	190.0	110.9	105.4	112.8	138.7	134.5	137.5	215.7	207.1	234.6
	30d	132.0	118.5	127.6	197.6	216.0	195.3	171.4	173.0	188.6	114.4	107.0	112.9	138.2	136.0	140.5	207.9	216.1	236.6
	60d	114.0	108.7	129.0	180.0	207.1	193.0	146.0	164.8	187.5	97.6	106.4	112.5	119.0	129.2	140.0	184.0	206.0	237.8
	90d	116.4	119.2	131.7	185.0	220.3	198.0	157.0	173.4	195.7	100.0	103.0	115.5	123.0	138.1	143.1	191.5	217.7	245.7
	120d	126.1	121.0	130.3	203.2	222.6	194.1	175.0	179.9	194.2	110.7	108.3	117.6	136.7	142.1	145.1	211.5	231.7	247.6
Flavanoids	fresh	28.2	25.0	26.2	43.1	44.1	40.7	37.0	39.1	37.9	21.5	21.1	20.6	29.0	30.0	27.9	34.5	34.4	32.3
((+)-catechine eq. mg/L)	30d	28.0	24.1	23.3	42.4	40.9	38.4	34.7	34.6	35.8	21.8	20.4	19.6	29.2	28.0	25.4	33.7	32.3	30.9
	60d	27.3	23.2	22.8	41.7	41.3	36.1	35.0	32.0	33.2	20.4	18.6	20.9	28.8	25.0	24.1	33.1	29.5	30.6
	90d	24.6	22.0	21.9	41.6	40.8	35.0	33.9	31.9	32.4	19.3	18.4	18.7	27.2	24.8	23.3	32.4	28.4	30.5
	120d	21.2	19.5	21.4	34.7	40.5	33.8	28.6	30.9	30.9	17.0	18.2	17.6	24.2	23.4	22.7	28.0	27.4	29.6
TRAP (mM Asc. Eq.)	fresh	1.144	1.020	1.190	1.280	1.315	1.345	1.313	1.262	1.390	1.069	0.979	1.137	1.120	1.071	1.159	1.549	1.484	1.558

	30d	1.078	1.048	1.088	1.283	1.324	1.212	1.207	1.271	1.269	0.998	0.986	0.996	1.074	1.031	1.048	1.479	1.486	1.461
	60d	1.075	1.033	1.108	1.290	1.302	1.246	1.248	1.257	1.263	1.044	0.943	1.052	1.057	1.057	1.050	1.547	1.481	1.525
	90d	1.030	0.983	1.103	1.220	1.251	1.231	1.150	1.188	1.244	0.970	0.953	1.051	1.030	1.011	1.053	1.480	1.330	1.513
	120d	1.034	0.998	1.007	1.152	1.338	1.148	1.260	1.248	1.189	0.947	1.000	0.959	1.049	1.103	0.943	1.406	1.471	1.441
Beer colour (IOB)	fresh	6.7	7.4	8.0	5.2	5.2	5.1	6.9	6.8	6.7	7.0	6.9	6.4	5.5	6.3	5.8	5.6	5.8	6.2
	30d	6.7	7.6	8.1	5.3	5.3	5.6	7.1	6.9	7.1	7.1	7.1	6.7	5.5	6.5	6.0	5.7	6.2	6.3
	60d	7.2	8.2	8.5	5.8	6.0	5.6	7.5	7.6	7.9	7.3	7.6	7.0	5.8	7.1	6.6	6.0	6.5	6.8
	90d	7.6	8.4	8.7	5.8	6.1	5.7	8.2	7.8	8.2	7.9	7.9	7.5	6.6	7.2	7.0	6.6	7.2	6.9
	120d	8.1	8.4	8.8	5.9	6.1	5.9	8.4	8.2	8.7	8.0	8.2	7.6	6.6	7.3	7.3	6.8	7.3	7.0
Cold haze (EBC)	fresh	0.21	0.73	0.28	0.83	0.51	1.09	0.55	0.52	0.63	0.36	0.34	0.53	0.44	0.41	0.46	0.91	1.16	1.69
	30d	0.24	1.07	1.45	10.50	6.23	10.27	1.37	1.02	2.29	0.40	0.44	0.81	0.42	0.46	0.71	1.96	1.75	1.71
	60d	0.27	4.33	3.11	9.11	12.66	11.30	1.79	1.99	4.14	0.45	0.66	0.97	0.38	0.82	2.72	1.44	2.21	2.42
	90d	0.97	8.27	5.04	13.22	16.69	15.31	2.37	2.62	5.63	0.72	0.81	1.60	0.64	1.28	4.52	2.21	3.28	3.15
	120d	2.92	8.26	6.29	22.98	25.81	19.25	4.22	5.46	6.80	1.30	2.40	2.63	1.20	4.91	6.53	3.28	2.91	3.61
Permanent haze (EBC)	fresh	0.18	0.56	0.23	0.35	0.36	0.35	0.41	0.38	0.35	0.32	0.29	0.37	0.41	0.35	0.39	0.76	0.94	0.89
	30d	0.21	0.54	0.22	0.81	0.53	0.45	0.57	0.43	0.43	0.32	0.31	0.42	0.35	0.31	0.40	0.73	1.01	0.80
	60d	0.27	0.75	0.24	3.19	2.28	0.61	1.09	0.77	0.81	0.38	0.38	0.45	0.34	0.62	0.46	1.29	1.15	0.80
	90d	0.27	1.02	0.35	2.64	0.54	1.32	1.18	0.64	1.16	0.47	0.44	0.65	0.36	0.30	0.69	1.03	1.08	0.94
	120d	0.17	0.71	0.50	0.68	1.32	1.91	1.09	1.13	1.46	0.48	0.81	1.01	0.30	0.49	0.94	0.88	0.98	1.03

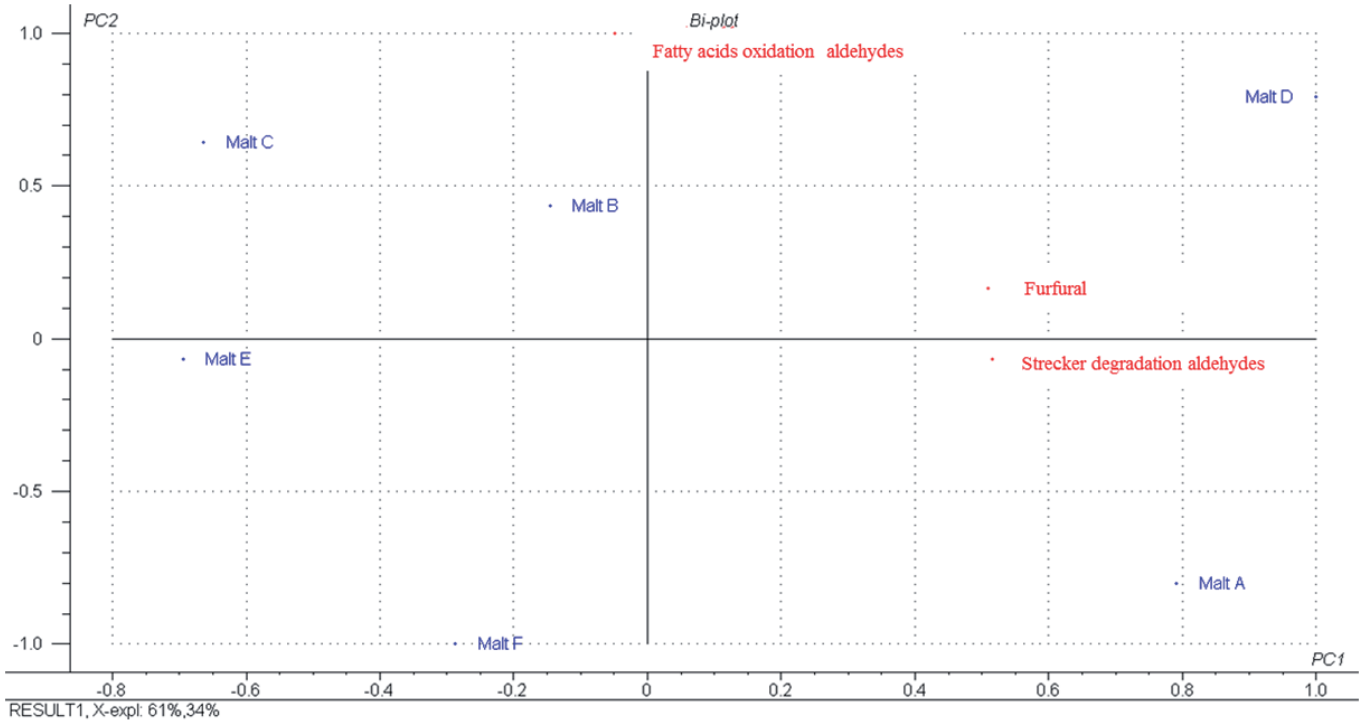

Fig. 1 Bi-plot of PCA of the aldehydes content in pilsner malts

Fig. 2 Bi-plot of PCA of analytical data obtained on the fresh lager beers

Component identification: t-ich: *trans*-isocohumulone; c-ich: *cis*-isocohumulone; t-ih: *trans*-isohumulone; c-ih: *cis*-isohumulone; t-iah: *trans*-isoadhumulone; c-iah: *cis*-isoadhumulone; T/C-ratio: *trans*/*cis* iso-α-acids ratio; ALD1: 2-methylpropanal; ALD2: 2-methylbutanal; ALD3: 3-methylbutanal; ALD4: methional; ALD5: benzaldehyde; ALD6: phenylacetaldehyde; ALD7: furfural; ALD8: hexanal; ALD9: *trans*-2-nonenal; ALD: sum aldehyde markers; RDF: real attenuation; ADF: apparent attenuation

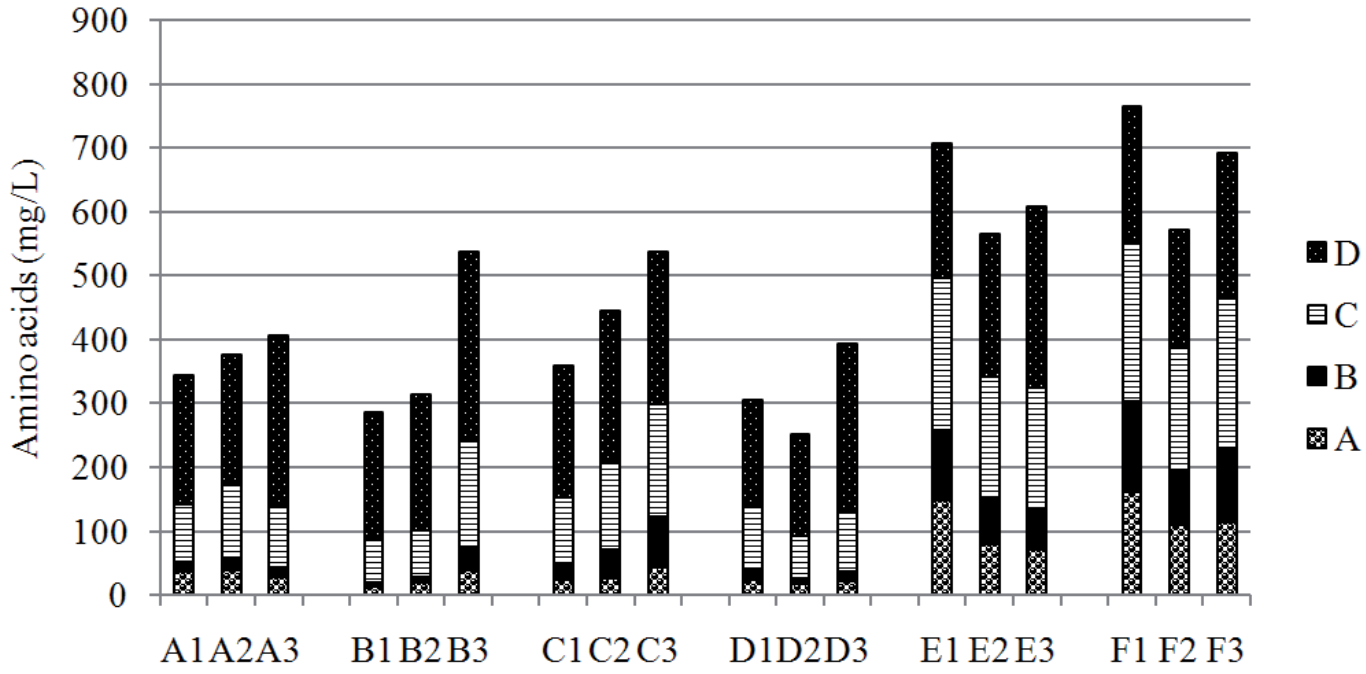

Fig. 3 Amino acids profile of the six lager beers, according to the amino acids groups A–D as defined by Jones and Pierce [43]

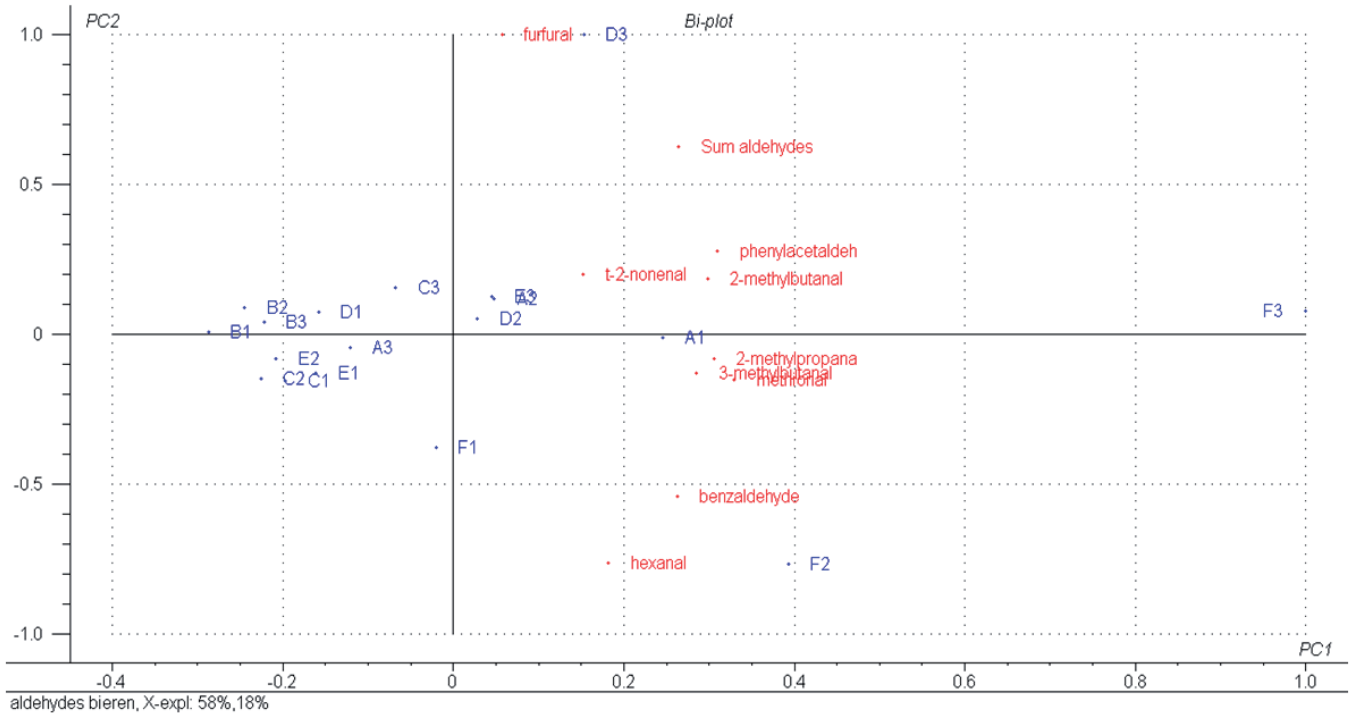

Fig. 4 Bi-plot of PCA of the aldehydes content in fresh commercial lager beers from six different breweries

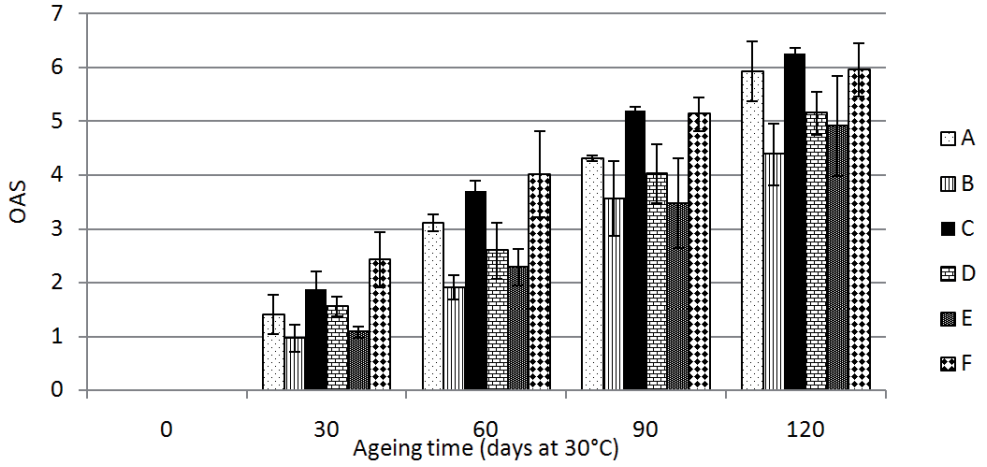

Fig. 5 Mean overall-aging score as a function of ageing time of the lager beers

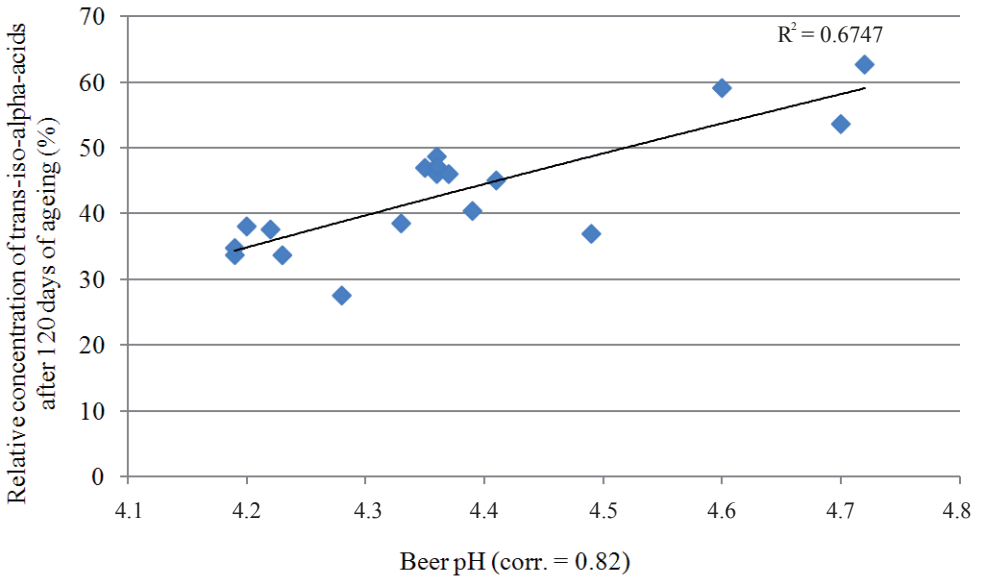

Fig. 6 Correlation between beer pH and relative concentration of *trans*-iso-α-acids after 120 days of forced ageing at 30 °C (corr.: pearsons correlation coefficient)

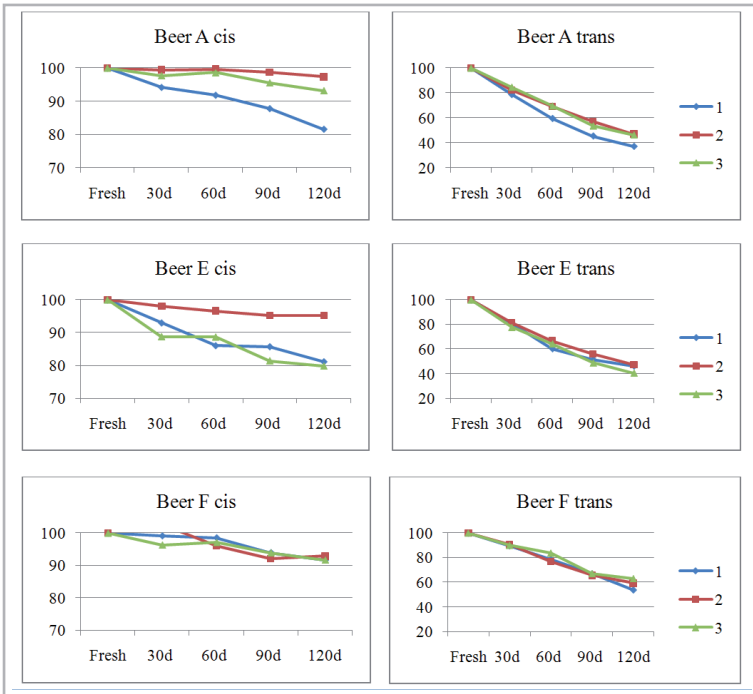

Fig. 7 Relative concentrations (% of initial level in fresh beer) of *trans*- and *cis*-iso-α-acids in commercial lager beers as a function of forced ageing at 30 °C

(compound identification: *trans*: *trans*-isocohumulone + *trans*-isohumulone + *trans*-isoadhumulone;

cis: *cis*-isocohumulone + *cis*-isohumulone + *cis*-isoadhumulone)

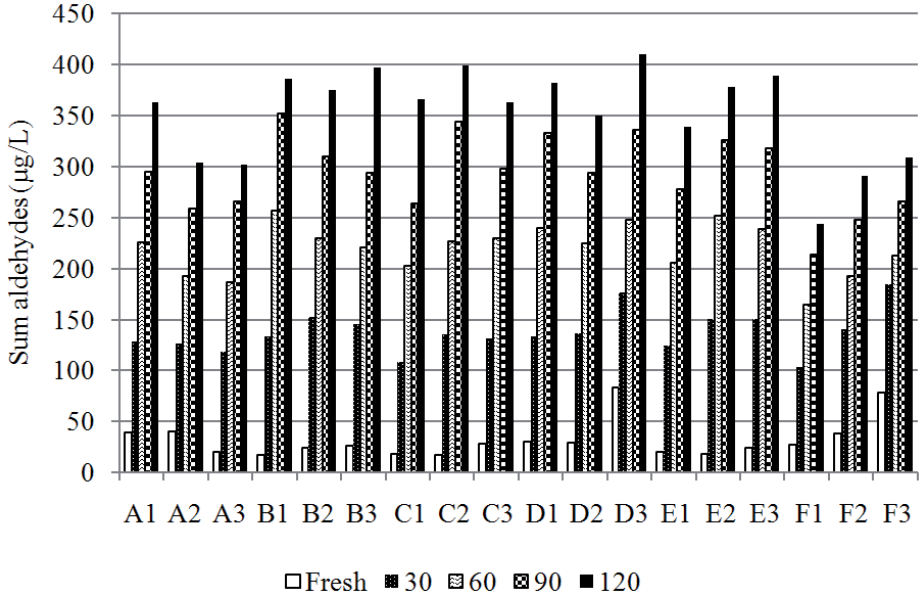

Fig. 8 Concentration of aldehyde markers (µg/L) as a function of aging of six commercial lager beers A–F
(fresh: fresh beer samples; 30, 60, 90, 120: storage time in days at 30 °C)

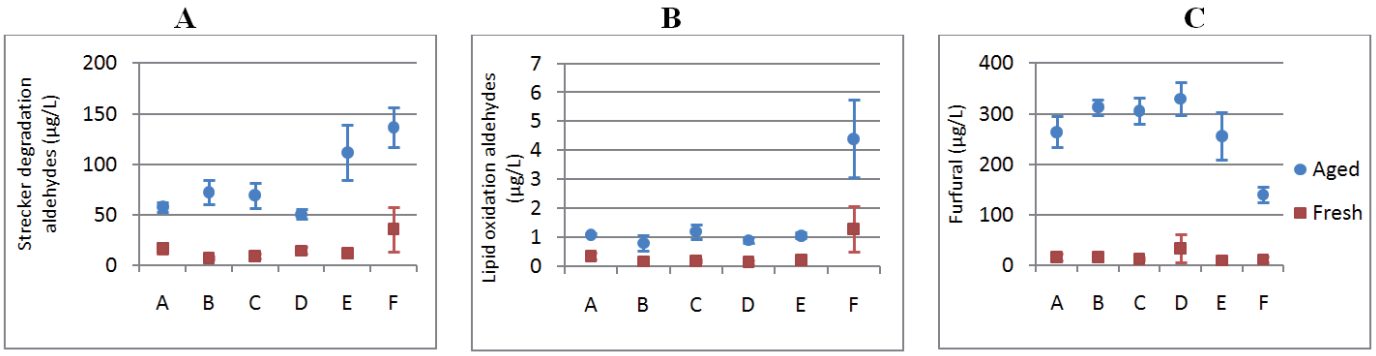

Fig. 9 Levels of aldehydes (µg/L) after 120 days ageing (30 °C) of six commercial lager beers A–F (a: Strecker degradation aldehydes; b: lipid oxidation aldehydes; c: furfural)

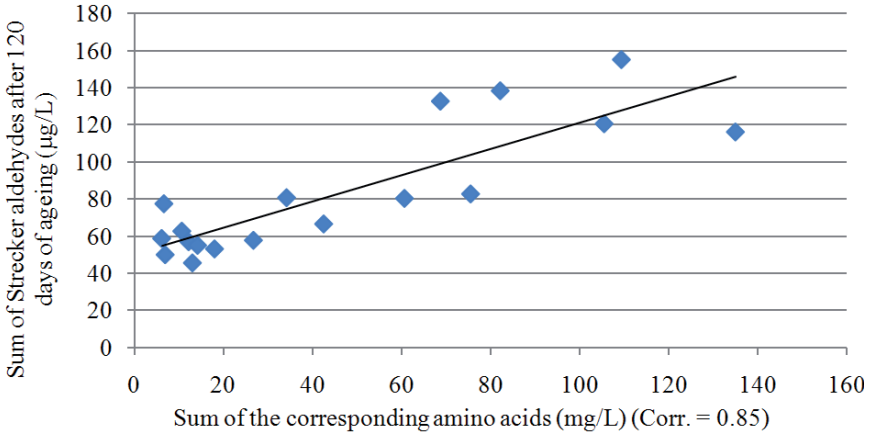

Fig. 10 Concentration of Strecker aldehydes measured after 120 days of beer aging (30 °C), plotted as a function of the concentration of the corresponding amino acids in the fresh beers

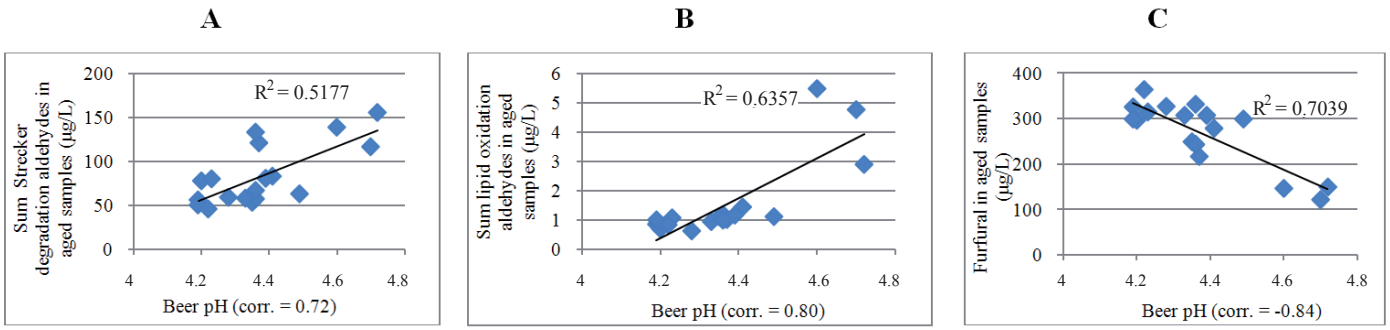

Fig. 11 **Concentration of the**
a) Strecker aldehydes,
b) lipid oxidation aldehydes,
c) furfural measured after 120 days aging plotted as a function of beer pH

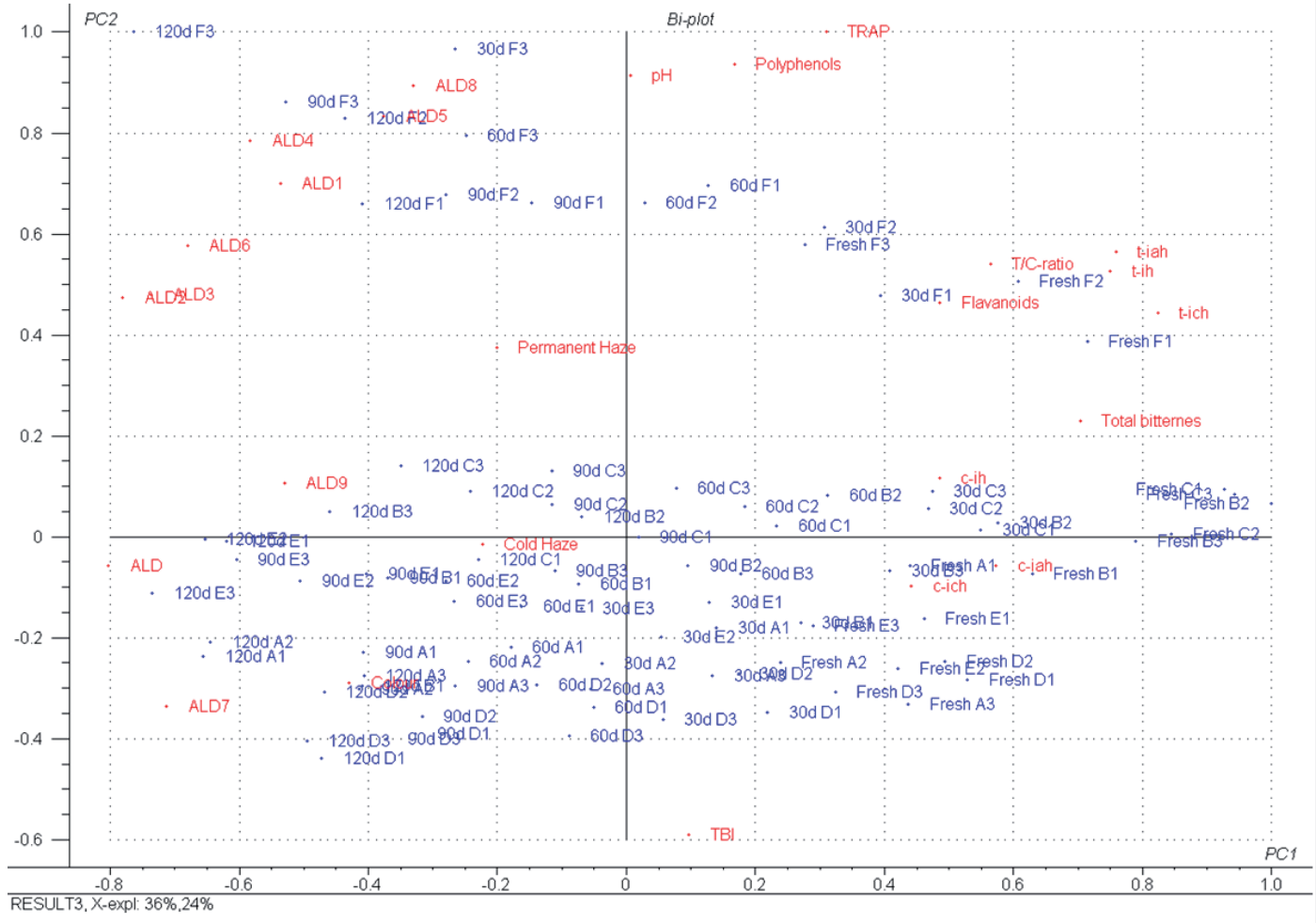

Fig. 12 **Bi-plot of PCA on the analytical data on fresh and forced aged lager beers (PC1 explained variance: 36 %; PC2 explained variance: 24 %)**

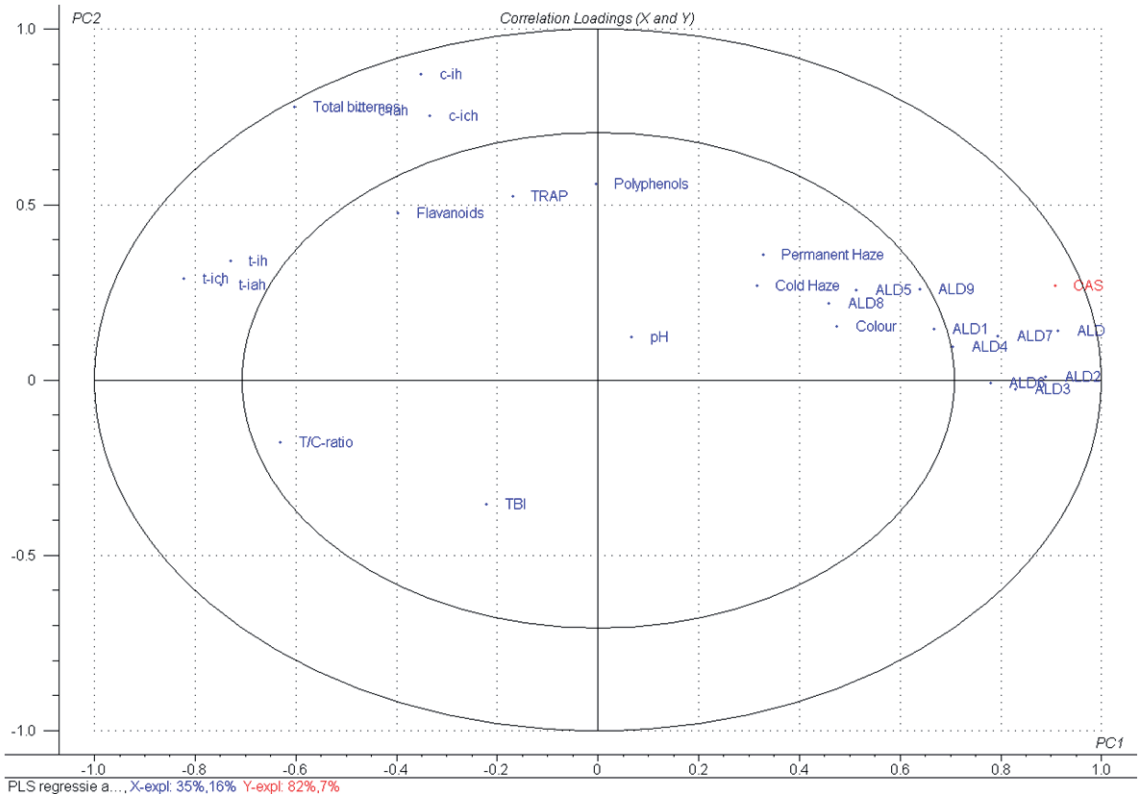

Fig. 13 **Correlation loading plot of PLSR analysis of fresh and aged commercial lager beers, based on analytical data (X matrix) compared with OAS (Y matrix)**

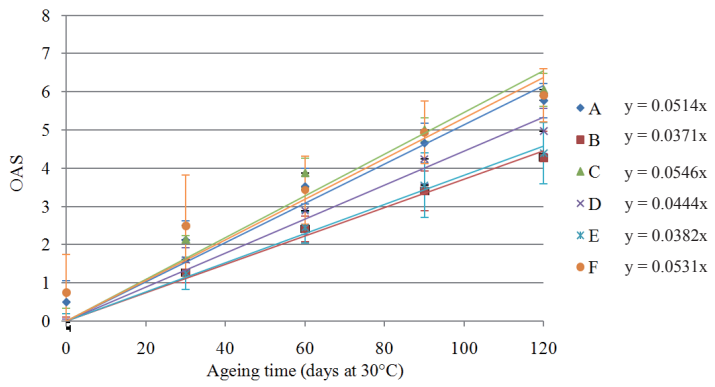

Fig. 14 **Ageing models (calculated OAS) of the six commercial lager beers based on the analytical parameters**

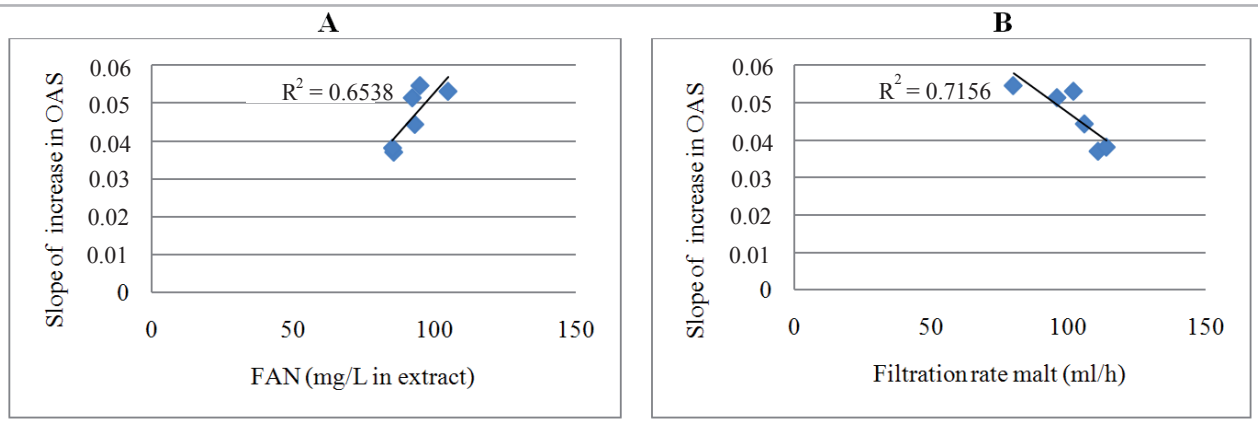

Fig. 15 **Correlation between reaction rate of ageing (increase in OAS) and a) FAN of malt and b) filtration rate of malt**