Food Chemistry 114 (2009) 1206–1215

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Contribution of staling compounds to the aged flavour of lager beer by studying their flavour thresholds

Daan Saison *, David P. De Schutter, Bregt Uyttenhove, Filip Delvaux, Freddy R. Delvaux

Centre for Malting and Brewing Science, Faculty of Bioscience Engineering, K.U. Leuven, Kasteelpark Arenberg 22, Box 2463, 3001 Heverlee, Belgium

ARTICLE INFO

Article history:
Received 28 July 2008
Received in revised form 17 September 2008
Accepted 31 October 2008

Keywords:
Beer
Flavour stability
Flavour
Threshold
Sensory analysis
Flavour interactions
Carbonyl compounds

ABSTRACT

The effect of 26 staling compounds on the aged flavour of a Belgian lager beer was studied. Strikingly, thresholds were regularly found to be substantially lower than those previously reported, and it appeared important to consider threshold values as indicative rather than absolute because of very large variations in sensitivity of individuals. In addition, a masking effect of isoamyl acetate and various interactions were observed between flavour compounds, which can considerably influence their flavour activity, even at sub-threshold concentrations. Cardboard flavour was essentially caused by (E)-2-nonenal. Additionally, methional, 3-methylbutanal, 2-furfuryl ethyl ether, β-damascenone and acetaldehyde were confirmed as key contributors to the aged flavour and to a lesser extent, (E,E)-2,4-decadienal, phenylacetaldehyde, 2-methylpropanal, diacetyl and 5-hydroxymethylfurfural. Finally, the addition of a selection of compounds could fairly well reproduce the flavour of aged lager beer, indicating that these compounds account for the major part of this flavour.

1. Introduction

Beer flavour is the result of a complex interaction between hundreds of chemical compounds and even more taste and olfactory receptors. Compounds that impart taste can be sensed directly on the tongue, while aroma will refer to any volatised compounds that can be perceived either through the nose or retro-nasally through the back of the mouth. Perception of either taste or aroma or a combination of both is described as flavour. However, chemical compounds imparting this flavour are not in chemical equilibrium in fresh beer and many chemical reactions take place during storage, resulting in a decrease of fresh flavour notes and the appearance of typical aged flavours. This lack of flavour stability is of great concern for brewers as it is important that a commercial beer is consistent and satisfies the expectations of the consumer at all times. Despite extensive research, it remains very difficult to understand and control the underlying reactions of the development of aged flavour (Vanderhaegen, Neven, Verachtert, & Derdelinckx, 2006).

About 30-40 years ago, sensory changes during storage were described and it appeared that various flavour notes, such as cardboard, ribes, sweet and caramel, increased and that bitterness and fruity/estery notes decreased (Dalgliesh, 1977; Meilgaard, 1972). Additionally, great progress was made in understanding the sensory impact of individual flavour compounds and interactions (Meilgaard, 1975a; Meilgaard, Elizondo, & Moya, 1970). From an analytical point of view, the concentration increase of (E)-2-nonenal, giving cardboard flavour, was considered as the principal reason for aged flavour, since other compounds linked to ageing, mainly aldehydes, were found to stay well beyond their thresholds (TH). In the past 30 years, however, only few reports have handled sensory changes in beer during storage, but advances in analytical equipment and methods have revealed the increase of many compounds and now it is evident that numerous chemical reactions occur simultaneously, e.g. oxidation of fatty acids and higher alcohols, Maillard reactions, Strecker degradation, aldol condensation, furanic ether formation, degradation of hop bitter acids and acetate esters, terpenoid oxidation, glycoside hydrolysis and synthesis of volatile esters and dimethyl trisulphide (Vanderhaegen et al., 2006). Notwithstanding all improvements, the contribution of the compounds formed in these reactions to aged flavour remains unclear, since sensory data are mostly missing or inconsistent. Additionally, it is remarkable that concentrations of ageing compounds are mostly substantially lower than THs reported in literature. This could have two explanations: either THs were not determined in a solution that provided information relevant to the studied beers or these compounds act together, thereby evoking stale character at sub-threshold concentrations (Techakriengkrai, Paterson, & Taidi, 2006). For example, the contribution of methional and phenylacetaldehyde

* Corresponding author. Tel.: +32 16 32 96 27; fax: +32 16 32 15 76.
E-mail address: daan.saison@biw.kuleuven.be (D. Saison).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.10.078

to aged flavour was first thought to be insignificant (Meilgaard, 1975a), but seemed of remarkable importance later on (da Costa et al., 2004). Similar assumptions could be made for 3-methylbutanal, 2-methylbutanal and 2-methylpropanal, since newly determined THs were found to be close to their levels in aged beer (Thum, Miedaner, Narziss, & Back, 1995). Noteworthy is that the TH value of an added substance is dependent on its endogenous concentration (Brown, Clapperton, Meilgaard, & Moll, 1978) and the overall flavour of the beer being investigated (Engan, 1972).

At present, there is more to aged flavour than meets the eye. On the one hand, it is obvious that the fresh flavour profile is disturbed by the appearance of various aged flavours and, on the other hand, an increase or decrease of many compounds is observed during ageing. However, it remains difficult to explain observed sensory changes based on analytically determined chemical compounds.

Therefore, the aim of this study was to elucidate the precise impact of chemical compounds, that were already linked to beer ageing, on beer flavour. This was performed by determination of their flavour thresholds in a Belgian lager beer. Additionally, the effect of some mixtures of compounds was studied in order to get a better insight of possible interactions. Afterwards, the contribution of the selected staling compounds to aged flavour was evaluated by comparing the obtained thresholds with their concentration evolution in three lager beers during ageing. Finally, an attempt was made to explain the observed sensory changes during ageing of a lager beer by the increase of the studied staling indicators.

2. Materials and methods

2.1. Reagents

The following chemicals were purchased from Sigma (St. Louis, MO, USA) with the highest purity available. Food grade chemicals included: acetaldehyde (⩾99%), hexanal (⩾97%), 2-methylbutanal (⩾90%), methional, phenylacetaldehyde (⩾90%), benzaldehyde (⩾98%), (E,E)-2,4-decadienal, 5-methylfurfural (⩾98%), acetylfuran (⩾99%), dimethyltrisulphide (98%), diacetyl (⩾95%) ethyl isovalerate (⩾98%), ethyl-2-methylbutyrate (⩾98%), ethyl lactate (⩾98%), ethyl pyruvate (⩾97%), isoamylacetate (⩾97%); Non food grade included: 3-methylbutanal (97%), ethyl nicotinate (99%), 2-methylpropanal (⩾98%), (E)-2-nonenal (97%), furfural (99%), 5-hydroxymethylfurfural (⩾99%), β-damascenone, γ-nonalactone (⩾97%). Non-food grade methyl isobutyl ketone (⩾99%) was purchased at Prolabo (Paris, France) and non-food grade 2-furfuryl ethyl ether at Narchem Corporation (Chicago, IL, USA).

2.2. Threshold determination of added substamces

Thresholds were determined by the ascending method of limits test as described in EBC Analytica (method 13.9). Beers were prepared no more than 24 h prior to tasting, by adding increasing amounts (2–64 μl) of an ethanol solution containing a specified amount of the tested compound(s) and decreasing amounts of pure ethanol (62–0 μL) to 250 ml bottles. Reference beers were prepared by adding 64 μl of pure ethanol. Afterwards, beers were recapsulated and stored in a cold room (0 °C). The tasters that took part in the experiment were between 20 and 35 years old and were partly trained members of the CMBS tasting panel and partly non-trained persons who attended the tasting sessions regularly. Because of their regular attendance, the latter group was familiar with the test, which resulted in personal THs similar to those obtained with the trained assessors. Consequently, differences in personal THs were only defined by variations in sensitivity between tasters. The tests were performed with fully carbonated beer served at 7–8 °C in 30 ml beakers (Omnilab, Bremen, Germany) between 2 and 6 p.m. The tasters were informed of the nature of the additive and a standard solution was presented at the entrance of the tasting room. If the compound could be obtained in food grade form, tasters were allowed to taste and smell (flavour TH). Otherwise, differentiation was only performed by the odour of the beers (odour TH). Panellists were presented six 3-alternate-forced choice (3-AFC) tests and their 'best estimate threshold' (BET) was calculated on the basis of the geometric mean of the highest concentration missed and the next higher adjacent concentration. In order to obtain correct BETs, and since the range of these varied widely between individuals, eight concentrations of the dilution series (factor 2) were prepared instead of six. In this way, six 3-AFC tests could initially be presented to the taster, but when the highest concentration of the additive was not indicated correctly or when all triangular tests were correct, a test with a higher or lower concentration of the additive was presented. The group TH was calculated as the geometric mean of the BETs. Tasters were also asked to describe the perceived flavour in the test samples of the beers. Each TH was determined with at least 18 tasters (average 20.2 tasters). Finally, in order to obtain a better insight of the obtained threshold values, several principal flavour compounds and physical and chemical characteristics of the used beer are presented in Table 1. Additionally, endogenous concentrations of the studied staling compounds are given in Table 5 where lager A (initial) represents the beer that was used for the TH determinations.

2.3. Determination of flavour compound concentrations

Most flavour compounds were determined by headspace solid phase micro extraction (SPME) coupled to gas chromatography–mass spectrometry (GC–MS), as described by Saison, De Schutter, Delvaux, and Delvaux (2008). On the one hand, on-fibre derivatisation with pentafluorobenzylhydroxylamine (PFBHA) was used for the determination of aldehydes and ethyl pyruvate, while the other compounds were extracted by a normal SPME procedure, followed by a GC–MS analysis on a Trace GC Ultra gas chromatograph coupled to a dual stage quadrupole MS (both from Thermo, Austin, TX, USA). Analyses were carried out in triplicate and the results were examined with Xcalibur software (Thermo, Austin, TX, USA).

Acetaldehyde and the compounds mentioned in Table 1 were measured by headspace (HS) GC. Detection of diacetyl and 2,3-pentanedione was performed by electrochemical detection (ECD), and the other compounds by flame ionisation detection (FID). Five ml of filtered beer were transferred to a vial and subsequently analysed with a calibrated Autosystem XL gas chromatograph with a HS autosampler (HS40; Perkin Elmer, Norwalk, USA) and equipped

Table 1
Characteristics of the lager beer that was used for the threshold determinations.

	Lager A
Original gravity (°Pl)	11.72
Apparent extract (°Pl)	2.07
Ethanol (v/v%)	5.12
CO_2-content (g/L)	5.0
Color (EBC)	6.5
Bitterness (EBU)	26
pH	4.24
Dimethyl sulphide (ppm)	0.02
Ethyl acetate (ppm)	14.5
Ethyl caproate (ppb)	80
Isoamyl acetate (ppb)	473
Propanol (ppm)	13.0
Isobutanol (ppm)	8.73
Isoamyl alcohol (ppm)	58.9
Diacetyl (ppb)	13.5
2,3-Pentanedione (ppb)	8.2
Acetaldehyde (ppb)	952

with a Chrompack-Wax 52 CB column (length: 50 m; 0.32 mm ID; film thickness: 1.2 μm; Varian, Palo Alto, CA, USA). Samples were heated for 16 min at 60 °C in the HS autosampler before injection (needle temperature: 70 °C). Helium was used as the carrier gas. The oven temperature was kept at 50 °C for 7.5 min, increased to 110 °C at 25 °C/min and held at that temperature for 3.5 min. The FID and ECD temperatures were kept constant at 250 and 200 °C, respectively. Analyses were carried out in duplicate and the results were examined with Perkin Elmer Turbochrom Navigator software.

2.4. Sensory analysis

Sensory tests were carried out with a trained panel of eight members. A fresh and a forced aged beer (3 weeks at 40 °C) were presented first, followed by randomly presented beers with added staling compounds (all in black glasses). The latter were prepared by adding 10 μl of an ethanol solution (containing defined amounts of the tested compounds corresponding with the observed concentration increase after forced ageing) to 250 ml bottles. Besides an evaluation of some fresh flavour aspects and the general ageing character, stale flavour was also evaluated for nine aspects (cardboard, metal, solvent, old hops, ribes, Maillard, stale-sulphury, acetaldehyde (green apple) and Madeira) by giving a score from 0 to 8. A score of 0 meant that the particular flavour aspect was not present, while a score of 8 indicated that this aspect was extremely strong. Finally, a global appreciation score was given on a scale from 1 to 9.

3. Results and discussion

3.1. Threshold determinations

In order to obtain a better insight of the chemical background of aged beer flavour, the sensory impact of 26 compounds regularly linked to ageing, and representing different classes of chemical reactions, was studied. Therefore, THs were determined for the selected compounds. In Table 2, TH values are presented, together with TH values found in the literature and flavour descriptors toward each compound. The presented values are group THs which give an indication of the average sensitivity of the tasters for the compound. However, since variations in the tasters' BETs were often so large, frequency distributions of the BETs for all compounds are also presented in Fig. 1. In practice, this distribution is often of much greater interest than the group TH and its confidence limits (Meilgaard, 1991).

When THs of several linear aldehydes were determined, they appeared to be considerably lower (10×) than previously reported for acetaldehyde and 3 and 4 times for (E,E)-2,4-decadienal (TT24DD) and hexanal, respectively. The TH of (E)-2-nonenal (T2N) was somewhat below the lowest levels found so far. The very low endogenous concentration of acetaldehyde might explain the first observation (Table 1). In addition, low concentrations of esters in the reference beer created a matrix with limited presence of masking compounds.

THs of Strecker aldehydes were generally lower than those reported in Mexican and US lager beer (Meilgaard, 1975a). However, the author used a different method at that time and some compounds were only tasted at 2–3 concentrations with only 4–5 tasters (indicated as values in brackets) and were thus only determined approximately. Later on, the TH determination of benzaldehyde was assigned to several laboratories, using different beers and the ascending method of limit test. The TH was determined 11 times and large variations were observed (330–2360 ppb) with an average of around 1000 ppb (Meilgaard et al., 1980). The TH determined in the studied beer (515 ppb) would thus have been located at the lower limit of the range. Nevertheless, the THs of 2-methylpropanal (2-MP), 2-methylbutanal (2-MB), 3-methylbutanal (3-MB) and phenylacetaldehyde (PheAA) in German lager beer, as determined by Thum et al. (1995), were similar to the obtained values. Moreover, it was already suggested that the PheAA and the methional THs were less than 1 and 0.5 ppb, respectively (da Costa et al., 2004).

The obtained THs of the Maillard reaction products confirm that furan derivatives have high THs, especially when a hydroxyl group is present. Since furfural and 5-hydroxymethylfurfural (5-HMF) were not available in food grade form, only odour THs were determined. However, when lower concentrations were also tasted in 3-AFC tests by 2–3 panellists, it was obvious that lower flavour THs could be obtained. Beer is slightly heated in the mouth, which gives many volatile compounds the opportunity to vaporise above the tongue towards the olfactory organ. This difference was tested for T2N and the odour TH (0.19 ppb) appeared to be about 6 times higher than the flavour TH. The observed difference ratio is, however, greatly dependent on the compound under study. On the other hand, diacetyl had a low TH (17 ppb) in the studied beer with low endogenous diacetyl content (13.5 ppb). The TH of diacetyl has been shown to be highly dependent on the endogenous concentration (Brown et al., 1978) and THs of 70–150 (Meilgaard, 1982) and 14–61 ppb (Kluba et al., 1993) were reported for beers with 30–300 ppb and unreported diacetyl contents, respectively.

Degradation of hop bitter compounds during ageing can lead to the release of methyl isobutyl ketone (MIBK), which has a relatively high TH compared to structurally related aldehydes. Additionally, 2-methylbutyric and 3-methylbutyric acid can be formed and further esterified to ethyl esters. Their THs are several orders of magnitudes lower than those of the corresponding acids and appear to be slightly higher than those reported by Williams and Wagner (1978) in Canadian lager and ale beers. THs of the other ethyl esters were considerably higher, which might be explained by the increasing polarity of present side chains, especially for ethyl lactate containing a hydroxyl group. Moreover, boiling points (224, 144 and 154 °C for nicotinate, pyruvate and lactate ethyl esters, respectively) appeared to be of less importance than the polarity of side chains for the TH of ethyl esters. This could be explained by the volatility of a compound in a watery medium, which is not only determined by its boiling point, but also by its aqueous solubility. Therefore, hydrophobic compounds escape more easily from the medium than do hydrophilic ones.

The TH of 2-furfuryl ethyl ether (FEE) was comparable to, but slightly higher than, the previously found values (Harayama, Hayase, & Kato, 1995; Vanderhaegen et al., 2003). This was probably because only the odour of FEE was considered. The same could be suggested for the odour THs of β-damascenone and γ-nonalactone of 203 and 607 ppb respectively, compared to 2.5 (Suzuki, Wanikawa, Kono, & Shibata, 2006), 25–50 (De Schutter, Saison, Derdelinckx, & Delvaux, 2007) and 150 ppb (Moll, 1994) for the flavour TH of β-damascenone and 11.2 (Suzuki et al., 2006) and 80 ppb (Yamashita et al., 2006) for the flavour TH of γ-nonalactone. Since no information is given about the surprisingly low values in Japanese lager beer by Suzuki et al. (2006), it is difficult to explain these large differences. The TH of dimethyl trisulphide (DMTS) was extremely low (0.027 ppb), as for most sulphur compounds, and was slightly higher than THs determined in Finnish lager beers (Arkima, Jounela-Eriksson, & Leppänen, 1981), but lower than the TH in UK lager (Peppard & Laws, 1971) and Canadian ale beer (Williams & Gracey, 1982). Finally, a low TH of 510 ppb was obtained for isoamyl acetate (IAA) (endogenous concentration: 473 ppb) compared to a TH range of 500–1700 ppb reported in beers containing 1–3 ppm IAA (Meilgaard, Reid, & Wyborski, 1982).

When the frequency distributions of Fig. 1 are studied, very large variations are observed between the individual BETs. For DMTS, the difference between the lowest and the highest concen-

Table 2
Determined flavour and odour thresholds of the selected staling compounds, accompanied by their flavour description and, additionally, threshold values found in literature.

	Determined TH (ppb)	TH Literature (ppb)	Flavour description
Linear aldehydes			
Acetaldehyde	1114	<12,500[c], 10,000–20,000 [k]	Green apple, fruity
Hexanal	88	350[a]	Bitter, winey
(E)-2-nonenal	0.03	<0.05[c], 0.05[j], 0.11[a], 0.5[b]	Cardboard, papery, cucumber
(E,E)-2,4-decadiënal	0.11	0.3[a]	Deep-fried, papery
Strecker aldehydes			
2-Methylpropanal	86*	65[e], <500[c], (1000)[a]	Grainy, varnish, fruity
2-Methylbutanal	45	35[e], 1250[a]	Almond, apple-like, malty
3-Methylbutanal	56*	46[e], (600)[a]	Malty, cherry, almond, chocolate
Benzaldehyde	515	1000[l], (2000)[a]	Almond, cherry stone
Phenyl acetaldehyde	105	<1[q], 100[e], (1600)[a]	Hyacinth, flowery, roses
Methional	4.2	<0.5[q], 40[d], (250)[a]	Cooked potatoes, worty
Maillard reaction products			
Furfural	15,157*	25,000–50,000[t], 150,000[a]	Caramel, bready, cooked meat
5-Hydroxymethylfurfural	35,784*	1,000,000[a]	Bready, caramel
5-Methylfurfural	1174	8000[f], 20,000[a]	Almond, marzipan
Acetylfuran	513	80,000[a]	Nutty, almond, burnt
Diacetyl	17	14–61[u], 70–150[k], 100[c], 150[a]	Buttery
Hop degradation products			
Methyl isobutyl ketone	2560*	(5000)[a]	Varnish
Ethyl-2-methylbutyrate	27	6–8[g]	Fruity, apple, sweetish, candy
Ethyl-3-methylbutyrate	91	15–20[g], 1300[a]	Artificial strawberry, fruity, candy, violet, sweetish
Ethyl esters			
Ethyl nicotinate	4555*	(6000)[a]	Medicinal, solvent, anis
Ethyl pyruvate	22,525	85,000[a]	Peas, freshly cut grass
Ethyl lactate	353,553	250,000[a]	Fuity, buttery
Furan ethers			
2-Furfuryl ethyl ether	11*	2.5[o], 6[v]	Solvent
Polysulphides			
Dimethyl trisulphide	0.027	0.012–0.02[h], 0.1[m], 0.15[i,n]	Onion, rotting fruit, red cabbage, sulfury
Oxidation carotenoids/glycoside hydrolysis			
β-Damascenone	203*	2.5[p], 25–50[r], 150[w]	Coconut, tobacco, red fruits
Lactones			
γ-Nonalactone	607*	11.2[p], 80[s]	Coconut, vanilla, glue, rancid
Acetate esters			
Isoamyl acetate	510	500–1700[k], 1600[a], 2300[c]	Banana, estery

[a] Meilgaard (1975).
[b] Jamieson and Van Gheluwe (1970).
[c] Palamand and Hardwick (1969).
[d] Anderson and Howard (1974).
[e] Thum et al. (1995).
[f] Meilgaard et al. (1978).
[g] Williams and Wagner (1978).
[h] Arkima et al. (1981).
[i] Stewart and Russel (1981).
[j] Van Eerde and Strating (1981).
[k] Meilgaard (1982).
[l] Meilgaard et al. (1980).
[m] Peppard and Laws (1971).
[n] Williams and Gracey (1982).
[o] Harayama et al. (1995).
[p] Suzuki et al. (2006).
[q] da Costa et al. (2004).
[r] De Schutter et al. (2007).
[s] Yamashita et al. (2006).
[t] Ahrenst-Larsen and Hansen (1963).
[u] Kluba et al. (1993).
[v] Vanderhaegen et al. (2003).
[w] Moll (1994).
* Odour Threshold.

tration tasted was only 16-fold, while this was 256-fold for other compounds. Additionally, it was noticed that tasters, who were very insensitive for one compound, could be very sensitive for another. This was most remarkable for diacetyl and confirms earlier observations (Meilgaard et al., 1982). These results confirm that it is important to use THs as indicative rather than absolute values for the flavour impact of a compound. In practice, each taster will perceive the same beer differently and will perceive some aged flavour notes in sub-(group)TH concentrations, even without considering possible synergistic or additive effects.

3.2. Interactions between flavour compounds

Although the TH of a compound gives a good indication of its flavour impact, it would be insufficient and oversimplified to consider the overall beer flavour as the sum of contributions made by

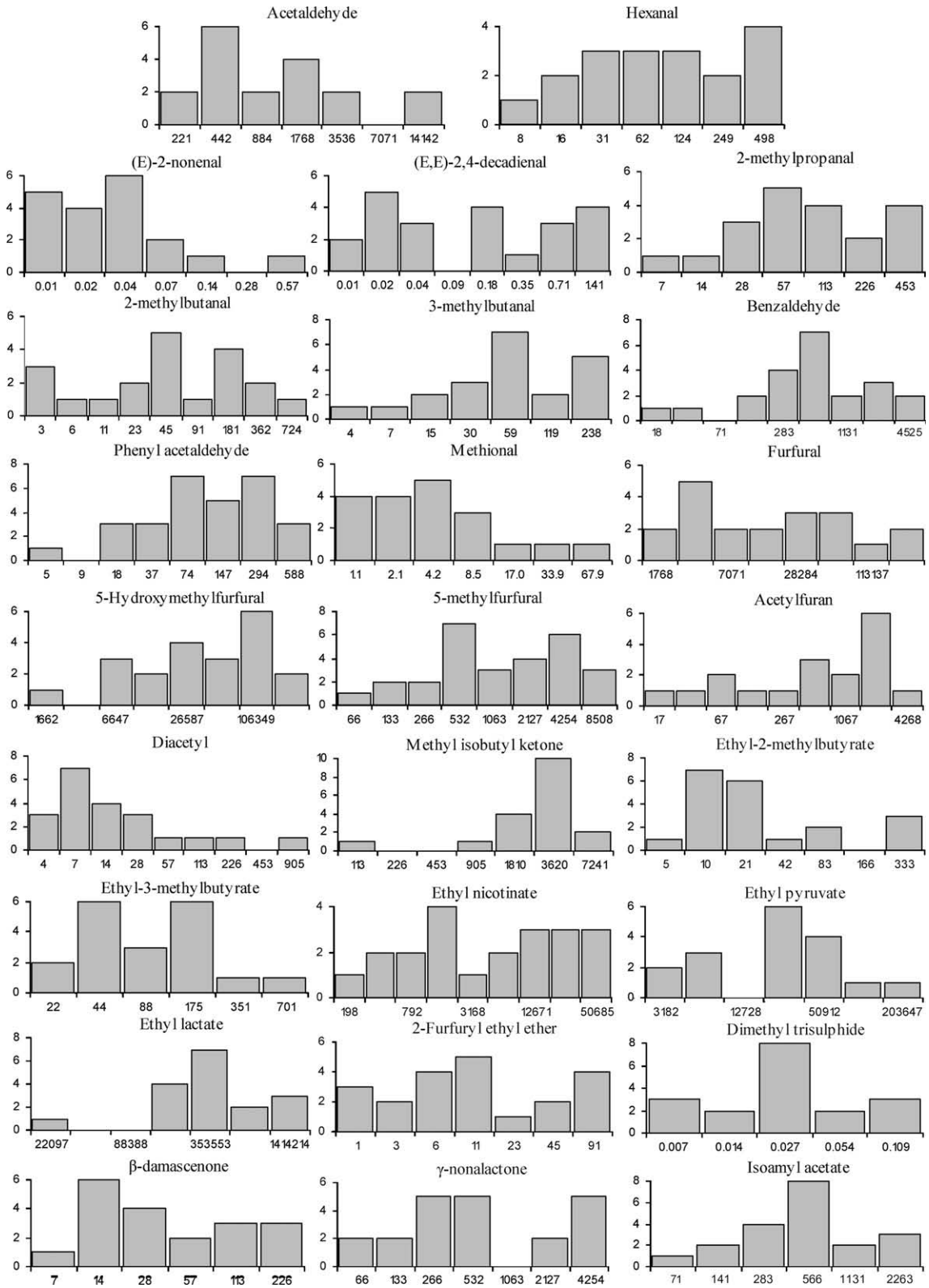

Fig. 1. Frequency distributions of the best estimate thresholds of the tasters for the selected staling compounds (*y*-axis: numbers of tasters; *x*-axis: added concentration of the compound).

each individual compound, since several interactions affecting flavour perception can occur. Therefore, the impact of mixtures of compounds was evaluated by determining their THs. Compounds were added in the ratio of their individual THs in order to evaluate the interactions at the same level of sensory activity. The THs of the mixtures (TH_{mixt}) were expressed as% of their calculated TH, assuming independency of the compounds. This can also be regarded as the individual TH of each compound in the presence of the other compound(s), expressed as the % of its individual TH (TH_{ind}). Four possible interactions might occur. The compounds

can exhibit their flavour independently, which means that each compound has to be present at 100% of its individual TH in order to taste a difference (TH_{mixt} = 100% TH_{ind}). They can counteract each other (antagonism) (TH_{mixt} > 100% TH_{ind}), or they can exhibit an additive (TH_{mixt} = 50% TH_{ind}), or a synergistic effect (TH_{mixt} < 50% TH_{ind}), (Guadagni, Buttery, Okano, & Burr, 1963; Meilgaard, 1975b). Nine combinations were tested, eight 2-compound combinations of which four with similar and four with distinct flavour and structure, and finally one 3-compound combination. Results are presented in Table 3.

When a mixture of the highly flavour active unsaturated linear aldehydes T2N and TT24DD was tested, a strong synergistic effect was observed. This is in accordance with a previous study where the addition of a combination of unsaturated linear aldehydes (T2N, (E)-2-hexenal and (E)-2-heptenal) to wine showed a threefold increase in intensity (Cullere, Cacho, & Ferreira, 2007). However, in this respect, care should be taken when considering flavour interactions. It can be expected that similar compounds will exhibit an additive effect, but compounds with different structures would not necessarily be expected to behave in the same way (Guadagni et al., 1963). Meilgaard (1975b), on the other hand, hypothesized that the degree of chemical similarity between compounds is irrelevant. Substances of a like flavour might be perceived additively, while those with very distinct flavours are independent, and substances with partly similar flavour notes are partly additive.

The combination of 2-MP and 3-MB showed a partial additive effect, which is in accordance with the proposed hypothesis. A comparable, but stronger additive effect has also been observed for a mixture of 2-MP, 2-MB and 3-MB in wine (Cullere et al., 2007). Acetylfuran and 5-MF, in contrast, despite similar flavour characteristics, showed very strong antagonism, while ethyl-2-methylbutyrate (Et2MB) and ethyl-3-methylbutyrate (Et3MB) exhibited a very strong synergistic effect. The latter was unexpected despite their similar flavour, since a previous study showed that the addition of a combination of Et2MB and Et3MB appeared to be rather subtractive than additive (Williams & Wagner, 1978). When combinations of compounds with distinct flavour and structure were considered, all kind of interactions were observed, i.e. independent, slightly suppressive, additive and strongly synergistic. The combination of 3 compounds showed a partly additive effect, and could be approximately estimated by the interactions of the binary mixtures. It appeared that the hypothesis of Meilgaard (1975b) cannot be applied to all mixtures and, more importantly, that predicting flavour interactions is a very ambitious project.

In general, a higher complexity of flavour perception was observed for mixtures, since flavour descriptors varied more between tasters than for single-component additions. Additionally, descriptors for a mixture often differed considerably compared to the flavour of the individual compounds, as already observed by Palamand and Hardwick (1969). In this respect, the combination of 2-MP and 3-MB was described as flowery, fruity, caramel, burnt and sweet, in comparison with grainy, varnish and fruity for 2-MP and malty, cherry, almond and chocolate for 3-MB.

The study of the effect of interactions between compounds on flavour is very complex and much more research is necessary. However, with respect to flavour stability, it can be concluded that important interactions might occur that may cause staling compounds to affect flavour at sub-TH concentrations.

Table 3
Threshold values of mixtures of compounds in the ratio of their individual thresholds (TH) and the corresponding flavour description.

	TH of mixture (%)	Flavour description
2 Compounds		
Similar flavour and structure		
(E)-2-nonenal and (E,E)-2,4-decadienal	24	Cardboard, papery, rancid fatty
2-Methylpropanal and 3-methylbutanal	73	Flowery, fruity, caramel, burnt, sweet
Acetylfuran and 5-methylfurfural	584	Marzipan, almond, sweet
Ethyl-2-methylbutyrate and ethyl-3-methylbutyrate	22	Sweet, candy, winey
Distinct flavour and structure		
2-Methylbutanal and methional	98	Cooked potato, sweet, caramel
2-Methylbutanal and phenyl acetaldehyde	121	Candy, flowery
Methional and phenyl acetaldehyde	63	Cooked potato, flowery, honey
2-Methylbutanal and ethyl-3-methylbutyrate	14	Winey, candy, caramel, fruity
3 Compounds		
2-Methylbutanal, methional and phenyl acetaldehyde	75	Malty, worty, cooked meat, chocolate, bread crust, potato

3.3. Masking effects

Besides formation of many distinct compounds during ageing, degradation of acetate esters can occur, resulting in a decrease of fresh flavour notes (Vanderhaegen et al., 2006). Acetate esters determine the fresh flavour of beer greatly and might be able to mask the perception of other flavour compounds. Therefore, the appearance of aged flavour notes can be accelerated upon acetate ester degradation. Hence, the potential masking effect of IAA on 2-MB, methional and PheAA was studied. This was performed by determining THs of aldehydes in beer with an extra added amount of IAA (to the test, as well as the reference beer). Comparing the THs with those determined in beer without addition might give an idea of masking effects exerted by IAA.

The results presented in Table 4 indicate that masking effects may not be underestimated as THs of 2-MB and methional were raised by 250% and 40%, respectively, when the reference beer contained an extra 300 ppb of IAA. The TH of PheAA, on the other hand, was unaffected by an extra added amount of IAA and the masking effect thus appeared to be compound-dependent. Apart from the observed elevation of THs, it was remarkable that no further increase was observed when sub-(300 ppb) and supra-TH (600 ppb) concentrations were added. Nevertheless, it can be concluded that the presence of higher IAA concentrations can affect the TH value considerably, indicating once again that the TH of a compound is highly dependent on the reference beer and that masking effects might play an important role in decelerating the appearance of aged flavours.

3.4. Evaluation of the contribution of staling compounds to aged flavour

3.4.1. Approach

The determination of THs gives an idea of the flavour activity and it has been shown that TH values vary widely among com-

Table 4
Threshold values (TH) of 3 compounds in a reference beer with varying concentrations of isoamylacetate (IAA).

	TH Lager	TH Lager + 300 ppb IAA	TH Lager + 600 ppb IAA
2-Methylbutanal	45	157	161
Methional	4.2	5.8	5.8
Phenylacetaldehyde	105	110	99

pounds. Since concentrations in beer vary accordingly, the concept of the flavour unit (FU) can be used to predict the contribution of a component to the overall beer flavour and it is calculated as the ratio of the concentration of a compound and its TH value (Meilgaard, 1975b). Hence, the evolution of the concentrations and FUs of the selected compounds was monitored during forced ageing of three different Belgian lagers (3 weeks 40 °C). Results are presented in Table 5 (lager A was used as the reference beer for TH determinations). When considering FUs, it is interesting to keep in mind that flavour compounds possibly start to be of interest from a 0.2 FUs increase or decrease compared to the original concentration and that an influence on beer flavour begins to be perceived at 0.5 FUs increase or decrease, but the taster can rarely identify the compound or group responsible. At 1 FU increase or decrease, the cause may be identified, and at 2 FUs the character of the beer is quite altered (Meilgaard, 1975b). Again, these values give only an indication and should not be considered absolute, since the sensitivity of individuals for a compound can vary considerably. Consequently, for a specific compound, more sensitive tasters will perceive a flavour alteration at lower FU increases or decreases, and the opposite occurs for less sensitive tasters. This was further examined by calculating the FU that could be detected by the most sensitive taster (the ratio of its BET and the corresponding group TH) for each individual compound. It appeared that, on average, a difference could be perceived by the most sensitive tasters when an increase of 0.17 FUs was observed. Additionally, since beer is a mixture of several hundreds of compounds, interactions between compounds, which can alter the TH value considerably, may not be neglected. Consequently, studying the TH of a compound and its FU evolution during ageing may not be sufficient for evaluating its impact on aged flavour. Therefore, an additional approach was followed in order to obtain a broader insight of the flavour impact of the selected compounds. For this approach, after ageing and analysing lager A, beers were prepared, starting from fresh lager A, by adding that amount of a compound or combination of compounds that corresponds with the observed increase in concentration during ageing. Afterwards, the beers were tasted by an expert tasting panel. The combinations of the added compounds were chosen in order to clarify the contribution of different chemical ageing reactions. These combinations are indicated in Table 5 and the results of the tastings are given in Fig. 2.

After 3 weeks of ageing at 40 °C, several aged flavour notes were observed; particularly the cardboard flavour was apparent, but also ribes, solvent, Maillard and stale-sulphury notes were perceived. In this respect, the perception of berries, cassis, raisins and/or honey is described as ribes; caramel, burnt, bread and/or butter is described as Maillard and straw, and hay and/or onion as sulphury. Since metal, old hops and acetaldehyde flavour notes were only present at very low intensities, they were not shown in the spider plots. The aged beer was given as a reference and its flavour pattern was therefore repeated in the three spider plots. Although the fresh beer was also presented as a reference, its flavour pattern is not shown because hardly any aged flavour notes were perceived. Finally, an increase of about 5 units is seen for the ageing score and a decrease of 3 for the global appreciation score after ageing. In the following, the contribution of the selected staling compounds will be estimated from both points of view.

3.4.2. Contribution of staling compounds to aged flavour

In all three lagers, an increase of about 1 FU was observed for acetaldehyde during ageing, which indicates a great contribution

Table 5
Threshold value (TH), concentration (conc) and flavour units (FU) of the studied compounds in 3 fresh and forced aged (3 weeks at 40 °C) Belgian lager beers.

	TH (ppb)	Lager A				Lager B				Lager C			
		Initial		Aged (3w40 °C)		Initial		Aged (3w40 °C)		Initial		Aged (3w40 °C)	
		Conc (ppb)	FU	Conc (ppb)	FU	Conc (ppb)	FU	Conc (ppb)	FU	Conc (ppb)	FU	Conc (ppb)	FU
Acetaldehyde	1114	952	0.85	2163	**1.94**	202	0.18	1307	**1.17**	505	0.45	1463	**1.31**
Hexanal[a]	88	0.7	0.01	1.8	0.02	0.4[A]	0*	1.3	0.02	0.6	0.01	1.6	0.02
(E)-2-nonenal[a]	0.03	0.09	**2.93**	0.15	**4.84**	0.05	**1.58**	0.10	**3.35**	0.05	**1.80**	0.10	**3.30**
(E,E)-2,4-decadiënal[a]	0.11	0.02[A]	0.20	0.06[A]	0.53	0.01[A]	0.12	0.02[A]	0.18	0.01[A]	0.11	0.02[A]	0.20
2-Methylpropanal[b]	86	7.9	0.09	37.7	0.44	5.2	0.06	43.4	0.50	2.2[A]	0.03	25.7	0.30
2-Methylbutanal[b]	45	2.7	0.06	6.4	0.14	2.1	0.05	6.8	0.15	0.7[A]	0.02	3.9	0.09
3-Methylbutanal[b]	56	36	0.64	64	**1.14**	24	0.43	76	**1.36**	12	0.21	32	0.58
Benzaldehyde[b]	515	0.3[A]	0*	0.6[A]	0*	0.3[A]	0*	0.5[A]	0*	0.3[A]	0*	0.4[A]	0*
Phenyl acetaldehyde[b]	105	22	0.21	51	0.49	15	0.14	51	0.48	8	0.08	16	0.15
Methional[b]	4.2	2.7	0.65	5.9	**1.41**	1.8	0.42	5.6	**1.34**	0.4[A]	0.10	1.7	0.40
Furfural[c]	15157	25	0*	457	0.03	9	0*	359	0.02	17	0*	322	0.02
5-Methylfurfural[c]	1174	0.7[A]	0*	4.5	0*	0.6[A]	0*	2.4	0*	1.2	0*	3.6	0.00
Acetylfuran[c]	513	5.8[A]	0.01	15.7	0.03	6.8	0.01	14.4	0.03	4.9[A]	0.01	13.7	0.03
Diacetyl	17	13.5	0.79	20.7	**1.21**	7.9	0.46	18.5	**1.09**	5.6	0.33	12.1	0.71
Methyl isobutyl ketone	2560	5.4[A]	0*	6.0[A]	0*	7.8	0*	8.6	0*	2.5[A]	0*	3.1[A]	0*
Ethyl-2-methylbutyrate	27	0.61	0.02	0.66	0.02	0.54	0.02	0.66	0.02	0.28	0.01	0.42	0.02
Ethyl-3-methylbutyrate	91	0.54	0.01	0.95	0.01	0.72	0.01	1.02	0.01	0.28	0*	0.83	0.01
Ethylnicotinate	4555	6.5[A]	0*	15.3[A]	0*	6.4[A]	0*	18.7	0*	3.3[B]	0*	33.1	0.01
Ethylpyruvate	22525	118	0.01	159	0.01	25	0*	63	0*	104	0*	218	0.01
2-Furfuryl ethyl ether[d]	11	1.9	0.17	16.1	**1.46**	2.3	0.21	19.2	**1.74**	1.6	0.14	15.3	**1.39**
Dimethyl trisulphide	0.027	n.d.	0*	0.012[B]	0.44	n.d.	0*	0.009[B]	0.33	0.011[B]	0.39	0.004[B]	0.16
β-Damascenone[e]	203	128	0.63	398	**1.96**	42	0.20	334	**1.64**	157	0.77	419	**2.06**
γ-Nonalactone	607	25	0.04	32	0.05	20	0.03	31	0.05	22	0.04	35	0.06
Isoamyl acetate	510	473	0.93	427	0.84	703	**1.38**	734	**1.44**	1452	**2.85**	1276	**2.50**

* FU < 0.005.
[A] Approximate concentration, since the obtained value is: lower than the limit of quantification, but higher than the limit of detection.
[B] Approximate concentration, since the obtained value is: below the limit of detection Saison et al. (2008).
[a] Compounds incorporated in the mixture of Linear aldehydes.
[b] Compounds incorporated in the mixture of Strecker aldehydes.
[c] Compounds incorporated in the mixture of Maillard reaction compounds.
[d] Compounds incorporated in the mixture of 2-Furfurylethylether.
[e] Compounds incorporated in the mixture of β-damascenone.

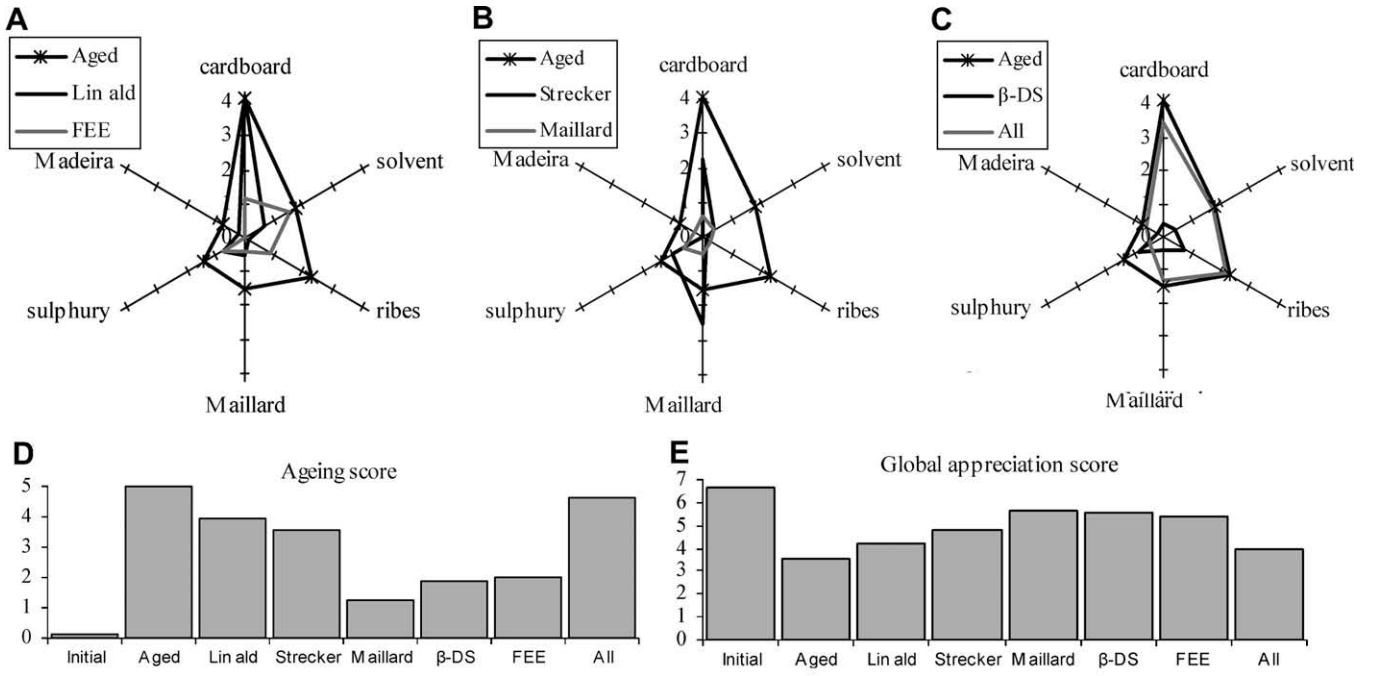

Fig. 2. Results of sensory analysis of aged lager (3 weeks at 40 °C) and fresh beer (Initial) with added concentrations of staling compounds corresponding to the observed increase after ageing (composition and concentrations of mixtures are given in Table 5). A, B and C represent intensities of aged flavour notes in spider plots and D and E give the ageing and the global appreciation scores. (Lin ald: linear aldehydes; FEE: 2-furfuryl ethyl ether; Strecker: Strecker aldehydes; Maillard: Maillard reaction products; β-DS: β-damascenone).

of acetaldehyde to the aged flavour. However, it is surprising that no acetaldehyde notes were found in the aged beer. This is probably because of its non-characteristic and mild flavour (Meilgaard et al., 1982) and acetaldehyde might contribute to other aged flavour notes (Palamand & Hardwick, 1969) and might act as a supporting background for other staling compounds. The contribution of hexanal, on the other hand, is probably negligible since its FUs did not even exceed 0.02 in either lager. The concentration of T2N was already present above its TH in the fresh beer although it could not be tasted. Additionally, an increase of at least 1.5 FUs was observed in the lager beers after forced ageing, indicating a large contribution of T2N to the perceived aged flavour. The highest concentration of TT24DD, however, was only at 0.53 FUs in aged lager A, with an observed increase of 0.33 FUs. This indicates that TT24DD can have an effect on aged flavour for a specific group of tasters, since added concentrations as low as 0.01 ppb could be tasted by some individuals (Fig. 1). On the other hand, it was shown that TT24DD and T2N act in a synergistic way (Table 3) and the presence of TT24DD can thus be important for intensifying the cardboard flavour in aged beer, supporting previous observations of Meilgaard, Ayma, and Ruano (1971). Addition of a mixture of hexanal, T2N and TT24DD confirmed these findings since a strong cardboard flavour was perceived upon addition, which was of comparable intensity to that in the aged beer (Fig. 2A).

2-MP was only present in very small concentrations in all fresh lagers, but a strong increase (0.27–0.44 FUs) was observed during ageing in the three lager beers. Next to an expected contribution for the more sensitive tasters, it is very probable that 2-MP will be important through interactions with other compounds, since a decrease of the group TH of 27% was already observed in the presence of 3-MB (Table 3). This also indicates the substantial contribution of the latter, since an increase in FUs as high as 0.93 was observed. The increase of 2-MB during ageing, on the other hand, was much smaller (0.07–0.1 FUs). This was even more pronounced for benzaldehyde which probably will not contribute at all to aged flavour (ΔFU ca. 0.0004). The increase of PheAA during ageing (ΔFU 0.07–0.34) indicates a slightly lower but comparable contribution, as was seen for 2-MP and its combination with methional proved already to lower the group TH by about 37% (Table 3). Finally, the effect of methional on aged flavour will be considerable, as deduced from its increase in FUs (ΔFU 0.31–0.92) during ageing. When a mixture of all Strecker aldehydes was added, their contribution to the aged flavour appeared to be very large and it was remarkable that both cardboard and Maillard flavour notes were perceived (Fig. 2B). The modest sulphury note could be explained by methional and the beer was described as malty and worty which is in accordance with previous studies (da Costa et al., 2004; Perpete & Collin, 1999). Needless to say, interactions between the Strecker aldehydes were very important for the observed flavour impact.

The furan compounds, furfural, 5-MF and acetylfuran, probably will not impart flavour, as derived from the observed evolution of their FUs. This is even more pronounced when the antagonistic effect between 5-MF and acetylfuran is considered and addition of a mixture of these compounds confirmed the very limited flavour impact (Fig. 2B). The concentration of 5-HMF could not be measured with on-fibre derivatisation followed by SPME GC–MS, since all concentrations were lower than the method detection limit of 5.7 ppm. However, the concentration of 5-HMF was estimated to be about 4 ppm from the chromatograms corresponding to 0.1 FUs in aged lager A. In a previous study, 5-HMF concentrations were estimated to be about 7 ppm (0.2 FUs) after 2 weeks at 37 °C and 16.6 and 36.6 ppm (0.5 and 1.0 FUs) after 28 days at 37 °C (Techakriengkrai et al., 2006). It therefore seems that the contribution of 5-HMF to aged flavour should be taken into account. In the studied lagers, the effect will, however, probably be minimal after 3 weeks at 40°C. The last observed Maillard reaction product, diacetyl, is expected to contribute to the aged flavour, since increases of 0.38–0.92 FUs were observed and it has already been seen that diacetyl can give a slightly burnt, malt aroma at low concentrations (Palamand & Hardwick, 1969).

The increase of MIBK during ageing appeared to be negligible with respect to aged flavour. Similar conclusions could be drawn for Et2MB and Et3MB from their FU evolution. However, observed synergistic effects were very large when one or both compounds were involved. Notwithstanding these observations, it is likely that the contribution of these esters is minimal in lagers in the observed ageing period. The importance of other studied ethyl esters for the appearance of the aged flavour was found to be even less. Ethyl lactate could not be measured with the employed techniques, but a previous study showed concentrations of 69 ppb in fresh beer and 474 ppb after 4 weeks at 37 °C (Harayama et al., 1995), corresponding with an increase of only 0.001 FUs.

During ageing of the three lagers, an increase of about 1.5 FUs was observed for FEE and a considerable flavour contribution is expected. An additional test in which lager A was tasted after addition of 14.2 ppb FEE confirmed this assumption (Fig. 2A) and showed that mainly a stale solvent flavour was perceived. However, additionally, and as previously observed for the cardboard flavour (Harayama et al., 1995), it was remarkable that the cardboard and ribes flavours were intensified upon FEE addition.

Since the method detection limits were close to the determined TH, and mostly higher than the concentrations in beer, it was difficult to make accurate assumptions for the flavour impact of DMTS. However, it can be seen that the DMTS TH was not exceeded in any of the lagers and that FUs of around 0.4 can be reached, indicating a modest contribution.

The contribution of β-DS, on the other hand, was already apparent in fresh beer and the concentration increased further during ageing (ΔFU ca. 1.35). This indicates a strong contribution of β-DS to aged beer flavour, confirming assumptions made in a previous study (Gijs, Chevance, Jerkovic, & Collin, 2002). In contrast, addition of the increased amount of β-DS to beer showed a limited effect. Its effect could more or less be regarded as that of an important background flavour that can intensify the perception of varying flavour notes and this can probably be attributed to its non-characteristic and mild flavour. In contrast to previous statements (Gijs et al., 2002; Suzuki et al., 2006), and as deduced from its FU evolution during ageing, it appeared that γ-nonalactone will contribute to the aged flavour.

Finally, decreases of 46 and 176 ppb IAA were observed in lagers A and C, respectively, but a rise of 31 ppb in lager B. The latter indicates that a chemical condensation reaction between acetate and isoamyl alcohol might also occur during ageing. A decrease of IAA could lead to a loss in fruitiness and consequently, to a loss of masking flavours. However, it is difficult to estimate the effect on aged flavour, since no decrease of THs was observed when the IAA concentration of the reference beer was lowered from about 1070 to 770 ppb. On the other hand, a decrease of the IAA concentration, from 770 to 470 ppb, lowered the THs of 2-MB and methional considerably (Table 4). Finally, the loss of fruitiness during ageing cannot only be explained by a decrease of acetate esters since it was seen that masking can also act oppositely and the addition of mixtures of staling compounds to fresh lager resulted in a marked decrease of fruitiness (results not shown).

3.4.3. Final remarks on sensory analysis

Apart from the already discussed beers with added compounds, a final beer to which all 5 studied mixtures were added was tasted in order to estimate the resemblance that can be obtained between the truly forced aged beer and the artificially prepared aged beer from the compounds under study (Fig. 2C). The addition of the staling compounds seemed to substantially reproduce the aged flavour, and it can be concluded that the presented compounds account for the major part of the aged flavour perceived in the studied beer. Even the ribes and the Madeira flavour notes, which could hardly be perceived in the beers with one of the individual mixtures, were detected upon addition of the combination of all compounds. The sulphury note was the only flavour that could not be explained at all by the added mixture and DMTS and other sulphur compounds that were not previously linked to ageing are probably the cause. Despite the observation that Strecker aldehydes and FEE can support the cardboard flavour, the simultaneous addition of the mixtures brought about a cardboard flavour that was less harsh than when only linear aldehydes were added. This can probably be attributed to masking effects of other staling compounds (Cullere et al., 2007). From the ageing and global appreciation scores (Fig. 2D and E), it can be seen that the contribution of the linear aldehydes and Strecker aldehydes to the aged flavour was the largest, followed by FEE and β-damascenone. The combination of the compounds approximated the scores for the aged beer. Finally, it has to be mentioned that metal, old hops and acetaldehyde flavours were not perceived and that the tasters described the aged flavour of the truly aged beer as the most complex and varied. In addition, forced ageing at 40°C does not generate the same relative level increase of staling compounds compared to natural ageing, the latter rendering relatively less cardboard character (Vanderhaegen et al., 2006). This means that the addition of the selected compounds can account for the most important part of aged flavour, but that research towards the identification of new ageing compounds remains important.

4. Conclusion

Threshold determinations of 26 compounds that were already linked to ageing were regularly found to be substantially lower than those previously reported. In addition, TH values were observed to be rather indicative than absolute as a measure of the flavour activity of a compound, since large variations in sensitivity were observed between individuals. Moreover, various interactions can occur between flavour compounds which can considerably influence their flavour activity. Because of the varying and seemingly arbitrary nature of the observed interactions, no predicting assumptions could be made considering interactions between compounds. However, with respect to aged flavour, interactions appeared to have an important contribution, even when compounds were present at sub-TH concentrations. Next to the observed interactions, a masking effect of IAA on staling compounds was observed, indicating that a higher concentration in fresh beer can play an important role in decelerating the appearance of aged flavours.

In conclusion, the cardboard flavour was essentially caused by T2N, but the supporting effects of TT24DD, FEE and Strecker aldehydes could not be underestimated. Acetaldehyde exceeded its TH after ageing and was suggested to support aged flavour as a background flavour rather than a key flavour compound. The addition of a mixture of Strecker aldehydes contributed to Maillard, sulphury and cardboard notes. Particularly, methional and 3-MB and to a lesser extent 2-MP and PheAA, were found to be important. Furan compounds and ethyl esters were of minor importance, except for possibly the contribution of 5-HMF. Diacetyl, on the other hand, might well be able to contribute Maillard-like flavour notes. Finally, FEE and β-damascenone were found to be essential staling compounds, the former as a contributor to the stale solvent flavour and both as supporting compounds in determining the broad spectrum of aged flavour. Altogether, it was found that the aged flavour could be reproduced fairly well by addition of the selected staling compounds, indicating that these compounds account for the major part of the aged flavour of lager beers.

Acknowledgements

Daan Saison gratefully acknowledges the granting of a Ph.D fellowship from the Institute for the promotion of Innovation by Science and Technology in Flanders (IWT Flanders). In addition, the authors wish to thank the tasters for their attendance at the numerous tastings.

References

Ahrenst-Larsen, B., & Hansen, H. L. (1963). Gaschromatographische Untersuchungen über die Geschamcksstabilität von bier. *Brauwissenschaft, 16*, 393–397.

Anderson, R. J., & Howard, G. A. (1974a). The origin and occurence of volatile sulphur compounds in British ales and lagers. *Journal of the Institute of Brewing, 80*, 357–370.

Anderson, R. J., & Howard, G. A. (1974b). The origin and occurence of volatile sulphur compounds in British ales and lagers. *Journal of the Institute of Brewing, 80*, 357–370.

Arkima, V., Jounela-Eriksson, P., & Leppänen, O. (1981). The Influence of volatile sulphur compounds on beer flavour. In *European brewery convention – flavour symposium* (pp. 89–102).

Brown, D. G. W., Clapperton, J. F., Meilgaard, M. C., & Moll, M. (1978). Flavor thresholds of added substances. *Journal of the American Society of Brewing Chemists*, 73–80.

Cullere, L., Cacho, J., & Ferreira, V. (2007). An assessment of the role played by some oxidation-related aldehydes in wine aroma. *Journal of Agricultural and Food Chemistry, 55*(3), 876–881.

da Costa, M. S., Goncalves, C., Ferreira, A., Ibsen, C., de Pinho, P. G., & Ferreira, A. C. S. (2004). Further insights into the role of methional and phenylacetaldehyde in lager beer flavor stability. *Journal of Agricultural and Food Chemistry, 52*(26), 7911–7917.

Dalgliesh, C. E. (1977). Flavour stability. In *Proceedings of the European brewery convention congress* (pp. 623–659).

De Schutter, D. P., Saison, D., Derdelinckx, G., & Delvaux, F. R. (2007). β-Damascenone, a suitable heat load indicator during wort boiling? In *Proceedings of the European brewery convention congress*.

Engan, S. (1972). Organoleptic threshold values of some alcohols and esters in beer. *Journal of the Institute of Brewing, 78*(1), 33–36.

Gijs, L., Chevance, F., Jerkovic, V., & Collin, S. (2002). How low pH can intensify beta-damascenone and dimethyl trisulfide production through beer aging. *Journal of Agricultural and Food Chemistry, 50*(20), 5612–5616.

Guadagni, D. G., Buttery, R. G., Okano, S., & Burr, H. K. (1963). Additive effect of sub-threshold concentrations of some organic compounds associated with food aromas. *Nature, 200*, 1288–1289.

Harayama, K., Hayase, F., & Kato, H. (1995). Contribution to stale flavor of 2-furfuryl ethyl ether and its formation mechanism in beer. *Bioscience Biotechnology and Biochemistry, 59*(6), 1144–1146.

Jamieson, A. M., & Van Gheluwe, J. E. A. (1970). Identification of a compound responsible for cardboard flavor in beer. In *Proceedings of the American society of brewing chemists* (pp. 192–197).

Kluba, R., de Banchs, N. M., Fraga, A., Jansen, G., Langstaff, S., Meilgaard, M. C., et al. (1993). Sensory threshold determination of added substances in beer. *Journal of the American Society of Brewing Chemists, 51*(4), 181–183.

Meilgaard, M. C. (1972). Stale flavor carbonyls in brewing. *Brewers Digest, 47*, 48–57.

Meilgaard, M. C. (1975a). Flavor chemistry of beer; part I: Flavor interaction between principal volatiles. *MBAA Technical Quarterly, 12*(2), 107–117.

Meilgaard, M. C. (1975b). Flavor chemistry of beer: Part II: Flavor and threshold of 239 aroma volatiles. *MBAA Technical Quarterly, 12*(3), 151–168.

Meilgaard, M. C. (1982). Predicition of flavor differences between beers from their chemical composition. *Journal of Agricultural and Food Chemistry, 30*(6), 1009–1017.

Meilgaard, M. C. (1991). Testing for sensory thresholds of added substances. *Journal of the American Society of Brewing Chemists, 49*(3), 128–135.

Meilgaard, M. C., Ayma, M., & Ruano, J. I. (1971). A study of carbonyl compounds in beer. IV. Carbonyl compounds identified in heat-treated wort and beer. *Proceedings of the American Society of Brewing Chemists, 5*, 219–229.

Meilgaard, M. C., Babcook, D. R., de Banchs, N. M., Berndt, R., Bernstein, L., Canales, A. M., et al. (1978). Flavor testing. *Journal of the American Society of Brewing Chemists, 36*(3), 127–129.

Meilgaard, M. C., de Banchs, N. M., Berndt, R., Bernstein, L., Blachske, E. F., Canales, A. M., et al. (1980). Sensory analysis. *Journal of the American Society of Brewing Chemists, 38*(3), 99–107.

Meilgaard, M. C., Elizondo, A., & Moya, E. (1970). A study of carbonyl compounds in beer – 2. Flavor and flavor thresholds of aldehydes and ketones added to beer. *MBAA Technical Quarterly, 7*(3), 143–150.

Meilgaard, M. C., Reid, D. S., & Wyborski, K. A. (1982). Reference standards for beer flavor terminology system. *Journal of the American Society of Brewing Chemists, 40*(4), 119–128.

Moll, M. (1994). *Beers and coolers*. New York: Springer-Verlag. p. 510.

Palamand, S. R., & Hardwick, W. A. (1969). Studies on the relative flavor importance of some beer constituents. *Technical Quarterly, 6*(2), 117–127.

Peppard, T. L., & Laws, D. R. J. (1971). Hop derived sulphur compounds and their effect on beer flavour. In *Proceedings of the European brewery convention congress* (pp. 91–104).

Perpete, P., & Collin, S. (1999). Contribution of 3-methylthiopropionaldehyde to the worty flavor of alcohol-free beers. *Journal of Agricultural and Food Chemistry, 47*(6), 2374–2378.

Saison, D., De Schutter, D. P., Delvaux, F., & Delvaux, F. R. (2008). Optimisation of a complete method for the analysis of volatiles involved in the flavour stability of beer by solid-phase microextraction in combination with gas chromatography and mass spectrometry. *Journal of Chromatography A, 1190*(1–2), 342–349.

Stewart, G. G., & Russel, I. (1981). The influence of yeast on volatile sulphur compounds in beer. In *European brewery convention – flavour symposium* (pp. 173–187).

Suzuki, M., Wanikawa, A., Kono, K., & Shibata, K. (2006). *Factors affecting the formation of gamma-nonalactone and its contribution to the flavor and aroma of aging beer*. Asia Pacific Section, Tasmania: The Institute of Brewing and Distilling.

Techakriengkrai, I., Paterson, A., & Taidi, B. (2006). Relationship of sensory staleness in two lagers to headspace concentrations of trans-2-nonenal and three staling aldehydes. *Journal of the Institute of Brewing, 112*(1), 36–40.

Thum, B., Miedaner, H., Narziss, L., & Back, W. (1995). Bildung von "Alterungscarbonylen" – mögliche Mechanismen un Bedeutung bei der Bierlagerung. In *Proceedings of the European brewery convention congress* (pp.491–498).

Van Eerde, P., & Strating, J. (1981). Trans-2-nonenal. In *European brewery convention – flavour symposium* (pp. 117–121).

Vanderhaegen, B., Neven, H., Coghe, S., Verstrepen, K. J., Verachtert, H., & Derdelinckx, G. (2003). Evolution of chemical and sensory properties during aging of top-fermented beer. *Journal of Agricultural and Food Chemistry, 51*(23), 6782–6790.

Vanderhaegen, B., Neven, H., Verachtert, H., & Derdelinckx, G. (2006). The chemistry of beer aging – a critical review. *Food Chemistry, 95*(3), 357–381.

Williams, R. S., & Gracey, D. E. F. (1982). Beyond dimethyl sulfide: The significance to flavor of thioesters and polysulfides in Canadian beers. *Journal of the American Society of Brewing Chemists, 40*(2), 68–71.

Williams, R. S., & Wagner, H. (1978). The isolation and identification of new staling related compounds from beer. *Journal of the American Chemical Society, 36*(1), 27–31.

Yamashita, H., Kühbeck, F., Hohrein, A., Herrmann, M., Back, W., & Krottenthaler, M. (2006). Fractionated boiling technology: Wort boiling of different lauter fractions. *Monatsschrift für Brauwisschenschaft*, 130–147.