A New Validation of Relevant Substances for the Evaluation of Beer Aging Depending on the Employed Boiling System

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ABSTRACT

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During the last years changes in aging indicators have been observed, supposedly resulting from modern brewing technology. The Research Brewery Weihenstephan (Forschungsbrauerei Weihenstephan) offers excellent opportunities for comparing different modern wort boiling systems under semi-industrial conditions. Employing three different boiling systems, nine brews were produced. The resulting worts were compared regarding the most common wort parameters. Furthermore the influence of the different boiling systems on aging indicators in the resulting beers was analyzed using a newly developed mass spectrometry-based method. The decrease in the total amount of aging indicators in industrial beers over the last years is very likely the result of lower thermal intake in modern brewhouse equipment. The total amount of aging indicators is sufficient to describe the differences in modern boiling systems. In summary, 2-furfural dominates all other indicators in terms of thermal influence. 2-Furfuryl ethyl ether can be suggested as good indicator of aging as postulated by Eichhorn, whereas β-damascenone is questionable as an aging indicator. Supplementary experiments were carried out to investigate the role of the aging indicators as stale flavour components. Because of synergistic effects, many stale flavour compounds act as aroma compounds and not only as indicators.

Key words: Aging indicators, beer flavour, boiling systems, brewhouse, synergistic effects, wort.

INTRODUCTION

The wort boiling process is an essential step during wort production. Different objectives have to be achieved during the wort boiling process. This comprises evaporation of water and unwanted volatiles, isomerisation of humulones, fixation of wort composition by inactivation of enzymes and removal of proteins. The thermal load should be as low as possible at the same time. The quality of the boiling step is therefore described by the ratio of obtained positive reactions (isomerisation, evaporation) to the thermal load. Hereby, key criteria are evaporation of dimethylsulfide (DMS) and adjustment of coaguable nitrogen[2]. Modern wort boiling systems have a homogeneous temperature distribution in wort and a higher degree of efficiency. They own a higher evaporation efficiency which means that the required evaporation of volatiles is conducted with lower overall evaporation and hence lower thermal impact. Lower thermal impact results in better flavour stability of the final beer. By control of single parameters such as steam temperature, wort temperature, wort circulation, and others, it is possible to fix a wort composition which is needed for individual beer styles.

Flavor stability of beer is mainly dependent on the oxygen uptake during production, filtration and bottling[8,9,27,33,40,41,59]. In addition, thermal treatment of wort[12–14,47], pH-value[17,19,21,28], and storage conditions[4,21,24,69] have been shown to have a significant influence on flavor stability. The analysis of aging indicators described by Eichhorn[15] and Lustig[35] to determine oxidative and thermal processes (aging) in beer has successfully been used for several years. During the last years changes in aging indicator concentrations in commercial beers have been observed, supposedly the result of modern mashing regimes, new wort boiling systems[25,31,32,64,70], improved yeast management[63] and better filling technology, which all influence aging stability[3]. Many of these technical and technological improvements were achieved based on data of the aging indicators. The total amount of aging indicators in forced-aged commercial lager beers decreased during the last years, as shown in Table I. These commercial beers were analysed in the institute's laboratory. In addition, single components showed no changes, or only little changes, during forced aging and therefore those components were not appropriate any more as aging indicators. The total amount of aging indicators in forced aged beers (1 day shaking, 4 days at 40°C) was 260 µg/L (n = 27) on average in 1998. In 2005, the average of total aging indicators was only 168 µg/L (n = 97). This effect could not be observed at beers brewed in the institute's pilot-scale brewing plant. This plant has not been technically changed or updated since 1998. Thus the analysis can be judged as stable and the observed shift is most probably due to technical or technological improvements at the breweries.

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To obtain more information on the influence of boiling technology on aging indicators on the one hand and on the impact of aging indicators on stale flavour on the other hand, two series of experiments were conducted in this work. Different boiling systems were employed at the research brewery Weihenstephan and the resulting worts and beers were analyzed. In a second series of experiments additive flavour effects of aging indicators on the sensory threshold of the substances were evaluated.

The importance of the aging stability of the beer is clearly shown by further investigations on new technologies, e.g. fractionated wort boiling technology[73].

MATERIAL AND METHODS

All experiments were carried out in the research brewery of the Technische Universität München-Weihenstephan[74]. The research brewery offers the possibility to compare different wort boiling systems at identical peripheral conditions such as grinding, mashing, lautering, trub separation, fermentation, maturation and storage. For this project three different wort boiling systems were employed. The three systems are shown in Table II. A schema of the used systems is given in Figs. 1 and 2. Bold lines in the figures indicate the wort flow. Boiling trials were carried out three times for each of the systems and showed good reproducibility. The following wort parameters were analyzed according to MEBAK[38,50]: Thiobarbituric Index (TBI), Dimethylsulfide (DMS), Dimethylsulfide-Precursor (DMS-P) respectively and Coaguable Nitrogen (coag. N).

Different components were analyzed using GC mass spectrometry. The preparation of samples was made in accordance to the standard aging indicator analysis[15,35]. The GC method was as follows: Initial temperature 40°C hold for 4 min, final temperature 220°C hold for 30 min, 5°C/min, flow 1.2 mL/min Helium as carrier gas, 2 µL injection volume, splitless mode, split ratio 8. The measurement of single substances was conducted using the selected ion monitoring modus with simultaneous measurement of the whole mass spectrum. The GC instrument was a Thermo Trace GC Ultra DSQ. A Varian VF-5ms (0.25 mm internal diameter, 60 m length) was used as the column. The coefficient of variation of the analysis of aroma compounds was less than 5%. The assortment of the analyzed substances was made depending on their relevance as aging components[5-7,16-18,23,30,36,39,40,42-46,48,49,51-53,55,56,59,66-68,71,72] and on commercial availability.

Table I. Increasing of aging indicators from fresh to force aged beer in 1998 and 2005 [µg/L].

	Category	1998	2005
2-Acetyl furan	A[a]	7.4	2.3
2-Furfural	T[b], A	111.2	77.7
2-Phenyl ethanal	O[c], A	9.1	3.7
2-Propionyl furan	A	7.3	0.5
3-Methyl butyraldehyde	O, A	5.6	2.5
5-Methyl furfural	A	1.2	0.8
Benzaldehyde	O, A	1.1	0.2
Diethyl succinate	A	0.5	0.2
γ-Nonalactone	T, A	20.6	12.7
Ethyl nicotinate		15.5	6.2
Ethyl phenyl acetate	A	0.6	0.2

[a] Aging indicator.
[b] Thermal indicator.
[c] Oxygen indicator.

The boiling systems shown in Table II are described by Back[2] and Kattein and Herrmann[29]. All systems were employed with boiling regimes that are typical for their industrial use. The resulting differences in overall evaporation are typical for the industrial use of these systems and resulted in varying wort qualities. Hot trub separation was identical for all applied systems in a whirlpool (15 min). The values for aging indicators and aroma components were not calculated on overall evaporation because the concentration of those substances in wort and beer has to be compared under practical conditions.

Aging indicator analysis is often criticized for failing to describe the effective aging aroma; it only indicates it. This is because the threshold of the single substances is far higher than the concentrations that can be found in beer[11]. On the other hand many synergistic and additive effects are known between single substances in complex food matrices in general[20,34,37]. Similar effects were assumed for aging indicators in beer.

Combinations of 22 substances were investigated in the matrix beer for such combinatory effects. Preliminary experiments were made to determine sensory potent combinations. The mixture of components was made according to beer-typical concentration ratios. The employed substances are listed in Table III. Threshold determination was done according EBC method 13.9[65]. Purity of chemicals was >95%.

RESULTS AND DISCUSSION

The different batches of one system were comparable as most of the parameters deviated less than 15%, which was the normal range of technical and analytical fluctuations.

Table II. Overview over the boiling systems and procedures employed.

System A	System B	System C
Internal Heater	External heater	Dynamic low pressure boiling (internal heater)
Preheating 95°C – 12 cycles/h (max. heating power)	Preheating 98°C – 12 cycles/h (max. heating power)	Preheating 95°C – 12 cycles/h (max. heating power)
1. Boiling 25 min (80 % of max. heating power) – 8 cycles/h	Boiling (102°C on outlet of heat exchanger) 60 min; 12 cycles/h	5 cycles Pressure build-up / expansion
2. Boiling 15 min (40 % of max. heating power) – 8 cycles/h		Heating up to 103°C / expanding to 99.5°C
3. Boiling 25 min (80 % of max. heating power) – 8 cycles/h		
Overall evaporation: 7%	Overall evaporation: 6%	Overall evaporation: 4.5%

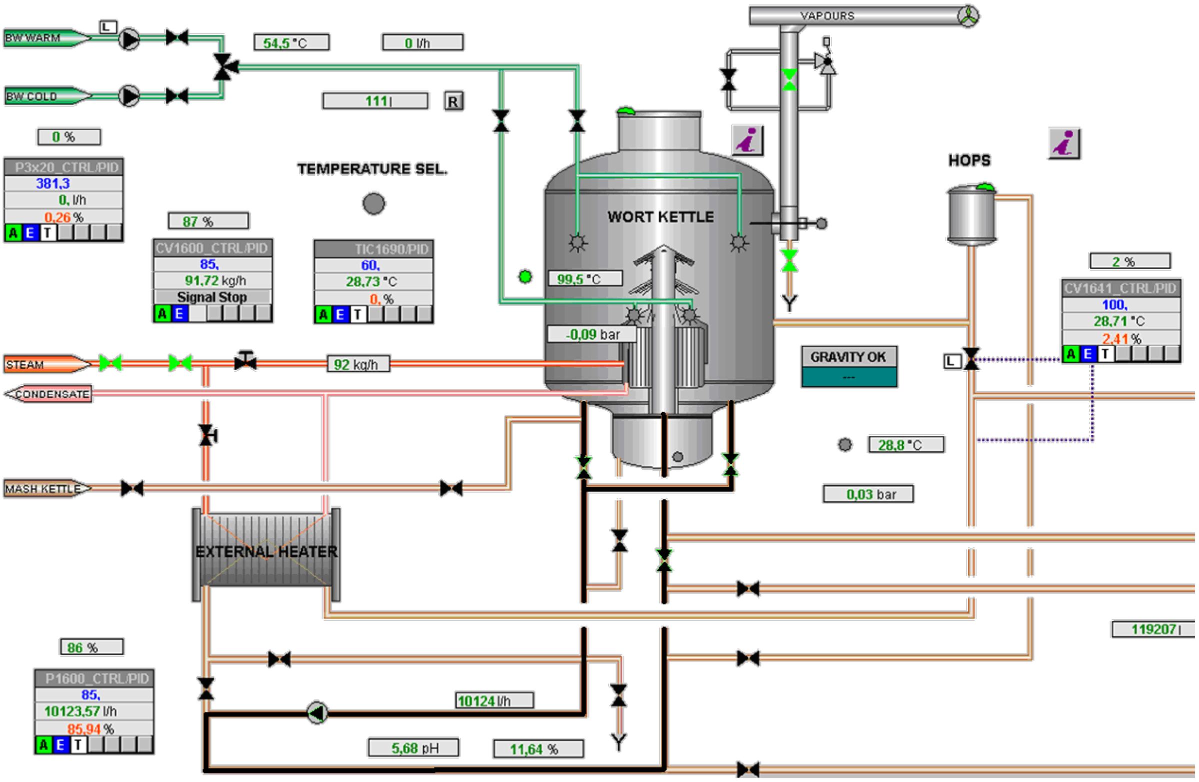

Fig. 1. Boiling System A (Internal Heater) and C (Dynamic Low Pressure Boiling): Bold lines indicate wort flow.

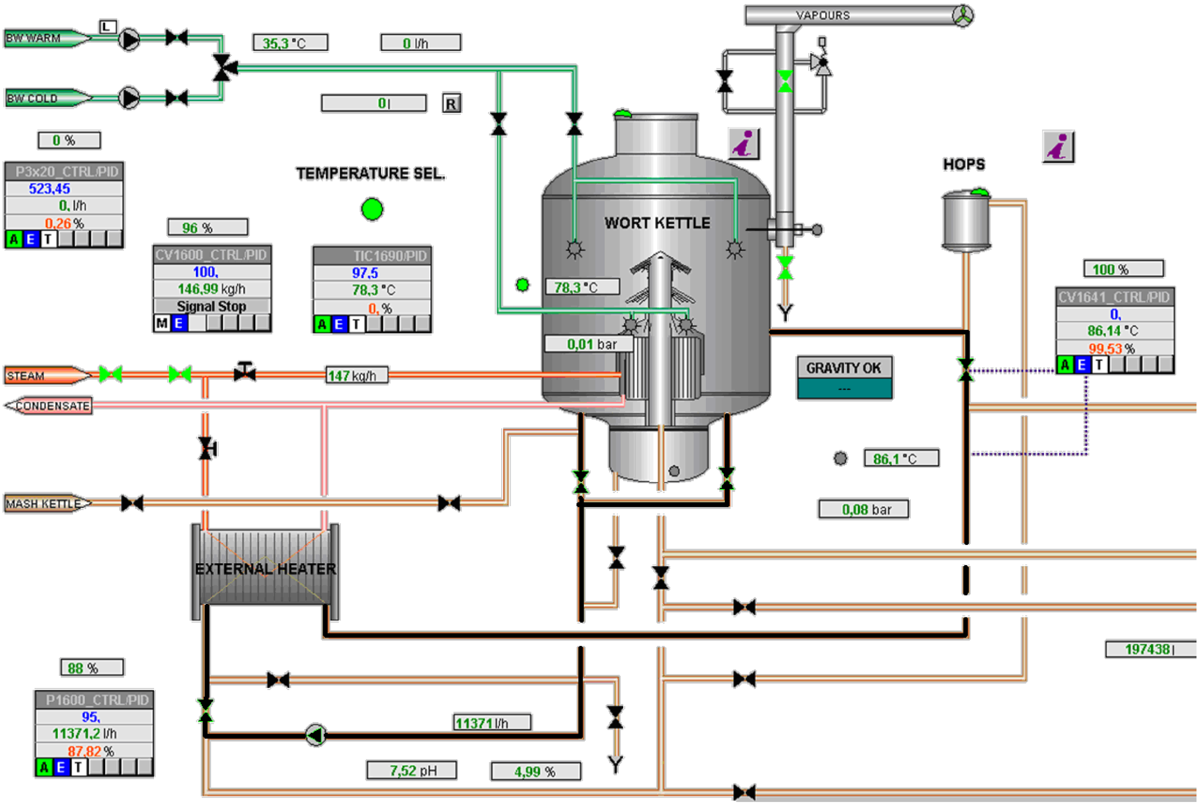

Fig. 2. Boiling System B (External Heater): Bold lines indicate wort flow.

The Thiobarbituric Index (TBI) was formulated by Thalacker and Kaltwasser[62]. They concluded that the TBI is an appropriate indicator for thermal load of the wort[61], which was confirmed by De Schutter et al.[13]. In Table IV the average values of three brews for the TBI are shown. All systems showed a moderate increase of the TBI. System B had a slightly higher increase during wort boiling, which can be due to the higher temperature in the external boiler. According to the German Institute for Standardization (DIN), the deltaTBI should be less than 15[1], because a high deltaTBI is associated with poorer flavour stability[58]. Only the internal boiler system, which is the system with the lowest heat load, was below 15.

Table V compares the content of coaguable nitrogen (coag. N) of the three systems. The data showed no difference and the systems were below 30 mg/L after boiling. High values of coag. N (>35 mg/L) can result in lower colloidal stability and affect yeast performance during fermentation[2]. All systems reduced the coag. N in a sufficient way.

The third parameter to describe the boiling process in a sufficient way is the content of free dimethylsulfide (DMS). It should be lower than 100 ppb[57], otherwise it creates a vegetable-like off-flavour. In Table VI, it can be seen that all systems showed good evaporation efficiency. Even the slightly higher values of free DMS in the kettle-full worts were reduced to 22 ppb. Also the differences in the concentration of DMS-P diminished during boiling.

The wort aroma components after boiling are shown in Table VII. Because of the early hop addition, only small amounts of linalool and other terpene derivates were detected. Hop derived flavour compounds are usually recognized as positive aroma components[22,26,35]. System C showed higher amounts of Strecker aldehydes resulting from the lower overall evaporation. The higher sum of Strecker aldehydes was mainly due to the high amount of 2-phenylacetic aldehyde, which has the highest boiling point. During fermentation the yeast will reduce this aldehyde to 2-phenylethanol and therefore the difference is diminished. Higher amounts of lipid oxidation products were also due to the lower evaporation of System C. Remarkable was the high value for 2-furfural in System C. This can be explained by the higher thermal load during the boiling process. In contrast to an external boiler, where the maximum temperature of 102°C is only in the heat exchanger, the temperature in System C rose to 103°C for several minutes (dynamic low pressure boiling). This hypothesis is supported by the concentration of 2-acetylfurane, which was also higher in System C. However, according to De Schutter et al.[13], 2-furfural is not appropriate as a heat indicator, because of its pH induced formation during wort boiling. Higher concentrations of alcohols, such as 2- and 3-methylbutanal, 1-pentanol etc., are due to the lower overall evaporation of this system.

Table VIII shows the average values of beer aroma components for the three systems. There were no remark-

Table III. Overview of the employed substances for combined use in sensorical experiments.

2-Methyl butyraldehyde	Ethanal (acetaldehyde)
3-Methyl butyraldehyde	Benzaldehyde
2-Furfural	3-Methyl 2-butanone
5-Hydroxy 2-methyl furfural	γ-Nonalactone
Amyl aldehyde	2-Methyl butyric acid ethyl ester
Hexanal	2-Phenyl acetic aldehyde
(E)-2-Hexenal	Succinic acid diethyl ester
Heptanal	Nicotinic acid ethyl ester
2,4-Hexadienal	2-Acetyl furan
(E,Z)-2,6-Nonadienal	2-Furfuryl ethyl ether
(E)-2-Nonenal	β-Damascenone

Table IV. Average values for Thiobarbituric Index (TBI) for the three systems.

TBI	Wort kettle filled	End of boiling / after whirlpool	Δ
System A	25	39	14
System B	23	40	17
System C	26	41	15

Table V. Average value of coaguable nitrogen for the three systems.

Coaguable N [mg/L]	Wort kettle filled	End of boiling / after whirlpool
System A	74	27
System B	76	29
System C	80	27

Table VII. Wort aroma components [µg/L] after boiling for the three systems (average of three brews).

	System A	System B	System C
3-me-Butanal	66.6	48.3	63.3
2-me-Butanal	19.3	15.0	17.1
Methional	18.3	23.3	19.0
Benzaldehyde	2.3	3.7	6.5
2-Phenyl acetic aldehyde	87.9	94.3	151.5
Total of Strecker-Aldehydes	201.2	184.7	238.4
Pentanal	0.9	1.0	1.6
2-Pentanone	0.6	0.5	1.1
Hexanal	3.6	4.5	7.8
Heptanal	1.1	1.3	2.3
(E)-2-(Z)-6-Nonadienal	0.7	0.5	0.6
2-Furfural	149.5	178.4	248.8
2-Acetyl furan	2.4	2.5	5.9
γ-Nonalactone	3.3	5.6	5.2
3-me-Butanol	4.6	14.4	11.1
2-me-Butanol	4.4	3.4	9.1
1-Pentanol	1.0	2.2	15.7
1-Hexanol	0.5	1.5	9.7
1-Octanol	0.1	0.1	1.0
2-Phenyl ethanol	165.4	185.0	129.7
Linalool	2.0	1.1	2.1

Table VI. Average values for DMS and DMS-P for the three systems.

	DMS [µg/L]		DMS-P [µg/L]	
	Wort kettle filled	End of boiling / after whirlpool	Wort kettle filled	End of boiling / after whirlpool
System A	288	22	250	46
System B	246	21	271	46
System C	231	20	342	57

able differences in beer aroma components. A comparable fermentation can thus be assumed.

The aging indicators for the three systems are shown in Table IX. The total amount of aging indicators was nearly the same for System A and System C, for both fresh and force-aged beers. System B shows higher values even in the fresh beers. The increased total amount of aging indicators for System B was mainly caused by a high concentration of methional. Methional and phenylacetic aldehyde, which were also higher in the beers made with System B, are known to be indicators for thermal stress[60]. System B also showed the highest total amount of aging indicators. This difference in System B was caused mainly by 2-furfural. The difference between aged and fresh beers brewed with System A and C was very similar. The system with the lowest thermal impact (System A, internal boiler) showed the lowest amounts of Strecker aldehydes and the smallest increase of aging indicators during the forced-aging procedure.

Table VIII. Beer aroma components (average value of 3 brews) in [µg/L] in fresh beers.

	System A	System B	System C
1-Hexanol	8.1	11.0	9.2
1-Heptanol	4.8	3.8	5.1
1-Octanol	17	18	18
1-Decanol	7.9	7.4	8.2
2-Phenyl ethanol	12813	12673	12562
Isobutyl acetate	27	29	32
Hexyl acetate	2.5	3.2	2.1
Heptyl acetate	2.6	2.0	2.9
Octyl acetate	3.6	4.0	3.8
2-Phenyl ethyl acetate	353	309	377
Ethyl butyrate	71	65	62
Ethyl hexanoate	97	87	92
Ethyl octanoate	203	161	205
Ethyl decanoate	24	16	23
Hexanoic acid	1428	1423	1582
Octanoic acid	4418	3954	4355
Nonanoic acid	13	15	16
Decanoic acid	509	359	557
Dodecanoic acid	29	15	16
α-Terpineol	traces	traces	traces
Linalool	1.2	1.6	1.9
Nerol	3.1	3.3	3.2

In addition to the total amount of aging indicators, the following results shown in Table IX are notable. The concentration for benzaldehyde was low for all systems. This shows that mashing, storage and filling with all of the experiments were comparable and did not lead to high oxygen uptake. Strecker aldehydes increased the most for System B, whereas the aldehydes resulting from lipid oxidation (hexanal, hexenal, heptanal, E-2-nonenal) increased the least for this system. Also the values in fresh beer were the lowest for System B, which indicates a good evaporation efficiency. Those lipid oxidation products have a high sensory impact but very low influence on the total amount of aging indicators. The β-damascenone showed low values in all of the force-aged beers, which makes its suitability as aging indicator questionable. The single flavour threshold of β-damascenone, in a German lager beer, was 154 µg/L, a concentration which was 100-fold the β-damascenone concentration of the aged beers. 2-Furfurylethylether showed a clear increase during aging for all beers. This appears to verify the postulation of Eichhorn, who classified this substance as potent analytical storage indicator[38]. The obtained flavour threshold was 34 µg/L, thus a direct contribution is questionable. But sub-threshold effects of β-damascenone and 2-furfurylethylether with other beer volatiles cannot be excluded.

The beers were also tasted by a trained taste panel, but no significant differences and no preferences could be detected in fresh and force aged beers.

For several combinations of aging indicators additive and synergistic effects were found. Fig. 3 shows the threshold for 2-methyl butanal and 3-methyl butanal in beer, both as single substances and in combination. For 2-methyl butanal the threshold as single substance was 156

Table IX. Comparison of aging indicators in fresh and forced aged beers of the three systems.

	System A			System B			System C		
Aging indicators [µg/L]	Fresh	Forced	Δ	Fresh	Forced	Δ	Fresh	Forced	Δ
2-Methyl butanal	4.1	6.5	2.4	5.6	8.1	2.5	3.2	5.8	2.6
3-Methyl butanal	7.7	9.7	2	12	14.9	2.9	7.1	11.8	4.7
Methional	13	27	14	41	66	25	11	26	15
Benzaldehyde	1.1	1.1	0	0.9	1.9	1	1	1.1	0.1
2-Phenyl acetic aldehyde	5.3	7	1.7	10.4	15.4	5.1	6.1	7.2	1.1
Sum Strecker aldehydes	31.2	51.3	20.1	69.9	106.3	36.5	28.4	51.9	23.5
2-Furfuryl ethyl ether	0.2	3.7	3.5	0.1	3.8	3.7	0.2	3.7	3.5
Ethyl nicotinate	2.3	6.8	4.5	3.4	8.4	5	2.5	6.9	4.4
2-Furfural	2.5	34.4	31.9	5.7	47.8	42.1	4.2	32.4	28.2
γ-Nonalactone	16	22.3	6.3	16	20	4	15.1	20.8	5.7
Hexanal	0.6	1.1	0.5	0.2	0.5	0.3	0.6	1.1	0.5
Hexenal	1.2	1.7	0.5	0.8	0.8	0	0.8	1.6	0.8
Heptanal	0.37	0.54	0.17	0.24	0.27	0.03	0.4	0.55	0.15
Hexadienal	0.04	0.05	0.01	0.05	0.07	0.02	0	0.08	0.08
5-Methyl furfural	5	5.6	0.6	4.8	5.6	0.8	4.8	6.2	1.4
2-Propionyl furan	0.07	0.08	0.01	0.04	0.05	0.01	0.3	0.4	0.1
Diethyl succinate	0.1	0.1	0	0.05	0.07	0.02	0	0.08	0.08
E-2-nonenal	0.007	0.026	0.019	0.007	0.022	0.016	0.007	0.026	0.019
Ethyl phenyl acetate	0.28	0.33	0.05	0.3	0.28	neg.	0.45	0.63	0.18
β-Damascenone	1	1	0	0.8	0.9	0.1	1	1	0
2-Acetyl furan	3.4	3.7	0.3	3.4	3.6	0.2	3.6	4	0.4
Total	64	133	68	106	198	93	62	131	69

µg/L and for 3-methyl butanal 57 µg/L. A combination of both substances in a 2.2:1-ratio (2-methyl butanal:3-methyl-butanal) showed significantly reduced thresholds of 30 µg/L for 2-methyl butanal and 14 µg/L for 3-methyl butanal respectively. The thresholds for the single substances were usually not exceeded in the beers, whereas the combined threshold could be found in many of the aged lager beers. As a result, these two components can act as substances which directly contribute to the stale flavour and thus are not only indicators for stale flavour.

Linear aldehydes, derived from fatty acid degradation processes, possess high thresholds as single substances in beer. In combination, those substances showed high additive and synergistic effects. Thresholds of single substances and their combinations (same concentration for every substance) are shown in Fig. 4. Even the combined threshold was above the normal concentration in beer, which is about 2 µg/L in force aged beers. On the other hand not every possible combination of aging indicators was tested, which means that some more effects of linear aldehydes on stale taste would be expected.

Some combinations did not only show synergistic effects, but also changes in the sensory perception of components in combination. (E)-2-nonenal was described by the tasters as "cardboard" as a single substance. (E)-2-(Z)-6-nonadienal was described as cucumber like. The combination provoked a sweet fruity flavour which was perceived by the tasters. This observation could explain the different existing opinions about the influence of (E)-2-nonenal on beer taste. The combinatory effects of the thresholds of (E)-2-(Z)-6-nonadienal and (E)-2-nonenal are shown in Fig. 5. Threshold of both components was at 23 ng/L for (E)-2-(Z)-6-nonadienal respectively 2 ng/L for (E)-2-nonenal in a mixture with a 10:1 ratio ((E)-2-(Z)-6-nonadienal:(E)-2-nonenal) between both aroma components. Dalgliesh[10] showed that a beginning stage of the stale flavour is a berry-like stage, which decreases as cardboard flavour increases. One explanation for this observation could be due to an additive effect such as with (E)-2-nonenal. As was seen, the flavour of the mixture was fruity. A further increase could decrease the fruity perception and increase the cardboard flavour.

Combinatory effects lead to sensorial perceptibility of aging indicators. Therefore those aging indicators should also be recognized as aging aroma components. This confirms the results recently published by Saison et al.[54], who also found that the thresholds of compounds in mixtures were lower than the single compound thresholds. The only aging indicator that was perceptible as a single substance was γ-nonalactone with a threshold of 26 µg/L.

CONCLUSIONS

There are recognizable differences in modern boiling systems regarding the development of aging indicators. Systems with a low evaporation efficiency result in beers with a higher total amount of aging indicators. Systems with low thermal indicators show increased values regard-

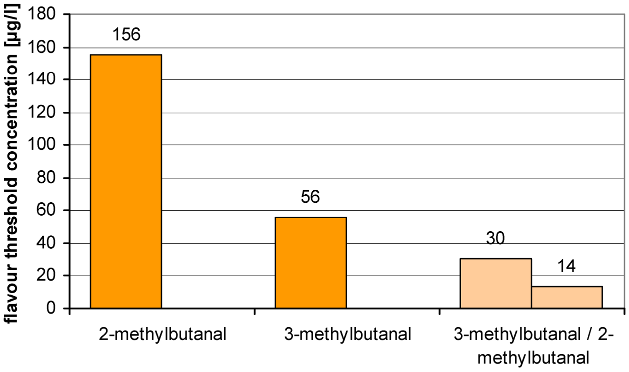

Fig. 3. Threshold of 2- and 3-methyl butanal and of a 2.2:1-mixture.

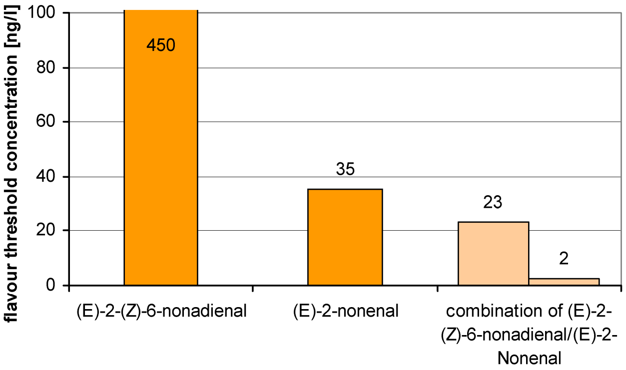

Fig. 5. Threshold of (E)-2-(Z)-6-nonadienal and (E)-2-nonenal and of a mixture of both.

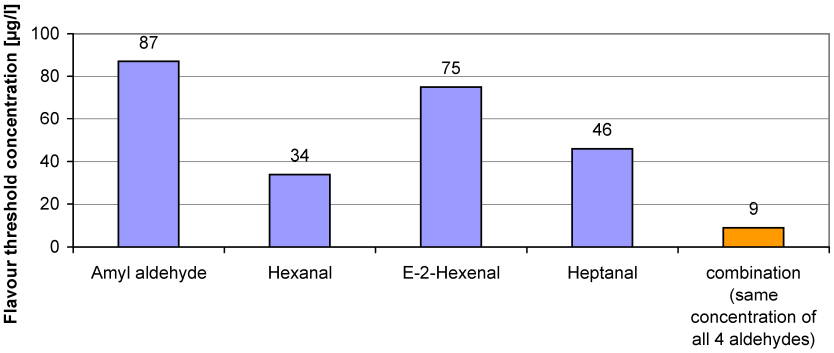

Fig. 4. Threshold of some linear aldehydes and of an equivalent mixture of these aldehydes.

ing products of fatty acid degradation, which are also known as evaporation indicators. As the employment of modern boiling systems leads to decreasing amounts of aging indicators in beer, the observed decrease of the total amount of aging indicators in industrial beers over the last years is very likely to be the result of the lower thermal impact with modern brewhouse equipment.

2-Furfuryl ethyl ether can be suggested as good analytical indicator of aging as postulated by Eichhorn, whereas β-damascenone is questionable as an aging indicator. Sensory experiments showed that many aging indicators are also aging aroma components, as their threshold in a beer matrix is lower than the threshold found in the literature, because of the interactive effects of aroma compound mixtures.

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