

Flavour-improved alcohol-free beer – Quality traits, ageing and sensory perception

Gernat, D. C.; Brouwer, E. R.; Faber-Zirkzee, R. C.; Ottens, M.

DOI

[10.1016/j.fbp.2020.07.014](https://doi.org/10.1016/j.fbp.2020.07.014)

Publication date

2020

Document Version

Final published version

Published in

Food and Bioproducts Processing

Citation (APA)

Gernat, D. C., Brouwer, E. R., Faber-Zirkzee, R. C., & Ottens, M. (2020). Flavour-improved alcohol-free beer – Quality traits, ageing and sensory perception. *Food and Bioproducts Processing*, 123, 450-458. <https://doi.org/10.1016/j.fbp.2020.07.014>

Important note

To cite this publication, please use the final published version (if applicable). Please check the document version above.

Copyright

Other than for strictly personal use, it is not permitted to download, forward or distribute the text or part of it, without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license such as Creative Commons.

Takedown policy

Please contact us and provide details if you believe this document breaches copyrights. We will remove access to the work immediately and investigate your claim.

Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Food and Bioproducts Processing

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


Flavour-improved alcohol-free beer – Quality traits, ageing and sensory perception

D.C. Gernat^a, E.R. Brouwer^b, R.C. Faber-Zirkzee^b, M. Ottens^{a,*}^a Department of Biotechnology, Delft University of Technology, van der Maasweg 9, 2629 HZ Delft, The Netherlands^b Heineken Supply Chain, Burgemeester Smeetsweg 1, 2382 PH Zoeterwoude, The Netherlands

ARTICLE INFO

Article history:

Received 9 March 2020

Received in revised form 15 July 2020

Accepted 19 July 2020

Available online 25 July 2020

Keywords:

Alcohol-free beer

Zeolite

Aldehydes

Wort flavour

Ageing

Sensory evaluation

Adsorption

ABSTRACT

The increasing popularity of alcohol-free beers (AFBs) fosters the industry interest in delivering the best possible product. Yet, a remaining sensory defect of AFBs is the over-perception of wort flavour, caused by elevated concentrations of small volatile flavour compounds (i.e. aldehydes). Previously, molecular sieves (hydrophobic ZSM-5 type zeolites) were found most suitable to remove these flavours by adsorption with high selectivity from the AFBs. In this work, a flavour-improved beer is produced at pilot-scale using this novel technology, and its chemical composition, sensory profile and stability are evaluated against a reference. Aldehyde concentrations in the flavour-improved product were found 79–93% lower than in the reference. The distinct difference was confirmed with a trained sensory panel and could be conserved even after three months ageing at 30 °C. Future work will focus on the process design to scale up this technology.

© 2020 The Authors. Published by Elsevier B.V. on behalf of Institution of Chemical Engineers. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Due to increasing awareness, the importance of responsible drinking as well as other motivators such as religion or fitness, one of the fastest growing categories in the beverage industry are currently low-alcohol and alcohol-free drinks (Liguori et al., 2018; Blanco et al., 2016). Nonetheless, alcohol-free beers still lack behind their regular counterparts, due to several sensory defects that can occur as a consequence of their altered production process (Blanco et al., 2016; Brányik et al., 2012; Mangindaan et al., 2018). Therefore, a new process to produce a flavour-improved alcohol-free beer (AFB) has been developed recently (Gernat et al., 2020a): Zeolites, a type of molecular sieve, are used to adsorptively remove wort off-flavours from biologically produced alcohol-free beers, resulting in a product with a significantly reduced amount of Strecker aldehydes, which are commonly known to cause the undesired wort off-flavour in AFBs. To be specific, 2- and 3- methylbutanal (2-MB and 3-MB) as well as methional (Met) have most

often been related to the wort flavour perception, but recent studies found more compounds such as 2-methylpropanal (2-MP) to be involved (Beal and Mottram, 1994; Perpète and Collin, 1999; Piornos et al., 2019).

Strecker aldehydes are formed through a heat-induced reaction between an amino acid and a reducing sugar (Schonberg and Moubacher, 1952), but also alternative pathways through numerous reactive carbonyls are possible (Rizzi, 2008; Hidalgo et al., 2013; Hidalgo and Zamora, 2004; Delgado et al., 2015). It is therefore difficult to prevent their formation. Previous researchers in brewing have addressed this matter in different ways, for instance by addition of masking compounds, removal through fermentation or co-separation with ethanol during dealcoholisation by thermal or membrane technologies. So far, either the degree in wort flavour decrease or the selectivity has been limiting (Gernat et al., 2020b). The advantage of using an adsorbent and specifically in particular zeolites for the separation task is their 2-fold selectivity based on molecular size and hydrophobicity. In this way, only small (volatile), hydrophobic compounds are removed from the product.

In this work, we present the quality traits of this novel product, as well as its sensory characteristics. Furthermore, the

* Corresponding author.

E-mail address: m.ottens@tudelft.nl (M. Ottens).<https://doi.org/10.1016/j.fbp.2020.07.014>0960-3085/© 2020 The Authors. Published by Elsevier B.V. on behalf of Institution of Chemical Engineers. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Table 1 – Flavour detection thresholds in alcohol-free beer determined by logistic regression and adjusted for false positives (Piornos et al., 2019; Saison et al., 2009).

Compound	Flavour/Odour	Detection threshold ($\mu\text{g/L}$) in AFB	
		Orthonasal	Retronasal
2-MP	Fruity, grainy, nutty, chocolate	4.32	0.86
2-MB	Fruity, sweet, almond, malt	23.4	8.99
3-MB	Malty, nutty, chocolate	0.61	0.44
Met	Boiled potato, metallic, wort	0.47	0.73

Table 2 – Specification of original extract, alcohol content, pH and colour in feed.

Parameter	Feed
Original extract ($^{\circ}\text{P}$)	12.12
Ethanol (% v/v)	0.015
pH	4.2
Colour (EBC)	8.54
Total iso- α -acids (mg/L)	<0.4

ageing process of the AFB is studied for selected compounds. The chemical and physical analysis presented in this work includes a comprehensive representation of chemical groups and quality indicators. Particular focus lies, however, on four common Strecker aldehydes, whose flavour descriptors and orthonasal (forward or sniffing-smell) and retronasal (backward or mouth-smell) thresholds in AFB are given in Table 1. Furthermore, furfural (FF) as a representative for compounds formed by heat-induced reactions and trans-2-nonenal (t2N), a common indicator for ageing were studied in more detail (Saison et al., 2009).

2. Materials and methods

2.1. Chemicals

The adsorbent ZSM-5 G-360 was purchased from ACS Materials (United States). To prepare solutions in the laboratory, either Milli-Q grade water (Merck, Millipore, United States) or analytical grade, absolute ethanol (VWR International BV, The Netherlands) was utilized. The pre-isomerized iso- α -acid solution was obtained from Joh. Barth & Son (Germany). If not mentioned otherwise, other chemicals were purchased from Sigma Aldrich (United States).

2.2. Preparation of wort-flavour reduced beer

The adsorbent was prepared by soaking it in 70% ethanol for 1 h and applying three consequent washing steps with water, to remove less volatile hydrophobic compounds that might have adsorbed during storage. It was then dried at 220°C until a stable weight was reached. After cooling, 4 kg of dry adsorbent were filled into a column with a diameter of 20 cm and adjustable height (Evolve 200, Astrea Bioseparations Ltd, United Kingdom) and purged with sterile filtered nitrogen gas for 3 h to remove as much oxygen as possible. Finally, it was wetted by flushing the column with oxygen-free process water. As a substrate, an AFB was produced by fermenting hop-free wort at $2\text{--}4^{\circ}\text{C}$, with the composition outlined in Table 2. Due to the low fermentation temperature, the formation of ethanol was inhibited. The resulting unhopped, alcohol-free product was stabilized (polyvinylpyrrolidone and silica) and filtered through a Beer Membrane Filtration System (Pentair, The Netherlands). The batch was then split

into two vessels with a liquid volume of 200 L, respectively, adding a slight over-pressure to avoid foaming. One of the vessels was then connected to the zeolite-filled column, avoiding the introduction of air as much as possible. The AFB contained in this vessel was then circulated over the column and samples were taken frequently. After 42 h, the tank was disconnected and both AFBs (flavour-improved and reference) were again filtered, standardized to 5.3°P (1°P equals 10 g sugar per kilogram wort), pasteurized with 50 PU (1 PU equals pasteurization for 1 min at 60°C) and bottled into 0.3 L bottles made from brown glass.

2.3. Ageing of samples

The bottled products were stored at 30°C in a dry, dark room for a maximum of 4 months. Each month, two bottles were sampled to analyze for ageing indicators. After 3 months samples were tested by a sensory panel as described in Section 2.9.

2.4. Determination of original extract, oxygen content, pH and colour

The original extract and pH of a product were determined by a digital density metre of the oscillation type (Xample 510, Anton Paar, Austria) and a pH metre (Accumet Basics AB15, Fisher Scientific, The Netherlands), respectively. The oxygen content was analyzed with the Haffmans CO_2/O_2 Gehaltemeter, type c-DGM (Pentair, The Netherlands) according to the supplier instructions. To measure the product colour, the EBC method 9.6 was employed, i.e. spectrophotometrically (Spectrostar Nano, BMG Labtech, Germany) and converted to the EBC unit by Eq. (1), where $A_{430\text{nm}}$ is the absorption at 430 nm and d the dilution factor.

$$\text{Colour [EBC]} = A_{430\text{nm}} \cdot 25 \cdot d \quad (1)$$

2.5. Analysis of volatile aldehydes

Aldehydes, other than acetaldehyde, were analyzed with an adapted method of Vesely et al. (Vesely et al., 2003) were the sample was concentrated by headspace solid-phase micro-extraction on a PDMS/DVB fibre (57327-U, Supelco, United States) and then injected to a gas chromatograph (Agilent 7890A) equipped with a $30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$ VF17MS column and a mass spectrometer as the detector in negative ionization mode (Agilent 5975C MSD). In order to improve the selectivity O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine was used as derivatisation agent.

2.6. Analysis of other volatiles

The concentration of ethanol was determined with an enzymatic method with the test kit obtained from Thermo

Table 3 – Overview on tastings of flavour-improved (A) and reference (B) AFBs performed with the descriptive panel.

Session	Products included	Storage	# of panellists
1	(I) Base AFBs (A and B)	Fresh	13
2	(II) Base AFBs (A and B) (III) Base AFBs spiked with fruity flavour mix (A and B) (IV) Base AFBs spiked with fruity flavour mix and iso- α -acids to adjust to 15 BU ^a (A and B)	Fresh	13
3	(V) Base AFBs (A and B)	Aged	11

^a BU (bitterness unit) = 50 · A_{275nm}, where A_{275nm} is the absorbance at 275 nm of a beer iso-octane extract.

Fisher Scientific (The Netherlands) according to the recommended EBC method 9.3.1 (Analytica, 2013). Diacetyl and 2,3-pentanedione are quantified with an adapted method of Ruehle et al. (2013) by headspace gas chromatography (7820A, Agilent Technologies, The Netherlands) equipped with a fused silica WCOT CP Sil CB wide bore column (50 m × 0.53 mm × 1 μ m) and detected with an electron capture detector. To increase accuracy, 2,3-hexanedione was used as internal standard.

Acetaldehyde, ethyl acetate, isoamyl acetate and isoamyl alcohols were analyzed by headspace gas chromatography (7820A, Agilent Technologies, The Netherlands) equipped with a flame ionization detector (7890B, Agilent Technologies, The Netherlands) according to EBC method 9.39 (Analytica, 2000). The compounds were separated over a polar capillary narrow bore column (DBWaxETR, 60 m × 0.32 mm ID, 1 μ m fused silica) and 4-heptanon and 1-butanol were added to each sample as internal standard.

2.7. Analysis of non-volatile compounds

The total of fermentable sugars was calculated from the summation of glucose, fructose, sucrose, maltose and maltotriose. These sugars were quantified by UPLC (Water Acquity, Milford, United States) equipped with a RI detector and a BEH Amide column 1.7 μ m (2.1 × 150 mm) as described in EBC method 9.27 (Analytica, 1997). To determine the free-amino nitrogen (FAN), a CDR Beerlab test kit from FoodLab (Italy) was used according to the procedure described by the manufacturer.

2.8. Analysis of foam stability and turbidity

The foam stability was determined at room temperature in a NIBEM-T metre from Pentair (The Netherlands) (Analytica, 2004). Following MEBAK method 2.141.2, the sample's turbidity was analyzed (MEBAK-EV, 2013).

2.9. Sensory evaluation

To evaluate the improvement in the flavour of the AFB that has been contacted with the selective adsorbent, three different sensory evaluation sessions were held. In the first session, the unhopped base products, i.e. the flavour-improved (I A) and the reference (I B) were tasted. To understand the impact of the adsorptive removal step on the final product, the base AFBs (both flavour-improved A and reference B) were spiked with a fruity/estery flavour mix (sample III) as well as the fruity flavour mix and iso- α -acid solution (sample IV) and compared to the base AFB (sample II) in the second session. Furthermore, the base AFBs were again tasted after 3 months ageing at 30 °C in a third session. The overview of all tast-

ings is given in Table 3. The sensory evaluation of produced AFBs was performed with a trained sensory descriptive panel consisting of a total of 16 trained assessors with a modified Quantitative Descriptive Analysis (QDA) (Stone et al., 2004). First, an attribute list was determined during a group discussion. Attribute intensities were quantified in duplicate on a 100-point line scale during two individual sessions. Panellists were aligned in their line scale usage and the samples were offered one-by-one in randomized order. In a subsequent session, panellists were seated in individual sensory booths and received 100 mL of each sample, presented in black-coated glasses that are coded with three-digit codes. They were given approximately 10 minutes to evaluate each sample, allowing to neutralize their palate in between. Per session, the panel evaluated a maximum of six samples.

2.10. Statistical analysis

The standard deviation of the sample σ and the standard error σ_m of each measurement were determined according to Eqs. (2) and (3), respectively. The propagated error σ_{mQ} was determined as described elsewhere (Young, 1962). Thereby, the statistical error of the sample, as well as the systematic error of the regressed calibration parameter, were taken into account. All errors in the result section represent the standard propagated error, if not mentioned otherwise.

$$\sigma = \sqrt{\frac{1}{(n-1)} \sum_{i=1}^n (x_i - \bar{x})^2} \quad (2)$$

$$\sigma_m = \frac{\sigma}{\sqrt{n}} \quad (3)$$

The data collection for the sensory evaluation is done in EyeQuestion 4.11.32. EyeOpenR (part of EyeQuestion) is used for statistical analysis. EyeOpenR is based on the statistical language R. The analysis is verified and documented by Qi Statistics Ltd.

3. Results and discussion

3.1. Flavour-improved product

During the adsorptive removal through recirculation of the alcohol-free beer over the zeolite-filled column, samples were taken frequently to monitor the reduction in wort flavour over time. The normalized results are depicted in Fig. 1. The concentration decreases exponentially over time, equilibrating at a plateau of 6–10% of their initial concentration, thus distinctly decreasing the wort flavour in the product. According

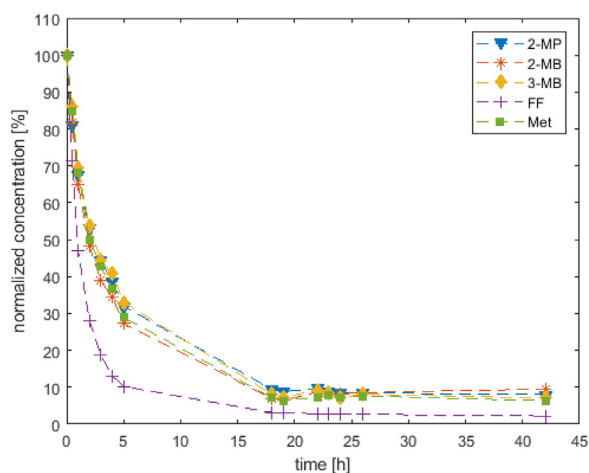


Fig. 1 – Evolution of aldehyde concentrations during the adsorptive removal step.

to previously reported data (Gernat et al., 2020a), however, the theoretical capacity of the adsorbent should allow for a decrease of free aldehydes to near zero. There could be several reasons for this observation, but, when considering the absolute concentration range ($<15 \mu\text{g/L}$) and the fact that the oxygen level was significantly increased during the trial ($\sim 210\text{--}1000 \mu\text{g/L}$) one could suspect that oxygen caused the formation of new Strecker aldehydes for instance through the formation of reactive dicarbonyls. Furfural's concentration shows a different behaviour, decreasing to 2% of its original amount, while still further reducing in concentration after 42 h. Contrary to the Strecker aldehydes, 2-MP, 2-MB, 3-MB and Met, whose formation are closely related to oxygen (Wietstock et al., 2016), furfural originates from the reaction of a pentose with an amine or amino group to an imine called Schiff base, which at $\text{pH} < 5$ further reacts to 3-deoxyosone. During a condensation reaction, ring formation occurs and furfural is the product (Vanderhaegen et al., 2006a). Thus, since oxygen is hence not involved in the formation mechanism of furfural, the observed difference to Strecker aldehyde gives evidence to support the theory of oxygen being the cause for the lower limit of Strecker aldehyde removal.

Overall, however, the concentration in wort flavours is significantly decreased. Fig. 2 shows the absolute concentration of the selected aldehydes in the end-product of the treated product and the reference. The achieved reduction amounts to 79–93%. This reduction is less pronounced than what was measured during the test, due to the intermediate pasteurization step, where the aldehyde concentration is slightly increased again (data not shown).

To gain a full understanding of the effect of the adsorbent on the product quality, a thorough analysis of numerous quality indicators was performed. The most informative data is summarized in Table 4. Both products were adjusted to a similar original extract to make the analysis comparable. Due to the process-related handling, the oxygen concentration in the flavour-improved product was increased significantly. However, when scaling up, it is expected that with a more sophisticated set-up, the introduction of oxygen can be avoided. The measurements confirm that the optical appearance of the aroma-improved AFB is unchanged. It exhibits the same colour and turbidity as the reference and even has a slightly improved foam stability. Relatively polar compounds such as sugars and ethanol are not removed and also the FAN content is similar to the reference. According to

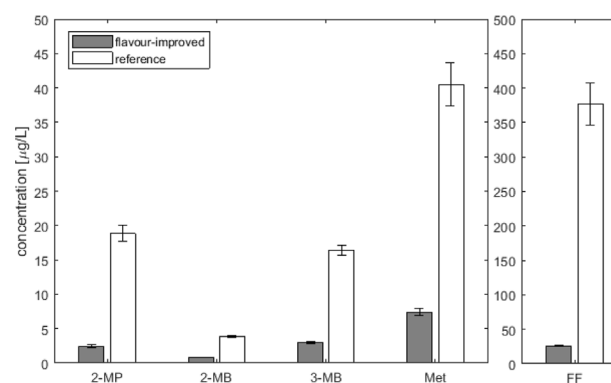


Fig. 2 – Concentration of 2-methylpropanal, 2- and 3-methylbutanal, methional and furfural in alcohol-free beer directly after pasteurization.

expectation, the adsorbent removes small volatile compounds such as acetaldehyde, diacetyl or ethylacetate. Isoamylacetate ($<0.05 \text{ mg/L}$), amyl alcohols ($<1.1 \text{ mg/L}$) and 2,3-pentanedione ($<2 \mu\text{g/L}$) were found below their respective detection limits for both samples and could hence not be included in the analysis.

To confirm that the analytical results are also perceivable during consumption and to establish whether the reduction in wort flavour is significantly lower as intended, a QDA was performed with a trained descriptive panel. In the first set of tasting, only the hop-free base products (I A and I B) were tasted by the panellists. The outcome is represented in the diagram of Fig. 3. The panellist found a clear difference in the perceived wort flavour (-8 points) between the two samples. Particularly, raisins and rye bread flavour diminished, which is desired for AFBs. Furthermore, the bitter and sour (after) taste was reduced. As a consequence, the treated sample was found more watery and exhibited a lower odour and total intensity ($-6/-5$ points).

To assess which impact the flavour improvement has on the final product, i.e. a product exhibiting fruity beer flavour (not containing wort flavour) and bitterness, the second set of tastings was executed. For this purpose, the base AFBs (II A and II B), a flavoured AFB (III A and III B) and a flavoured AFB adjusted to 15 BU in bitterness (IV A and IV B) were tasted. The results for the QDA are listed in Table 5. At first glance, it is prominent that the difference in wort flavour between matching samples persists, although it is perceived less pronounced. For instance, if sample II A and II B are compared, they differ in 3 points in wort flavour contrary to the 8 points when the same samples (IA and I B) were tasted in a separate session. It should be noted that samples I and II are the same sample, but tasted in different sessions. The relative different perception of wortiness reduction is not related to the uncertainty of the sensory evaluation. It rather means that the presence of fruity flavours and bitterness in one or more samples tasted in the same session influences the panellist's focus on the wort character. Nonetheless, a significant difference in particular in rye bread flavour is detected. In between samples II, III and IV one can clearly observe the masking effect of adding a fruity flavouring and bitterness with respect to the wort character of the products. Interestingly, the panellists perceived flavoured samples of the improved base higher in tropical fruit flavour than the reference. As a consequence, flavour dosing could be reduced in the final product to achieve the same sensory profile.

Table 4 – Specifications and their standard deviation of bottled products (reference and flavour-improved).

		Flavour-improved	Reference
Adjusted	OE (% m/m)	5.23 ± 0.02	5.30 ± 0.02
Process related	Oxygen (µg/L)	61.1 ± 3.2	16.3 ± 1.3
Unchanged	Colour (EBC)	3.4 ± 0.0	3.3 ± 0.0
	Ethanol (% V/V)	0.01 ± 0.00	0.01 ± 0.00
	FAN (mg/L)	88 ± 2	90 ± 2
	pH	4.76 ± 0.02	4.74 ± 0.02
	Total fermentable sugars (g/L)	34.0 ± 1.5	33.9 ± 1.5
	Turbidity (EBC)	0.2 ± 0.0	0.1 ± 0.0
	Turbidity after 7 days at 57 °C	0.1 ± 0.0	0.2 ± 0.0
Increased	Foam Stability (s)	256 ± 5	217 ± 4
Decreased	Acetaldehyde (mg/L)	1.4 ± 0.1	2.3 ± 0.1
	Diacetyl (µg/L)	3.9 ± 0.3	15.9 ± 1.1
	Ethylacetate (mg/L)	<0.2 ± n/a	0.3 ± 0.0

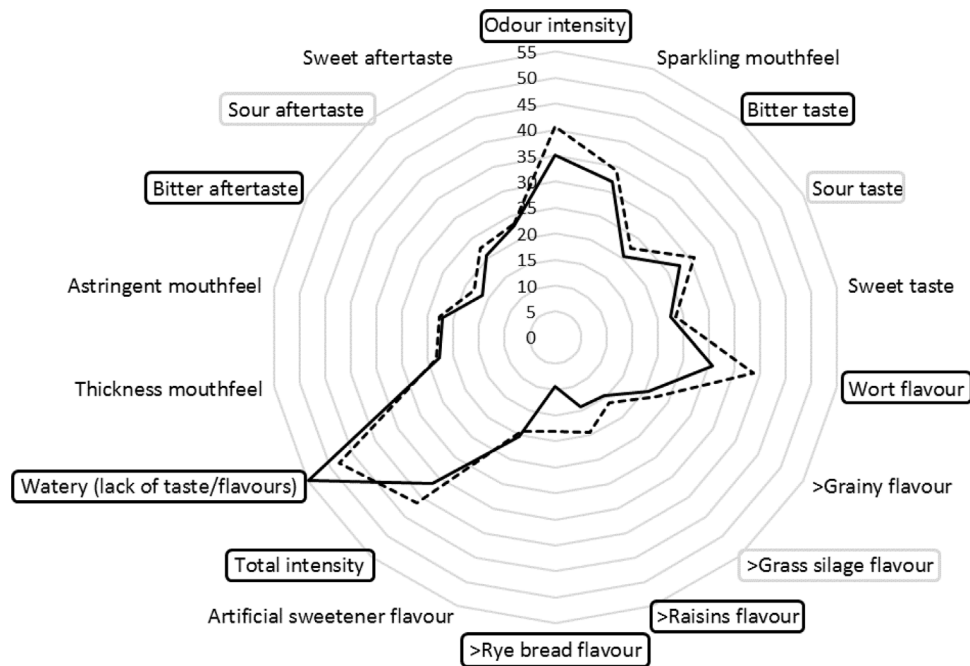


Fig. 3 – Sensory profile of base AFB, flavour-improved (solid line) and reference (dashed line). Attributes who differ with a significance level of 95% are circled in black and with a significance level of 90% in grey.

Table 5 – Sensory attributes quantified by the descriptive panel.

	II A	II B	III A	III B	IV A	IV B	p-Value
Odour intensity	37	40	39	40	38	38	0.14
Sparkling mouthfeel	31	31	32	32	31	29	0.19
Bitter taste	24	24	24	24	35	35	0.00
Sour taste	29	29	28	29	30	29	0.72
Sweet taste	22	23	22	22	21	22	0.19
Wort flavour	34	37	28	32	29	31	0.00
Grainy flavour	24	25	18	22	18	21	0.00
Grass silage flavour	16	19	14	17	18	16	0.45
Raisins flavour	12	17	9	12	9	9	0.04
Rye bread flavour	14	17	5	12	5	11	0.00
Fruity/estery flavour	26	25	39	34	37	35	0.00
Peardrop flavour	23	22	34	31	34	32	0.00
Tropical fruit flavour	8	7	20	12	18	15	0.00
Artificial sweetener flavour	16	14	14	15	12	12	0.23
Total intensity	41	41	43	40	43	43	0.02
Watery (lack of taste/flavours)	41	41	41	42	39	39	0.35
Thickness mouthfeel	22	22	21	22	21	22	0.48
Astringent mouthfeel	22	22	22	19	24	23	0.02
Bitter aftertaste	20	20	20	21	34	33	0.00
Sour aftertaste	24	24	23	23	25	22	0.04
Sweet aftertaste	22	21	22	20	19	19	0.01

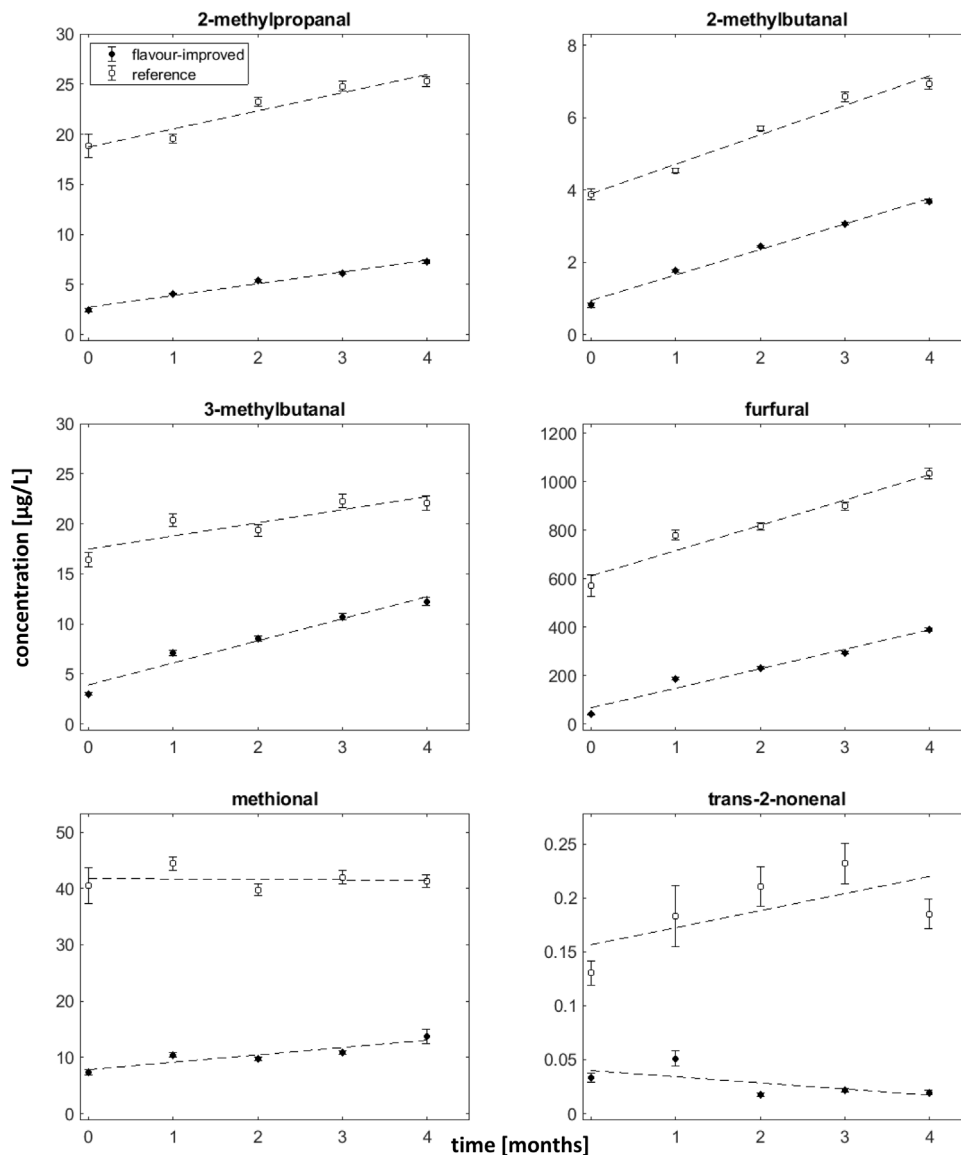


Fig. 4 – Concentration of selected compounds over a course for 4 months for treated (dark) and reference product (white).

Table 6 – Formation rate of aldehydes in flavour-improved and reference base beer during storage at 30 °C and comparison with literature range.

Compounds	Formation rate (µg/month)		Approximated formation rate ^a range from Jaskula-Goiris et al. (2011) and Baert (2015) (µg/month)
	Improved	Reference	
2-MP	1.2 ± 0.1	1.8 ± 0.2	4.7–28.8
2-MB	0.7 ± 0.0	0.8 ± 0.1	0.6–1.9
3-MB	2.2 ± 0.3	1.3 ± 0.4	1.4–3.2
FF	53 ± 9	69 ± 15	63–99
Met	1.3 ± 0.3	–0.1 ± 0.7	0.9–2.1
t2N	–0.006 ± 0.004	0.016 ± 0.026	0.003–0.033

^a Visual estimation from plotted data.

3.2. Ageing

Aldehydes are not only known wort off-flavours in AFBs, but also related to ageing during storage. There is a vast variety of literature available, investigating flavours and their chemical pathways involved in ageing of beers (Vanderhaegen et al., 2006a; Baert et al., 2012). In this study, we focus on a selected

set of aldehydes. The most recent studies in regular beer indicate that *de novo* formation of aldehydes during the ageing process is limited and that the most dominant mechanism causing the increase in aldehyde concentration over time is the release from cysteine and bisulphite adducts (Baert et al., 2018; Baert et al., 2015). To our knowledge, this is the first study to measure ageing in AFB. Fig. 4 and Table 6 depict the

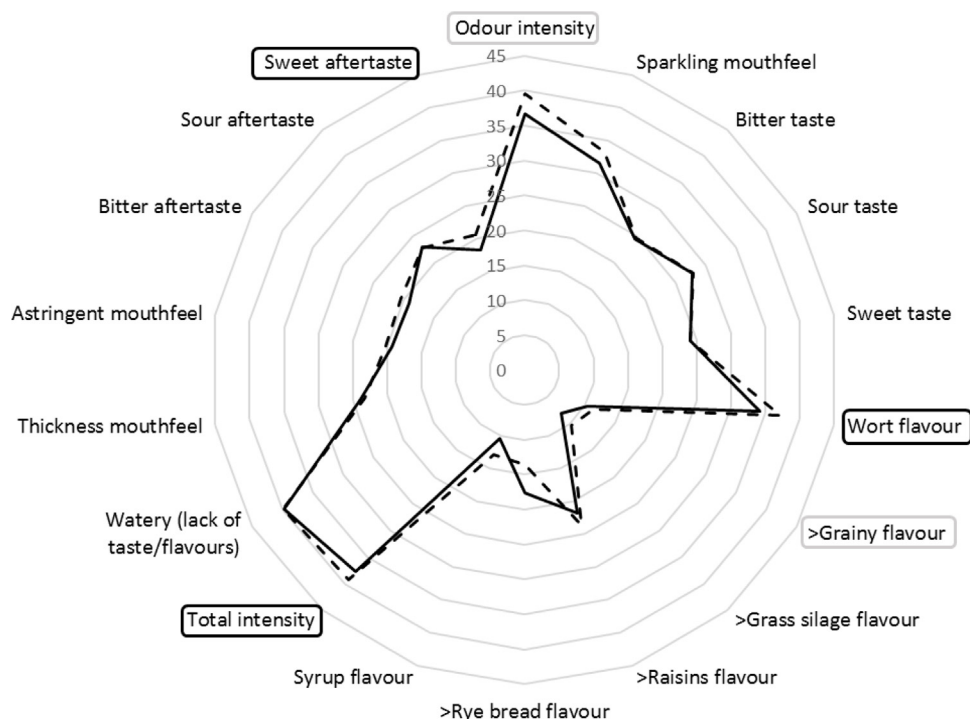


Fig. 5 – Sensory profile of base AFB, flavour-improved (VA, solid line) and reference (VB, dashed line) aged for 3 months at 30 °C. Attributes differing with a significance level of 95% are circled in black and with a significance level of 90% in grey.

time-wise concentration profile of the flavour-improved and reference beer for all studied aldehydes during four months and their regressed formation rates, respectively. The first remarkable observation is that the formation rate, i.e. the slope, of the flavour-improved and the reference AFB are relatively similar in the case of 2-MP, 2-MB and FF, although the formation rate of the treated AFB is generally lower. This means that the difference in concentration between the improved and reference AFB will remain similar or even increase over the storage time. This is also the case for t2N, but here the treated product slightly decreases in the ageing compound. This is different for 3-MB: here the formation rate in the improved product is higher and hence the difference is lessening during storage. Methional's concentration is inclining in the improved product, however, barely seems to change in the reference. The reason behind this different behaviour can only be speculated and are hence ground for further research. Considering that the starting concentration of methional in the reference is the highest of all measured Strecker aldehydes, one could postulate that it already is in equilibrium with its educts. Another possible explanation is that it is simultaneously formed and converted to degradation products such as methanethiol and acrolein as previously reported in literature (Gijs et al., 2000; Wainwright et al., 1972; Ballance, 1961).

Since the beer is fermented at low temperatures resulting in minimal yeast activity, the studied beer has several properties, which makes this case interesting to study and compare with data reported in literature:

1. The starting concentration in the fresh beer is higher than in regular beer, particularly in the reference product.
2. The ethanol content is <0.01 vol.% in ethanol.
3. No sulphite is produced by yeast.
4. The fermentable sugar concentration is higher than in regular beer since they are not consumed during fermentation.

Furthermore, no compounds originating from hops are contained in the AFB. Jaskula-Goiris et al. (2011) studied the beer stability of pale lager beers from different breweries. Surprisingly, despite the numerous differences in the beer composition, the formation rate in aldehydes is quite comparable (see Table 6). They found that staling was positively correlated with the residual FAN content, and thus the concentration of amino acids in beer. In their study, fresh beers contained between 49.1 and 141.2 mg/L FAN, where in our products comparable levels of 88–90 mg/L were determined. This observation supports the hypothesis that aldehyde formation is strongly linked to the presence of amino acids in beer. Other authors report that unhopped beer barely produces any aged flavour (Hashimoto and Eshima, 1979), contrary to what is observed in this analysis.

When comparing the sensory profile of the aged products with each other, as shown in Fig. 5, the difference between the flavour-improved and the reference product generally become less distinct, i.e. in odour/total intensity and wort flavour is still found to be significant between the two samples, however, this difference is less pronounced. The panellists also cannot perceive clear differences in raisins and rye bread flavour anymore. Attributes that were found slightly divergent in the fresh product such as the bitter taste and aftertaste are now perceived similar. At a closer look, it is observed that the identified attributes slightly differ from the tasting of the fresh product. While the artificial sweetener flavour is not recognized anymore, the sweet aftertaste of the sample is becoming more pronounced in the reference beer and a syrup flavour is noted by the panellists.

In literature, it is reported that over the whole course of ageing, sweetness and toffee-like or caramel aroma increases and bitterness decreases (Vanderhaegen et al., 2006b; Dalglish, 1977). Ribes and cardboard flavour may appear during the ageing process, but after reaching a peak at about 4 weeks disappear again (Baert et al., 2012; Zufall et al., 2005). Thus, the above findings resonate well with the expectations from liter-

ature. Although the difference in the actual concentration in wort flavour between the flavour-improved and the reference product is still very similar as in the fresh product, the same degree of wort flavour reduction is not reflected in the sensory evaluation. One reason for this might be that the curve of concentration and response in flavour intensity follows a sigmoidal shape, hence is nearly linear in the range close to the flavour threshold, but, at higher concentrations this response declines and might even saturate (Breslin, 1996). The same absolute difference in concentration in two samples is hence less obvious at higher concentrations. Additionally, synergetic effects can enhance this phenomenon (Singh et al., 2019).

4. Conclusion

In this work, a flavour-improved AFB was produced with a significantly decreased concentration in characteristic wort flavours, resulting in a clean-tasting base beer. This difference was confirmed during sensory evaluation with a trained panel. Due to the high selectivity of the adsorbent, only small volatile compounds were removed, while other parameters such as colour, foam stability or sugar content were similar. Addition of fruity flavours and bitterness decreased the relative perceived difference in wort flavour between the improved AFB and the reference, but a significantly lower wortiness remained. Even after three months of forced ageing, quality differences were still detectable. Future work will focus on designing a scalable and economically feasible process unit operation and investigate the regenerability of the adsorbent. Furthermore, more detailed investigations of the ageing behaviour for instance with hopped AFB are necessary to improve the understanding of the involved mechanisms.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge Heineken Supply Chain B.V. for the funding provided for this project and the opportunity to carry out the pilot scale tests at their facilities.

References

- Analytica, 1997. EBC 9.27: Fermentable Carbohydrates in Beer by HPLC (IM).
- Analytica, 2000. EBC 9.39: Dimethyl Sulphide and Other Lower Boiling Point Volatile Compounds in Beer by Gas Chromatography.
- Analytica, 2004. EBC 9.42.1: The Determination of the Foam Stability of Beer Using the NIBEM-T Meter.
- Analytica, 2013. EBC 9.3.1: Ethanol in Alcohol-Free and Low Alcohol Beers: Enzymatic Method (IM).
- Baert, J.J., 2015. Unravelling the Role of Free and Bound-state Aldehydes in Beer Flavor Instability. Faculty of Bioscience Engineering, KU Leuven, Leuven.
- Baert, J.J., et al., 2012. On the origin of free and bound staling aldehydes in beer. *J. Agric. Food Chem.* 60 (46), 11449–11472.
- Baert, J.J., et al., 2018. Exploring aldehyde release in beer by 4-vinylpyridine and the effect of cysteine addition on the beer's pool of bound aldehydes. *J. Am. Soc. Brew. Chemists* 76 (4), 257–271.
- Baert, J.J., et al., 2015. Further elucidation of beer flavor instability: the potential role of cysteine-bound aldehydes. *J. Am. Soc. Brew. Chemists* 73 (3), 243–252.
- Ballance, P.E., 1961. Production of volatile compounds related to the flavour of foods from the Strecker degradation of DL-methionine. *J. Sci. Food Agric.* 12 (7), 532–536.
- Beal, A.D., Mottram, D.S., 1994. Compounds contributing to the characteristic aroma of malted barley. *J. Agric. Food Chem.* 42 (12), 2880–2884.
- Blanco, C.A., Andres-Iglesias, C., Montero, O., 2016. Low-alcohol beers: flavor compounds, defects, and improvement strategies. *Crit. Rev. Food Sci. Nutr.* 56 (8), 1379–1388.
- Brányik, T., et al., 2012. A review of methods of low alcohol and alcohol-free beer production. *J. Food Eng.* 108 (4), 493–506.
- Breslin, P.A.S., 1996. Interactions among salty, sour and bitter compounds. *Trends Food Sci. Technol.* 7 (12), 390–399.
- Dalglish, C.E., 1977. Flavour stability. Proceedings of the European Brewery Convention Congress, 623–659.
- Delgado, R.M., Zamora, R., Hidalgo, F.J., 2015. Contribution of phenolic compounds to food flavors: Strecker-type degradation of amines and amino acids produced by o- and p-diphenols. *J. Agric. Food Chem.* 63 (1), 312–318.
- Gernat, D.C., et al., 2020a. Selective off-flavor reduction by adsorption: a case study in alcohol-free beer. *Food Bioprod. Process.* 121, 91–104.
- Gernat, D.C., Brouwer, E., Ottens, E., 2020b. Aldehydes as wort off-flavours in alcohol-free beers – origin and control. *Food Bioprocess Technol.* 13 (2), 195–216.
- Gijs, L., et al., 2000. 3-Methylthiopropionaldehyde as precursor of dimethyl trisulfide in aged beers. *J. Agric. Food Chem.* 48 (12), 6196–6199.
- Hashimoto, N., Eshima, T., 1979. Oxidative degradation of isohumulones in relation to flavour stability of beer. *J. Inst. Brew.* 85 (3), 136–140.
- Hidalgo, F.J., Delgado, R.M., Zamora, R., 2013. Intermediate role of α -keto acids in the formation of Strecker aldehydes. *Food Chem.* 141 (2), 1140–1146.
- Hidalgo, F.J., Zamora, R., 2004. Strecker-type degradation produced by the lipid oxidation products 4,5-epoxy-2-alkenals. *J. Agric. Food Chem.* 52 (23), 7126–7131.
- Jaskula-Goiris, B., et al., 2011. Detailed multivariate modeling of beer staling in commercial pale lagers. *Brew. Sci.* 64 (11–12), 119–139.
- Liguori, L., et al., 2018. Production of low-alcohol beverages: current status and perspectives. In: Grumezescu, A.M., Holban, A.M. (Eds.), *Food Processing for Increased Quality and Consumption*. Academic Press, pp. 347–382 (Chapter 12).
- Mangindaan, D., Khoiruddin, K., Wenten, I.G., 2018. Beverage dealcoholization processes: past, present, and future. *Trends Food Sci. Technol.* 71, 36–45.
- MEBAK-E.V., 2013. 2.14.1.2. Haze Formation Optical Method. Freising, Germany.
- Perpète, P., Collin, S., 1999. Contribution of 3-methylthiopropionaldehyde to the warty flavor of alcohol-free beers. *J. Agric. Food Chem.* 47 (6), 2374–2378.
- Piornos, J.A., et al., 2019. Orthonasal and retronasal detection thresholds of 26 aroma compounds in a model alcohol-free beer: effect of threshold calculation method. *Food Res Int.* 123, 317–326.
- Rizzi, G.P., 2008. The Strecker degradation of amino acids: newer avenues for flavor formation. *Food Rev. Int.* 24 (4), 416–435.
- Ruehle, G., et al., 2013. Headspace gas chromatography/electron capture detector analysis of total vicinal diketones in beer. *J. Am. Soc. Brew. Chemists* 71 (4), 274–275.
- Saison, D., et al., 2009. Contribution of staling compounds to the aged flavour of lager beer by studying their flavour thresholds. *Food Chem.* 114 (4), 1206–1215.
- Schonberg, A., Moubacher, R., 1952. The Strecker degradation of α -amino acids. *Chem. Rev.* 50 (2), 261–277.
- Singh, V., et al., 2019. Competitive binding predicts nonlinear responses of olfactory receptors to complex mixtures. *Proc. Natl. Acad. Sci. U.S.A.* 116 (19), 9598–9603.

- Stone, H., et al., 2004. Sensory evaluation by quantitative descriptive analysis. In: *Descriptive Sensory Analysis in Practice*.
- Vanderhaegen, B., et al., 2006b. The chemistry of beer aging – a critical review. *Food Chem.* 95 (3), 357–381.
- Vanderhaegen, B., et al., 2006a. The chemistry of beer aging – a critical review. *Food Chem.* 95 (3), 357–381.
- Vesely, P., et al., 2003. Analysis of aldehydes in beer using solid-phase microextraction with on-fiber derivatization and gas chromatography/mass spectrometry. *J. Agric. Food Chem.* 51 (24), 6941–6944.
- Wainwright, T., McMahon, J.F., McDowell, J., 1972. Formation of methional and methanethiol from methionine. *J. Sci. Food Agric.* 23 (7), 911–914.
- Wietstock, P.C., Kunz, T., Methner, F.-J., 2016. Relevance of oxygen for the formation of Strecker aldehydes during beer production and storage. *J. Agric. Food Chem.* 64 (42), 8035–8044.
- Young, H.D., 1962. *Statistical Treatment of Experimental Data*. M.-H.B.C. Inc., United States of America.
- Zufall, C.R.G., Gasparri, M., Franzquitz, J., 2005. Flavour stability and ageing characteristics of light-stable beers. In: 30th European Brewery Convention, Fachverlag Hans Carl, Nürnberg, Germany.