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“The interaction effect between vibrations (50 Hz, 15m/s²) and temperature (5°C, 30°C, 45°C), simulating truck transport, on the flavor stability of beer”

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ABSTRACT

BACKGROUND: Beer flavor stability is important to brewers due to the increased global demand for beer. Increasing export lead to prolonged periods of transportation and storage and cause fresh flavor deterioration. Therefore, the effect of different temperatures in combination with vibrations on beer quality is examined. Beer was exposed to vibrations

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(50Hz, 15m/s², simulating transport) at 5°C, 30°C and 45°C for 22, 38 and 90 hours and (half the samples) aged for 60 days at 30°C.

RESULTS: Results indicated decreased oxygen concentrations due to elevated temperature and vibrations. There was no effect ($P>0.05$) on color and limited effect of temperature and vibrations on iso- α -acids. The parameters temperature and vibrations have a significant influence ($P<0.05$) on aldehydes concentrations, i.e. total aldehydes, and especially '2-methylpropanal', '2-methylbutanal' and 'furfural'.

CONCLUSIONS: The impact of vibrations on the aldehydes concentrations was substantial when subjected to elevated temperature. Furthermore, a forced aging test of shorter duration than traditional methods might be developed.

Keywords

Beer flavor stability, vibrations, temperature, beer quality

Introduction

Controlling beer quality is crucial in the brewing industry since export is becoming more important due to market globalization¹. The taste, aroma, mouthfeel and appearance of beer change over time and results in an irreversible and undesirable quality degradation. Variable storage and transport conditions, which the brewer is not always able to control, are important contributors to the beer aging process (next to the raw materials and the beer brewing process). In particular, exposure to elevated temperature and light attribute to a decrease in beer quality according to literature^{1,2}. However, Janssen et al. (2014)³ suggested vibrations during transport might also negatively influence the beer quality. In a first vibration experiment, the authors indicated not only the accelerated growth of molds due to vibrations contaminating food products, but also the development of turbidity in beer. Since turbidity is only one parameter related to beer quality, an essential in-depth analysis on the influence of vibrations on the sensorial and chemical quality of beer is required and will be, as a consequence, the topic of this paper.

Flavor instability, a complex phenomenon that even after 30 years of research is not completely understood, causes beer to age and induces diverse chemical reactions to generate compounds that alter the sensorial properties of beer². Several parameters can indicate beer aging, i.e. a change in aldehydes, iso- α -acids, permanent haze and color^{1,2,4,5}. Furthermore, quality degradation of beer is mostly initiated by oxidative reactions that affect the formation or degradation of flavor active components^{1,6,7}. Therefore, the freshness of beer decreases with higher temperatures, increasing the rate of reaction and thereby increasing the amount of oxidative and non-oxidative chemical reactions that take place^{1,2}. Beer quality and stability are also sensitive to light. Exposure of beer to light causes the development of an offensive

taste and a skunky odor termed the “lightstruck flavor”. The sensitivity of iso- α -acids to the light can be indicated as the main contributor to photodegradation of beer or its ‘lightstruck flavor’⁸.

The influence of transport vibrations and shocks on the beer flavor quality is a research gap that needs to be explored. Therefore, it is essential to identify the vibrations beer bottles are exposed to and, subsequently, the impact of the vibrations on the beer flavor quality. In prior research, the exact vibrations beer bottles are exposed to are identified⁹. Moreover, vibration patterns diverge over the different modes of transport (i.e. truck, ships) but are also altered by the packaging and stacking of beer crates. Two hypothesizes might describe this relation between vibrations and the beer flavor quality:

Hypothesis 1: vibrations and shocks increase molecular energy and, as a consequence, increase the reaction rate of the ageing mechanism (reaction kinetics)

Hypothesis 2: vibrations and shocks could raise the uptake of oxygen from the beer bottleneck into the beer (more flavor active components or off-flavors can be formed/degraded)

The objective of the current study was to experimentally identify the influence of vibrations (in combination with temperature) on the beer quality. By performing an extensive chemical analysis of the beer, not only the impact of the vibrations during transport on beer aging can be assessed, the results can also be benchmarked with the single influence of elevated temperature. Also, the influence of the duration of the vibrations on the beer quality was studied. The scope of the current explorative study is limited to the influence of vibrations and temperature on a limited number of beers brewed in Belgium.

Material and methods

Experimental design

In the current research, the influence of one selected vibration in combination with different temperatures and the influence of duration of vibrations on the beer quality is studied. A design of experiments was built prior to executing the experiment and is described.

i. Temperature

Current practice by Belgian and foreign breweries is to transport and store beer under uncooled conditions and, therefore, temperature is a major contributor to flavor instability of (exported) beer. During transport, container temperature can rise up to 55°C under influence of direct sunlight¹⁰ with only the beer packaging that can delay but not prohibit the beer from heating up¹¹. In the current vibration experiment, the following temperatures were selected 5°C (i.e. the optimal beer temperature), 30°C and 45°C (i.e. an extreme temperature observed during transport), and the ambient temperature of 25°C ± 2°C. The third temperature of 30°C, in between the two extrema of 5°C and 45°C, was selected in order to investigate the (possible) nonlinear effect of the beer aging mechanism. Additionally, after the vibration experiment, half of all vibrated and non-vibrated samples were aged for 60 days at 30°C, while the other half of the samples were stored during the same period in 0°C in order to simulate storage. Simulating storage (conform the method used by Lehnhardt et al. (2018)¹², Jaskula-Goiris et al. (2011)¹³ and Malfliet et al. (2008)¹⁴) was developed in order to see if there is a difference in beer flavor quality with and without vibrations after a prolonged storage. It was possible that delayed aging reactions take place after the vibration experiment is completed (and when exposed to an elevated temperature).

ii. Duration of the vibrations

Beer is mainly transported by truck within the European mainland and by ship when exported overseas. The duration of transport within Europe can be limited to 3 to 4 days before

reaching its destination, while by ship the duration can be extended to 30 days. However, the vibration load on cargo during ship transport is less severe when compared with trucks (lower in acceleration $< 1 \text{ m/s}^2$ (0-peak))¹⁵. Therefore, the duration of the vibration experiment for individual setups in the current research was fixed to 22 hours (e.g. transport from Belgium to the Netherlands), 38 hours (e.g. transport from Belgium to Italy) and 90 hours (e.g. transport from Belgium to Greece). Three durations were chosen in order to investigate the (possible) nonlinear effect of the duration on the beer aging.

iii. Vibrations

The input vibrations, the stacking and packaging of the beer bottles determine the major contributing frequencies beer bottles are exposed to¹⁶. However, vibration experiments are regularly conducted by imposing vibrations of one specific frequency and acceleration (due to the specifications of the shaking equipment)¹⁷. Therefore, the optimal vibration signal (frequency) for the current experiment was identified based on both the occurrence of the vibration pattern during transport and the sensitivity of the beer flavor quality to the specified frequency. Therefore, in the current experiment that experimentally tests the interaction effect between temperature and vibrations, test samples were subjected to vibrations of 50 Hz and 15 m/s^2 (0-peak) in order to expose the test samples to extreme conditions (conform the method used by Paternoster et al. (2017)¹⁶).

iv. Beer samples

Fruit flavored beers are less susceptible to beer aging (due to the pH value), as well as specialty beers with refermentation after bottling due to the yeast strains in the beer that may counteract the aging mechanism (i.e. yeast may take up oxygen and thereby counteract the aging mechanism)¹⁸. Therefore, a blond beer without refermentation after bottling was chosen to execute the current test (chemical characteristics in Table 1). The batch of beer used in the experiment was brewed in the KULeuven technology campus Ghent to optimally control the brewing process and to identify the chemical parameters. Since beer aging relates to the

uptake of oxygen, half of all samples were bottled inhibiting oxygen under the metal cap (bottled with O₂ scavengers) while in the other samples no measures were taken to prevent oxygen under the metal cap (bottled by hand without overfoaming). This methodology enables to identify the influence of oxygen in the beer aging process when being exposed to vibrations. In Table 1, an overview is presented of the oxygen concentrations (TPO, HSO, DO) measured directly after bottling.

Table 1

v. Chemical tests

The samples were analyzed in the KULeuven technology campus Ghent and chemically profiled. The chemical parameters [Specifications in Appendix (A)] used in this study are:

- Oxygen concentrations: (TPO – Total package oxygen –, HSO – Headspace oxygen –, DO – Dissolved oxygen –)

Determination of oxygen concentrations:

Oxygen in bottled beer is determined using the 'Haffmans Inpack TPO/CO₂ meter Type c-TPO'. The measurement is performed in a sealed bottle and the principle is based on O₂ dependent fluorescence of the sensor. The instrument measures dissolved oxygen (DO) and headspace oxygen (HSO). The total package oxygen (TPO) is calculated as the sum of DO and HSO values. All values are calculated taking into account the beer volume in the bottle and are given in µg/l.

- Beer color: IOB method: 9.1¹⁹;
- Iso-α-acids: (*trans*-isocohumulone, *cis*-isocohumulone, *trans*-isohumulone, *cis*-isohumulone, *trans*-isoadhumulone, *cis*-isoadhumulone, total iso-α-acids, T/C-ratio)

UPLC determination of iso-α-acids:

The selected iso-α-acids (that contribute to beer bitterness) are good marker aldehydes related to beer ageing as shown in the literature²⁰. UPLC separations of iso-α-acids were performed on an Acquity UPLC (Waters, Milford, USA), consisting of a

PDA detector, column heater, sample manager, binary solvent delivery system and an Acquity UPLC HSS C18 1.8 μ m column (2.1 i.d. $\times$ 150 mm; Waters, USA). Data reprocessing was done using Empower 2 software. Chromatographic conditions were: eluent A: milli-Q water adjusted to pH 2.80 with H₃PO₄ (85%, Merck, Darmstadt, Germany); eluent B: HPLC-grade CH₃CN (Novasol, Belgium). Elution: isocratic using 52% (v/v) solvent B and 48% (v/v) solvent A. Analysis time: 12 min. Flow rate: 0.5 mL.min⁻¹. Column temperature: 35°C. UV detection: 270 nm (iso- α -acids). The *trans/cis* iso- α -acids ratio (T/C-ratio) was based on the measured concentrations of *trans*- and *cis*-isocohumulone and *trans*- and *cis*-isohumulone, i.e. the sum of the two *trans*-parameters divided by the sum of the two *cis*-parameters²⁰.

- Aldehydes: (2-methylpropanal, 2-methylbutanal, 3-methylbutanal, hexanal, furfural, methional, benzaldehyde, phenylacetaldehyde, (E)-2-nonenal, total aldehydes)

GC-MS (gas chromatography mass spectrometry) determination of aldehydes:

The selected aldehydes [flavor attributes of aldehydes in Table A.1 in Appendix (A)] are good marker aldehydes related to beer ageing as shown in the literature²¹. Volatile aldehydes in beer were determined according to Vesely et al. (2003)²², using headspace-solid phase microextraction (HS-SPME) with on-fibre PFBOA (*o*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine) derivatization and capillary gas chromatography/mass spectrometry (CGC/MS) (Dual Stage Quadrupole (DSQ™ II) GC/MS system, Interscience Benelux). The DSQ™ II was coupled to a Thermo Trace GC Ultra (Interscience Benelux) equipped with a CTC-PAL autosampler (including SPME sampling), a split/splitless injector with a narrow glass inlet liner (0.5 ml volume), and a RTX-1 fused-silica capillary column (40 m $\times$ 0.18 mm i.d. $\times$ 0.2 μ m film thickness, Restek, Interscience Benelux). The complete set of samples is analysed under the same conditions. Furthermore, recalibration of the device was regularly conducted during

the analysis. Data reprocessing was done by the XCalibur™ data system (Thermo Electron Corporation).

vi. Experimental setups

The vibration experiment in this study was conducted in collaboration with the company Bosal (Research and development subsidiary, Dellestraat 20 - 3560 Lummen [Belgium]). Bosal is a leading manufacturer of exhaust systems and provided the project with a dedicated experimental environment in which a large pneumatic shaker was available that was used for executing the vibration test (type MTS 258.05, max. amplitude 50 mm, max. force 50 kN) (Figure 1). A thermostatic cabinet was built over the shaker frame and enabled to perform a vibration experiment while exposing the beer samples to a decreased (5°C) or an elevated temperature (30°C, 45°C). In total, 24 bottles of beer were installed in an aluminum shaker frame, specifically built for this experiment. Furthermore, 24 bottles of beer were placed next to the shaker but inside the insulated cabinet to expose the samples to the same heat as the beer samples on the shaker. Before the start of every setup of the experiment, the climate cabinet was conditioned for two hours. A plastic crate with 24 beer bottles, the reference samples, was placed next to the shaker (inside the dedicated experimental room but outside the climate cabinet) and was exposed to the ambient temperature (25°C ± 2°C). An accelerometer was attached to the beer bottleneck and to the shaker plate to iteratively adapt the generated vibration signal to the preferred pattern or frequency. Since the facilities of Bosal were located approximately one hour driving from the laboratory of KULeuven technology campus Ghent, the samples were transported in an isomo box filled with cool elements to protect from heat. An overview of all setups is presented below.

- *Setup 1*: Frequency 50 Hz / Acceleration 15 m/s² (0-peak) during 90 hours at 5°C
- *Setup 2*: Frequency 50 Hz / Acceleration 15 m/s² (0-peak) during 90 hours at 30°C
- *Setup 3*: Frequency 50 Hz / Acceleration 15 m/s² (0-peak) during 90 hours at 45°C

- *Setup 4*: Frequency 50 Hz / Acceleration 15 m/s² (0-peak) during 90 hours at 45°C
- *Setup 5*: Frequency 50 Hz / Acceleration 15 m/s² (0-peak) during 22 hours at 45°C
- *Setup 6*: Frequency 50 Hz / Acceleration 15 m/s² (0-peak) during 22 hours at 45°C
- *Setup 7*: Frequency 50 Hz / Acceleration 15 m/s² (0-peak) during 38 hours at 45°C

After the vibration experiment, half of all vibrated and non-vibrated samples were aged for 60 days in 30°C, while the other half were stored at 0°C to simulate storage conditions after transport. Setup 3 was repeated (Setup 4) in order to increase the total sample size, and to further examine the effect of vibrations at 45°C on the variability of the chemical components and to enable a thorough statistical analysis.

Figure 1

Data Analysis

Multiple parameters may have an influence on the chemical output parameters, i.e. the influence of temperature and vibrations, the duration of vibrations, the aging after the vibration test, and the influence of samples with and without inhibiting oxygen after bottling. Therefore, a mixed model that is able to integrate and estimate the influence of random effects (e.g. bottles together in climate cabinet, bottles together in the setup and crate) was built in the statistical software JMP PRO12 (significance level of 5%).

Results

i. Oxygen concentration

An uptake of oxygen in beer results in the formation or degradation of flavor active components. As a consequence, the concentration of dissolved oxygen can be indicative of the flavor change that is taking place or that took place in the past. In the current experiment, the oxygen concentration decreased significantly for both samples with higher and lower initial oxygen content when increasing the exposed temperature (Table 2). Table 2 indicates that apart from temperature, vibrations further facilitate the uptake of oxygen, which indicates there is strong evidence hypothesis 2 is correct (and which does not induce the incorrectness of hypothesis 1). Similar results were found for HSO and TPO measurements. The measured oxygen concentrations for DO, HSO and TPO of samples with higher and lower initial oxygen content reach similar levels after the vibration treatment in combination with an elevated temperature at 45°C. After 60 days of aging at 30°C, a significantly decreased oxygen concentration was measured for all tested samples including the reference samples (data not shown). In conclusion, oxygen concentrations have significantly decreased under a regime of vibrations and temperature.

Table 2

ii. Color

A change in beer color is a visual characteristic that can be attributed to the formation of Maillard reaction products and the oxidation of polyphenols during beer aging. In the current experiment, no statistically significant change of the measured beer color was observed when samples of the different setups were compared directly after the vibration experiment (Table 2), nor after the vibration experiment and aged at 30°C for 60 days.

iii. Iso- α -acids

Literature reports of the decrease in concentration of iso- α -acids when beer is subjected to an increased temperature^{2,23}. However, the results of the current experiment indicate that the impact of the exposure to an elevated temperature and vibrations in a short period of time (90 hrs.) is limited with respect to total iso- α -acids. Moreover, the mixed model that was made to evaluate the explanatory power of the tested variables indicated that only temperature ($p=0.025$) has a small but significant effect on the total iso- α -acids. A more detailed investigation of the results of the individual *trans*- and *cis*-iso- α -acids (Table 3) indicates that the *cis*-isomers are stable and the *trans*-isomers are sensitive to elevated temperature (especially *trans*-isocohumulone and *trans*-isohumulone at 45°C). There was no statistically significant effect observed due to vibrations on individual iso- α -acids. The T/C-ratio, on the contrary, also takes into account the stable and unstable nature of the *cis*- and *trans*-isomers²⁰ and, therefore, is a better indicator than total iso- α -acids for the possible harmful effect of temperature and vibrations^{20,24}. With respect to the T/C-ratio, the single effect of temperature ($p=0.0002$) and the quadratic effect of temperature is significant ($p=0.0017$). The quadratic effect of temperature on beer quality is described by the Arrhenius equation and the influence of temperature on reaction kinetics (experimentally assessed by various authors in the literature^{1,2}). The single effect of vibrations on the T/C ratio is not significant ($p=0.1317$) (significance level of 5%). The interaction effect between temperature and vibrations is significant ($p=0.0473$) in the mixed model, although the significance of the interaction effect should be interpreted with caution since the main effect, vibrations, was not significant. After the aging period of 60 days at 30°C, the results indicate no statistically significant differences between the samples when comparing the T/C-ratio over the different setups. Additional information on the results of the total iso-alpha-acids concentrations and T/C-ratio can be found in Table B.1 in Appendix (B).

Table 3

iv. Aldehydes

In contrast to color and iso- α -acids, aldehydes indicate to be more sensitive when exposing the tested beer samples to the different temperatures in combination with vibrations. Moreover, the aldehydes '2-methylpropanal', '2-methylbutanal' and 'furfural' change considerably in concentration under an elevated temperature and vibrations while the other aldehydes do not increase significantly over all setups (information on the aldehyde concentrations in Table 4 and Table B.1 in Appendix (B)). In Figures 2a and 2b, the concentration of the total aldehydes measured directly after the vibration experiment and after an aging period of 60 days in 30°C is displayed. Also, a more detailed view of the results of '2-methylpropanal', '2-methylbutanal' and 'furfural' is presented in Table 4. Figure 4a indicates the exponential effect of exposure to elevated temperature and the additive effect of vibrations that further increase aldehyde concentrations. The effect of vibrations on the aldehydes concentration is visible at all temperatures (5°C, 30°C and 45°C) and all durations (90hrs, 38 hrs. and 22hrs.). Furthermore, the figure also indicates that the higher the exposure of the temperature is, the more pronounced is the effect of vibrations on beer quality. The latter indicates the possible presence of an interaction effect between temperature and vibrations.

Figure 2a

Figure 2b

Table 4

The results of Figure 2a demonstrate a significant increase of the total aldehydes concentration due to vibrations when the samples are exposed to an elevated temperature of 45°C in combination with vibrations during 90 hrs. However, after an aging period of 60 days at 30°C, no significant difference is observed between the total aldehydes concentrations of the reference samples (ambient temp. 25°C) and the other samples (Figure 2b). Figure 2b and

Table 4 indicates that the total aldehydes concentration possibly saturates, i.e. the samples that have been vibrated under a regime of 45°C for four days only increase marginally in total aldehydes concentration after aging for 60 days. The latter also implies that the total aldehydes concentration reached after 60 days of exposure to 30°C can be approximated by 90 hours of exposure to vibrations and an elevated temperature of 45°C. As a consequence, there is potential to further develop the latter method into a beer aging test that can be used to assess the chemical stability of beer. In current methods, beer is predominantly aged at 30°C for two to four months, since the taste of beer exposed to 30°C best represents storage conditions in contrast to aging over a shorter time span with a higher temperature. A shorter duration of the test improves the number of analyses the beer scientist is able to perform, and, therefore, results in a significant reduction of time and money. However, further analysis is required to validate the possibility to substitute the beer aging method.

In order to statistically analyze the effect of oxygen, temperature, and vibrations on the total aldehydes content, a mixed model was built in which the batch is the random effect. Since the variation of the measured total aldehydes concentration becomes larger when temperature increases, the logarithm of the concentration of aldehydes was identified as the output variable in order to increase the performance and the correctness of the presented model. In Table 5a, the fixed-effects parameter estimates of the initial model are illustrated. The single effect of temperature, the quadratic effect of temperature and the single effect of vibrations are described as significant by the model (significance level of 5%). The effect of oxygen ($p = 0.4571$) and the interaction effect between temperature and vibrations ($p = 0.8528$) are not assessed as significant by the model. The nonsignificant effect of oxygen was not expected before initiating the vibration experiment. However, the descriptive analysis of the beer aging parameters already indicated the latter relation. The interaction effect between temperature and vibrations is intuitively expressed by the plot of the results in Figure 2a. Due to the

increased variance in aldehydes concentration when the temperature rises, an interaction effect between temperature and vibrations is not addressed as significant by the model. Furthermore, the optimal model (Table 5b) and the 'effect summary' indicates that temperature (and the exponential effect) is the predominant parameter that describes the concentration of aldehydes. In other words, vibrations have a significant effect on the total aldehydes concentration, but the importance of the parameter is considerably lower compared to temperature. Beer aging due to vibrations is only prominent with respect to aldehydes when the samples are subjected to an elevated temperature (45°C). Similar results and the same significant parameters were found for the aldehydes '2-methylpropanal', '2-methylbutanal' and 'furfural'.

Table 5a

Table 5b

Discussion

A variety of beer parameters, related to beer quality, are analyzed in the current experimental research. Furthermore, the results of the study were extensively benchmarked with the experiments performed prior to the current study^{13,16}. Nonetheless, due to the uniqueness of the research topic, the authors indicate that further research is recommended on (1) the development of turbidity in beer under a regime of vibrations and on (2) the influence of shocks on beer quality. In the current experiment, the measured oxygen concentrations decreased (both HSO and DO) due to vibrations, but there was no effect observed on color nor on the iso- α -acids. This is noteworthy since literature indicates that oxidation can be noticed by a change in color and a decrease in the *trans*- and *cis*-iso- α -acids^{20,23}. As a consequence, additional research is recommended on the uptake of oxygen due to vibrations. The development of turbidity in beer can be one of the causes of the uptake of oxygen (and the increased interaction between proteins and polyphenols²⁵) and, therefore, should be subjected to further research. Also, the influence of shocks, i.e. transient phenomena that damp out over time, on beer quality should be researched since beer bottles are frequently exposed to shocks during truck transport (depending on the packaging)¹⁶. From a physics point of view, a transient phenomenon is completely different compared to a vibration and, as a consequence, the impact on beer quality might also diverge. The impact of shocks on the flavor stability of beer might be an interesting research track, complementary to the current study.

Additionally, the statistical analysis indicated that the individual effect of temperature and vibrations are significant ($P < 0.05$), while the effect of oxygen and the interaction effect are not. The insignificant effect of oxygen was expected due to the high standard deviation of the

measured oxygen concentrations after bottling (Table 1). Therefore, additional experiments on the influence of oxygen with respect to vibrations and the flavor quality of different beer types are still recommended. Furthermore, with respect to the interaction effect between vibrations and temperature, the former findings still allow to conclude that the impact of vibrations on the aldehyde concentrations was substantial when subjected to an elevated temperature, and, therefore, predominantly elevated temperatures (during transport and storage) should be avoided. The authors of the current study recommend to further validate the results with real-life flavor quality measurements due to the inherent difficulties of simulating transport (i.e. mechanical specifications of the shaker and the different vibration patterns measured on bottled beer¹⁶).

Conclusions and recommendations

The main conclusions of the experimental study are summarized as follows.

- A significant decrease in oxygen concentrations (TPO, HSO, and DO) is observed due to the exposure to both temperature and vibrations, which indicates that beer aging reactions were initiated.
- No difference in color was detected for beer samples that were subjected to an increased temperature or vibrations.
- The *cis*-iso- α -acids are stable and do not change when exposed to temperature and vibrations. The *trans*-iso- α -acids are more sensitive (especially *trans*-isocohumulone and *trans*-isohumulone) and induce both the total iso- α -acids and the T/C-ratio to decrease in concentration, respectively percentage. Only temperature and not vibrations are observed as a significant explanatory parameter with respect to total iso- α -acids and the T/C-ratio.
- Three aldehydes, i.e. '2-methylpropanal', '2-methylbutanal' and 'furfural' (and additionally the total aldehydes concentration), are sensitive to vibrations in combination with temperature. The temperature, the quadratic effect of temperature, and vibrations have a significant impact on the total aldehydes concentration. However, the importance of vibrations is considerably lower compared to temperature. The interaction effect between vibrations and temperature is not identified as significant ($P > 0.05$) due to the increased variability at a higher temperature.

Since the impact of vibrations on beer quality is only substantial when subjected to an elevated temperature, reducing temperature during transport and storage should be the main focus to brewers. Furthermore, there is potential to further develop a beer aging method that is shorter in time and duration than traditional methods (with respect to aldehydes).

Appendices

- A. Overview of the measurement errors of the performed chemical analyses and additional information on the chemical tests. (Table A.1)

Table A.1

- B. A more detailed investigation of the results of the aldehyde compounds, total iso-alpha-acids, TC-ratio (after storage for 60 days at 0°C - fresh beer - and after aging for 60 days at 30°C) (Table B.1)

Table B.1

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	Chemical test/parameter	Systematic error*	Flavor description
Oxygen	TPO, HSO, DO	1ppb +/- 2%	Oxydized flavor
Color	Absorption 430 nm	0,062 (EBC)	/
Iso- α -acids	<i>trans</i> -isocohumulone	0.017 mg/L	Beer bitterness
	<i>cis</i> -isocohumulone	0.011 mg/L	Beer bitterness
	<i>trans</i> -isohumulone	0.008 mg/L	Beer bitterness
	<i>cis</i> -isohumulone	0.018 mg/L	Beer bitterness
	<i>trans</i> -isoadhumulone	0.002 mg/L	Beer bitterness
	<i>cis</i> -isoadhumulone	0.019 mg/L	Beer bitterness
Aldehydes	2-methylpropanal	1.61 μ g/L	Grainy, varnish, fruity
	2-methylbutanal	0.60 μ g/L	Almond, apple-like, malty
	3-methylbutanal	2.01 μ g/L	Malty, chocolate, cherry, almond
	Hexanal	0.20 μ g/L	Bitter, winey
	Furfural	4.37 μ g/L	Caramel, bready, cooked meat
	Methional	0.72 μ g/L	Cooked potatoes, warty
	Benzaldehyde	0.30 μ g/L	Almond, cherry, stone
	Phenylacetaldehyde	1.24 μ g/L	Hyacinth, flowery, roses
	(E)-2-nonenal	0.0052 μ g/L	Cardboard, papery, cucumber
*Systematic errors in experimental observations come from the measuring instruments. The systematic error referred to in the current paper is identified as the accuracy of the measurement or the offset or zero setting error in which the instrument does not read zero when the quantity to be measured is zero.			

Table A.1: Measurement errors of the performed chemical analyses

Table B.1: A more detailed investigation of the results of the aldehyde compounds, total iso-alpha-acids, TC-ratio (measured after vibration test and after **additional aging** for 60 days at 30°C)

Avg. (STD)	3-methylbutanal [10 ⁻⁶ g/L]	hexanal [10 ⁻⁶ g/L]	methional [10 ⁻⁶ g/L]	benzaldehyde [10 ⁻⁶ g/L]	phenylacet-aldehyde [10 ⁻⁶ g/L]	(E)-2-nonenal [10 ⁻⁶ g/L]
90hrs. in 25°C NO shaking	9.72 (0.69)	0.80 (0.29)	14.84 (6.77)	1.29 (0.24)	42.89 (12.02)	0.05 (0.02)
90hrs. in 5°C NO shaking	8.84 (0.04)	0.57 (0.10)	12.25 (2.72)	1.48 (0.14)	37.64 (9.72)	0.02 (0.01)
90hrs. in 5°C WITH shaking	8.51 (0.39)	0.36 (0.03)	22.40 (4.10)	1.25 (0.19)	61.25 (21.66)	0.02 (0.01)
90hrs. in 30°C NO shaking	9.57 (0.72)	0.58 (0.18)	11.73 (2.43)	1.17 (0.09)	37.74 (5.48)	0.03 (0.01)
90hrs. in 30°C WITH shaking	9.90 (0.86)	0.43 (0.04)	24.75 (5.30)	1.70 (0.43)	78.72 (13.21)	0.02 (0.00)
90hrs. in 45°C NO shaking	11.47 (0.62)	0.65 (0.07)	20.10 (6.42)	1.27 (0.14)	49.62 (17.46)	0.04 (0.01)
90hrs. in 45°C WITH shaking	10.85 (1.09)	0.62 (0.06)	25.47 (4.52)	1.35 (0.29)	62.68 (16.80)	0.03 (0.01)
Avg. (STD)	total aldehydes [10 ⁻⁶ g/L]	total aldehydes (Additional aging) [10 ⁻⁶ g/L]	total iso-alpha-acids [10 ⁻³ g/L]	total iso-alpha-acids (Additional aging) [10 ⁻³ g/L]	T/C-ratio [%]	T/C-ratio (Additional aging) [%]
90hrs. in 25°C NO shaking	115.27 (22.85)	342.63 (57.96)	13.96 (0.33)	13.12 (0.80)	18.93 (0.25)	13.06 (1.95)
90hrs. in 5°C NO shaking	85.57 (12.08)	387.60 (30.35)	14.04 (0.14)	13.52 (0.37)	19.24 (0.32)	12.28 (0.15)
90hrs. in 5°C WITH shaking	121.09 (26.18)	309.36 (16.40)	14.24 (0.27)	12.86 (0.80)	19.46 (0.16)	13.62 (0.25)
90hrs. in 30°C NO shaking	109.43 (7.86)	301.22 (16.76)	13.84 (0.21)	12.80 (1.04)	18.84 (0.14)	12.96 (0.29)
90hrs. in 30°C WITH shaking	182.78 (19.01)	310.61 (22.08)	13.90 (0.33)	12.53 (0.19)	18.88 (0.17)	13.37 (0.27)
90hrs. in 45°C NO shaking	290.35 (44.65)	380.5 (27.95)	13.46 (0.42)	13.01 (0.46)	19.92 (0.23)	11.67 (0.35)
90hrs. in 45°C WITH shaking	402.66 (30.41)	444.67 (39.69)	13.53 (0.34)	13.78 (2.08)	16.39 (0.14)	11.56 (0.31)

* There was no statistically significant difference observed between the samples with high and low oxygen content, and, therefore, all samples are included in the former graphs. Furthermore, only an effect was observed with respect to the three presented aldehydes (and predominantly the fresh beer samples).

Table 1: Oxygen concentration after bottling – chemical characteristics beer samples

Avg. (STD)	Samples with higher initial oxygen content [ppb]		Samples with lower initial oxygen content [ppb]	
TPO after bottling	1265 (185)		523 (382)	
HSO after bottling	1207 (180)		472 (380)	
DO after bottling	58 (44)		51 (38)	
Chemical characteristics	pH:	4.44	Eorg. (°P)	12.43
	Alc. (v/v%)	5.11	RDF (%)	63.71
	Alc. (w/w%)	4.00	ADF (%)	77.00
	Er (°P)	4.70	Cal. (kJ/100 ml)	188.19
	Ea (°P)	2.86		
Legend: TPO (Total Package Oxygen), HSO (Headspace Oxygen), DO (Dissolved Oxygen), pH (acidity), Alc. (Alcoholic volume or weight in %), Er (Real extract), Ea (Apparent extract), Eorg. (Original extract), RDF (Real degree of fermentation), ADF (Apparent degree of fermentation), Cal. (Caloric content)				

Table 2: A more detailed investigation of the results of the DO, HSO and TPO measurements of samples with higher/lower initial oxygen content, and color (measured after vibration test)

Avg. (STD)	DO (samples with higher initial oxygen content) [ppb]	HSO (samples with higher initial oxygen content) [ppb]	TPO (samples with higher initial oxygen content) [ppb]	DO (samples with lower initial oxygen content) [ppb]	HSO (samples with lower initial oxygen content) [ppb]	TPO (samples with lower initial oxygen content) [ppb]	Color* [EBC]
90hrs. in 25°C NO shaking	31 (8)	258 (48)	289 (48)	19 (12)	129 (74)	148 (75)	8.08 (0.77)
90hrs. in 5°C NO shaking	48 (6)	286 (25)	334 (25)	26 (11)	164 (76)	190 (77)	8.27 (0.15)
90hrs. in 5°C WITH shaking	23 (4)	230 (46)	253 (46)	22 (11)	156 (41)	177 (42)	8.35 (0.10)
90hrs. in 30°C NO shaking	36 (10)	259 (43)	295 (44)	10 (2)	89 (7)	99 (7)	8.24 (0.17)
90hrs. in 30°C WITH shaking	17 (1)	153 (4)	169 (4)	12 (1)	97 (21)	109 (21)	8.33 (0.10)
90hrs. in 45°C NO shaking	16 (6)	102 (42)	118 (42)	9 (4)	65 (26)	74 (26)	8.40 (0.12)
90hrs. in 45°C WITH shaking	9 (2)	72 (28)	81 (28)	8 (2)	49 (25)	56 (25)	8.43 (0.11)

* There was no statistically significant difference observed between the samples with high and low oxygen content with respect to color, and, therefore, all samples are included in the column of color.

Table 3: A more detailed investigation of the results of the individual *trans*- and *cis*-iso- α -acids (measured after vibration test)

Avg. (STD)	<i>Trans</i> - isocohumulone [10 ⁻³ g/L]	<i>Cis</i> - isocohumulone [10 ⁻³ g/L]	<i>Trans</i> - isohumulone [10 ⁻³ g/L]	<i>Cis</i> - isohumulone [10 ⁻³ g/L]	<i>Trans</i> - isoadhumulone [10 ⁻³ g/L]	<i>Cis</i> - isoadhumulone [10 ⁻³ g/L]
90hrs. in 25°C NO shaking	1.26 (0.02)	6.26 (0.11)	0.78 (0.03)	4.34 (0.11)	0.23 (0.07)	1.13 (0.06)
90hrs. in 5°C NO shaking	1.24 (0.02)	6.25 (0.03)	0.80 (0.02)	4.34 (0.05)	0.28 (0.03)	1.15 (0.05)
90hrs. in 5°C WITH shaking	1.28 (0.03)	6.37 (0.17)	0.82 (0.01)	4.38 (0.10)	0.21 (0.07)	1.18 (0.05)
90hrs. in 30°C NO shaking	1.21 (0.01)	6.23 (0.12)	0.77 (0.01)	4.30 (0.06)	0.19 (0.06)	1.14 (0.05)
90hrs. in 30°C WITH shaking	1.24 (0.07)	6.36 (0.36)	0.80 (0.06)	4.41 (0.23)	0.21 (0.06)	1.15 (0.05)
90hrs. in 45°C NO shaking	1.07 (0.03)	6.11 (0.13)	0.68 (0.04)	4.24 (0.17)	0.24 (0.02)	1.12 (0.06)
90hrs. in 45°C WITH shaking	1.06 (0.03)	6.16 (0.14)	0.66 (0.02)	4.28 (0.14)	0.24 (0.01)	1.15 (0.05)

* There was no statistically significant difference observed between the samples with higher and lower initial oxygen content, and, therefore, all samples are included in the former graph.

Table 4: A more detailed investigation of the results of '2-methylpropanal', '2-methylbutanal', and 'furfural' (measured after vibration test and after **additional aging** for 60 days at 30°C)

Avg. (STD)	2-methyl-propanal [10 ⁻⁶ g/l]	2-methyl-propanal (Additional aging) [10 ⁻⁶ g/l]	2-methyl-butanal [10 ⁻⁶ g/l]	2-methyl-butanal (Additional aging) [10 ⁻⁶ g/l]	Furfural [10 ⁻⁶ g/l]	Furfural (Additional aging) [10 ⁻⁶ g/l]
90hrs. in 25°C NO shaking	17.75 (2.03)	73.39 (6.54)	3.06 (0.26)	5.17 (0.64)	24.86 (4.68)	189.07 (27.57)
90hrs. in 5°C NO shaking	11.63 (0.45)	82.43 (12.85)	2.65 (0.09)	5.47 (0.28)	10.46 (0.59)	199.65 (14.22)
90hrs. in 5°C WITH shaking	12.34 (0.88)	64.43 (1.90)	2.63 (0.18)	4.74 (0.16)	12.33 (1.40)	177.14 (13.32)
90hrs. in 30°C NO shaking	18.83 (1.09)	70.75 (2.45)	3.05 (0.19)	4.74 (0.17)	26.73 (1.35)	166.63 (5.74)
90hrs. in 30°C WITH shaking	23.74 (1.03)	67.51 (2.54)	3.61 (0.32)	4.96 (0.25)	39.93 (1.97)	172.35 (23.40)
90hrs. in 45°C NO shaking	59.22 (6.85)	92.84 (7.10)	4.35 (0.16)	5.94 (0.52)	143.62 (17.77)	253.36 (28.68)
90hrs. in 45°C WITH shaking	79.64 (7.69)	95.15 (8.92)	4.89 (0.36)	6.04 (0.61)	217.14 (15.04)	314.14 (57.02)

* There was no statistically significant difference observed between the samples with high and low oxygen content, and, therefore, all samples are included in the former graphs. Furthermore, only an effect was observed with respect to the three presented aldehydes (and predominantly the fresh beer samples).

Table 5a: Mixed model – total aldehydes after vibration experiment (first model - JMP output)
(beer samples analyzed after vibration test)

Fixed Effects Parameter Estimates							
Term	Estimate	Std. Error	DFDen	T Ratio	Prob> t	95% Lower	95% Upper
Intercept	3.98	0.16	5.0	24.13	< 0.01	3.56	4.40
Oxygen	-0.02	0.02	28.1	-0.75	0.46	-0.06	0.03
Temperature	0.04	< 0.01	5.0	9.42	< 0.01	0.03	0.05
(Temperature)^2	< 0.01	< 0.01	5.0	2.85	0.04	8.29e-5	< 0.01
Vibrations	-0.16	0.05	5.0	-2.91	0.03	-0.30	-0.02
Temperature x Vibrations	< 0.01	< 0.01	5.0	-0.20	0.85	-0.01	0.01

Table 5b: Mixed model – total aldehydes after vibration experiment (optimal model - JMP output)
(beer samples analyzed after vibration test)

Fixed Effects Parameter Estimates							
Term	Estimate	Std. Error	DFDen	T Ratio	Prob> t	95% Lower	95% Upper
Intercept	3.98	0.15	6.0	26.80	< 0.01	3.62	4.35
Temperature	0.04	< 0.01	6.1	10.35	< 0.01	0.03	0.04
(Temperature)^2	< 0.01	< 0.01	6.0	3.12	0.02	< 0.01	< 0.01
Vibrations	-0.16	0.05	6.1	-3.22	0.02	-0.28	-0.04
Random Effects Covariance Parameter Estimates					Effect Summary		
Covariance Parameter	Estimate	Std. Error	95% Lower	95% Upper	Source	LogWorth	PValue
Batch	0.02	0.01	-0.01	0.04	Temperature	4.35	< 0.01
Residual	0.02	< 0.01	0.01	0.03	Vibrations	1.75	0.02
					Temperature)^2	1.69	0.02

Figure 1 Experimental equipment (beer samples in the climate cabinet // beer samples on the shaker frame in the climate cabinet // beer samples in ambient temperature)

Figure 2a: Total aldehydes as a function of temperature, vibrations and time (after the vibration experiment the samples were stored for 60 days at 0°C -fresh beer-)

Legend: A: samples stored for 90hrs. at 25°C / B: samples stored for 90hrs. at 5°C / C: samples stored for 90hrs. at 5°C while shaking / D: samples stored for 90hrs. at 30°C / E: samples stored for 90hrs. at 30°C while shaking / F: samples stored for 90hrs. at 45°C / G: samples stored for 90hrs. at 45°C while shaking / H: samples stored for 38hrs. at 45°C / I: samples stored for 38hrs. at 45°C while shaking / J: samples stored for 22hrs. at 45°C / K: samples stored for 22hrs. at 45°C while shaking

* There was no statistically significant difference observed between the samples with higher and lower initial oxygen content, and, therefore, all samples are included in the former graph. The average with confidence intervals (minimum and maximum value) was calculated for each setup.

Figure 2b: Total aldehydes as a function of temperature, vibrations and time (after the vibration experiment the samples were stored for 60 days at 30°C)

Legend: A: samples stored for 90hrs. at 25°C / B: samples stored for 90hrs. at 5°C / C: samples stored for 90hrs. at 5°C while shaking / D: samples stored for 90hrs. at 30°C / E: samples stored for 90hrs. at 30°C while shaking / F: samples stored for 90hrs. at 45°C / G: samples stored for 90hrs. at 45°C while shaking / H: samples stored for 38hrs. at 45°C / I: samples stored for 38hrs. at 45°C while shaking / J: samples stored for 22hrs. at 45°C / K: samples stored for 22hrs. at 45°C while shaking

* There was no statistically significant difference observed between the samples with higher and lower initial oxygen content, and, therefore, all samples are included in the former graph. The average with confidence intervals (minimum and maximum value) was calculated for each setup.