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Quality reduction of beer stored in transfer tubes of a dispensing system – an investigation with sensory and GC/MS analyses

Dispensing systems are essential in the beer supply chain and form one of the last steps to its final link – the consumer. The maintenance and hygiene of dispensing systems are crucial as they influence the beer quality directly. Dispensing systems comprise various equipment and components necessary to pour a beer in the quality desired by the brewer. The key components with the most prominent product contact are beer transfer tubes. Depending on the construction properties of a gastronomy's premises, long distances have to be covered frequently, resulting in significant filling volumes. For decades, brewers, sommeliers and connoisseurs have known that long hold-back times of beer in the beer tubes reduces quality. In such a case, discarding the whole beer tube's volume is necessary before serving the first tapped beer to meet the quality standards of brewers, gastronomers, and consumers. This study investigates sensory and analytically aroma changes of three different beer styles during storage in a beer transfer tube. For this purpose, an experimental dispensing system with six beer hoses was constructed. The beer storage in the transfer tubes was conducted at two different temperatures, 5 °C and 20 °C. After particular time intervals, the beer was poured and analysed for aging indicators, flavours by GC/MS resp and a trained tasting panel. The results show significant influences on the aroma profile after three hours of storage. The panel identified a change in the aroma profile to flavours usually associated with oxidation and aging. In the comparison of the two storage temperatures, higher temperatures significantly worsen the aroma profile. These findings were supported by an increase in aging indicators determined by GC/MS analysis.

Descriptors: dispensing systems, beer transfer tubes, beer quality, sensory analysis, GC/MS

1 Introduction

Modern dispensing systems consist of various components. The harmony of their construction provides dispensed beer with the quality desired by the brewer. One main component is the beer transfer tube that ensures the transport of the beer from the keg to the tap. For the majority of dispensing systems, they are the components with the most substantial contact with the beer. Producers mainly use polymers for manufacturing beer hoses. Following the DIN 6653-1 [1], primary materials are polyamide, polyethylene, or ethyl vinyl acetate. Depending on the construction properties of gastronomy premises, beer tubes have to cover long distances (exemplary calculations see Table 1). Thus, filling volumes of several liters quickly arise in the beer tubes. The beer itself can also function as a contaminant when it stays too long in the tubes or if insufficient cleaning occurs.

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For decades every brewer has known about the problematic hygienic situation in dispensing systems. In restaurants or pubs, beer transfer tubes are often not the main cleaning focus. The fact that dispensing systems are not sterile nor germ-free will not cause problems as long as cleaning cycles are adequate. However, microbial contamination can potentially form biofilms that create severe hygienic problems (see Fig. 1 on page 12). The reasons are either non-consciousness of this problem or economic considerations. Many components of dispensing systems are not visible due to their construction and installation in wall ducts or insulations. Furthermore, cleaning procedures are expensive and time-consuming, making a frequent execution unattractive at first glance.

Tab. 1 Exemplary beer volumes in a single beer tube with different diameters and different transfer distances; values depend on average assumptions and standard room heights

	Transfer distance	Volume in beer transfer tube / amount of beer that has to be discarded [l]	
	[m]	avg. diameter 7 mm	avg. diameter 10 mm
Keg cooler	3	0.12	0.24
Cellar cooling chamber	10	0.38	0.79
2 floors	12.5	0.48	0.98
3 floors	15	0.58	1.18

Fig. 1 **Exemplary images of spoiled beer transfer tubes showing biofilm formation and fouling on the hose surface with beer contact**

Another fundamental problem is the filling volume of beer that stays in the beer hose for a significant time (e. g. after the closing hour). In a poorly cleaned system, this beer interacts with the residual contamination in the beer tubes, does not meet the standards of quality anymore, and becomes unfit for consumption. In German-speaking countries, this phenomenon is also called "Nachtwächter" (night watchman) and is known to every brewer and informed beer consumer. When the gastronomy reopens, the barkeeper is expected to discard this beer volume. The image loss by selling such a beer is severe for the gastronomy and the brewery and often results in negative economic effects. The consumption of such a beer can even constitute a human health risk.

Additionally, food auditors fine gastronomies when they serve such a spoiled beer to customers. The beer does not meet the requirements of a fresh product anymore as it contains off-flavours (e.g., diacetyl or aging aromas) due to microbial spoiling and oxidation. According to European legislation (e.g., EC No 178/2002) [2], serving such a beer fulfills the combined facts of disgust and consumer deception, as a guest expects a fresh and uncontaminated product.

The microbiological influence on beer spoiling and the appearance of corresponding off-flavours were the research object in many publications [3–6]. However, the effect of quality reduction of beer stored in beer transfer tubes depends not only on spoilage by microorganisms. There is also a formation of off-flavours in new or well-cleaned systems. Different publications evidenced that the aging of beer depends on several ambient conditions [7–9]. Higher temperatures, light, and oxygen intake result in faster aging without additional microbial influence. In dispensing systems, there are often no installations of secondary cooling systems that keep the beer transfer tubes and the beer in a cold condition. The increased gas permeability of polymers and potential gas inlet at connectors can increase the oxygen concentration in the beer. Besides, there is a simultaneous CO_2 decrease due to thermodynamical laws where always an equilibrium of the partial pressure between two systems appears [10, 11]. From this oxygen intake into the beer, oxidation of the components results, which causes faster aging and the appearance of off-flavours.

This research study examined the change in the flavour profiles of beer stored in beer transfer tubes. Firstly, the construction of a specific dispensing system with six beer hoses formed the centerpiece of the experimental investigations. In the beer transfer tubes, beer was storable at two different temperatures and for particular time intervals. Additionally, an examination of the potential impact of beer styles by using three different beers showed influencing parameters as well as the intrinsic potential of the resp. beer style to mask those changes. The analysis of the flavour change occurred by three different analytical approaches. Firstly, the O_2- and CO_2-concentration analysis in a lager beer showed the thermodynamical activities at certain storing times. Secondly, a tasting panel examined the ageing-flavours of the samples via sensory analysis. Ultimately, an instrumental analysis investigated corresponding aroma components with a GC/MS-analysis. The main aim was to investigate how the beer flavour and thermodynamical properties change while being stored in beer tubes. The results display how dispensing systems and the tapping conditions in gastronomy are improvable. Herefrom, considerations on possible installations and alternative material use for beer transfer tubes are derivable.

Tab. 2 **Specific properties of the selected beers**

Beer style	Lager	Wheat beer	Pilsener
Original gravity [%]	11.5	12.6	11.4
ABV [%]	5.1	5.4	4.9
IBU [-]	21	14	40
Type of fermentation	Bottom fermented	Top fermented	Bottom fermented

2 Material and Methods

2.1 Beer selection

The experiments were conducted with three different beer styles that are available commercially (properties see Table 2). The style variation was necessary to highlight the impact of differently brewed beers and ingredients. Besides, this selection also enabled an immediate application of the study's results in practice. The first used style was a German Lager with the lightest aroma profile and no masking effects by other aroma components. Here, it could be assumed that any appearing off-flavours are already detectable with sensorial methods at a low threshold. The focus of the sensorial studies was on oxidation and aging aroma. The second beer style was a Bavarian wheat beer, which possessed increased masking effects due to heavy aroma components, e.g., isoamyl acetate. Following the literature, such aroma compositions can provide increased taste thresholds for off-flavours. The third beer style was a Pilsener from Northern Germany, consisting of more bitter units. In comparison to the Lager, it could be assumed that the bitter components show a different aging behaviour with accelerated effects on oxidation of the hop-derived ingredients.

During the use of all beers in the experiments, it was ensured that the beer was fresh, within the expiration date and from the same batch.

2.2 Experimental dispensing system

The experimental setup (see Fig. 2) comprised six hoses manufactured of the polymer polyethylene. Three hoses were stored cold at 5 °C, and the other three warm at 20 °C to achieve typical gastronomical environmental conditions. The beer transfer tubes were connected to a single keg and thus fed with the same beer. An additional valve at the keg enabled the pouring of fresh beer as a reference for the analysis. All beer tubes were provided with light protection to prevent the influence of ambient light on the beer. This feature is close to practical installations where beer transfer tubes usually are protected from light (e.g., insulation or cable pits). The taps were equipped with compensators, which made it possible to control the volume flow. The entire setup was cleaned before and after every trial, making microbial influence negligible.

For the sensorial analysis, the beer samples were dispensed by the tap. The beer samples for the CO_2- and O_2 measurements were directly streamed from the beer hoses via a valve positioned before the tap. This bypass avoided CO_2 loss and O_2 intake during dispensing and enabled direct flow to the measurement device.

2.3 Determination of CO_2 and O_2 content

The beer could directly flow into the measurement device via the mentioned bypass valve, enabling inline measurement. Here, the CboxQC from Anton Paar (Graz, Austria) facilitated a combined analysis of CO_2 and O_2. The entire filling volume of the respective beer transfer tube was used to ensure a stable and precise analysis. The CO_2 content in the beer was measured in g/l, while the O_2 content was determined in mg/l.

The selected measurement device enables a high standardisation as it complies with the current brewing technology methods according to ASBC, EBC and Mebak. A big advantage of the device was the easy connectivity to the experimental dispensing system. Thus, it was ensured that the beer sample passed directly into the measuring chamber and was not falsified by an intermediate transfer due to additional gas exchange. Due to the advantages listed above, further measurements of CO_2 and O_2 were not performed

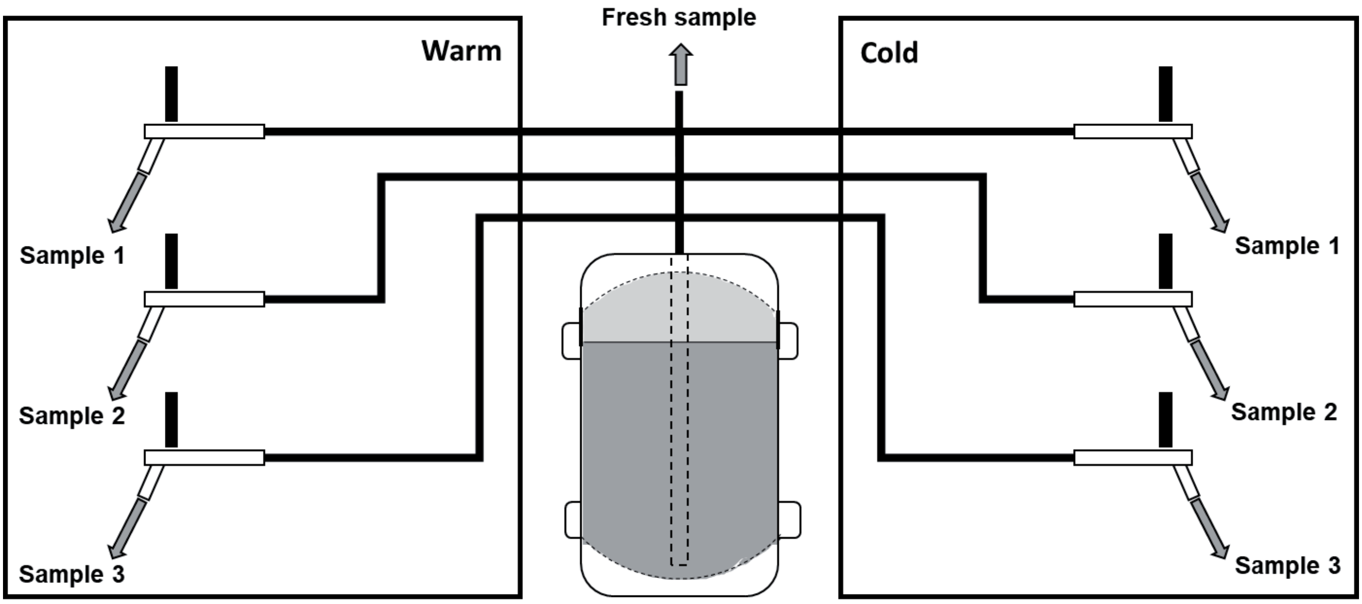

Fig. 2 **Experimental dispensing system**

during the experimental investigations.

2.4 Sensory analysis

2.4.1 Panel and sample setup

The tasting panel consisted of 21 certified DLG (Deutsche Landwirtschafts-Gesellschaft e. V.) certified tasters and seven introduced tasters. All of these underwent a weekly training for an extended period. The participation per tasting ranged from 5 to 10 tasters. In each session, samples were analysed in biological triplicates.

2.4.2 Aging and acceptancy test (Eichhorn)

The degree of aging was evaluated by three attributes: smell, taste, and bitterness. These were rated on a scale from 1 (fresh, no aging impression) to 2 (moderate aging), 3 (strongly aged) and 4 (extremely aged) [12]. Acceptancy, according to Eichhorn was evaluated on a scale from 100 % to 0 %, where 100 % represented full acceptancy and 0 % no acceptancy.

2.4.3 Intensity rating of aging attributes

The typical aging-related attributes bready, berry, honey, cardboard, sherry, and dull/moldy were rated on a scale from 0 (not perceivable) to 5 (very intense). Each sample was tested by six or seven trained panelists.

2.4.4 Triangle test

In a fully randomized test design, 7 tasters participated in one sessions. The samples were tempered to 12 °C before tasting.

2.5 Analysis of aging compounds by HS-SPME-GC/MS

2.5.1 Chemicals

The chemicals, o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA; ≥ 99 %), ethyl 2-methyl propanoate (99 %), ethyl 2-methylbutanoate (99 %), ethyl 4-methylpentanoate (≥ 97 %), β-damascenone (≥ 98 %), γ-nonalactone (98 %),ethyl 2-phenylacetate (99 %), ethyl nicotinate (99 %), 2-methylpropanal (≥ 99.5 %), 2-methylbutanal (95 %), 3-methylbutanal (97 %), 2-phenylacetaldehyde (≥ 90 %), methional (≥ 97 %), benzaldehyde (≥ 99.5 %), pentanal (≥ 97.5 %), hexanal (98 %), heptanal (95 %), (E)-2-nonenal (97 %) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethyl 3-methyl butanoate (Fluka Analytical, ≥ 99.7 %), dimethyl trisulfide (SAFC, ≥ 98 %), furfuryl ethyl ether (Fluorochem, 95 %), and 2-furfural (Fluka Analytical, ≥ 99.0 %) were purchased as indicated.

2.5.2 SPME procedure

Analysis of aging compounds in beer was performed according to Lehnhardt et al. [12]. In brief, 5 mL of a sample was placed in a 20-mL headspace vial. Using a CAR-PDMS-DVB fiber, the headspace was analyzed by solid-phase microextraction (SPME) after derivatisation with PFBHA. The fiber was injected splitless at 250 °C into a GC (GC-Ultra 1300, Thermo Scientific Inc., Waltham, MA, USA) coupled to a single quad mass spectrometer (ISQ, Thermo Scientific Inc., Waltham, MA, USA) via a transfer line that was heated to 250 °C. The GC was equipped with a DB-5 column (length: 60 m, inner diameter: 0.25 mm, film thickness: 0.25 µm). Helium was used as carrier gas (flow rate: 1.85 mL/min). The initial temperature was 60 °C, held for 4 min. Heating at 5 K/min was undertaken until a final temperature of 250 °C was reached and held for 3 min. Ionisation was performed in EI mode in a full scan mode (m/z 35-350). The dwell time was 0.02 s. Each sample was measured in triplicate except for the control samples (drawn from every experiment analogous to sensory analysis). Peak detection and integration were done in Xcalibur 3.1.66.10 (Thermo Scientific Inc., Waltham, USA).

2.6 Statistical analysis

One-way analysis of variance (ANOVA) was performed to uncover statistical differences at α ≤ 0.05 of aging indicators within sample

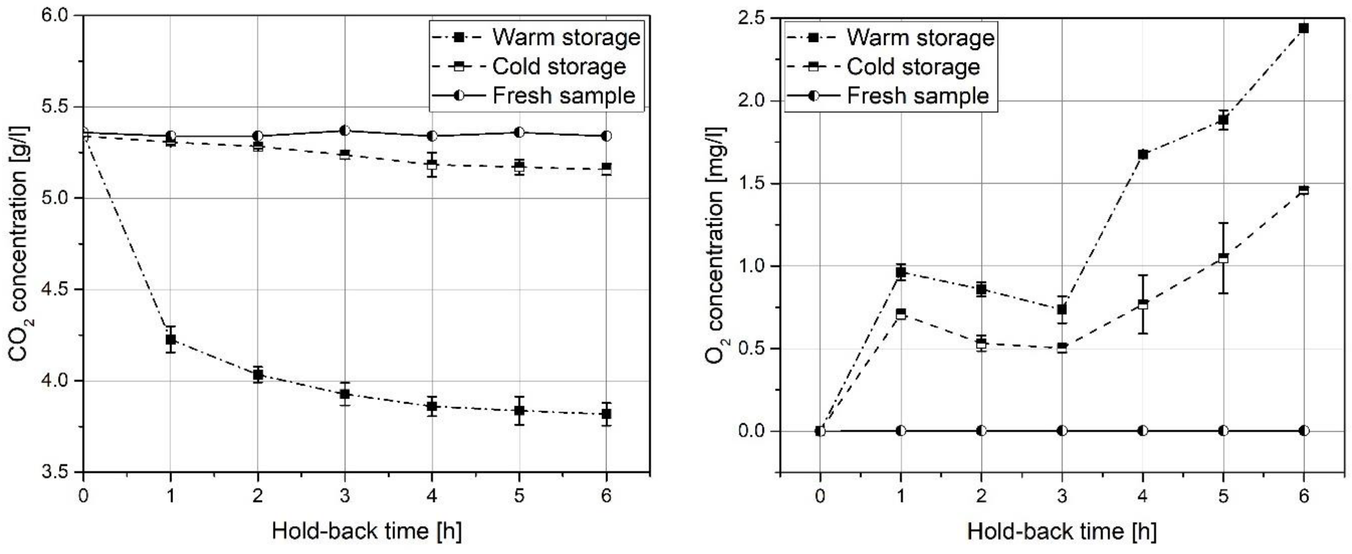

Fig. 3 **CO_2 reduction in the stored beer (A); O_2 intake (B)**

sets. All data analysis was carried out in JMP Pro 14 (SAS Institute Inc., Cary, NC, USA).

3 Results and Discussion

3.1 Analysis of CO_2 and O_2 in Lager beer

The determination of the CO_2- and O_2-content in the beer after specific hold-back times in the beer transfer tube was conducted with the Lager beer only. For a precise and reproducible analysis of the relevant gas concentrations, this beer style showed the highest suitability as it had been filtered and was the lightest of all selected styles. The beer hold-back time in the hoses was selected due to the general downtimes of a dispensing system in gastronomy. Additionally, the beers were stored under warm and cold conditions to highlight possible differences between chilling systems and standard uncooled tube systems. Two subfigures illustrate the results in figure 3.

The results show significant trends in CO_2 and O_2 contents at different retention times. Under warm storage conditions, a substantial decrease in the CO_2 content in the beer of approximately 1 g/l was observable within one hour. The content fell after one hour below 4 g/l, which undoubtedly led to a significant decline in the beer quality. While the amount of removed CO_2 after 1 h is more than 2 g/l, the the remaining curve is asymptotic and only additional 1 g/l are released from the beer. In contrast, the CO_2 decrease of beer stored in cooled beer transfer tubes was not severe. Only a slight reduction of approximately 0.2 g/l was observed after 6 h.

Concerning the O_2 intake, a steady increase in both storing temperatures was detected. Although, the warm stored beer again resulted in a higher O_2 increase with significant values. Remarkably, a near-constant resp. slightly decreasing concentration was observed between one and three hours, assuming oxidation processes took place. One possible explanation for this behaviour is the formation of reactive oxygen species (ROS), namely hydroxyl radicals, via the Fenton-Reaction. Oxygen ingress and its consumption appear to be in a state of equilibrium in this period [7]. Especially in beer tubes, the ratio between the available volume and the tube's surface is beneficial for a high O_2 intake and faster oxidation.

Generally, warmer liquids show less solubility of CO_2 due to the Henry solubility constant H_{cp}, highlighted in equation 1 [13, 14].

$$H_{cp} = \frac{c_a}{p_a} = \frac{H_{cc}}{R \cdot T} \quad \text{(Eq.1)}$$

The gas concentration in the liquid is c_a and p_a is the gas's partial pressure. H_{cc} is the dimensionless Henry solubility, R is the gas constant and T the temperature. Considering the equation, the Henry constant will be reduced with an increased temperature, indicating less CO_2 solubility. This explains why warm stored beer in the lines releases CO_2 significantly (see Fig. 3 A). In contrast, the O_2 intake into the beer is higher regarding warmer storage temperatures. This finding is remarkable since the Henry equation is also applicable to O_2. It can therefore be assumed that this deviation was either due to the measuring device or related to the faster transfer of O_2 through the tube material at warmer temperatures.

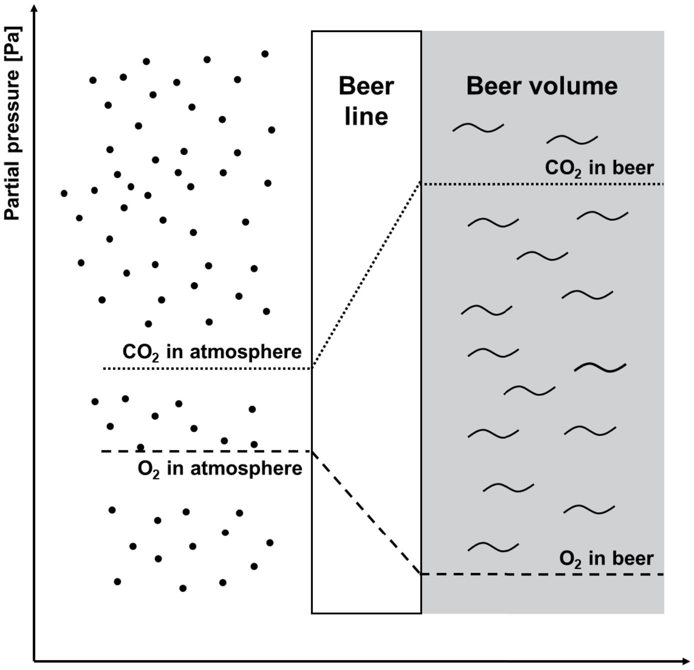

Fig. 4 **Schematic drawing of the gas diffusion through a polymer beer hose – illustration adapted for tubes from Müller [10]**

In addition, it must be noted that the initial concentration of O_2 was initially zero; therefore, oxidation effects already occurred after the first uptake into the beer. This aspect also indicates the decrease in O_2 uptake in the time interval between 1 and 3 h. Thus, it could be assumed that the O_2 dissolved in the cold beer has already contributed to the oxidation of the ingredients and was, therefore, no longer measurable.

Also, the influence of the tube and its specific polymer can be assumed. There are two ways of gas diffusion through a polymer [15]. The pore effect describes the diffusion of gases and steams according to pressure and partial pressure gradients. Secondly, there is the solution-diffusion-effect, explaining the solving of gases and steams in polymers and diffusion at lower concentrations. Besides, other factors have to be considered critically regarding the results here. A high diffusion at the tube's connector can significantly affect the O_2 intake. Figure 4 illustrates the presence of O_2 and CO_2 solved in the beer, in the surrounding air and with the polymer as a barrier between the concentration gradient [10].

3.2 Sensory analysis

3.2.1 Eichhorn point aging and acceptance

Figure 5 shows the Eichhorn scores for smell, taste, bitterness and acceptancy. The control samples for each beer style were rated as fresh indicated by low Eichhorn scores and high values for acceptancy. Keeping the samples in line in cold conditions resulted in a slight to moderate aging impression after a short time for the lager and pilsner samples. Under these conditions, the wheat beer samples appeared to be more stable.

For warm conditions, a similar behaviour was observed. Already after 6 h the lager sample showed moderate to strong aging

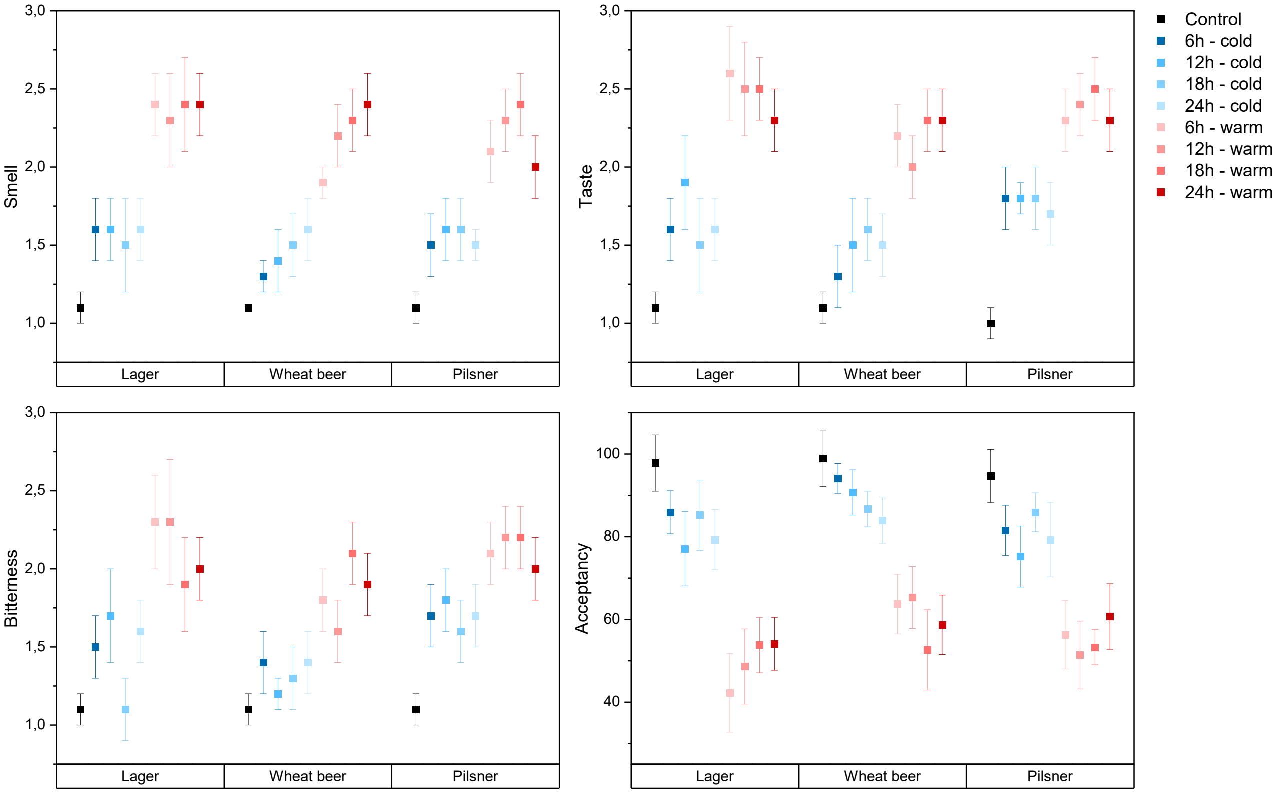

Fig. 5 Eichhorn scores (from 1 to 4) for smell, taste, bitterness and Eichhorn acceptancy (0 to 100 %) for three different beer styles kept in tube for 6, 12, 18, and 24 h and control in cold and warm conditions

characteristics, while the wheat and pilsner samples were rated with higher values for acceptancy. This indicates that the effect of masking aging impressions due to a more complex sample is higher than an elevated aging potential due to non-volatile precursors.

3.2.2 Aging attributes

To further differentiate the aging impressions, a descriptive analysis was applied. Figure 6 shows the sensory profile of a lager, wheat and pilsner beer respectively; control and samples kept in the draught hose for 12 h and 24 h in cold and warm environment. It is to be noted that every beer style behaves differently. In most samples, there was an observable aging impression in aroma after 6 h in the beer transfer tube.

The aging profile of the lager proofed to be most susceptible, especially in warm samples, where intense typical aging impressions such as bready, berry, honey, cardboard and even sherry were found. Cold samples showed a tendency towards sweetish attributes such as berry and honey

In the wheat beer, sweetish impressions such as berry, honey and sherry were pronounced in warm samples. In the cold samples on the contrary, bready and cardboard aroma showed the greatest impact.

In the pilsner sample, that was kept warm berry impressions were rated highest. In the cold samples, there was a tendency towards the descriptor bready.

3.2.3 Triangle tests

Further, the goal was to determine after which time perceivable differences in flavour of lager beer were significant. Therefore, triangle tests were performed with shorter times in the tubes (see Table 3). In warm samples, after 2 h in the hose significant changes (p = 0.001) were perceivable, while in cold samples significant changes (p = 0.001) resulted only after 3 h.

3.3 GC/MS – aging indicators

Since aging characteristics were perceived in the analysed samples by sensory analysis, volatile aging indicators were measured by GC-MS in order to evaluate possible correlations. One-way ANOVAs were used to check if aging indicators showed statistical differences ($\alpha \leq 0.05$) within the sample set. For the lager beer samples, all analysed compounds showed statistical differences. In

Tab. 3 **triangle test to determine the time in tube until perceivable differences (n = 7)**

sample	correct answers	wrong answers	p-value
Cold 1 h	4	3	> 0.05
Warm 1 h	4	3	> 0.05
Cold 2 h	4	3	> 0.05
Warm 2 h	7	0	0.001
Cold 3 h	5	2	0.05
Warm 3 h	6	1	0.01

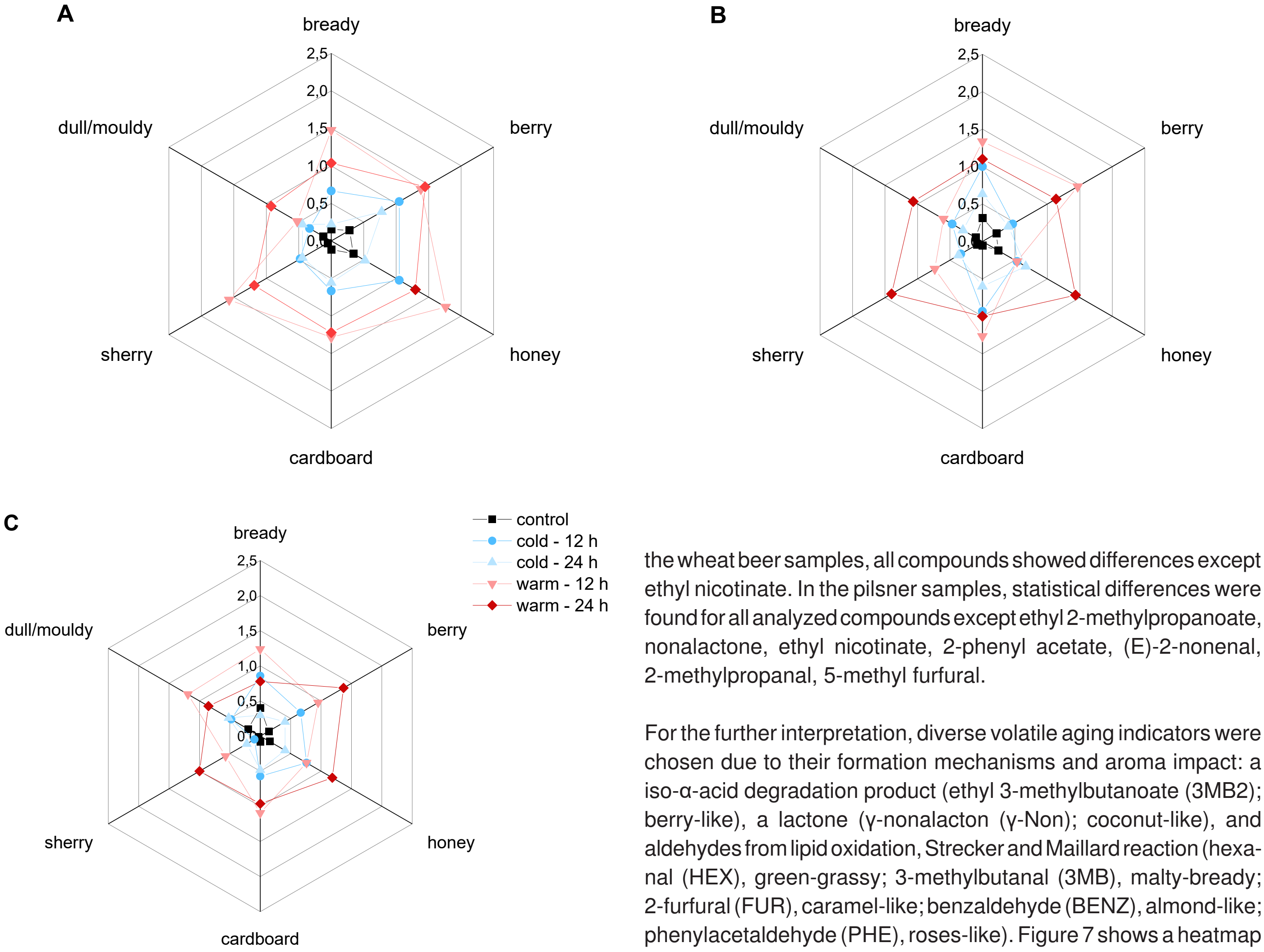

Fig. 6 **Sensory profile of a lager beer (A), a wheat beer (B) and a pilsner (C); control and samples kept in the draught hose for 12 h and 24 h in cold and warm environment**

the wheat beer samples, all compounds showed differences except ethyl nicotinate. In the pilsner samples, statistical differences were found for all analyzed compounds except ethyl 2-methylpropanoate, nonalactone, ethyl nicotinate, 2-phenyl acetate, (E)-2-nonenal, 2-methylpropanal, 5-methyl furfural.

For the further interpretation, diverse volatile aging indicators were chosen due to their formation mechanisms and aroma impact: a iso-α-acid degradation product (ethyl 3-methylbutanoate (3MB2); berry-like), a lactone (γ-nonalacton (γ-Non); coconut-like), and aldehydes from lipid oxidation, Strecker and Maillard reaction (hexanal (HEX), green-grassy; 3-methylbutanal (3MB), malty-bready; 2-furfural (FUR), caramel-like; benzaldehyde (BENZ), almond-like; phenylacetaldehyde (PHE), roses-like). Figure 7 shows a heatmap of z-scores of the concentrations of these compounds.

In the lager samples, for 3MB2, γ-Non, 3MB, and FUR show the highest concentrations after 12 h in cold and warm conditions.

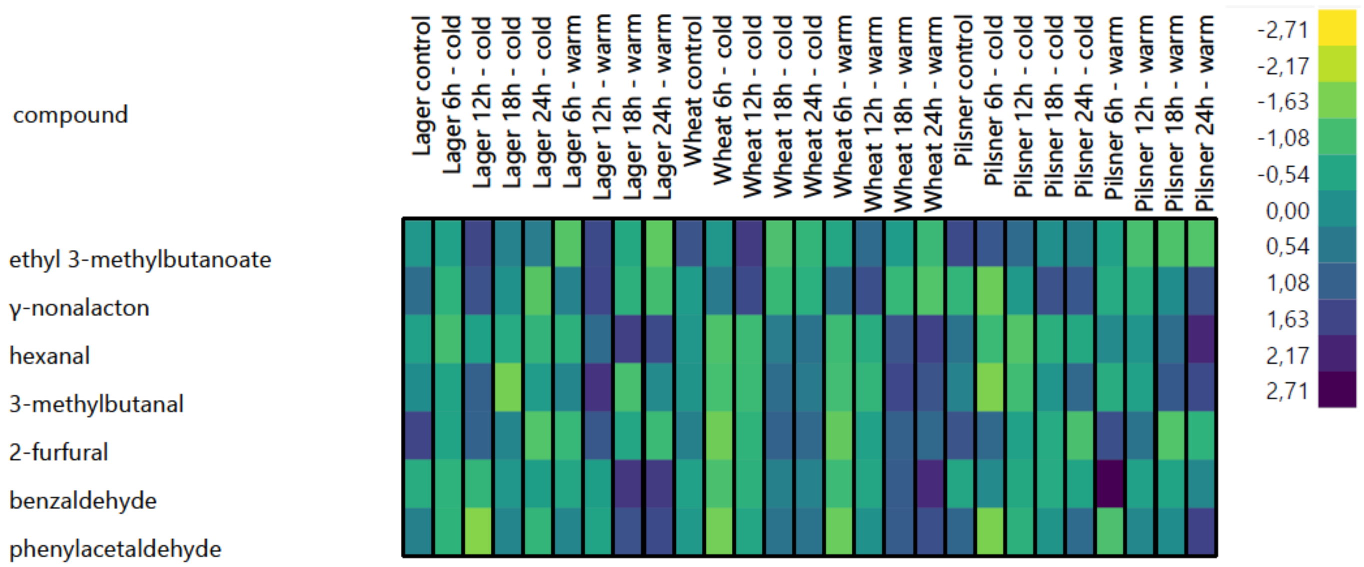

Fig. 7 **Heatmap concentrations (z-scored) of chosen aging indicators (ethyl 3-methylbutanoate (3MB2), γ-nonalacton (γ-Non), hexanal (HEX), 3-methylbutanal (3MB), 2-furfural (FUR), benzaldehyde (BENZ), phenylacetaldehyde (PHE)) for 3 beer styles**

Longer times lead to lower concentrations of these compounds. For HEX, BENZ, and PHE the highest concentrations were found in the 18 h and 24 h samples in warm conditions. Especially, PHE is known to play a role in the honey aroma in aged beer samples [16]. As described above, these samples showed a high intensity in the descriptors sherry, berry, and honey.

In the wheat beer samples, 3MB2 and γ-Non, also show their maxima after 12 h. All aldehydes increased with higher temperatures and longer hold-back times. Again, BENZ and PHE go well together with the high intensity of sherry and honey aromas.

In the pilsner samples, 3MB2 showed a time- and temperature-dependent decrease, which is contrary to the pronounced berry aroma in these samples. On the other hand, γ-Non is continuously formed. Similarly, all aldehydes except FUR show the same pattern. For FUR, a continuous decrease is observed but no increase in furfurylethyl ether was found (data not shown). This indicates that FUR is lost via another pathway.

4 Conclusion

This study scientifically investigated the night watchman (german: Nachtwächter) in dispensing systems for beer. This phenomenon is well-known in gastronomy but has never been studied with scientific methods. Thus, a combined CO_2- and O_2-detection of beer in the beer transfer tubes, a sensorial analysis with a trained panel and GC/MS-analysis were conducted.

Firstly, the results of the gas detection showed a significant decrease in CO_2 in warm storage conditions (20 °C). At the same time, warm conditions resulted in the highest O_2 ingress into the beer. Furthermore, beers kept under warm conditions showed increased amounts of aging- and oxidation-related off-flavours compared to cold storage conditions. Among other non-measured substances that play an additional role in beer flavour, 3-methylbutanal, furfural, hexanal and phenylacetaldehyde were determined as beer hose ageing indicators.

Accordingly, the aroma profiles of warm samples showed significant oxidation and aging attributes that were already detected after 6 h. Triangle tests unravelled these sensory differences even earlier. After 2 h of warm storage resp. 3 h of cold storage, significant differences in the sensory properties were present.

After all, this study highlighted the importance of immaculate installation and suitable materials to avoid quality reduction within the dispensing system. The results contribute to understanding the mechanisms and points in time of beer aging in hoses. From this, essential findings can also be concluded concerning materials employed or new types of installation. It can be determined, for example, that a light-protected and continuously temperature-controlled dispensing system can keep the beer in the lines fresh for a longer duration. New materials might include multi-layered polymer tubes or completely new compounds. Thus, similar to specific food packaging, the concentration gradients of CO_2 and O_2 can be minimized. For particularly complex systems designed for large dispensing volumes, fixed piping with stainless steel lines may even be considered. Future studies should focus on multilayer beer transfer tubes, barrier layers to avoid oxygen intake, cooling systems, heat exchangers and power packs.

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