



ORIGINAL RESEARCH ARTICLE

Investigation of aroma carryover risk in a pilot plant and on industrial scale when bottling aromatised and subsequent regular wines on the same filling line

Jörg Gottmann¹, David Müller³, Jochen Vestner¹, Jens Schuster³ and Ulrich Fischer^{1,2*}

¹ Dienstleistungszentrum Ländlicher Raum (DLR) Rheinpfalz, Institute for Viticulture and Oenology, Breitenweg 71, 67435 Neustadt an der Weinstraße, Germany

² Rhineland-Palatinate University of Technology (RPTU) Kaiserslautern-Landau, Department of Chemistry, Erwin-Schrödinger-Straße 52, 67663, Kaiserslautern, Germany

³ Kaiserslautern University of Applied Sciences, Institut für Kunststofftechnik Westpfalz (IKW), Carl-Schurz-Str. 10-16, 66953 Pirmasens, Germany



*correspondence:
ulrich.fischer@dlr.rlp.de

Associate editor:
Valeriu Cotea



Received:
3 January 2024

Accepted:
4 June 2024

Published:
5 July 2024



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ABSTRACT

Aromatised wine-based beverages and regular wines are commonly bottled on the same bottling line. Sealings installed in the bottling line absorb added aroma compounds from the aromatised wine-based beverages and a subsequent release of the absorbed aroma compounds from the sealings was monitored during cleaning sequences and in subsequent products. Such unintentional carryover is associated with the risk of violating the legal ban of any aromatisation of regular wine. If cleaning is carried out according to good manufacturing practice (GMP) and traces of aroma compounds in the subsequently bottled wine show no sensory significance, this unintended aroma carryover will be considered by the German Federal Ministry of Nutrition and Agriculture as technically unavoidable and has no legal consequences. The risk of aroma absorption and aroma carryover have been investigated in different foods (orange juice, milk, and wine) and simulated in model systems using a beaker, but no real bottling scenario had been investigated till now.

To fill the knowledge gap we investigated the potential aroma carryover in a pilot plant, and in two different wineries during three bottlings of aromatised wine-based beverages and subsequent regular sparkling wine. Monitoring of installed sealings revealed the uptake of seven aroma compounds and further release of aroma compounds during a recommended cleaning sequence and subsequent contact with wine. A sensory significant aroma carryover into the subsequent wine that had been circulating for 24 hours in the pilot plant was observed only for α -ionone, which exceeded its odour detection threshold in white wine after 8 hours. Regarding the three bottlings of aromatised wine-based beverages and the subsequent sparkling wine, we recorded the uptake of γ -decalactone, eugenol, and *trans*-cinnamaldehyde in the sealings, and their further diminishment during the implemented cleaning of the wineries and subsequent bottling of a sparkling wine. During the short residence time of the product in the bottling line, no sensory and analytical differentiation of the pre-bottled and bottled sparkling wines was observed.

KEYWORDS: Lactones, α -ionone, EPDM, migration, CIP, oxidative cleaning agents, ozone, aroma carryover, scalping

INTRODUCTION

The much-discussed risk of aroma carryover from aromatised wine-based beverages such as mulled wines into regular wines bottled later on the same filling line, and the possible associated legal consequences, represent a risk for small wineries in particular (Vetter, 2012; LUA, 2014; BMEL, 2019; Beierle *et al.*, 2020; Freund, 2022). These wineries may not be able to afford a second filling line reserved for bottling mulled wine once a year, or ensure the high industrial standards associated with cleaning-in-place (CIP) procedures requiring high temperatures of cleaning agents, or they may lack knowledge of the risk of aroma carryover during the bottling of aromatised wines.

In case of wine, even the unintentional carryover of aroma compounds from aromatised wine-based beverages to regular wine has legal consequences. Aroma addition to wine is not listed in the European regulation No 2019/934, which lists the allowed oenological procedures, and is consequently prohibited (European Parliament, 2019). Sensory conspicuous wines are analytically examined by food control authorities to confirm aroma addition. Aromatisation is usually done with synthetic aroma concentrates containing, among other compounds, chiral aroma compounds in a racemic ratio (50:50), enabling evidence of fraud to be detected via enantioselective analysis (Mosandl, 2004; Langen *et al.*, 2013; Lampe, 2015; Langen *et al.*, 2016). However, realistically, given the continuous analytical progress, aroma carryover will become increasingly detectable and will be measured more frequently in the future. Therefore, the German Association of the Flavour Industry (Deutscher Verband der Aromenindustrie, DVAI) stated that any analytical detection in subsequently bottled products should not necessarily be equated with an active addition, and consequently should not per se be accompanied by a complaint or devaluation of the product. Moreover, they suggest that further factors are required to assess a complaint, such as the respective influence of the carried over aroma compounds on the product (DVAI, 2016). In 2019 the German Federal Ministry of Nutrition and Agriculture (Bundesministerium für Ernährung und Landwirtschaft, BMEL) developed in cooperation with food control authorities and the wine industry, a guideline for the precaution and evaluation of aroma carryover in wine bottling. Aroma compounds that are analytically suspicious but have no sensory significance for the bottled wine are treated as technically unavoidable (BMEL, 2019). Sensory significance can be evaluated using the odour detection thresholds (OTs) of individual aroma compounds. The values of OTs depend on the selected matrix and can vary by up to four orders of magnitude. To evaluate sensory significance in our previous study we recommended the use of OTs determined in wine (Gottmann *et al.*, 2022b).

The generic absorption of aroma compounds into polymer materials such as food packaging is widely known as ‘scalping’ and impacts food quality (Arora *et al.*, 1991; Licciardello *et al.*, 2009; Wietstock *et al.*, 2016). In case of reused packaging, the desorption of these absorbed aroma

compounds from the polymer into later foods is known as carryover and can have a sensory impact (Nielsen, 1994; Safa and Bourelle, 1999). The excessive aroma addition in the range of mg/L in aromatised wine-based beverages facilitate high absorbance rates into sealing polymers in model experiments, which are associated with the high risk of carryover of nonpolar aroma compounds. Using a model system, we confirmed the high risk of carryover of nonpolar compounds, and showed an 8 % carryover of the initial concentration of the high nonpolar α -ionone into a subsequent model wine free of any initial α -ionone (Gottmann *et al.*, 2021). A systematic incorporation of filling materials into ethylene propylene diene monomer rubber (EPDM), which is the most used sealing material in bottling lines, reduced the aroma absorption by at least 50 %. But even with the highly chemical resistant fluorine-based rubber (FKM), which reduced absorption by 85–99 %, overall absorption did not reach a value of zero (Gottmann *et al.*, 2022a). FKM sealings are limited to static sealings and are significantly higher in price, which is why EPDM sealings are mostly used in bottling lines with many moving parts that require flexible sealings. The removal of absorbed aroma compounds during cleaning is a function of time, temperature and, mechanical work, as well as the chemical nature and concentration of the applied cleaning agents (Sinner, 1960; Tamime, 2009). Our previous study using a model system investigated different cleaning agents and temperatures based on the removal of absorbed aroma compounds from EPDM sealings. In agreement with the literature, no difference was shown among agents and only fractions of the absorbed aroma compounds were removed. Thus, the transfer of the remaining compounds from the sealing into regular wine cannot be prevented. The highest removal of absorbed compounds was achieved at the highest temperature of 85 °C and a cleaning time of 60 min (Elss *et al.*, 2007; Eckstein, 2011; Vetter, 2012; LUA, 2014; Nishijima *et al.*, 2014; Freund and Seckler, 2016; Gottmann *et al.*, 2021).

Based on the numerous factors that potentially influence the bottling process, to date aroma carryover has only been investigated via simplified model systems using beakers and fixed volumes. A more practical approach was needed in order to investigate aroma carryover in a real-world scenario. This work aimed to expand on previously gained knowledge on aroma carryover using model systems to study the bottling of aromatised wines and regular wines on the same bottling line (Gottmann *et al.*, 2021; Gottmann *et al.*, 2022b; Gottmann *et al.*, 2022a). Therefore, we designed a test pipeline to study installed commercial and customised DIN 11851 sealings with DN 50. First, aroma carryover was monitored under controlled conditions in a pilot plant. Second, potential aroma carryover was evaluated during the bottling of aromatised and regular wine at an industrial scale in two wineries. The absorption and desorption of seven aroma compounds were monitored in this study: γ -decalactone, δ -decalactone, and γ -undecalactone (peach), ethyl 2-methylbutanoate (apple), α -ionone (raspberry), eugenol (clove-aroma), and *trans*-cinnamaldehyde (cinnamon). Previous studies have shown that these

compounds occur at high concentrations in aromatised beverages (up to 37,8 mg/L) and control authorities analysed their presence in regular wine to detect any fraudulent aromatisation (Gottmann *et al.*, 2022b). Furthermore, the sensory relevance of analytically determined concentrations of aroma compounds in the subsequent wines was evaluated within the promoted sensory concept.

MATERIALS AND METHODS

1. Chemicals

Aroma compounds were purchased from Sigma-Aldrich KGaA (Darmstadt, Germany) with purities as follows: ethyl 2-methylbutanoate (99 %), α -ionone (≥ 96 %), γ -decalactone (≥ 98 %), δ -decalactone (≥ 98 %), γ -undecalactone (≥ 98 %), eugenol (99 %), and *trans*-cinnamaldehyde (≥ 99 %). Sodium hydroxide (98 %) and citric acid (99.5 %) were obtained from Merck KGaA (Darmstadt, Germany) and D-glucose (p.a.), D-fructose (> 99.5 %), L-tartaric acid (≥ 99 %), D-malic acid (≥ 99.5 %), L-lactic acid (≥ 95 %), sodium chloride (≥ 99 %), and glycerin (≥ 99 %) from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Ethanol (96 %) was purchased from Berkel AHK (Ludwigshafen, Germany).

2. Polymer materials

EPDM sealings according to DIN 11851 with DN 50 - also called dairy thread - were manufactured by the Institut für Kunststofftechnik Westpfalz (IKW) at the University of Applied Sciences, Kaiserslautern. The customised polymer material was composed of 100 parts per hundred rubber (phr) KEP 510 (Weber and Schaer, Hamburg, Germany), 150 phr kaolin “Kaoprime 11P“ as additive filling material (Thiele Kaolin Company, Sandersville, USA), 15 phr white oil from (WHC GmbH, Schweitenkirchen, Germany), 8 phr Peroxan DC (Thermo Fischer, Kandel, Germany), and 2 phr TAC-70 XP (Pergan, Bocholt, Germany). A commercial DIN 11851 EPDM sealing (70 EPDM 291) and an FKM sealing (75 Fluoroprene XP41) that had been optimised in terms of its aroma absorption by the supplier (Freudenberg, Weinheim, Germany) were used for comparison. The weight of the three investigated seals were as follows: 5.7 g per sealing for customised EPDM with kaolin, 4.6 g per sealing for EPDM (70 EPDM 291), 8.3 g per sealing for FKM (XP41). These commercial and more customised sealing materials were classified earlier in separate model study (Gottmann *et al.*, 2022a).

3. Direct quantitation of absorbed aroma compounds in polymer material with TD-GC-MS

Thermal desorption coupled to gas chromatography and mass spectrometry (TD-GC-MS) enabled the direct analysis of ethyl 2-methylbutanoate, α -ionone, γ -decalactone, δ -decalactone, γ -undecalactone, eugenol and *trans*-cinnamaldehyde from sealings (Gottmann *et al.*, 2021). The analytical system consisted of an Agilent 6890 GC equipped with an Agilent 5975 C MS, thermo desorption unit (TDU), cold injection system (CIS), and a multi-purpose

sampler (MPS) provided by Gerstel (Mühlheim, Germany). For calibration, four stamped untreated polymer samples were placed in a TDU liner with glass wool, and 1 μ L of each calibration standard solution (0.1–300 μ g/g) was pipetted directly into the TDU liner. Calibration standards were prepared in methyl tert-butyl ether (MTBE). As an internal standard 3-methylbutyl acetate (9 μ g/L) was added.

Separation of volatiles was achieved with a ZB-WAXplusTM column of 30 m \times 0.25 mm internal diameter and 0.5 μ m film thickness (Phenomenex, Germany). The oven programme started at 40 °C for 2 min followed by a 3 °C/min increase to 60 °C. The temperature rose further by 20 °C/min until 180 °C was reached, followed by a slower increase of 5 °C/min up to 240 °C, which was maintained for 9 min. The total runtime was 35.6 min. The transfer line temperature was set to 250 °C and the helium carrier gas flow rate was constant at 1.2 mL/min. The MS was operated in electron impact ionisation mode with an electron energy of 70 eV, the source temperature was set to 230 °C, and total ion chromatograms were recorded over m/z 30–300. The initial TDU temperature of 30 °C was held for 1 min and heated up at a rate of 720 °C/min to the final extraction temperature of 150 °C and kept constant for 30 min in splitless mode. The TDU transfer line was kept at 280 °C. The cryo-focusing temperature was set to 0 °C and a CIS-liner with Tenax TATM filling was chosen. After thermal extraction, the CIS was heated at a rate of 12 °C/min to 250 °C and held for 5 min. The GC inlet was run in solvent vent mode. The total flow was set to 24 mL/min while the purge flow to split vent was set to 20 mL/min at 0.01 min, corresponding to a split ratio of 1:20. The gas saver was set to 15 mL/min after 20 min until the end of the GC analysis. The vent flow was 25 mL/min with a vent pressure of 20 kPa until 0.01 min. Chromatograms obtained were processed with MassHunter Qualitative Analysis software (ver. B.05.00, Agilent Technologies) and PARADISE (ver. 3.9).

4. Quantitation of aroma compounds in wine with HS-SPME-GC-MS

Ethyl 2-methylbutanoate, α -ionone, γ -decalactone, δ -decalactone, γ -undecalactone, eugenol, and *trans*-cinnamaldehyde were quantitated in wine using an established headspace solid-phase micro extraction (HS-SPME)-GC-MS method for volatiles in Riesling wine (Schwinn *et al.*, 2019; Gottmann *et al.*, 2022b). The analytical system consisted of a Finnigan TRACE DSQ GC-MS (Thermo Fisher Scientific, Waltham, MA, USA) with a CombiPAL auto sampler (CTC Analytics, Zwingen, Switzerland). Eight milliliters of a saturated sodium chloride solution, 2 mL of wine and 10 μ L of the internal standard 2-heptanol (10 mg/L) were pipetted into a 20 mL headspace vial and closed with a screw cap. When the calibration limit was exceeded, samples were diluted with model wine according to Lampe (2015). Volatiles were separated on a ZB-5ms 30 m \times 0.25 mm fused silica column containing (5 %-Phenyl)-methylpolysiloxan with a film thickness of 0.5 μ m (Phenomenex, Aschaffenburg, Germany). Samples were pre-incubated at 40 °C for 10 min, followed by a headspace extraction with a polydimethylsiloxane (PDMS)

fiber for 20 min (FIB-P-100/10, CTC Analytics, Zwingen, Swiss). Volatiles were desorbed for 2 min from the SPME fiber at 240 °C in the GC injector using splitless mode and purging 2 min afterwards. The flow rate of helium carrier gas was kept constant to 1.0 mL/min. Oven temperature was held initially for 2 min at 30 °C, then increased to 150 °C at a rate of 3.5 °C/min, followed by an additional increase to 300 °C at a rate of 25 °C/min. Ionisation by electron impact mode with an electron energy of 70 eV, and total ion chromatograms were recorded over *m/z* 29–300. Extracted ion chromatograms were obtained using the following *m/z* values (quantifier; qualifier): ethyl 2-methylbutanoate (102; 85), α -ionone (136; 121), γ -decalactone (128; 85), δ -decalactone (99; 114), γ -undecalactone (128; 85), eugenol (164; 149) and *trans*-cinnamaldehyde (131; 132). Chromatograms were processed with Xcalibur 2.2 (Thermo Fisher Scientific). Linear calibration models were obtained for all compounds using a series of standards in model wine (10–1000 $\mu\text{g/L}$ for each compound).

5. Sensory evaluation

A “two-out-of-five test” was conducted according to Lawless and Heymann (2010) to identify a potential differentiation between the subsequent bottled regular wines and the last rinse water based on an aroma carryover from the earlier product. Nine judges that frequently participate in in-house-tastings were selected from the staff of the Institute for Viticulture and Oenology (DLR Rheinpfalz, Neustadt, Germany). Judges received five samples and had to identify the two deviating samples. Samples of 20 mL were presented to each judge in transparent INAO (Institute national de l’origine et de la qualité) wine glasses covered with plastic lids.

6. Experimental design

To investigate the absorption and desorption of aroma compounds within installed sealings, a test pipeline with 18 sealing seats according to DIN 11851 with DN 50 was designed (Supplementary Figure 1 A) in order to allow separate sealings to be removed during different stages of the experiments. Prior to analysis, the sealings were rinsed with deionised water and dried with cellulose tissue. Two 1 mm thick slices were cut out of the sealing and placed into a TDU liner. Before TD-GC-MS analysis, 1 μL of 3-methylbutyl acetate (9 $\mu\text{g/L}$ in MTBE) was automatically pipetted by the MPS into the TDU liner as an internal standard.

7. Pilot plant (Experiment 1)

The pilot plant consisted of a 300 L tank, a self-priming centrifugal pump Motek IEC 34 (Motek, Gatteo, Italy), two ball valves, and the test pipeline with 18 sealing seats. All parts were connected with three 1.5 m PE hoses (Supplemental Figure 1 B). The pump was set with a GPI flowmeter model 10 (GPI, Wichita, USA) to a pumping speed of 30 m/s through a relatively small diameter of 2 cm; the speed would decrease to 4.8 m/s due to the larger diameter of 5 cm in the hose of the model system. Regardless of the diameter, the volume per hour was 34000 L/h, which corresponds to 45000 bottles of 0.75 L per hour, which is a common figure for large fillers for beverages. To avoid carryover between individual experimental steps and later conducted experiments, the installed sealings in the tank, the pump and the valves, as well as the hoses, were exchanged after each work step. The white wine used in this experiment comprised 12 % v/v ethanol, 2 g/L residual sugar, 8.4 g/L titratable acidity, pH 3.1 and 15 mg/L free SO_2 . Parts of the wine were aromatised with 35 mg/L α -ionone, 14 mg/L

TABLE 1. Individual cleaning steps of the cleaning sequences conducted in the pilot plant in Experiment 1.

cleaning steps	recommended cleaning sequence ¹	short cold cleaning (ozone)	extended cleaning – recommended cleaning sequence ¹ and further 24 h cleaning
1	water (10 min; 20°C)	water (10 min; 20 °C)	water (10 min; 20°C)
2.1	water (30 min; 85 °C)	ozonized water (5 mg/L; 20 min; 20 °C)	water (30 min; 85 °C)
2.2	sodium hydroxide solution (1,5 % (w/v); 20 min; 20°C)	–	sodium hydroxide solution (1,5 % (w/v); 20 min; 20°C)
2.3	citric acid solution (1,5 % (w/v); 15 min; 20°C)	–	citric acid solution (1,5 % (w/v); 15 min; 20°C)
2.4	–	–	water (10 min; 20°C)
2.5	–	–	water (24 h; 20°C; no pumping)
3	water (10 min; 20°C)	water (10 min; 20°C)	water (10 min; 20°C)
Σ cleaning time	75 min	40 min	1535 min

¹Cleaning sequence recommended to mitigate aroma carryover during bottling wine (BMEL, 2019).

ethyl 2-methylbutanoate, 12 mg/L γ -decalactone, 10 mg/L δ -decalactone, 15 mg/L γ -undecalactone, 8 mg/L eugenol, and 8 mg/L *trans*-cinnamaldehyde. In the first step, 60 L of aromatised white wine circulated the pilot plant for 27 h; the next steps comprised cleaning sequences and then a second 24 h-long circulation of 60 L of the pure white wine without any added aroma. During the experiments the wine temperature was kept at 12 °C using a cooling circuit. Sealings were removed after each step of the three states of the experiment and analysed directly by TD-GC-MS. Wine samples were taken during the circulation of the aromatised and subsequent pure wine and analysed directly by HS-SPME-GC-MS. Three cleaning scenarios were executed in this experiment and are described in detail in Table 1. In each of the cleaning steps, 60 L of the cleaning medias were used. First, we conducted a cleaning process following the recommendations of the BMEL and repeated it once: water was heated up to 85 °C prior to the cleaning step involving steam and heated continuously during the cleaning. Second, the cleaning was shortened to a rinse step with cold ozonised water (5 mg/L), which was continually ozonised with a univog ozone generator (Zickler, Böchingen) incorporated into the pilot plant circuit. Third, in order to investigate long-duration cleaning, the recommended cleaning was extended to include an additional 24 h of flooding of the system with cold water and no pumping.

8. Bottling of aromatised wine-based beverages and a subsequent sparkling wine (Experiment 2-4)

Aroma carryover was investigated during the bottling process of aromatised wine-based beverages and subsequent bottling of wines on the same bottling line of two different wineries. The test pipeline with 18 sealing seats was installed after the membrane filter (0.45 μ m) placed before the bottling line. Sealings were sequentially removed and directly analysed with TD-GC-MS. During the bottling process, aromatised wines and subsequent regular wines were continuously sampled for analytical and sensory studies.

Two bottlings of aromatised wine-based beverages were executed by the first winery. The customised EPDM with kaolin and the commercial EPDM and FKM (XP41) were investigated in both experiments. In the first bottling (Experiment 2), three mulled wines were bottled on 4 consecutive days (~75000 L), and after cleaning of the bottling line a sparkling wine was subsequently bottled over 3 days (~65000 L). In the second bottling (Experiment 3), a peach-flavoured wine-based sparkling cocktail was bottled on 3 consecutive days (~60000 L), and after cleaning of the bottling line a sparkling wine was subsequently bottled over 2 days (~45000 L). The bottling speed was set to a volume of 3000 L/h, equaling 15000 bottles/h of 200 mL volume. The cleaning process applied between aromatised and regular wine bottling is a proprietary in-house-optimised cleaning sequence carried out at a flow-rate of 3000 L/h; hot water is applied at 85 °C, followed by acidic and alkaline agents, ozonized water, and steam at the end. The whole cleaning sequence lasted for 5 h. The cleaning agents and sequence

was identical to the cleaning of the pilot plant, only the total cleaning time lasted four times longer.

In the third bottling (Experiment 4), in the second winery, a pomegranate-flavoured sparkling wine cocktail was bottled on the first day (~30000 L) and a mango-flavoured one on the second (~20000 L). In this experiment, only the customised EPDM with kaolin and the commercial EPDM were installed in the test pipeline. After a thorough cleaning, a subsequent sparkling wine was bottled for 4 days (~150000 L). The cleaning applied between product changes consisted of the following rinsing steps: 60 min sodium hydroxide cleaner (2 % w/v) at a temperature of 60 °C; 60 min carbonised water (4 bar) at a temperature of 10 °C; 10 min water at 10 °C; 30 min phosphoric acid cleaner (1 % w/v) at 10 °C; 30 min water 10 °C; 60 min steaming. These cleaning steps were set to a flow of 20000 L/h.

9. Statistical analysis

Mean values of the aroma compounds determined in the polymers were compared using one-way analysis of variance (ANOVA, $p < 0.05$) with Tukey's honestly significant difference (HSD, $p < 0.05$) posthoc test. Values provided in the charts and tables are given as mean \pm standard deviation of the sample. These analyses were done using XLStat (Addinsoft, Paris, France).

RESULTS

1. Evaluation of aroma carryover in a pilot plant (Experiment 1)

The circulation of 60 L of aromatised wine in the pilot plant in all four experiments showed similar patterns over the monitored 27 hours. Aroma concentration in the aromatised wine (Figure 1) decreased for α -ionone (~50 %), γ -undecalactone (~40 %), ethyl 2-methylbutanoate (~80 %), and γ -decalactone (~15 %). Aroma absorption within installed sealings showed no significant differences in the pilot plant between the four experiments and mean values were calculated (Figure 2). The two installed EPDM sealings did not differ in their individual performance during the experiments, while the FKM (XP41) sealing reduced the absorption by 89–99 %, as reported earlier (Gottmann *et al.*, 2022a). The highest absorption of approximately 80 μ g per sealing was monitored for α -ionone in the EPDM sealings, which respectively showed the highest decrease in the aromatised wine. Cleaning based on the recommendations of the BMEL guideline removed up to 74 % of the absorbed aroma compounds from both EPDM sealings, while a shortened cold cleaning removed a maximum of 37 %. A further cleaning improvement up to 89 % removal was seen with an additional 24 hours flooding step after the recommended sequence. In the case of the FKM (XP41), the three cleaning protocols removed the initial low absorbed aroma components close to or even below the detection limits of the applied method. The polymer data obtained after the 24 h circulation of the subsequent pure white wine was used to further determine the differences in cleaning effects. After the shortened cleaning we observed the highest aroma

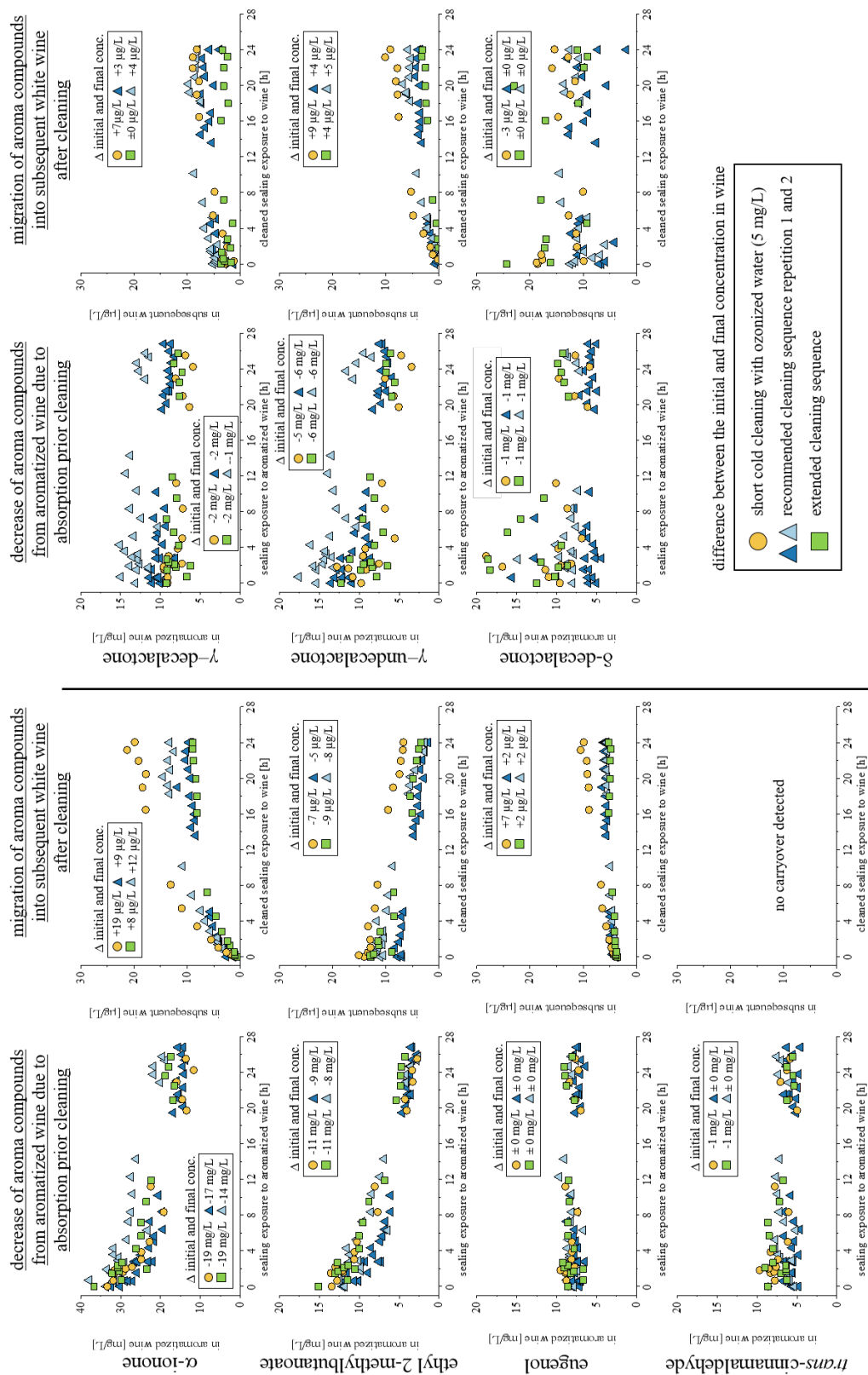


FIGURE 1. Time-dependent concentration of aroma compounds determined in the aromatized white wine (mg/L) and the subsequent white wine (μ g/L). Each wine was pumped in a circle in the pilot plant and a cleaning was conducted between these two circulations. The detailed cleaning sequences are described in Table 1.

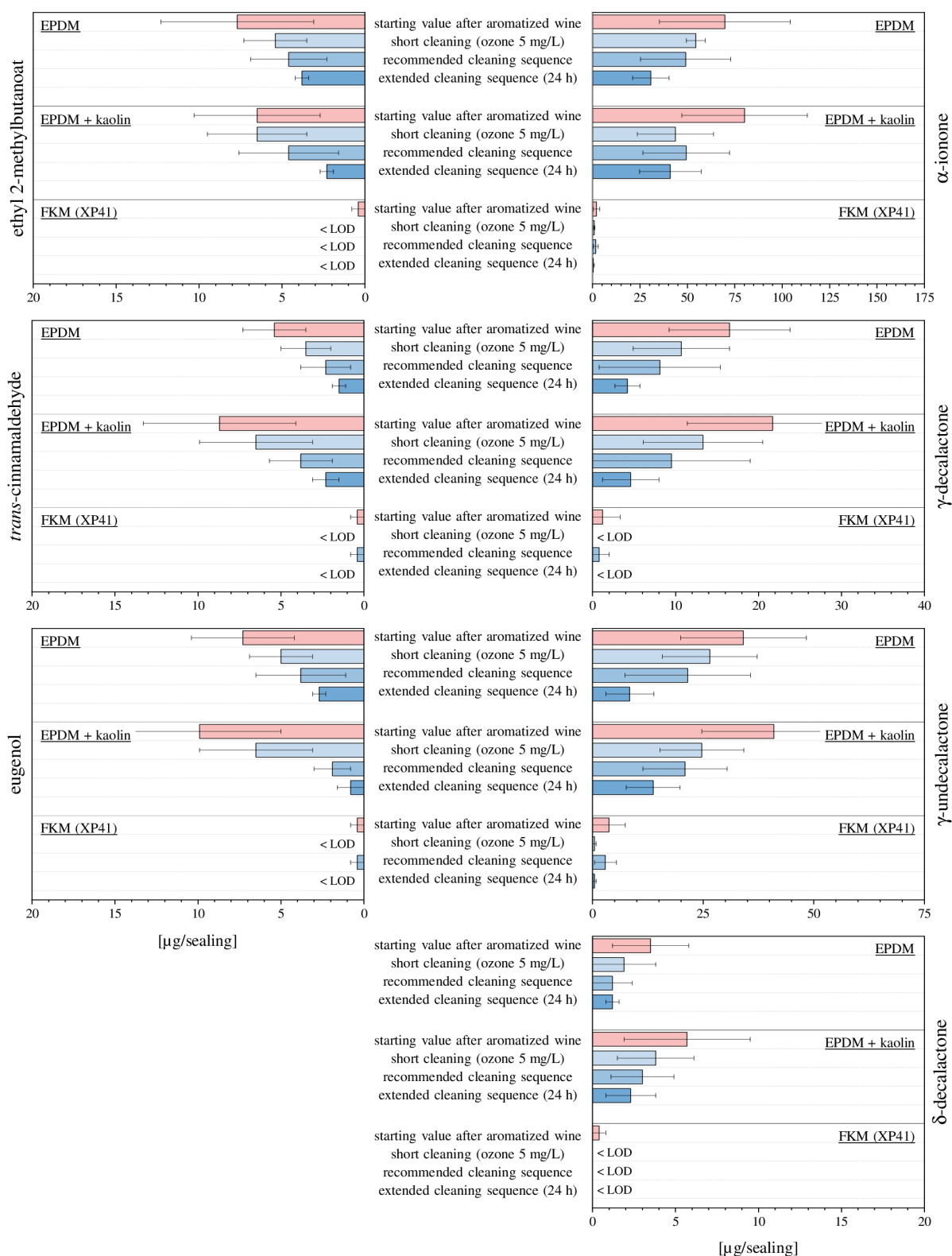


FIGURE 2. Aroma compounds absorbed from white wine by installed sealings in a pilot plant that remained in the sealing after three different cleaning protocols in the sealing. Individual cleaning sequences are described in detail in Table 1. Aroma compounds in the sealing polymer were determined directly by TD-GC-MS, values given as mean \pm standard deviation ($n = 5$).

transfer, while the lowest transfer occurred after the extended cleaning (Figure 1).

2. Bottling aromatised wines and regular wines on the same filling line (Experiment 2-4)

Within the scope of three bottlings of aromatised wines at an industrial scale, we investigated the real risk of aroma carryover during the subsequent bottling of a regular sparkling wine after a thorough cleaning of the bottling plant (Figure 3). High levels of eugenol addition (up to 10100 µg/L) to the three bottled mulled wines in Experiment 2, the addition of 28700 µg/L γ -decalactone to a peach-flavoured beverage in Experiment 3 and 51800 µg/L to a mango flavoured beverage in Experiment 4 facilitated high absorption into the installed EPDM sealings. The EPDM variants showed no differences in their absorption capacity. FKM (XP41) sealings reduced absorption to levels below the detection limit, and quantitative data was obtained for γ -decalactone only in the bottling of the peach-flavored beverage, remaining four times below the EPDM sealings (Figure 4A). The in-house, optimised and thorough cleaning sequence in the first two bottlings that lasted up to 5 h removed only minor portions of the absorbed eugenol (18–28 %), *trans*-cinnamaldehyde (0–12 %), and γ -decalactone (14–87 %). During the third aromatised bottling, we investigated the performed cleaning steps more closely. A strong effect was observed for the first cleaning step, which lasted one hour while the later steps with phosphoric acid or steam had no effect on the remaining absorbed compounds. Cleaning efficiency was assessed by the BMEL-recommended two-out-of-five sensory difference testing of the last rinse water and indeed confirmed that there was no risk of aroma carryover for a subsequent bottling (BMEL, 2019). During the subsequent bottling of the sparkling wines, only minor changes of the remaining compounds were found in all three experiments. Targeted analyses of seven markers in the subsequent sparkling wines revealed low

concentrations of 60 µg/L and 44 µg/L γ -decalactone in the second and third bottling respectively (Table 2). In advance the bottled wines could not be distinguished in an untargeted approach described by Sirén *et al.* (2019) (data not shown), as well as in two-out-of-five sensory difference testing with trained judges.

DISCUSSION

The potential risk of aroma carryover when bottling aromatised wine-based beverages and subsequent wines on the same bottling line has been postulated in various model experiments (Eckstein, 2011; Vetter, 2012; Freund and Seckler, 2016; Gottmann *et al.*, 2021). These simplified model systems with fixed volumes cannot be directly transferred to a bottling scenario in a winery in which small volumes of only 1000 L/h are bottled, and a continuous flow of a new product is guaranteed. This study attempts to close the gap between such models and the real world via an investigation of a pilot plant and three bottlings of aromatised wine-based beverages. As expected, our study revealed a continuous absorption of aroma compounds in the pilot plant as well as the real bottlings. In the aromatised wine, a high decrease in nonpolar α -ionone (~50 %) and γ -undecalactone (~40 %) resulted in a high absorption in the installed sealings. Moreover, a strong decrease in ethyl 2-methylbutanoate (~80 %) was observed, while the absorption level was moderate. We assume that the decrease of this ester is due to its high volatility, which led to its volatilisation and loss during the high turbulence in the tank. It is additionally worth noting that the three installed PE hoses, with a total length of 4.5 m and a total weight of 1.5 kg, together absorbed more than the installed sealings. A semi-quantitative analysis of the PE hoses showed an absorption rate that was 5 times higher than the installed EPDM sealings (data not shown). Therefore, high portions of the lost aroma compounds from the aromatised wine were found in the hoses and they were exchanged after each step.

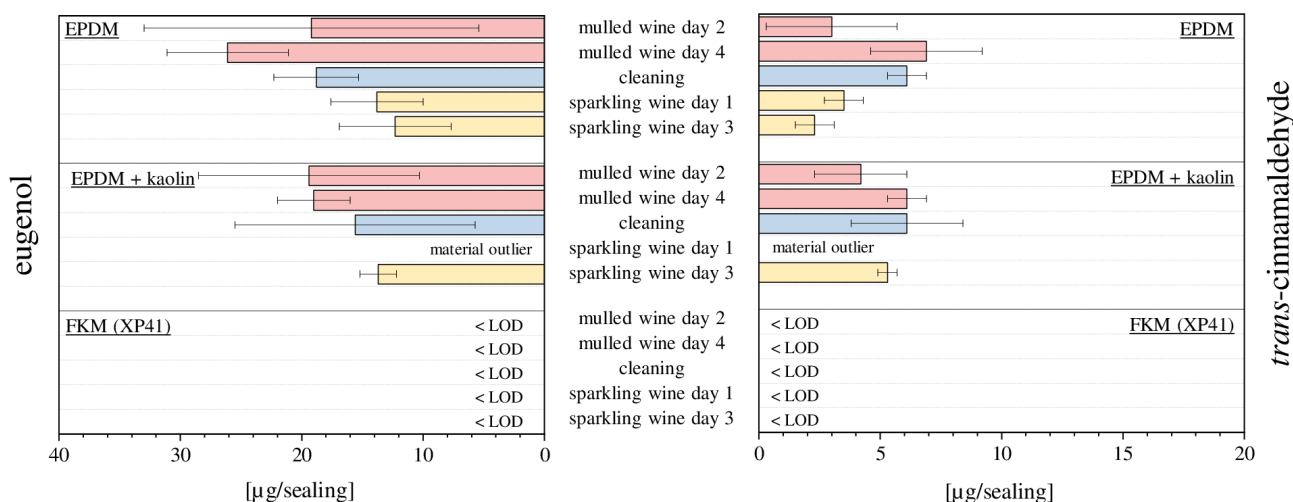


FIGURE 3. Aroma compounds absorbed by installed sealings during the bottling of mulled wines and their remaining concentrations after a conducted cleaning in between a product change and the subsequent bottling of sparkling wine (Experiment 2).

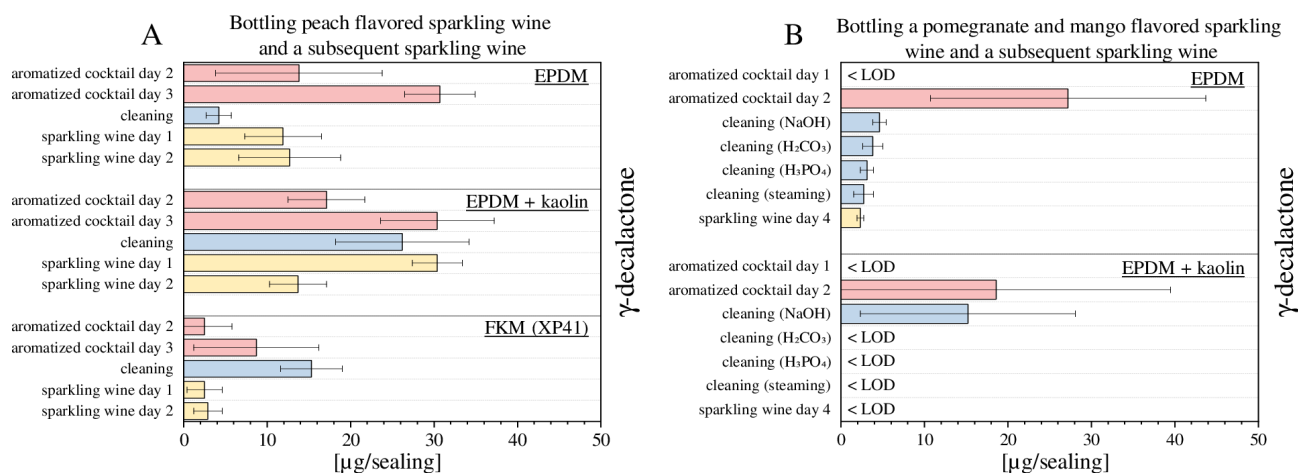


FIGURE 4. Aroma compounds absorbed by installed sealings during the bottling of aromatised products in two different wineries (Experiment 3–A and Experiment 4–B) and their remaining concentration after a cleaning in between a product change and the subsequent bottling of sparkling wine.

One of our previous studies showed a dependence in the exposed surface and the portions of absorbed aroma compounds (Gottmann *et al.*, 2021). Comparing our earlier model systems and the study of the installed DIN 11851 sealings, we notice a decrease in the absorption rate, which corresponded to a decrease in exposed surface area (Gottmann *et al.*, 2022a). At this point it should be noted that there was an increase in the determined variance of aroma compounds measured within

the installed polymer sealing samples. This variance can be attributed to a higher variability in the entire investigated system with small differences in the concentrations in the pilot plant, as well as a potential difference in the tightening of the sealing seats resulting in a difference in the exposed surface area. With the increased variation the two EPDMs did not differ significantly in their absorption. Thus we could not confirm our earlier improvement of the EPDM sealing

TABLE 2. Aroma concentration in aromatised wine based beverages and subsequent bottled sparkling wines. Bottled sparkling wines investigated towards aroma carryover in a sensory two-out-of-five difference test.

Aroma compound	Bottling of mulled wine (Experiment 2)		Bottling of peach flavoured beverage (Experiment 3)		Bottling of pomegranate and mango flavoured beverage (Experiment 4)			
	eugenol		<i>trans</i> -cinnamaldehyde		γ -decalactone			
	$\mu\text{g/L}$	OAV	$\mu\text{g/L}$	OAV	$\mu\text{g/L}$	OAV		
Odour detection threshold in white wine matrix ¹	135.0		1617.0		279.8		279.8	
Aromatized beverage bottle of								
1st day	10100	75	500	0.31	28700	103	3760	13
2nd day	2500	19	< LOD ²		25300	90	51870	185
3rd day	3100	23	290	0.18	23800	85	-	
4th day	6000	44	230	0.14	³		-	
Sparkling wine bottle of								
1st day	4	0.03 † ⁴	< LOD		58	0.21 †	39	0.14 †
2nd day	5	0.04 †	< LOD		55	0.20 †	41	0.15 †
3rd day	3	0.02 †	< LOD		54	0.19 †	44	0.16 †
3rd day	-		-		-		40	0.14 †

¹ Gottmann *et al.*, 2022b.

² below the detection limit of 10 $\mu\text{g/L}$ of the conducted HS-SPME-GC-MS method.

³ bottling ended after this bottle and the filling line was cleaned and the subsequent regular wine was bottled.

⁴ sensory comparison of the bottled and prebottled products in a two-out-of-five test; sensory significant differentiation indicated with (*) and non-significance indicated with (†).

with kaolin addition. Meanwhile, a decrease in absorption by at least a factor of 10 was observed for the FKM (XP41) (Gottmann *et al.*, 2022a).

The investigation of the three different cleaning sequences in the pilot plant revealed that a shorter cleaning with cold water and ozone addition (5 mg/L) for sanitisation of the bottling system is associated with a higher risk of aroma carryover. This is supported by results of our earlier model system that investigated individual cleaning steps and revealed that cleaning time and temperature had the highest impact, while the enhancement of the aroma removal with cleaning additives, such as ozone, can be neglected (Gottmann *et al.*, 2022a). Therefore, the shortened cleaning period failed to prevent prohibit the aroma carryover sufficiently. As a consequence, the highest concentration of polymers was determined after the shortened cleaning, followed by the highest aroma transfer recorded in the subsequently bottled wine. In the case of the extended and thorough cleaning with an additional 24 h flooding, resulted in a lower transfer of α -ionone and both γ -lactones into the subsequent wine (Figure 1). Reference to our previously published thresholds in wine revealed that α -ionone, with its low odour threshold of 10.6 $\mu\text{g/L}$ in wine, may exceed the sensory relevance of this experiment (Gottmann *et al.*, 2022b). It is important to keep in mind in this experiment that the wine circulated for a unusual 24 hours period, and the sensory relevance was only reached after an 8-hour circulation. At industry scale bottling, continuously fresh and unsaturated wine will pass through a plant, with contact time of wine with sealings being limited to few minutes only; therefore, these results cannot be directly applied to real bottling, but they show there is a potential risk of carryover.

The further assessment carried out during the industry-scale bottling of three aromatised wine-based beverages as well as that of the subsequent sparkling wines contributed to a better understanding of the carryover risk. This assessment was initially carried out in two wineries that continuously bottle regular wines and aromatised wines on the same bottling line, and which optimised their in-house cleaning sequences between the product changes. During the bottling of the aromatised product, we see a high absorption of the eugenol and *trans*-cinnamaldehyde from the mulled wines (Figure 3), and of γ -decalactone (Figure 4) from the two bottled fruity cocktails. However, only minor amounts of the absorbed compounds were desorbed from the sealing during the thorough cleaning sequences, and their concentrations changed only marginally during the subsequent bottling over several days. To prevent an aroma carryover during the bottling of aromatised and regular wines on the same filling line a sensory evaluation of the cleaning is recommended in the BMEL guidelines. The recommended sensory two-out-of-five difference test of the finally used cleaning water in comparison to the unused, clean water from the tap is easy to implement, and significant results can be already obtained by three judges only (BMEL, 2019). Based on the higher sensory sensitivity in water, a negative test result implies that there is no risk of sensory carryover during the bottling process of less

sensitive wine (Gottmann *et al.*, 2022b). This was confirmed by the results of the in-depth cleaning evaluation of the third bottling, which showed no sensory significant differentiation of the last cleaning water compared to tap water. The use of cold cleaning agents may also prevent aroma carryover but is very time consuming. The largest proportion of the aroma compounds desorbed already during the first hour. In a previous study, we addressed cleaning efficiency and showed a limiting effect of the cleaning agents on desorption from the polymer (Gottmann *et al.*, 2022a). We assumed that limited transport of aroma compounds is mainly enhanced by an increased temperature, but a total removal of the absorbed aroma compounds is not possible. The proposed limiting transfer process applies not only to the cleaning, but also to the subsequent bottling, whereby fractions of the absorbed compounds are transferred steadily into the subsequent product but are highly diluted within the bottled volume. In the case of bottling plants that cannot guarantee such a high volume or when a bottling process pauses for an hour, the increased contact time will potentially foster the transfer of aroma compounds and result in a higher risk of carryover.

In our experiments, only γ -decalactone was detectable in the subsequent sparkling wines. The determined γ -decalactone concentrations of 44 and 60 $\mu\text{g/L}$ reported the maximum natural concentration of 15.5 $\mu\text{g/L}$ reported in Fiano sweet wine by Genovese *et al.* (2007). It could thus be considered by food control authorities as suspicious, even though we did not conduct an enantioselective analysis to prove its synthetic origin. In light of our earlier discussion of analytically suspicious concentrations, sensory significance can be assessed by consulting published OTs. Our published γ -decalactone OT of 279.8 $\mu\text{g/L}$ in white wine indicates that the analysed concentrations of 44-60 $\mu\text{g/L}$ have no sensory significance and consequently it is classified as a technical not avoidable transfer (Gottmann *et al.*, 2022b). This more or less theoretical approach is supported by our sensory two-out-of-five difference test, which did not show any significant difference between the pre-bottled and bottled regular wines. As a result, at industry scale we were not able to confirm the carryover risk from the pilot plant. It should be noted that we studied the risk of aroma carryover in two wineries which had previously implemented a thorough and controlled cleaning sequence, allowing the bottling of both regular and aromatised wines on the same bottling line. Therefore, this study should be considered as an initial investigation of the actual risk of aroma carryover associated with the bottling of aromatised and regular wines in the same bottling plant. In the future, further studies should monitor smaller bottling lines and longer pauses between bottling processes to allow more aroma desorption to occur, as well as the impact of other sealing materials could help increase knowledge regarding the risk of aroma carryover.

CONCLUSIONS

Experiments conducted using an enlarged pilot plant corroborated the previously described risk of aroma carryover via absorption of aroma compounds into EPDM

and FKM sealings and their transfer into subsequently bottled regular wine. The carryover was larger when only using cold ozonised water for sanitisation and cleaning, and when applying shorter cleaning periods. Extending the cleaning sequence to 24 h improved desorption, but did not completely remove the absorbed aroma compounds from the studied sealings.

In two industrial-scale bottling lines, we monitored the cleaning efficacy of the subsequent bottling of aromatised and regular wines and detected aroma compounds in the analysed EPDM sealing material. Thus, a small potential risk of aroma carryover still exists. However, the large volumetric flow of the wine minimises the contact time between product and sealing surface and potential desorption. At the same time, the small concentration of desorbed aroma compounds was drastically diluted by the large volume passing through the bottling line per hour. As a result, we could not detect analytically or sensorially any aroma carryover from the sealings into the regular wine at industrial scale. Applying hot cleaning for a sufficient period of time and validating cleaning success using the proposed sensory method yields a high cleaning efficacy, allowing aromatised and regular wines to be bottled on the same filling lines without a risk of sensory relevant and fraudulent aroma carryover. These results indicate that it is unnecessary for one company to operate two bottling lines in, thus avoiding significant investments in machinery and staff.

ACKNOWLEDGEMENTS

This IGF Project 20220 N of the FEI is/was supported by AiF as part of the programme for promoting the Industrial Collective Research (IGF) of the German Ministry of Economics and Climate Action (BMWK), based on a resolution of the German Parliament.

We thank Michael Wacker for his technical GC support as well as the execution of the analyses. We thank Peter Herres Wein- und Sektkellerei for their cooperation in the bottling experiments.

REFERENCES

Arora, D. K., Hansen, A. P., & Armagost, M. S. (1991). Sorption of flavor compounds by low density polyethylene film. *Journal of Food Science* 56(5), 1421–1423.

Beierle, E., Bradshaw, I., Reuter, M., & Tomp, A. (2020). Produktwechsel ohne Aromaverschleppung? Qualitätsanspruch bei hoher Produktvielfalt. *Getränke!* 4, 36–40.

BMEL (2019). Leitfaden der guten fachlichen Praxis zur Verhinderung von technisch vermeidbaren Aromaverschleppungen bei Wein. Referat 414, Wein, Bier, Getränkewirtschaft.

DVAI (2016). Spuren von Aromastoffen in Lebensmitteln und Aromen. Deutscher Verband der Aromenindustrie e.V.

Eckstein, J. (2011). Durch geeignete Werkstoffauswahl Aromatransfer verhindern. *Pharma+Food* 9(9), 41–43.

Elss, S., Kleinhenz, S., & Schreier, P. (2007). Odor and taste thresholds of potential carry-over/off-flavor compounds in orange and apple juice. *LWT-Food Science and Technology* 40(10), 1826–1831. <https://doi.org/10.1016/j.lwt.2006.12.010>

European Parliament (2019). Regulation (EU) No 2019/934: supplementing Regulation (EU) No 1308/2013 of the European Parliament and of the Council as regards wine-growing areas where the alcoholic strength may be increased, authorised oenological practices and restrictions applicable to the production and conservation of grapevine products, the minimum percentage of alcohol for by-products and their disposal, and publication of OIV files.

Freund, M. (2022). Aromamigration aus Dichtungen Verarbeitung von Weinmischgetränken im Fokus. *Das deutsche Weinmagazin* (3), 27.

Freund, M., & Seckler, J. (2016). Aromaverschleppung im Wein. *Das deutsche Weinmagazin* (25), 25–29.

Genovese, A., Gambuti, A., Piombino, P., & Moio, L. (2007). Sensory properties and aroma compounds of sweet Fiano wine. *Food Chemistry* 103(4), 1228–1236.

Gottmann, J., Müller, D., Becker, A. M., Vestner, J., Schuster, J., & Fischer, U. (2022a). Improved sealing polymers and cleaning procedures to mitigate aroma carryover during bottling of aromatised and regular wines on the same filling line. *OENO One* 56(4), 41–54. <https://doi.org/10.20870/oeno-one.2022.56.4.5579>

Gottmann, J., Vestner, J., & Fischer, U. (2022b). Sensory relevance of seven aroma compounds involved in unintended but potentially fraudulent aromatization of wine due to aroma carryover. *Food Chemistry* 402, 134160. <https://doi.org/10.1016/j.foodchem.2022.134160>

Gottmann, J., Vestner, J., Müller, D., Schuster, J., & Fischer, U. (2021). Uptake and Release of Aroma Compounds by an Ethylene Propylene Diene Monomer Rubber Sealing Polymer: Investigating Aroma Carryover in a Model Wine System. *Journal of Agricultural and Food Chemistry* 69(38), 11382–11394. <https://doi.org/10.1021/acs.jafc.1c04565>

Lampe, U. (2015). Untersuchungen zur Authentizität von Weinaroma am Beispiel der γ -Lactone. *38th World Congress of Vine and Wine (Part 2)* 5, 6005.

Langen, J., Wang, C.Y., Slabizki, P., Wall, K., & Schmarr, H.G. (2013). Quantitative analysis of γ - and δ -lactones in wines using gas chromatography with selective tandem mass spectrometric detection. *Rapid Communications in Mass Spectrometry* 27(24), 2751–2759. <https://doi.org/10.1002/rcm.6736>

Langen, J., Wegmann-Herr, P., & Schmarr, H.G. (2016). Quantitative determination of α -ionone, β -ionone, and β -damascenone and enantiodifferentiation of α -ionone in wine for authenticity control using multidimensional gas chromatography with tandem mass spectrometric detection. *Analytical and Bioanalytical Chemistry* 408(23), 6483–6496. <https://doi.org/10.1007/s00216-016-9767-6>

Lawless, H. T., & Heymann, H. (2010). Sensory Evaluation of Food. Springer. <http://dx.doi.org/10.1007/978-1-4419-6488-5>

Licciardello, F., Del Nobile, M. A., Spagna, G., & Muratore, G. (2009). Scalping of ethyl octanoate and linalool from a model wine into plastic films. *LWT - Food Science and Technology* 42(6), 1065–1069. <https://doi.org/10.1016/j.lwt.2009.01.007>

LUA (2014). LUA-Bilanz Weinüberwachung Ergebnisse von Kontrollen und Untersuchungen der rheinland-pfälzischen Weinüberwachung 2014. Landesuntersuchungsamt Rheinlandpfalz.

Mosandl, A. (2004). Authenticity assessment: a permanent challenge in food flavor and essential oil analysis. *Journal of chromatographic science* 42(8), 440–449. <https://doi.org/10.1093/chromsci/42.8.440>

- Nielsen, T. J. (1994). Limonene and myrcene sorption into refillable polyethylene terephthalate bottles, and washing effects on removal of sorbed compounds. *Journal of Food Science* 59(1), 227–230. <https://doi.org/10.1111/j.1365-2621.1994.tb06936.x>
- Nishijima, W., Okuda, T., Nakai, S., & Okada, M. (2014). A green procedure using ozone for Cleaning-in-Place in the beverage industry. *Chemosphere* 105, 106–111. <https://doi.org/10.1016/j.chemosphere.2014.01.019>
- Safa, H. L., & Bourelle, F. (1999). Sorption–desorption of aromas on multi-use PET bottles. A test procedure. *Packaging Technology and Science: An International Journal* 12(1), 37–44. [https://doi.org/10.1002/\(SICI\)1099-1522\(199901/02\)12:1<37::AID-PTS452>3.0.CO;2-F](https://doi.org/10.1002/(SICI)1099-1522(199901/02)12:1<37::AID-PTS452>3.0.CO;2-F)
- Schwinn, M., Durner, D., Wacker, M., Delgado, A., & Fischer, U. (2019). Impact of fermentation temperature on required heat dissipation, growth and viability of yeast, on sensory characteristics and on the formation of volatiles in Riesling. *Australian Journal of Grape and Wine Research* 25(2), 173–184. <https://doi.org/10.1111/ajgw.12386>
- Tamime, A. Y. (2009). *Cleaning-in-place: dairy, food and beverage operations*. John Wiley & Sons.
- Sinner, H. (1960). Über das Waschen mit Haushaltwaschmaschinen: in welchem Umfange erleichtern Haushaltwaschmaschinen und geräte das Wäschehaben im Haushalt? Haus+ Heim-Verlag.
- Sirén, K., Fischer, U., & Vestner, J. (2019). Automated supervised learning pipeline for non-targeted GC-MS data analysis. *Analytica chimica acta: X* 1, 100005. <https://doi.org/10.1016/j.acax.2019.100005>
- Vetter, E. (2012). Aromamigration in elastomere Wekstoffe: Kleines Bauteil - große Wirkung. *DMW - Die Milchwirtschaft* 3(10), 380–386.
- Wietstock, P. C., Glattfelder, R., Garbe, L.A., & Methner, F.J. (2016). Characterization of the migration of hop volatiles into different crown cork liner polymers and can coatings. *Journal of Agricultural and Food Chemistry* 64(13), 2737–2745. <https://doi.org/10.1021/acs.jafc.6b00031>