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Research Paper

Biofilms in Water Hoses from the Food Processing Environment Harbor Diverse Microbial Communities

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ABSTRACT

Biofilms in drinking water distribution systems are harborage sites for diverse bacteria and fungi. The presence of opportunistic pathogens in these biofilms poses a potential health threat, in food processing environments, where there is still limited knowledge on biofilms. In the current study, we investigated the presence and composition of biofilms in eight months old water hoses from a meat processing environment.

First, we used optical coherence tomography (OCT) to directly visualize the biofilms on the inner wall of the water hoses. Next, we determined the bacterial and fungal load and the amount of biofilm matrix components (carbohydrates, proteins, eDNA). We further investigated the biofilm microbiota with 16S rRNA (bacteria) and ITS (fungi) sequencing.

Using OCT, we detected visible biofilms in two water hoses. In contrast, by targeting the microbial load and biofilm matrix components, biofilms were observed in 14 out of 15 tested water hoses. *Mycobacterium* and the fungal genus *Trichoderma* were highly abundant in the biofilms. Bacterial genera associated with meat spoilage such as *Pseudomonas*, unclassified *Microbacteriaceae,* and *Stenotrophomonas* were detected at low abundances. Furthermore, fungal and bacterial genera including opportunistic pathogens (e.g. *Legionella*, *Trichoderma*) were sparsely detected. Significant differences in the beta diversities of bacterial communities between water hoses from the different sampling points were detected. In this study, the biofilms indicate that the water is a potential source for cross-contamination in the food processing environment. Future research is necessary to understand the factors and mechanisms shaping the biofilm and microbial community in water hoses in food processing environments.

Microbiological safety of drinking water is important for public health, also ensuring hygiene in food processing environments and the safety of food products. It is well known that the microbiome of water consists of diverse and complex communities including bacteria, fungi and also protozoa (Proctor and Hammes, 2015). In food production, water is used in agriculture, in the primary production, for cleaning and disinfection of equipment, machines and floors, but also for processing of ingredients or food components and as a direct ingredient in food products. Therefore, water can be in direct and/or indirect contact with food (Bhagwat, 2019; WHO, 2019). Transportation and distribution of water occur via pipes or water hoses. The environmental conditions in water hoses or pipes are characterized by temperature fluctuations, shear stress, nutrient scarcity, the presence of disinfectants or even desiccation when the water supply system is not frequently in use. Microorganisms can adhere to the internal walls of pipes and water hoses and biofilm formation can occur improving the survival and persistence of these microorganisms embedded in the biofilm matrix under the influence of the environmental factors (Liu et al., 2016; Fish et al., 2017). Due to the limited accessibility to the hose interior surface, the biofilm can persist relatively undisturbed over time without cleaning measures disrupting and removing the biofilm. Biofilm presence in drinking water distribution systems, pipelines and reservoirs has been described by numerous studies (Lehtola et al., 2004; Fish et al., 2017; Learbuch et al., 2022;

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Moreno et al., 2024; Søborg et al., 2024; Tai et al., 2025). The microbial communities of biofilms in drinking water distribution systems are a diverse combination of bacteria and fungi with complex microbial interactions of those we only have limited knowledge (Afonso et al., 2019; Fernandes et al., 2019; Li et al., 2023). The phylum Proteobacteria is known to be highly prevalent in biofilms of the drinking water distribution system (Feazel et al., 2009; Proctor & Hammes, 2015; Voglauer et al., 2022). However, heterogeneity of biofilms and variability in bacterial abundances have been observed even on a small scale within a single water hose (Neu et al., 2019). Previous research investigated and demonstrated the presence of biofilms on the last meters of the water distribution system, namely in shower heads (Feazel et al., 2009), shower hoses (Soto-Giron et al., 2016; Proctor et al., 2018), hospital water supply or water hoses in a food processing environment (Voglauer et al., 2022). These studies confirmed the presence of (opportunistic) pathogens such as *Legionella*, *Pseudomonas,* or *Mycobacterium* in biofilms. Furthermore, biofilms on water pipelines shape the microbiome of passing drinking water and are a source for the distribution of antibiotic resistance genes (Zhang et al., 2018; Lin et al., 2025). Biofilms can also act as a reservoir for food spoilage bacteria such as *Microbacterium* or *Pseudomonas*, and after dispersal of these bacteria from mature biofilm, they can be spread through water flow and aerosols (Chan et al., 2019; Voglauer et al., 2022; Li et al., 2023; Alves et al., 2024). Ultimately, this leads not only to a threat for public health and reduction of water quality and safety (Wingender & Flemming, 2011) but also, in the scenario of a food processing environment, the reduction of the quality and shelf-life of food products (Karanth et al., 2023). Microorganisms such as bacteria and fungi present in drinking water system may further negatively impact the taste and odor of drinking water (Zhou et al., 2017; Zhao et al., 2024).

Our knowledge on biofilms and the bacterial and/or fungal microbiota in water hoses of food processing environments is still very limited. We previously investigated biofilms in water hoses and their bacterial community in a meat processing environment (Wagner et al., 2020; Voglauer et al., 2022), showing a frequent presence of biofilms in water hoses.

The aim of this study was to characterize the bacterial and fungal microbiota in water hoses isolated repeatedly from a meat processing facility. We sampled three times eight months old water hoses and performed optical coherence tomography (OCT) to get a visual impression on the inner surface of the hoses. In addition, we characterized the bacterial and fungal load and the biofilm matrix. To get an insight in the microbial diversity of water hoses in the food processing environment, we have explored the bacterial and fungal communities.

Methods

Sampling and study design. Water hoses which were in use in a meat processing facility for eight months were sampled three times.

The water hose samples, which were collected from six different sampling points labelled with numbers from 1 to 5 or *, were replaced after the sampling. Sampling A occurred in October 2021, Sampling B in June 2022, and Sampling C in January 2023 (Supplementary Table 1). Sampling point 5 could only be sampled twice (first and third sampling time points), and sampling point * was only sampled once (second sampling point). The water hoses were in operation daily, except the water hoses from sampling point 3 were only used once a week. After each sampling event, the sampled water hoses were replaced by new water hoses in the food processing facility. There was no cleaning schedule for the water hoses. The material of the water hoses was thermoplastic elastomers and polyester, and the diameter of the hose was 13 mm. All tested water hoses were in use with water in drinking water quality by the food processing operator. For visual inspection and sampling of the inner water hose walls, the water hoses were cut open and prepared for sampling on a length of 30 cm. First, the inner wall of the water hose was analyzed visually to note any deviations from an unused water hose and if there were optical inhomogeneities across the length of the water hose. Then, a sampling of the biomass was conducted with a cell scraper (length: 225 mm, blade width: 20 mm; Carl Roth) and Nylon® flocked swabs (552C, FLOQSwabs, COPAN) (Maes et al., 2017; Wagner et al., 2020). The samples were stored in 10 ml 0.25 × Ringer solution at −20 °C in 50−ml tubes. Negative controls (samples A6, B6, and C6) included the sampling equipment (i.e. swabs, scrapers, tubes) and reagents (Supplementary Table 2). No surfaces were sampled with the sampling tools of the negative controls. Additionally, slices of one centimeter were cut for further inspection using OCT.

OCT. The data presented in this paper were acquired with a home-built ultra-high resolution spectral-domain (UHR SD-) OCT setup. Equipped with a light source with a center wavelength λc around 800 nm and a bandwidth λ in the range of 150 nm, the system provides an axial resolution of less than 2 μm. For quantitative analysis, a refractive index of 1.4 was assumed for the biofilm to rescale the images to true axial dimension. This scaling factor was used to dimension the depth scale bars in all images.

DNA extraction. Processing of samples was done according to a previous study (Wagner et al., 2020). To each sample, 2 g of hydrated cation exchange resin (CER, Amberlite® HPR110, 20–50 mesh, Sigma-Aldrich) was added. Shaking of samples was done for 15 min at 500 rpm. Subsequently, centrifugation of the suspensions was carried out at 3,220 × g for 20 min at 20 °C. The supernatant was filtered through a 0.22−μm filter membrane (Filtropur S0.2, Sarstedt AG & Co KG). The sterile supernatant and the residual pellet were stored at −20 °C until DNA extraction or biochemical characterization of extracellular matrix components. For DNA extraction, the residual pellets were thawed at room temperature, and 5 ml 1 × PBS (Thermo Fisher Scientific) were added to separate the pellets from CER. The samples were mixed by vortex agitation for 1 min, followed by a 2 min break to allow the CER to settle. The supernatant was transferred to a fresh tube, and the procedure was repeated a second time. The bacterial cell pellet was recovered by 5 min centrifugation at 3,220 × g. DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen) according to the manufacturer's instructions with minor modifications. The elution step was carried out two times with 25 μl 70 °C H_2O for molecular biology.

Bacterial and fungal enumeration by qPCR. Bacterial and fungal cell equivalents were determined from all samples by using qPCR targeting the 16S rRNA gene (primers: 5′-CCT ACG GGA GGC AGC AG-3′, 5′-ATT ACC GG GCT GCT GG-3′) and fungal 18S rRNA gene (primers: 5′-GGR AAA CTC ACC AGG TCC AG-3′, 5′-GSW CTA TCC CCA KCA CGA-3′) as previously described (Dixon et al., 2019; Wagner et al., 2020). For a single qPCR reaction of the 16S rRNA gene qPCR approach, a total volume of 20 μl was used including 1 μl DNA template, 1 μl of each primer with a final concentration of 250 nM, 10 μl Brilliant III Ultra-Fast SYBR® Green qPCR master mix, and 7 μl sterile water. Amplification settings were: one cycle at 95 °C for 3 min, 40 cycles at 95 °C for 1 min and 60 °C for 20 s, and creation of a melting curve with one cycle at 95 °C for 1 min, 60 °C for 30 s, and 95 °C for 30 s. The qPCR analysis for 16S showed a high correlation coefficient (R^2 = 0.999) and an efficiency of 96.4%. Bacterial cell equivalents (BCE) were calculated from copy numbers using rrnDB estimating 5.3 16S rRNA gene copy numbers. Copy numbers of negative controls from the DNA extraction kit were removed from the final calculation. For a single qPCR reaction of the fungal 18S rRNA approach, a total volume of 20 μl was used including 1 μl DNA template, 1.2 μl (300 nM) of each primer, 0.8 μl (200 nM) of FungiQuant probe (5′-[FAM]-TGGTGCATGGCCGTT-[MGBQ5]-3′), 0.3 μl Taq polymerase, 0.8 μl (0.8 mM) dNTPs, 1.4 μl (3.5 mM) MgCl2, 2.00 μl of 10x Buffer, and 11.3 μl of sterile water. The following amplification settings were used: one cycle at 94 °C for 2 min followed by 45 cycles at 94 °C for 30 s and 60 °C for 1 min. Fungal cell equivalents (FCE)

were calculated from copy numbers using an estimated 150 18S rRNA gene copy numbers as an average of 150 18S rRNA gene copies per haploid genome occurs in yeast *Saccharomyces cerevisiae* (Kobayashi, 2011). Copy numbers of negative controls from the DNA extraction kit were removed from the final calculation. The qPCR analysis for 18S had a correlation coefficient (R^2) of 0.999 and an efficiency of 96.4%.

Sequencing. Sequencing of the V3-V4 region of the 16S rRNA gene for analysis of the bacterial community and of the ITS2 for analysis of the fungal community was carried out by Microsynth (Balgach, Switzerland). Illumina Nextera two-step PCR libraries were prepared, and paired-end sequencing (2 x 250 bp) was performed on an Illumina MiSeq platform.

Bioinformatics. Reads from 16S rRNA and ITS sequencing were already demultiplexed, and adapters had been removed by the sequencing company. The dataset used in this study can be found online in the NCBI data repository under the BioProject number PRJNA1274208 https://www.ncbi.nlm.nih.gov/. We performed a quality check of the reads received using FastQC v0.11.9 (Andrews, 2010) and MultiQC v1.0 (Ewels et al., 2016). Residual adapters were removed with Trimmomatic v0.39 (Bolger et al., 2014). Quality filtering of reads and trimming were performed with DADA2 (Callahan et al., 2016) implemented in QIIME2 v2024.5 (Bolyen et al., 2019). Taxonomy classification was done with the q2-feature-classifier plugin (Bokulich et al., 2018) using the SILVA 138.1 SSU-NR99 database (Quast et al., 2013) for the 16S rRNA data set and the UNITE v10 (99% similarity threshold level) database (Kõljalg et al., 2020; Abarenkov et al., 2024) for the ITS data set. Contaminant amplicon sequence variants (ASVs) were filtered from the data sets using the "isNotContaminant" function in the R package decontam v1.20.0 (Davis et al., 2018) using negative control samples (NC_A, NC_B and NC_C) for identification of contaminants. Subsequently, ASVs with 0 remaining counts were removed. Samples with a lower sampling depth of 1000 were removed from the 16S rRNA data set, and samples with a lower sampling depth of 800 were removed from the ITS data set. Removal steps and filtering steps resulted in the removal of 5 water hose samples from the 16S rRNA data set and 7 water hose samples from the ITS data set. A phylogenetic tree was created with the data from the 16S rRNA data set using mafft (Katoh et al., 2002) and fasttree2 (Price et al., 2010) in QIIME2. Both ASV tables (from the 16S rRNA and ITS data sets) were explored in R v4.4.1 using the packages phyloseq v1.48.0 (McMurdie & Holmes, 2013), dplyr v1.1.4 (Wickham et al., 2025), and tidyverse v2.0.0 (Wickham et al., 2019). Both data sets were rarefied to the lowest sampling depth (16S rRNA data set: 3829; ITS data set: 834) for alpha and beta diversity analysis. Alpha diversity indices (Observed, Chao1, Shannon, Simpson, InvSimpson, ACE, and Fisher's alpha) were analyzed using the R package vegan v2.6–8 (Oksanen et al., 2022). Beta diversity was visualized with the R package tsnemicrobiota v0.1.0 (Lindstrom, 2023) using the Bray-Curtis and a t-Distributed Stochastic Neighbor Embedding (t-SNE). Figures were created with ggplot2 v3.5.1 (Wickham, 2016) and ggpubr v0.6.0 (Kassambara, 2023).

Analysis of biofilm matrix components. Detection of carbohydrates. Evaporation of a 1 ml aliquot of each sample was performed for 1 h at 90 °C shaking at 300 rpm to concentrate carbohydrates in the sample solution. Carbohydrates were detected by applying a phenol–sulfuric acid method (Masuko et al., 2005). The absorbance (and color change as a result of the detection method) was measured at 490 nm with a plate reader (Biotek Synergy H1). Glucose was used as an internal standard for calculating a standard curve. The limit of blank (LOB) was 154 ng/50 µl.

Detection of extracellular DNA (eDNA). Precipitation of eDNA was carried out using an ethanol precipitation method according to Zetzmann et al. (2015). To each sample aliquot (500 µl), 0.1 × 3 M Na-Acetate (pH 5.2), 0.1 × 0.1 $MgCl_2$, and 2.5 × ice-cold ethanol absolute were added. Samples were vortex agitated, followed by a 24 h incubation period at −20 °C to precipitate the eDNA. Subsequently, the pellet was recovered by centrifugation at 20,817 × g for 15 min at 4 °C. The supernatant was removed by pipetting, and each pellet was washed with 1 × (of initial sample volume) 70% ethanol. Centrifugation was repeated a second time, the supernatant was removed, and the pellet was resuspended in 30 µl sterile H_2O. Samples were agitated for 10 min in a vortex adapter and briefly spined down. The concentration of eDNA was measured with a Qubit fluorometer using the Qubit dsDNA BR kit. The limit of blank (LOB) was 0.2 ng/µl.

Detection of proteins. First, proteins were precipitated at 4 °C overnight with 0.1 × (of sample volume) TCA/Acetone (1 g/ml) and 0.01 × 2% sodium deoxycholate (Rychli et al., 2016), followed by a centrifugation step at 20,817 × g at 4 °C for 30 min. The supernatant was carefully removed, and the pellet was washed with ice-cold acetone. The centrifugation step was repeated, and the supernatant was removed again. The samples were incubated with an open lid for 3 h at room temperature under a laboratory hood to air-dry the pellets. Then, the pellets were dissolved in 25 µl 50 mM Tris-HCl by flicking the tube, followed by a 3 h incubation period on ice. The concentration of proteins in the samples was measured with the Quant-iT protein assay kit (Invitrogen) according to the manufacturer's instructions with minor modifications. A spike solution was prepared with bovine serum albumin (final concentration: 1.25 ng/µl) and used to elevate the protein concentration in samples to overcome the detection limit of the protein quantification kit. The signal from the spike solution was measured in the same run, and the signal was subtracted from the signal of spiked true samples. The limit of blank (LOB) was 404 ng.

Statistics. The concentration of matrix components and bacteria or fungal cell equivalents for each water hose sample was calculated per cm^2 of the tested surface area (122.52 cm^2) (Supplementary Table 1). The concentration in negative controls was not adjusted to a certain surface area, since no surface was sampled (Supplementary Table 2). The bacterial or fungal load in negative controls was subtracted from the values of water hose samples before calculating the final BCE/cm^2 or FCE/cm^2. Data sets were tested for normality using the Shapiro-Wilk test (R function "shapiro.test"), and homogeneity of variances were tested using Levene's test (R function "leveneTest"). For calculating the statistical significance of alpha diversity indices between water hoses from the different sampling points, a Kruskal-Wallis test (R function "kruskal.test") was applied. A permutational analysis of variance (PERMANOVA) with 5,000 permutations was calculated using the "adonis2" function in the R package vegan 2.6–8 (Oksanen et al., 2022) to assess statistically significant ($p < 0.05$) dissimilarities between the communities. A two-sided t test (R function "t.test") or Mann-Whitney *U* test (R function "wilcox.test", two-sided), depending on the normality of the data, was applied for assessing statistical differences in BCE/cm^2, carbohydrates, proteins, and extracellular DNA between water hoses that were used daily or weekly (Supplementary Table 3).

Results

This study explored the occurrence of biofilms in eight months old water hoses isolated three times from a meat processing environment ($n = 15$). In addition, the bacterial and fungal communities in water hoses and their biofilms were investigated using 16S rRNA and ITS sequencing.

OCT measurements. OCT could only visualize biofilms in two hoses (B5, C3) with approximately 25 µm thickness (Fig. 1, Supplementary Fig. 1). Additionally, we could detect fibers sticking out of the hose in hoses C2 and C3 (Fig. 1B, Supplementary Fig. 1).

Biofilms in water hoses. For biochemical biofilm detection, the criteria were defined as the presence of bacteria and/or fungi, confirmed by 16S/18S rRNA qPCR, and the presence of at least two extracellular matrix components (Wagner et al., 2020). Bacteria were detected in all water hose samples Figure 2. The bacterial load ranged

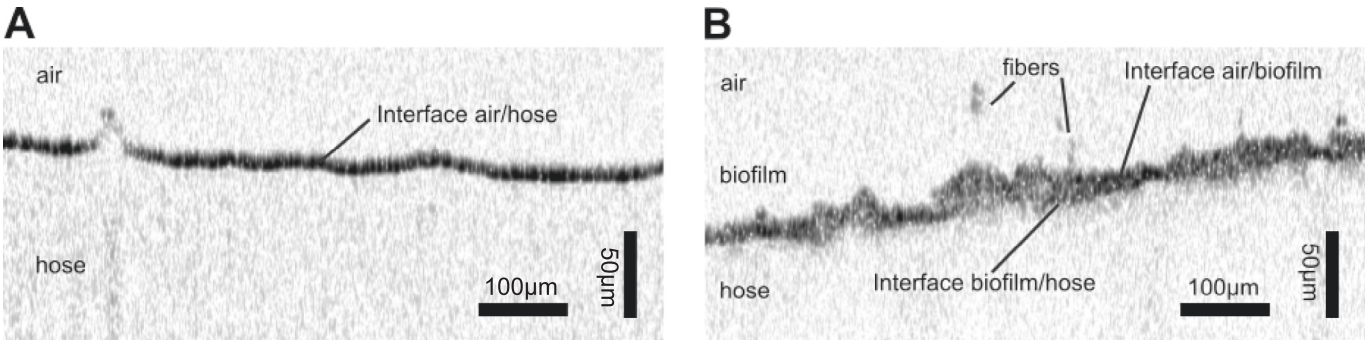

Figure 1. Representative OCT images of the inner surfaces of water hoses. The scalebar for thickness is corrected for a refractive index of $n = 1.4$, to relate to the thickness of the biofilm. A = sample C1 (no visible biofilm). B = sample C3 (visible biofilm).

from 0.93 to 5.93 $\log_{10}$ BCE/cm^2 (Supplementary Table 1). The lowest bacterial load was detected in the water hose C1 from sampling location 1, collected during the third sampling visit. The highest bacterial load was found in the water hose C3 from sampling location 3, which showed a visible biofilm using OCT. We could detect FCE in all samples, except in sample B2 Figure 2. The quantity of fungal cells ranged from 0.05 $\log_{10}$ 1 + FCE/cm^2 in water hose C1 to 4.32 $\log_{10}$ 1 + FCE/cm^2 in water hose C3 (Supplementary Table 1). BCE and FCE were also detected at low numbers in the negative controls.

Carbohydrates, measured as glucose equivalents, as part of the extracellular matrix components, were detected in all water hoses. Carbohydrate concentration ranged from 133.63 ng/cm^2 (water hose B2) to 9700.08 ng/cm^2 (water hose C5). We further evaluated the presence of two more biofilm matrix components – proteins and eDNA. Proteins were detected in 10 water hoses (A2, A3, B1, B2, B4, B*, C1, C3, C4 and C5), and their concentration ranged from 40.03 ng/cm^2 (water hose B1) to 1458.92 ng/cm^2 (water hose B*). eDNA was present in samples from 11 water hoses (A1, A2, A3, A4, B1, B3, B*, C1, C2, C3, and C5). The concentration of eDNA ranged from 203.44 ng/cm^2 in water hose C2 to 11875.61 ng/cm^2 in water hose B*. We also detected carbohydrates and proteins once at low levels in the negative controls. The sampling time point did not significantly ($p < 0.05$) impact the bacterial load ($p = 0.4317$), the fungal load ($p = 0.1637$), carbohydrate concentrations ($p = 0.3296$), protein concentrations ($p = 0.5268$), and eDNA concentration ($p = 0.6396$). Further, no significant differences were detected in the bacterial loads ($p = 0.247$), fungal loads ($p = 0.1211$), carbohydrate concentrations ($p = 0.5984$), protein concentrations ($p = 0.483$), or eDNA concentrations ($p = 0.257$) between water hoses from the different sampling locations. In addition, there were no significant differences in the bacterial loads ($p = 0.1704$), fungal loads ($p = 0.1011$), carbohydrate concentrations ($p = 0.4484$), protein concentrations ($p = 1.0000$), or eDNA concentrations ($p = 0.497$) between water hoses used daily or weekly.

Combining the results of bacterial presence and biofilm matrix component analyses, we detected biofilms in 14 out of 15 (93.3%) samples (Figure 2). Only the water hose from sampling location 5, collected during the first sampling event, did not harbor a detectable biofilm.

Bacterial communities in water hoses. In a first step, we investigated the composition of the bacterial community in 11 water hoses across all samples (Fig. 3). Due to a low number of reads and low sampling depth, we had to exclude four water hose samples (samples B2, B*, C1, and C2). The five most abundant bacterial genera across all samples were *Mycobacterium* (median relative abundance: 11.6%), unclassified *Comamonadaceae* (median relative abundance: 2.23%), unclassified *Rhodobacteraceae* (median relative abundance: 1.40%), *Rhodococcus* (median relative abundance: 1.33%), and *Ketobacter* (median relative abundance: 1.26%). Notably, ASVs assigned to the genus

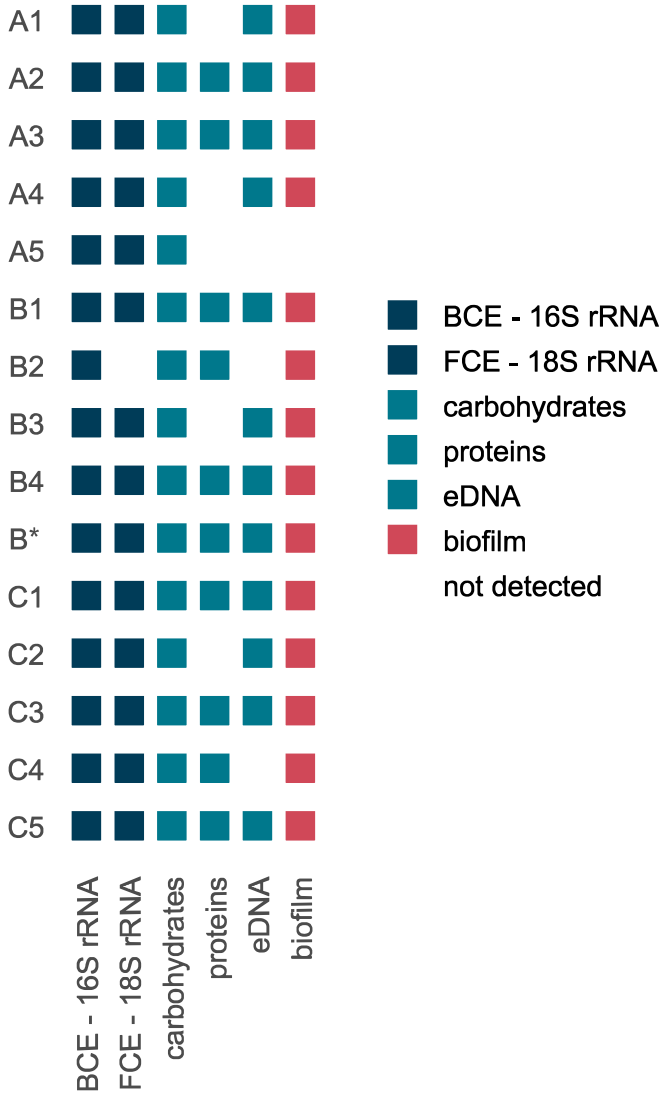

Figure 2. Biofilms in water hoses. The presence of bacteria (BCE – 16S rRNA), fungi (FCE – 18S rRNA), and the biofilm matrix components carbohydrates, proteins, and eDNA in samples from water hoses (A1-C5) is shown. If bacteria/fungi and at least two matrix components were detected, the samples were considered as biofilm positive.

Legionella (23rd most present genus) were detected in all water hoses. Grouping the water hoses from different sampling timepoints by their respective sampling points, the relative abundances of bacterial genera

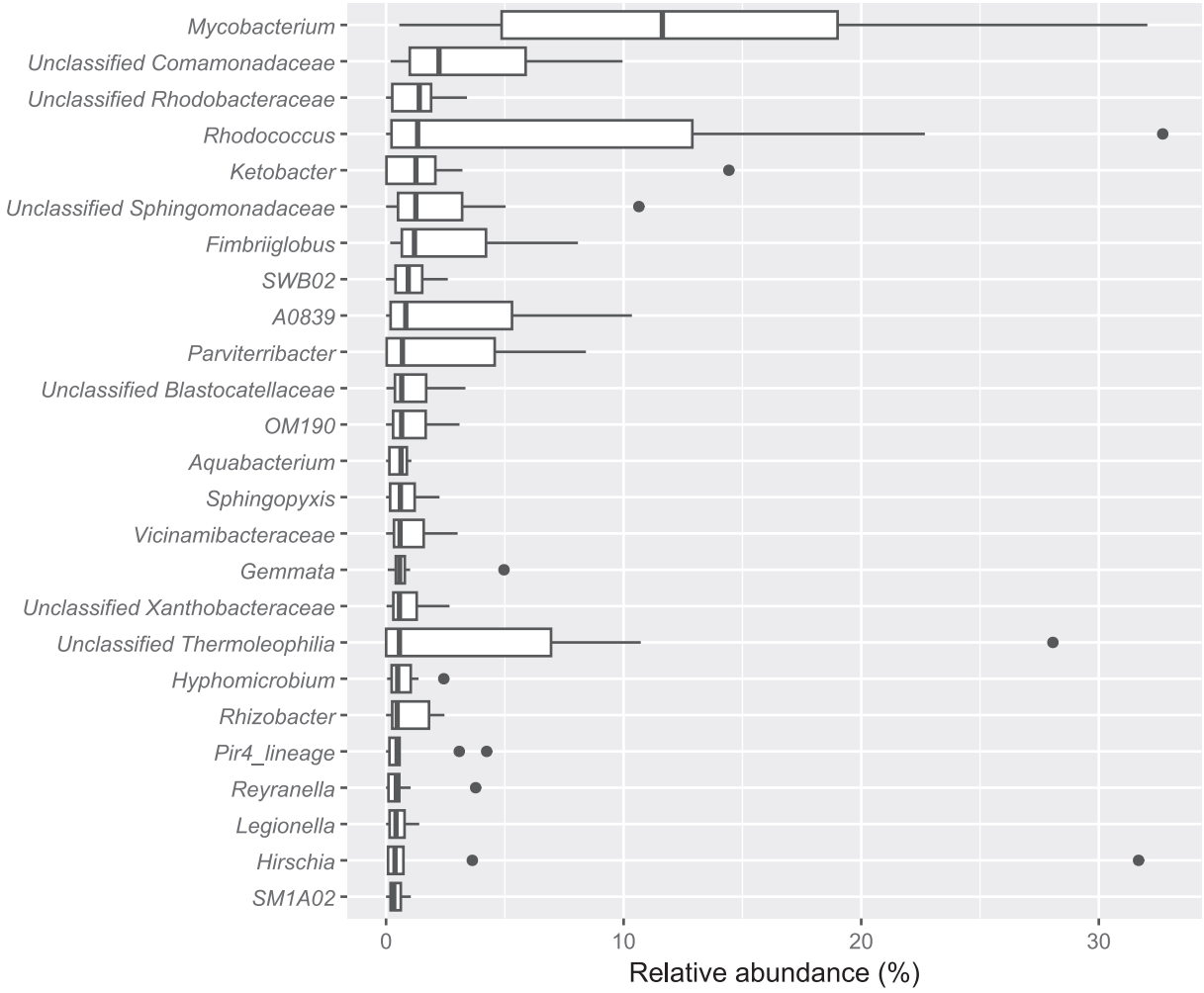

Figure 3. Top 25 bacterial genera. Boxplot showing the 25 most abundant bacterial genera (decreasingly ordered by median abundance) across all water hose samples.

varied partly by sampling point and partly by individual water hoses (Fig. 4). For example, *Rhodococcus* was highly prevalent in water hoses A5 and C5 (sampling point 5, timepoints A and C), but also high in abundance in water hose B1 (sampling point 1, timepoint B). *Nocardia* was highly abundant in water hose B4 (sampling point 4, timepoint B) and less prevalent in other samples. *Pseudomonas* was present mainly in water hose A5 and was not detected in water hoses A3, B1, and C4. In contrast, *Mycobacterium* was present in all water hose samples. We observed differences in the abundance of bacteria on the genus level between the water hoses (Fig. 4).

Microbial diversity from water hoses grouped by sampling points 1 – 5 was assessed with alpha diversity indices Observed ($p = 0.057$), Chao1 ($p = 0.060$), ACE ($p = 0.077$), Shannon ($p = 0.060$), Simpson ($p = 0.089$), InvSimpson ($p = 0.089$), and Fisher ($p = 0.057$) (Supplementary Fig. 2). No statistically significant differences were detected (Kruskal-Wallis test) between alpha diversities of the different sampling points. By trend, the highest richness was observed for water hoses from sampling point 3 (Supplementary Fig. 2). In addition, the bacterial community dissimilarity was statistically assessed by calculating a permutational multivariate analysis of variance (PERMANOVA). The bacterial communities were significantly ($p < 0.000$) different in the water hoses by beta-diversity analysis (Supplementary Fig. 3) e.g. the water hose from site 5 showed a higher abundance of Rhodococcus (Fig. 4).

Fungal communities in water hoses. The fungal community of only 9 water hoses was explored using ITS sequencing data, as 6 water hose samples were excluded due to a low number of reads. The diversity and richness of fungal genera were low. In total, four genera could be identified and two identifications on the family level were possible, while some fungal ASVs could not be classified ("Unclassified Fungi") (Fig. 5). The water hoses harbored the genera *Trichoderma*, *Sistotrema*, *Polyschema*, and *Asterostroma*. In addition, the families unclassified *Ascomycota* and *Acarosporaceae* were detected. For the detected *Acarosporaceae* ASVs, there was an uncertainty in the classification on the genus level indicated by "genus *incertae sedis*". In water hose C1 only, *Trichoderma* was detected, which was present in all samples except water hose B4. *Asterostoma* was found on sampling points 2, 3, and 4 but not at all sampling timepoints. *Polyschema* was absent in water hoses from sampling points 1 and 2 and present in several samples (but not all) from sampling points 3, 4, and 5. *Sistotrema* was only found in sample B4. Unclassified *Ascomycota* were detected in water hoses from sampling points 4 (except in water hose A4) and 5. Samples A3, B3 (sampling point 3), and sample B4 (sampling point 4) harbored additionally *Acarosporaceae*.

Discussion

Biofilms in water hoses can compromise water safety and in consequence impact hygiene standards in food processing facilities, where water hoses are typically used for equipment cleaning and sanitation of the processing environment, or as a water source for food production (Wingender & Flemming, 2004, 2011; Bhagwat, 2019).

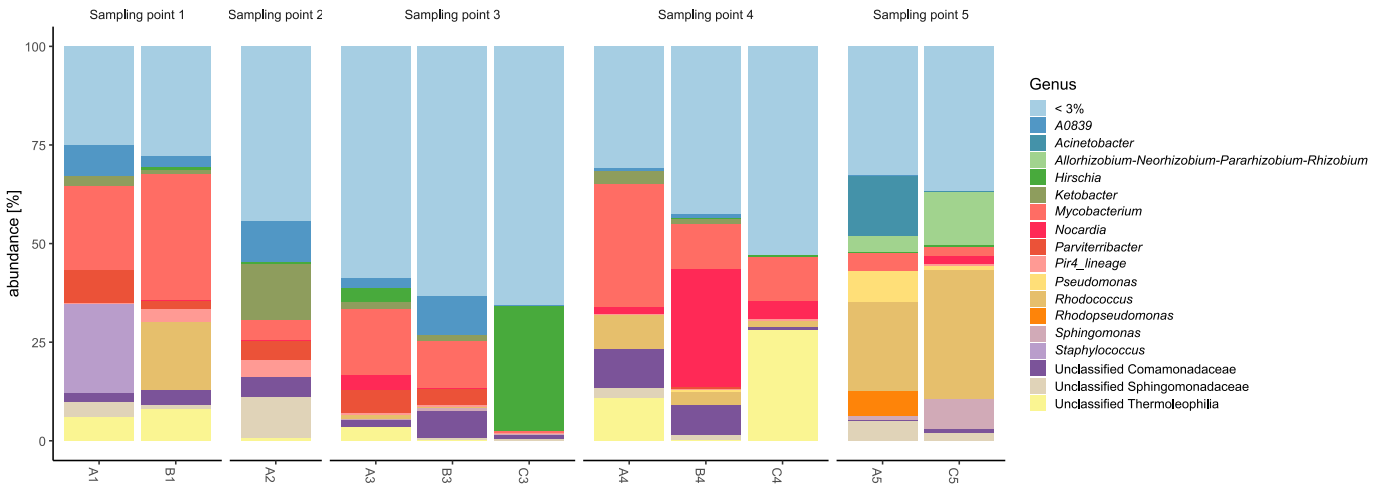

Figure 4. Abundance of bacteria on genus level. The relative abundance (%) of bacteria in samples collected from different sampling points is displayed in the bar chart in different colors.

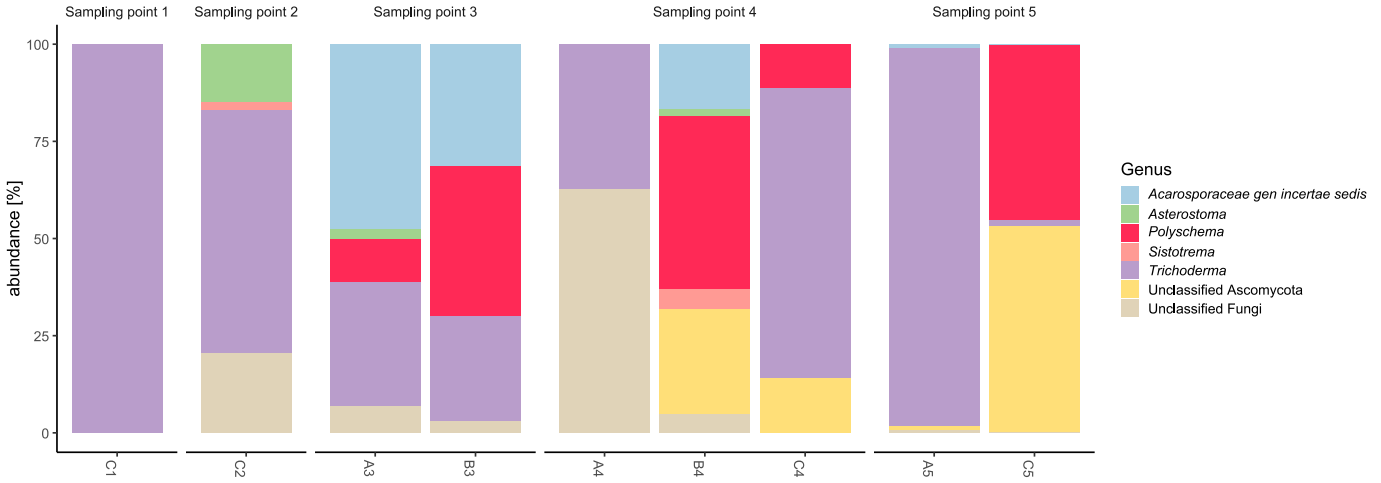

Figure 5. Abundance of fungi on genus level. The relative abundance (%) of fungi in samples collected from different sampling points is displayed in the bar chart in different colors.

In the current study, biofilms were detected in 14 out of 15 sampled water hoses, which is similar to a previous study where 100% of water hoses in a food processing facility tested positive for biofilms (Voglauer et al., 2022). By trend, we observed a lower bacterial load (0.93 $\log_{10}$ BCE/cm^2 to 5.93 $\log_{10}$ BCE/cm^2) in the tested water hoses than in one of our previous studies, where we reported a minimum bacterial load of 6.6 log BCE/cm^2 (Voglauer et al., 2022). A second study exploring biofilms in shower hoses found bacterial loads ranging from 4.6 to 8.8 log cells/cm^2 (Proctor et al., 2018). In this study, the bacterial load and concentration of biofilm matrix components varied between the different water hoses, indicating a diversity in the composition of the biofilm or the buildup, despite the similar age of the water hoses (8 months in operation). The duration of use of the water hoses did not correlate with the bacterial load and biofilm mass of samples in earlier studies (Wingender & Flemming, 2004; Proctor et al., 2018), which is supported by the fact that BCE/FCE and the biofilm structure were different in similarly aged samples of this study. Moreover, we could exclude that differences in the water source, hose material, or the temperature are the reason for biofilm diversity in the sampled hoses, since the water source and the material of the water hoses were the same for all sampled hoses, as well as the room temperature was very similar (10–12 °C). All water hoses were in use daily, except for water hoses from sampling point 3, which were only used once in a week. Interestingly, the amount of BCE/cm^2 and concentrations of matrix components in water hoses from sampling point 3 did not differ significantly from those that were in use daily. One further factor could be differences in the water pressure when the hoses were in operation. The water pressure was regulated individually based on the demand at the time of use; however, no exact data on this factor exist for this study.

The most abundant genera in the microbial community across all water hose samples included the genera *Mycobacterium* and *Rhodococcus* and the families *Comamonadaceae* and *Rhodobacteraceae*. Their members are widely distributed in the environment and are commonly found in soil and aquatic environments including fresh water and marine environments (Norton et al., 2004; Miller et al., 2007; Grenni et al., 2009; Gomila et al., 2010; Li and Zhou, 2015; Dogs et al., 2017; Deja-Sikora et al., 2019; Zhang et al., 2022; Lv et al., 2023; LeChevallier et al., 2024), and some members are known for their bioremediation and biotransformation abilities (Ge et al., 2015; Ivshina et al., 2022; Huang et al., 2024). *Mycobacterium* was the most abundant genus across all cumulated samples, similar to previous studies focusing on household showerhead biofilms (Feazel et al., 2009) and biofilms in hospital shower hoses (Soto-Giron et al., 2016). Previous studies con-

ducted under laboratory settings showed that *Mycobacterium* is able to build biofilms (Steed & Falkinham, 2006) but also to colonize established biofilms (Torvinen et al., 2007). The genus *Mycobacterium* includes opportunistic pathogens such as *M. avium*, which is frequently mentioned as a potential health risk in water sources (Whiley et al., 2012; Lande et al., 2019; Sousa et al., 2021). The microbiota in the water hose biofilms also harbored other opportunistic pathogens at low abundances such as *Legionella*, *Pseudomonas,* and *Neochlamydia,* which is in concordance with a study exploring biofilms in water hoses in a meat processing facility (Voglauer et al., 2022). Interestingly, *Legionella* were present in all water hoses except sample A5, while *Pseudomonas* was present in higher abundances in sample A5 and was less prevalent or absent in the other water hoses. A study of biofilm in shower hoses similarly observed that *Pseudomonas* rather cooccurred with *Mycobacterium* than with *Legionella*. A relationship between the total cell count and prevalence of certain genera demonstrated that *Pseudomonas* and *Mycobacterium* correlated with a lower total cell count and *Legionella* with higher cell counts (Proctor et al., 2018). However, water hose A5 did not have a particularly low or high amount of BCE in the current study, but we did not detect a biofilm in that sample. The absence of a biofilm may explain the absence of *Legionella* in water hose A5, as *Legionella* preferably colonized the bottom of a biofilm, providing protection against environmental factors, as shown in a laboratory study (Silva et al., 2024). We further investigated the presence of known meat spoilage bacteria, as their distribution would pose a potential risk for hygiene and the shelf-life of the products in the processing environment. *Pseudomonas*, unclassified *Microbacteriaceae*, *Stenotrophomonas*, *Enterococcus*, unclassified *Acetobacteraceae*, and *Stenotrophomonas* were present in some of the water hoses in mostly low to very low abundances. Other key players in meat spoilage such as *Brochothrix*, *Carnobacterium*, *Lactobacillus*, *Leuconostoc*, and *Weissella* (Nychas et al., 2008; Doulgeraki et al., 2012) were not detected. Comparing the prevalent genera of this study to the results of a previous study exploring the microbiota in biofilms from water hoses in a meat processing facility (Voglauer et al., 2022), we noticed several similarities. The families *Comamonadaceae*, *Sphingomonadaceae, Gemmataceae, Xanthobacteraceae*, and the genera *Aquabacterium*, *Legionella*, and *Reyranella* were prevalent in both studies. Nevertheless, there were still many differences in the detected genera and their abundance. Despite being connected to the same water supply and very similar environmental conditions at the different sampling points, dissimilarities in the microbial community were also observed among water hose samples of the current study. The microbial communities especially differed in samples from sampling point 3 and sampling point 5. Differences in water hoses from sampling point 3 could arise from being in operation only once per week compared to daily usage of the other water hoses, as desiccation or differences in shear stress may affect the microbial composition (Štovíček et al., 2017; Chen et al., 2023; Rožman et al., 2023). Differences in the water pressure when the water hoses were in operation may further influence the bacterial composition.

The presence of fungi was previously reported in studies on biofilms in the water distribution system including pipes (Siqueira et al., 2013) and shower hoses (Moat et al., 2016) or in drinking water (Al-gabr et al., 2014; Fernandes et al., 2019; Afonso et al., 2021). The fungal load in the biofilms was, by trend, lower than the bacterial load and ranged from 0 to 4.32 log FCE/cm^2 in the current study. Similar to another study (Moreno et al., 2024), many water hose samples generated a very low number of ITS reads and a low sampling depth after contamination removal (<800) and were excluded from further analysis. Bacteria were further identified as the main component of biofilms in a drinking water distribution system (Douterelo et al., 2018). The fungal community showed low diversity in all water hoses, especially in water hose C1, where only *Trichoderma* was detected. *Trichoderma* was absent in water hose B4 at sampling point 4, but prevalent in water hoses from the other sampling points. In previous research, *Trichoderma* was detected in surface water (i.e. rivers and lakes) and tap water (Al-gabr et al., 2014), in ground and surface water (Oliveira et al., 2013), or in a water system in hospitals (Warris et al., 2001; Pires-Gonçalves et al., 2008). While the involvement of *Trichoderma* in biofilms in coculture with *Azotobacter* was observed in the laboratory (Velmourougane et al., 2019), the inhibition of biofilm formation and bacterial growth in coculture scenarios was also observed (Santos et al., 2018; Velázquez-Moreno et al., 2023), underlining the complex bacterial-fungal interactions. Notably, *Trichoderma* has been involved in cases of human infections, especially of hospital patients (Román-Soto et al., 2019; Sal et al., 2022; dos Santos & dos Santos, 2023), implying a potential health risk for immunocompromised individuals. Other fungi were present in only a part of tested water hoses such as *Polyschema*, previously found in wetland soil (Xiao et al., 2025), and *Sistotrema*, involved in wood-decaying (Held & Blanchette, 2017; Gołębiewski et al., 2019; Cai & Zhao, 2023) and previously detected also in subseafloor samples (Navarri et al., 2016), human clinical samples such as in breast milk (Boix-Amorós et al., 2019) or in the eye as the causative agent of fungal keratitis (Chen et al., 2024). The fungal genus *Asterostroma* was reported as a wood-decaying/wood-inhabiting saprotroph detected in forests (Suhara et al., 2010; Deng et al., 2024), while *Acarosporaceae* were previously detected in different environmental habitats including arid habitats (Leavitt et al., 2018; Knudsen et al., 2025) or calcareous rock (Knudsen et al., 2023). Notably, *Acarosporaceae* were mainly present in water hoses from sampling point 3, which were only used once in a week and were therefore a drier environment than the other water hoses. Nevertheless, *Acarosporaceae* were also found in one water hose from sampling point 4 which was in use daily. Our data set included two further unclassified ASVs from the kingdom fungi and one from the phylum Ascomycota. The latter was previously detected in biofilms and water of groundwater supplied systems (Douterelo et al., 2018) and shower hose biofilms (Moat et al., 2016), highlighting the need for closing the huge research gap on fungi in diverse environmental niches. From our results, we could not conclude that fungal genera (except for *Trichoderma*) typically found in drinking water and associated supply systems (Oliveira et al., 2013; Afonso et al., 2021) such as *Aspergillus*, *Penicillium*, *Cladosporium,* or *Fusarium* were present.

The imaging of the water hoses done by OCT could only confirm the presence of biofilms in two cases. OCT is frequently used for biofilm imaging within the last years (Wagner & Horn, 2017). Even though it was not possible to detect biofilms on every hose with this technique, we could see fibers within the hose's inner surface, possible contributing to further biofilm growth. Further, it must be mentioned that not exactly the same site of the hose was visualized using OCT and sampled for biochemical evaluation of possible biofilms. OCT was able to detect fast thick layers of biofilms, while the biochemical detection method is more sensitive. Overall, we could show that OCT is suitable for detecting biofilms in water hoses.

In conclusion, we detected biofilms in 14 out of 15 water hoses being in use for eight months, highlighting that water hoses are an ideal environment for biofilm formation without significant forces disrupting the biofilm over time. The biofilms were heterogeneous despite the use of the same type of hose material, the same water source, and exposure to the same temperatures. Factors potentially explaining differences in the bacterial and fungal community could be the less frequent usage of water hoses from sampling point 3 and differences in the water pressure. Nevertheless, future research is required to understand the variables influencing the diversity in biofilm structure and community composition in water hoses. The biofilms harbored opportunistic bacterial and fungal pathogens and meat spoilage bacteria, though mostly in low abundance, indicating still a potential risk to food production via contamination of the processing environment through water flow and aerosols.

CRediT authorship contribution statement

Nadja Pracser: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Eva M. Voglauer:** Writing – review & editing, Visualization, Project administration, Methodology, Data curation, Conceptualization. **Sarah Thalguter:** Writing – review & editing, Validation, Methodology, Investigation. **Elisabeth Leiss-Holzinger:** Methodology, Investigation. **Andreas Zaiser:** Methodology, Investigation. **Martin Wagner:** Writing – review & editing, Supervision, Funding acquisition. **Kathrin Rychli:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

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

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Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.jfp.2025.100638.

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