Research article

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The impact of different hop compounds on the growth of selected beer spoilage bacteria in beer

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Beer spoiling lactic acid bacteria are a major reason for quality complaints in breweries around the world. Spoilage by a variety of these bacteria can result in haze, sediment, slime, off-flavours and acidity. As these bacteria occur frequently in the brewing environment, using certain hop products that inhibit the growth of these spoilers could be a solution to prevent problems. To investigate the impact of seven different hop compounds (α-acids, *iso*-α-acids, tetrahydro-*iso*-α-acids, rho-*iso*-α-acids, xanthohumol, *iso*-xanthohumol and humulinones) on the growth of six major beer spoilage bacteria (*Lactobacillus brevis. L. backi, L. coryniformis, L. lindneri, L. buchneri, Pediococcus damnosous*), two concentrations (10 and 25 mg/L) of each hop substance were added to unhopped beer. The potential growth of the spoilage bacteria was investigated over 56 consecutive days. A comparison of the results shows a strong inhibition of growth of all spoilage bacteria at 25 mg/L of tetrahydro-*iso*-α-acids closely followed by α-acids as the second most inhibitory substance. The results showed a high resistance of *L. brevis* to all hop compounds as well as an inhibition of *L. coryniformis* and *L. buchneri* at low concentrations of most hop components. In comparison with the control sample, *L. lindneri* showed increased growth in the presence of some hop compounds (rho-*iso*-α-acids, xanthohumol, *iso*-xanthohumol, humulinones). © 2020 The Authors. Journal of the Institute of Brewing published by John Wiley & Sons Ltd on behalf of The Institute of Brewing & Distilling

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Introduction

Hops (*Humulus lupulus*) are one of the microbiologically most important ingredients of beer *(1,2)*. Due to hop components that act as antibacterial agents, along with ethanol, CO_2, a low pH value and low nutritional status, beer is broadly stable with regard to microbiological spoilage *(3–6)*. However, spoilage by specially adapted beer spoilage bacteria is, to the regret of brewers, still a major reason for consumer complaints and/or discarded batches resulting in financial and production losses as well as reputational damage *(7)*. Beer spoilage bacteria, besides some gram-negative anaerobic species (*Megasphaera* spp., *Pectinatus* spp.), are mostly gram-positive lactic acid bacteria (LAB) *(4)* that have adapted to the harsh beer environment and to bittering hop compounds *(5)*. In the worst case, LAB spoilage results in slime formation, sediment, sour and hazy beer and/or off-flavours such as diacetyl *(6,8)*.

Adaptation to hop compounds is variable among LAB species and strains and can increase over time *(5,8)*. Beer spoilage bacteria are therefore categorised by their spoilage potential (obligate, potential and indirect beer spoilage bacteria) and where they appear in the brewing process (as primary or secondary contaminants). Obligate beer spoilage bacteria will cause spoilage in almost all beers, potential beer spoilage bacteria can cause spoilage under certain circumstances such as low concentrations of bittering substances and/or ethanol. Indirect beer spoilage bacteria can cause spoilage during the production process but cannot survive in beer (e.g. cold wort contamination) *(6)*. Primary and secondary contaminants refer to the stage in which the beer is contaminated, 'primary' during the production process and 'secondary' referring to contamination during packaging *(4,6)*.

Hop resistant LAB strains can pass on the ability to grow in the presence of hop compounds to hop-sensitive LAB strains. They do so via the horizontal transfer of plasmid encoded genes without being restricted to affiliated species *(9)*. Genes *horA* and *horC* are responsible for hop tolerance *(5,10)*. The presence of

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these genes has been found to correlate with the ability of LAB to spoil beer *(11)*. The release of plasmids (containing genes that support microbial fitness) is increased. Therefore, antimicrobial proprerties of beer promote plasmid excretion of genes containing hop resistance. Due to several factors (biofilm formation, occurrence, different contamination sources, etc.) presence of hop resistant strains in a brewery is always dangerous and almost unpreventable *(8)*. However, this means that, if found in a brewing environment, LAB should always be checked for the presence of these genes to establish the potential risk of spoilage *(8,10)*.

To inhibit the growth of certain spoilage bacteria, different hop products can be used in brewing and other food applications *(1,12,13)*. Kaltner et al. investigated α-acids (humulones), *iso*-α-acids, tetrahydro-*iso*-α-acids, rho-*iso*-α-acids and a combination of hexahydro-*iso*-α-acids and tetrahydro-*iso*-α-acids (ratio 30:50) for their inhibitory impact on the growth of *Lactobacillus brevis*, *L. lindneri* and *Pediococcus damnosus* at concentrations of 9.2-10.5 mg/L in beer *(12)*. It was reported that the most efficient hop compounds were α-acids followed by hexahydro-, tetrahydro-, rho- and *iso*-α-acids *(12)*. Buggey et al. *(14)* tested various hop components as well as their isomers against *L. brevis* at varying pH values. The results of this study showed reduced and non-isomerised hop acids had greater inhibitory effects than *iso*-α-acids, the main contributor to beer's bitterness *(14)*. In the production of beer, the main differences of products containing these compounds is their use for bitter sensory impression, microbiological inhibition, foam stability, light stability and, importantly, solubility in wort and/or beer *(1,15–17)*. The main focus in this study was microbiological inhibition, so there is no further consideration of technological applications or sensory aspects.

As reported in a recent study by Schneiderbanger et al., *L. brevis* is the most frequently found beer spoilage bacteria of all the LAB, occurring primarily in top but also bottom fermented beers *(7)*. *L. brevis* is categorised as an obligate beer spoilage bacterium as it is mostly found to be highly hop tolerant, appearing as a primary and secondary contaminant and causing haze, acidity and occasionally slime *(7,8)*. As regards its occurrence, *L. backi* (occurs proportionally almost the same in top and bottom fermented beers) is also found in many contamination incidents, during all stages of production, along with *L. lindneri* and *Pediococcus damnosus* (both mostly found in bottom fermented beers) *(7,8)*. In contrast to all other beer spoilage LAB, hop resistance was found for all *L. lindneri* strains reported to date *(18)*. Due to their hop sensitivity, *L. buchneri* and *L. coryniformis* are categorised as potential beer spoilage bacteria and predominantly found in low hopped bottom fermented beers *(7)*.

As these bacteria have been a major cause of spoilage in various beer types in recent years, this study aims to show the inhibitory impact of various hop compounds at two different concentrations on bacterial growth. In addition to those compounds previously investigated *(12,14)*, the effect of xanthohumol, *iso*-xanthohumol and humulinones was tested. To our knowledge, there has not been a study on the impact of xanthohumol, *iso*-xanthohumol and humulinones on the inhibition of beer spoilage bacteria. However, a study performed by Srinivasan et al. using xanthohumol, has shown good inhibition of the growth of protozoa *(19)*, gram-positive *Propionibacteria* and *Staphylococcus (20)*. Due to the growing interest in dry hopping, humulinones (oxidised α-acids) have also become more important due to their impact on taste. It has been reported that they are 66% as bitter as *iso*-α-acids *(21)*.

This study intends to increase the knowledge of inhibitory effects of hop compounds on the growth of frequently occurring LAB beer spoilers with differing spoilage potential and occurrence during beer production.

Materials and methods

Bacteria

All bacterial strains were stored at -80°C in the cryo stock of the Research Center Weihenstephan for Brewing and Food Quality of the TU München (Table 1).

Hop compounds

All hop compounds used in this study were provided by Simon H. Steiner, Hopfen GmbH, (Mainburg, Germany) and used as standardised extracts (Table 2). For reasons of taste, solubility and hopping technology, a concentration of 25 mg/L of tetrahydro-*iso*-α-acids, xanthohumol, *iso*-xanthohumol and humulinones is rarely found in beer. However, for better comparability between the substances tested, 10 and 25 mg/L was used in each trial. All of the hop compounds except *iso*-xanthohumol and humulinones are commercially available and can be used in beer production.

Hop free beer

An all-malt wort containing no hops (original gravity 11°P) was fermented with the yeast strain Frisinga – TUM 34/70® (*Saccharomyces pastorianus*) at 20°C for ten days until a final gravity of 1.7°P was reached. Wort was poured into 5 L fermentation bottles under aseptic conditions (with no additional aeration). The pitching yeast concentration was 20 million cells per mL. Fermentation took place in 5 L laboratory glass bottles (Schott GmbH, Mainz, Germany) with fermentation locks under atmospheric pressure. Fermentation bottles were shaken at 80 rpm on an orbital shaker (Diagonal, Münster Germany). Beer was filtered (pressure filter, pressure 2 bar, Pall EK, Seitz®, (Pall Corporation, New York, USA)) and the pH of the beer adjusted with sodium hydroxide (3 M) from pH 4.2 to 4.5. The beer was then filled in 50 ml swing-lock bottles and autoclaved at 121°C for 15 minutes. When the beer was cool, an aliquot of the extracts containing the corresponding hop compounds was added at 10 and 25 mg/L. Each sample was prepared in duplicate. Two further blank samples of each strain were prepared without any hop addition.

Table 1. Bacterial strains used in this study from the culture collection of the Research Center Weihenstephan for Brewing and Food Quality of the TU München, Freising, Germany

Strain abbreviation	Strain	Origin
TUM-BLQ-006	*Lactobacillus brevis*	Brewery isolate
TUM-BLQ-029	*Lactobacillus backi*	Brewery isolate
TUM-BLQ-003C	*Lactobacillus coryniformis*	Brewery isolate
TUM-BLQ-003L	*Lactobacillus lindneri*	Brewery isolate
TUM-BLQ-001	*Lactobacillus buchneri*	Brewery isolate
TUM-BLQ-012	*Pediococcus damnosous*	Brewery isolate

Table 2. List of hop extracts and hop compounds

Commercial hop product	Hop compound	Concentration [%]
AlphaExtract	*α-acids*	20
Isomerised hop extract 30%	*Iso-α-acids*	30
Tetra Iso-extract	Tetrahydro-*iso*-α-acids*	9
Rho Iso-extract 30%	Rho-*iso*-α-acids*	30
XanthoFlav™ extract on diatomaceous earth	Xanthohumol	1.3
Laboratory samples (produced by Martin Biendl at Hopsteiner, Simon H. Steiner)	*Iso-xanthohumol*	1.3
Laboratory samples (produced by Martin Biendl at Hopsteiner, Simon H. Steiner)	Humulinones	1.3

* light stable, chemically modified

Bacterial growth and inoculation

Bacterial strains were taken from -80°C cryo stocks and cultured twice anaerobically at 28°C in 100 ml of NBB-Broth (Döhler GmbH, Darmstadt, Germany) to check for growth in a hopped medium. After visual growth was detected (ca. four days) a loop of each sample was spread on NBB agar to check for purity and hop resistance genes. Agar plates were incubated anaerobically for three days at 28°C. Residual liquid samples (two for each spoilage organism) were centrifuged at 750 × g for 5 minutes. The supernatant was discarded, and the cell pellet was washed by adding 50 ml sodium chloride solution and vortexing for 30 seconds. Samples were centrifuged at 750 × g for 5 minutes and the supernatant was discarded. Cells were then suspended in unhopped beer and transferred to 50 ml white glass clip-lock bottles. To exclude the influence of oxygen, bottles were sealed, leaving no space for air in the bottle neck. Samples were incubated at 28°C for three days. The cell concentration of the different LAB cultures in unhopped beer was determined after incubation using a Cellometer Vision with SD25 counting chambers (Nexcelom Bioscience LCC, Lawrence, MA, USA). Sterile hop free beer samples containing the varying hop compounds at two concentrations (10 and 25 mg/L) were each inoculated with 20 CFU/ml of the individual spoilage microorganism. Incubation was performed in duplicate. In addition to the two concentrations (10 and 25 mg/L) of six different spoilers and seven different hop products, two positive growth controls for each spoiler and a negative growth control were prepared (a total of 203 samples).

PCR for *horA* and *horC*

All of the investigated bacteria strains were grown anaerobically at 28°C in NBB broth (Döhler, Darmstadt, Germany) prior to validating their ability to grow in the presence of hop compounds. Real time PCR was performed using these samples to validate the presence of the hop resistance genes *horA* and *horC* in each of the used strains.

Bacterial DNA was isolated using an InstaGeneTM matrix according to the manufacturer's instructions for bacteria (Bio-rad Laboratories, Munich, Germany). The DNA concentration was determined using the NanoDrop ND 1000 (Thermo Scientific Fisher, Wilmington, USA) and adjusted with Ampuwa® (Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany) to the initial concentration of 100 ng/μL.

Real time PCR for the detection of the presence of *horA* and *horC* was performed according to Koob et al. *(22)*. The following primers and probes were used in this study *horA*-F GGT CAA GGA ACT GTT GGC CA, *horA*-R TAA GAC CAA TGC GCC AAC CA, *horA*-probe FAM-TTC GGT TCC CAA AAC CGC AAC TTC G-BHQ1, *horC*-F TGA ATG CTC AAA TAT CGC AAT TG, *horC*-R CAC TTT GTT GCT GTG CGC TAA, *horC*-probe FAM-TAT CCC AAG CAC TTC CTA AGA TTGCAA ATG C-BHQ1 *(22)*. All nucleotides were synthesised by biomers.net GmbH, Ulm, Germany. All PCR-runs were carried out on a LightCycler® 480 Instrument II (Roche Diagnostics Deutschland GmbH, Mannheim, Germany), using 10 μl LightCycler® 480 Probes Master in a 20 μl volume assay with a 5 μl sample. The real time PCR was performedusing 400 nmol/L of forward and reverse primer and 200 nmol/L TaqMan probe. The Primers were added in aliquots of 0.8 μl and 10^{-5} mol/L, the probes were added in 0.4 μl aliquots of 10^{-5} mol/L. The initial heating at 95°C was held for 10 minutes, then 40 cycles were performed at 95°C for 10 seconds and 60°C for 30 seconds. The fluorescence was measured at the end of the 60°C step of each cycle *(22)*. *L. brevis* TUM-BLQ-006, *Lactobacillus backi* TUM-BLQ-029, *Lactobacillus lindneri* TUM-BLQ-003, *Pediococcus damnosous* TUM-BLQ-012, *Lactobacillus bucneri* TUM-BLQ-001 have *horA* and *horC* genes, whereas *Lactobacillus corynformis* TUM-BLQ-003C has only the horA gene.

HPLC analysis

HPLC analysis, based on the Analytica-EBC 9.47 method, was performed to verify the hop compounds at the applied concentrations. Components were detected at the following wavelength against most current ICS and ICE standards:

xanthohumol	:	370 nm
iso-xanthohumol	:	340 nm
iso-α-acids	:	270 nm
tetrahydro-*iso*-α-acids	:	270 nm
rho-*iso*-α-acids	:	270 nm
humulinones	:	270 nm
α-acids	:	270 nm

The HPLC system of Shimadzu Prominence Series was equipped with a column ZORBAX Eclipse XDB-C8 4.6 x 250mm 5 Micron and an SPD-20AV detector. LabSolutions software (Shimadzu) was used.

Detection of growth

Beer samples containing the varying spoilage organisms in 50 ml white glass clip-lock bottles were stored for 56 days at 28°C. Each day (including weekends) every sample was manually shaken for 30 seconds and the turbidity measured. Microbiological growth can be detected visually by customers due to turbidity and sediment in the bottles. The level of turbidity that customers that is

visually detectable is defined as 2 EBC-formazin units *(23)*. A Sigrist LabScat laboratory turbidity meter (Sigrist-Photometer AG, Ennetbürgen, Switzerland) was used to measure the EBC-formazin units of scattered light at 90° according to MEBAK 2.14.1.2 every day.

To be able to compare the inhibition of growth of different microorganisms by the hop compounds regardless of the rate of growth, positive growth samples containing no hops were created. An inhibition factor (μ) can be calculated for each spoilage microorganism (Formula 1). This factor is calculated by dividing the growth time in days (G_H) of a sample containing a hop compound by the growth time in days of the sample without hop addition (G_P). The inhibition factor is given by subtracting 1 from the result as this represents the time the microbes grow in the positive sample without inhibition.

$$\text{Formula 1:} \frac{G_H\ [days]}{G_P\ [days]} - 1 = \textit{Inhibition factor}\ \mu$$

G_H = Growth in the sample containing varying hop compounds [days]

G_P = Growth in the positive sample containing no hop compounds [days]

Beer analysis

The specific gravity, pH and degree of attenuation were determined using an Anton Paar DMA 5000 Density Meter with Alcolyzer Plus measuring module, pH measuring module, and Xsample 122 sample changer (Anton-Paar GmbH, Ostfildern, Germany).

Statistical evaluation

One-way ANOVA, Tukey test, PCA and Boxplots were created using Origin 2018b (OriginLab Cooperation Northampton, United States). Significance levels were chosen as $p < 0.01$.

Results and discussion

Microbiological spoilage by bacteria leads to customer complaints due to the presence of haze, off-flavours and/or acidity of the product *(6,8,18)*. In this study, the inhibition of common LAB beer spoilers by different hop compounds was evaluated. To simulate spoilage during packaging, 20 CFU/ml of six different spoilage organisms were inoculated into beer. Each sample was spiked individually with one of seven different hop components at two different concentrations (10 and 25 mg/L). Individual concentrations of hop compounds in the beer samples were verified by HPLC (data not shown). The average growth of the two positive samples (containing no hop compounds) was used to calculate the inhibition attributed to the individual hop compound at the two different concentrations. As shown in Table 3, the spoilers grew very differently in the unhopped beer samples.

It was found that the *L. lindneri* strain grew very slowly in the unhopped beer (Figure 1), which confirmed previous results from our laboratory and the literature, as growing samples containing *L. lindneri* is a very time consuming procedure *(18)*. It was reported that *L. lindneri* requires the hop compounds to be below a certain level of stress to increase the growth rate *(12,18)*, which was also shown by the results obtained in this study (Figure 1). Because of the different growth of *L. lindneri* in contrast to all the other investigated spoilage organisms, this species was not included when calculating the average inhibition of the different hop compounds. These results also support the findings of other authors that no *L. lindneri* strains are completely sensitive to hop compounds *(18,24)*. Figure 1 shows the results of all the *L. lindneri* trials. Growth of the *L. lindneri* strain was elevated by the presence of 25 mg/L of tetrahydro-*iso*-α-acids (Tetra), both concentrations of rho-*iso*-α-acids (Rho), xanthohumol (Xan), iso-xanthohumol (Iso-xan) and humulinones (Hum)). As the incubation time was 56 days and the positive growth samples of *L. lindneri* took 27 days, a maximum inhibition factor of 1.07 was found for 25 mg/L *iso*-α-acids and a factor of 1.0 for 10 mg/L tetrahydro-*iso*-α-acids. The greatest increase in growth was detected when adding 10 mg/L humulinones, resulting in a positive growth detection of 2 EBC turbidity units after 5 days. This suggests a potential application of these compounds in culture media with *L. lindneri*.

Beer spoilers are divided into different categories according to their spoilage potential and ability to grow and survive in beer. *L. coryniformis* and *L. buchneri* are categorised as potential beer spoilage bacteria with low hop resistance *(7)*. As Figure 2 shows, both strains were inhibited at 10 mg/L by a wide range of hop compounds, which supports the categorisation of these two

Table 3. Growth of six beer spoilage bacteria in the positive controls (beer samples containing no hops) measured by turbidity

Beer spoilage bacteria	Time to reach 2 EBC formazin units in positive control beer samples [days]	
	Sample 1	Sample 2
L. brevis	4	4
L. backi	6	6
P. damnosus	6	6
L. buchneri	4	4
L. coryniformis	4	4
L. lindneri	27	27

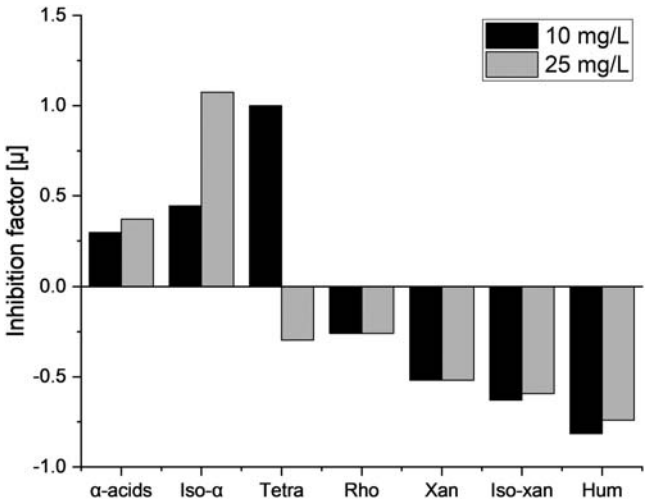

FIGURE 1. Calculated inhibition factor of the *L. lindneri* strain for the different hop compounds (α-acids, *iso*-α-acids (Iso-α), tetrahydro-*iso*-α-acids (Tetra), rho-*iso*-α-acids (Rho), xanthohumol (Xan), *iso*-xanthohumol (Iso-xan) and humulinones (Hum)).

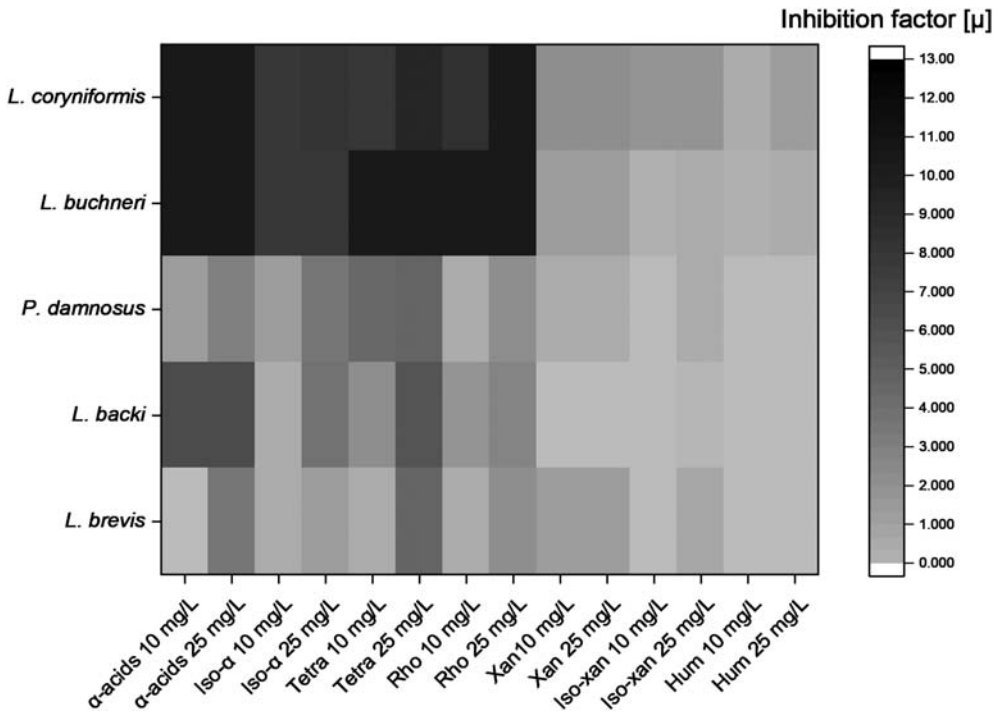

FIGURE 2. Comparison of the individual inhibition effect of the investigated hop compounds (α-acids, *iso*-α-acids (Iso-α), tetrahydro-*iso*-α-acids (Tetra), rho-*iso*-α-acids (Rho), xanthohumol (Xan), *iso*-xanthohumol (Iso-xan) and humulinones (Hum)) on each spoilage LAB strain.

spoilers as potential beer spoilage bacteria. Xanthohumol, *iso*-xanthohumol and humulinones showed almost no inhibitory impact on low hop resistant spoilers and high hop resistant obligate spoilers such as *L. brevis, P. damnosus* and *L. backi*. The *L. brevis* strain grew in all samples before the end of 56 days regardless of the concentration and the hop compound. The highest inhibition factor of 7 (28 days of inhibition, positive growth sample after 4 days) was found for *L. brevis* at 25 mg/L of tetrahydro-*iso*-α-acids. *L. backi* was found to be sensitive to α-acids (complete inhibition over 56 days by both concentrations) than the two other obligate beer spoilage bacteria (*L. brevis* and *P. damnosus*). *P. damnosus* was found to be more sensitive to *iso*-α-acids and tetrahydro-*iso*-α-acids (Figure 2).

An overall average inhibition factor for the single hop compounds was calculated for each concentration (Figure 3 and 4), omitting the results of *L. lindneri* as explained above. Figure 3 shows the comparison of all results for the application of 10 mg/L of the different hop compounds. Of the hop compounds, α-acids were found to have the highest mean of 7.3 of growth inhibition on the spoilage organisms (Figure 3). However, the wide

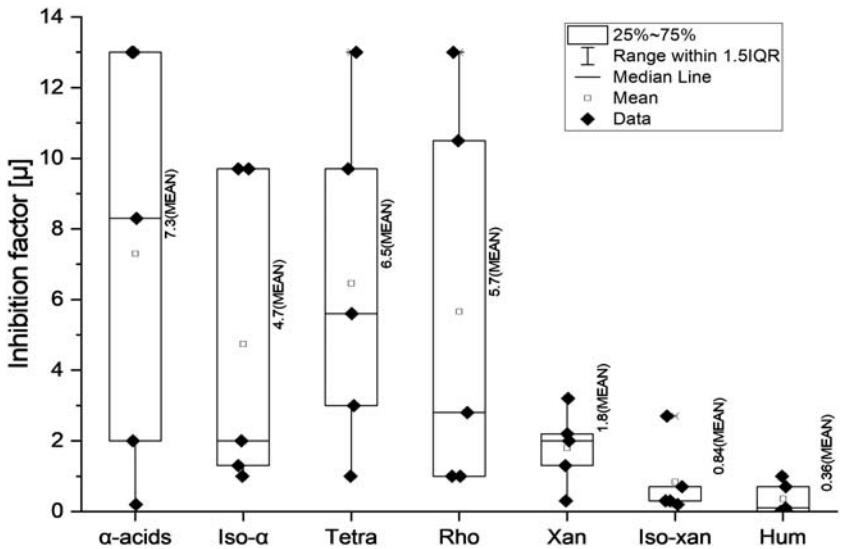

FIGURE 3. Average inhibition factor across all spoilers (excluding *L. lindneri*) calculated for the individual hop compound (α-acids, *iso*-α-acids (Iso-α), tetrahydro-*iso*-α-acids (Tetra), rho-*iso*-α-acids (Rho), xanthohumol (Xan), *iso*-xanthohumol (Iso-xan) and humulinones (Hum)) at a concentration of 10 mg/L. The mean of the individual hop compounds is stated to the right of the associated boxplot.

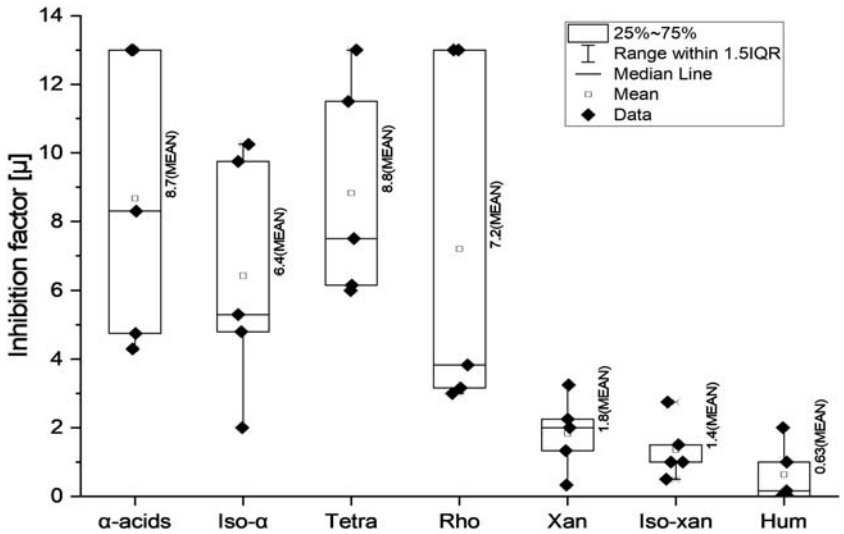

FIGURE 4. Average inhibition factor across all spoilers (excluding *L. lindneri*) calculated for the individual hop compound (α-acids, *iso*-α-acids (Iso-α), tetrahydro-*iso*-α-acids (Tetra), rho-*iso*-α-acids (Rho), xanthohumol (Xan), *iso*-xanthohumol (Iso-xan) and humulinones (Hum)) at a concentration of 25 mg/L. The mean of the individual hop compounds is stated to the right of the associated boxplot.

distribution of the data for α-acids of the single spoilage microorganisms, underlines the different sensitivities to α-acids. *Iso*-α-acids at a concentration of 10 mg/L showed a split between the low and relatively high growth inhibition throughout the selected spoilers. Furthermore, results of the hop compounds xanthohumol, *iso*-xanthohumol and humulinones showed very low inhibitory effects with inhibition factor means of 1.8, 0.83 and 0.38 respectively (Figure 3).

An increase in the concentration of the hop compounds to 25 mg/L resulted in a shift in the average maximum inhibitory effect as shown in Figure 4. On average, tetrahydro-*iso*-α-acids had the highest mean inhibitory factor of 8.8, closely followed by α-acids (mean 8.63), rho-*iso*-α-acids (mean 7.2) and *iso*-α-acids (mean 6.4). The three compounds - xanthohumol, *iso*-xanthohumol and humulinones - were found to have comparably low inhibition factors with means of 1.80, 1.40 and 0.63 respectively. The increase in *iso*-xanthohumol and humulinones from 10 to 25 mg/L showed a doubling of inhibition but still relatively low values compared with the other hop compounds (Figure 4). An increase in the concentration of xanthohumol had no measurable effect on bacterial growth. On average, for all other hop compounds, the increse in concentration to 25 mg/L only slightly increased inhibition. These findings suggest that 10 mg/L of tetrahydro-*iso*-α-acids, α-acids, rho-*iso*-α-acids and *iso*-α-acids already had a high inhibition potential for some strains. The concentration of 10 mg/L for tetrahydro-*iso*-α-acids is also much closer to the concentrations used in commercial beer production, whereas rho-*iso*-α-acids and *iso*-α-acids are often found at concentrations of 10 mg/L or higher.

When comparing these results at 10 mg/L with the results of Kaltner et al. who also used approx. 10 mg/L, an equal order of inhibition was found even though they did not use strains of *L. backi*, *L. coryniformis* and *L. buchneri* *(12)*. Kaltner et al. reported α-acids to be the most inhibiting at a concentration of 10 mg/L in contrast to - in inhibition effect order - tetrahydro-, rho- and *iso*-α-acids *(12)*.

To get a better overview of the spoilage potential of the individual species and the inhibition attributed to the hop substances at the two concentrations, a principle component analysis (PCA) was performed including all data except that from *L. lindneri*. The PCA could explain 90.84% of the variety in the data (Figure 5). The PCA draws a clear picture of the inhibition of the different spoilage microorganisms by the hop compounds. It shows that *L. coryniformis* and *L. buchneri* are sensitive to low concentrations of the hop compounds, followed by *L. backi* and *P. damnosus* with medium sensitivity, and the highly resistent *L. brevis*. These results support the findings of Riedl et al. who described *L. brevis* as the 'beer enemy number one' *(8)* and Schneiderbanger et al who found that *L. brevis* is responsible for most contamination cases in breweries *(7)*.

The different inhibitory effects of the hop compounds can be explained by their different chemical structures *(17,25)*. Humulones (α-acids) are lipophilic and also highly hydrophobic due to the prenyl side chains. This results in a low solubility in water but also high antibacterial activity due to a high affinity with cell membranes *(25)*. The effect on the membrane by *iso*-humulones was described by Simpson et al. *(17)* who reported a collapse of the H^+ gradient along the cell membrane. This leads to the inability of the cell to import nutrients and finally to cell death *(17)*. The hydrophobic character (hydrophobicity) of α-acids is strongly reduced by isomerisation, resulting in a much more soluble substance (*iso*-α-acids) but with less affinity to the cell membrane. As a consequence, *iso*-α-acids are found to have a lower antibacterial activity and therefore lower growth inhibition in contrast to α-acids *(12,17,25)*. The effect of α-acids and *iso*-α-acids on the cell is presumably the same, just with less affinity of *iso*-α-acids for the cell membrane *(25)*. Reducing the *iso*-α-acids to rho-*iso*-α-acids and tetrahydro-*iso*-α-acids increases the hydrophobicity, resulting in an increase in growth inhibition *(25)*. The much lower inhibition of growth of the beer spoilage bacteria by humulinones can be explained by their higher polarity in contrast to *iso*-α-acids *(26)*. However, the actual mechanism still needs to be investigated. Similarly, xanthohumol and *iso*-xanthohumol - even though they possess a prenyl group and antibacterial activity has been reported *(27)* - exhibited very low inhibition of growth. However, the strong inhibition by xanthohumol for the gram-positive bacteria species *Staphylococcus* was reported at higher concentration than 50 mg/L *(27)*, which is twice as much as used in this study.

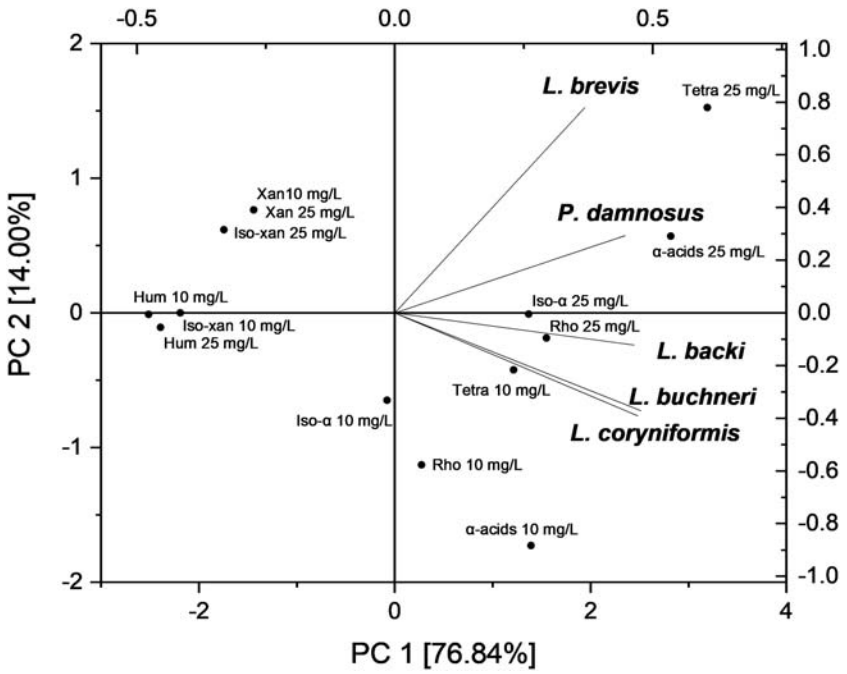

FIGURE 5. Comparison of the individual inhibition effect of the investigated hop compounds (α-acids, *iso*-α-acids (Iso-α), tetrahydro-*iso*-α-acids (Tetra), rho-*iso*-α-acids (Rho), xanthohumol (Xan), *iso*-xanthohumol (Iso-xan) and humulinones (Hum)) on each spoilage LAB strain compared using principle component analysis.

Conclusion

Hop compounds have very different effects on beer spoilage bacteria and can mostly improve microbiological stability. However, should a brewery plan to use a specific hop compound for the purpose of inhibiting a specific spoilage organism, it is important to know the identity of the spoiler as hop sensitivity varies greatly. Based on the results presented here, potential applications of hop products and improvements for increased biological stability can be suggested. The addition of hops to the whirlpool or for dry hopping can increase the α-acid content in beer *(12,28)*, resulting in higher microbiological and flavour stability *(12,29)*. The high inhibitory effect of tetrahydro-*iso*-α-acids but comparably lower dosage in commercial production due to their higher sensory bitterness, resulted in a suggestion by Simpson et al. of their use as a yeast washing agent *(30)*. The concentration of tetrahydro-*iso*-α-acids for yeast washing can be varied according to the sensitivity of the identified bacterial species if determined. However, other hop components such as α-acids, for example, could also be used for this purpose. Even at a level of 10 mg/L of tetrahydro-*iso*-α-acids, α-acids, rho-*iso*-α-acids and *iso*-α-acids, some strains exhibited high inhibition. With the exception of tetrahydro-*iso*-α-acids, these concentrations can be often found in commercially beers. If the contamination is not determined, a concentration of tetrahydro-*iso*-α-acids above 25 mg/L can be used to ensure microbiological stability.

Author contributions

Maximilian Michel: paper writing, data analysis, statistical calculations
Sandro Cocuzza: experimental design, paper writing, paper conception, data analysis
Martin Biendl: produced and provided hop substances, HPLC analysis
Frank Peifer: experimental design discussion of data critical review, supervised project
Sebastian Hans: produced unhopped wort, unhopped beer fermentation, strain maintenance - checking for horA/horC stability in test strains (potential loss due to plasmid loss was observed)
Yvonne Methner: microbial growth tests, visual daily analysis, data analysis
Friedrich Pehl: microbial growth tests, visual daily analysis, laboratory handling, data analysis
Werner Back: conception, data interpretation in accordance with former studies of his former group, Xanthohumol interpretation, provided microorganisms
Fritz Jacob: conception, provided chemical beer/wort analyses, discussion of data critical review, supervised project
Mathias Hutzler: ain conception, experimental design, paper writing, isolated most of the beer spoilage microorganisms, designed HorA and HorC real time PCR system

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Conflicts of Interest

The authors declare no conflict of interest.

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