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Original Article

# Inhibitory Effects of Iso-α and β Hop Acids Against *Pediococcus pentosaceus*

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**Abstract**

The goal of the research was to assess the inhibitory effects of hop extracts, iso-α and β acids, against *Pediococcus pentosaceus* bacteria, during a short incubation period, both in liquid selective media (high pH values) and beer wort fermentation (low pH values) and testing if the identified iso-α acid stress changes the activity of *S. cerevisiae boulardii* yeast and ethanol production. Flow cytometry analysis was used for bacterial and yeast cell viability. In relation to the antibacterial activity of β-acids, a lower viability of *Pediococcus pentosaceus* cells was observed after a short incubation period in selective media, under iso-α acid stress. In beer wort, for a mixed culture with *P. pentosaceus* bacteria and *S. cerevisiae boulardii* yeast, under iso-α acid stress conditions at pH 4.0-5.0, *Pediococcus pentosaceus* exhibited lower cell viability (20.7%) than in selective media (61.4%). Regarding iso-α hop acid on *S. cerevisiae boulardii* yeast, the results showed that iso-α does not change the *S. cerevisiae* activity but prevents the culture from being contaminated by *Pediococcus pentosaceus.* The results highlighted reliable inhibitory effects of iso-α and β-acids against *P. pentosaceus*, both at pH 6.0-7.0 and pH 4.0-5.0, which open the possibility of hops being used as a supplement to prevent beverage contamination with spoilage microorganisms.

***Keywords:*** cell viability; hop acids; inhibition; *Pediococcus pentosaceus*; *Saccharomyces cerevisiae*

## Introduction

Hop acids are one of the essential ingredients of beer, imparting its bitterness, flavour, and astringency. They have been known for thousands of years for their anti-inflammatory, antiseptic and sedative properties (Zanoli and Zavatti, 2008; Muthayan *et al*., 2011; Olsovska *et al*., 2016). Depending on the bacterial growth conditions, these bioactive compounds may exhibit either bacteriostatic or bactericidal activity (Muthayan *et al*., 2011; Cermak *et al*., 2017). Behr and Vogel (2009), demonstrated that the main factors affecting the antibacterial activity of hop compounds are the pH value and their ability to bind to the $Mn^{2+}$ cations (Sakamoto and Konings, 2003; Behr and Vogel, 2009; Zhao *et al*., 2017). Low pH enhances antibacterial activity while at high pH values hop acids lose their inhibitory effects (Zhao *et al*., 2017). The antibacterial activity of hop compounds decreases with high pH values, because hop acids are weak acids and only undissociated forms are active (Cleemput *et al*., 2009). Antibacterial hop compounds, mainly iso-α acids, have been described as ionophores, which exchange pH for cellular divalent cations and thus, dissipate ion gradients across the cytoplasmic membrane (Behr *et al*., 2007). An investigation by Buggey *et al*. (2001) showed that more hydrophobic, reduced iso-α acids have greater antimicrobial activity than their naturally occurring analogues. There are various studies pointing out that due to the antimicrobial properties, hop compounds could be utilized in commercial fuel bioethanol industries to control the contaminant bacteria. These bacteria produce lactic and acetic acid and under low pH values adversely affect the viability of yeast (Vaughan *et al*., 2005).

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In terms of beer spoilage microorganisms, lactic acid bacteria are the predominant spoilers, *Lactobacillus* and *Pediococcus* being the most commonly reported strains (Takahashi *et al*., 2014). Regarding *Lactobacillus* and *Pediococcus* bacterial strains, it has been reported that some isolates develop mechanisms that confer resistance to hop compounds, thus apparently facilitating growth in beer (Haakensen *et al*., 2009). Many studies revealed that beer represents an unfavourable growth media for most of the microorganisms because of its low pH, lack of nutrients, presence of hop derived compounds and alcohol (Pinto *et al*., 2004; Iijima *et al*., 2008). The contamination with lactic acid bacteria in the brewery environment is a problem when microorganisms are able to grow in beer and cause off-flavours or turbidity in the final product from metabolites and sediment production (Maifreni *et al*., 2015). It is well known that the contaminant microorganisms compete with the *Saccharomyces cerevisiae* strain for micro and macro-nutrients and produce inhibitory end products such as acetic acid and lactic acid (Obi, 2017). These substances, have been shown to increase "lag" times, decrease growth rates, reduce biomass yields and even lead to *Saccharomyces cerevisiae* death in some media (Bayrock and Ingledew, 2004; Skinner-Nemec *et al*., 2007).

Considering that at high pH values hop bitter acids lose activity and that some strains of *Pediococcus* bacteria develop hop-resistance mechanisms, this study aimed to evaluate the antibacterial activity of iso-α and β-hop acids against *Pediococcus pentosaceus* during a short incubation period in liquid selective media at pH 6.0-7.0.

Furthermore, some factors affecting the growth rate were studied, in order to determine the inhibitory effects of iso-α acids at low pH values and to identify if the iso-α acid stress changes the *S.cerevisiae boulardii* activity and ethanol production. Due to the iso-β-acid degradation during the wort boiling process and their very low values in beer, we chose to analyze the antibacterial activity of iso-α hop acids in beer wort fermentation, contaminated with *Pediococcus pentosaceus*.

## Materials and Methods

*Bacteria strains and culture conditions*

The microbial strains used in the experiment, were *Pediococcus pentosaceus* bacteria and *Saccharomyces cerevisiae* subsp. *boulardii* yeast, belonging to the Collection of Gembloux Agro Bio Tech University, Belgium. *Pediococcus pentosaceus* strain was inoculated in MRS liquid media at 30 °C, pH 6.5, until optical density $O.D._{600nm}$= 0.4 AbsU (*Pediococcus pentosaceus* culture A).

*Saccharomyces cerevisiae boulardii* strain was grown in selective liquid medium and incubated at 30 °C for 24 h, pH 6.5 (*S. cerevisiae boulardii* culture A). For testing the antibacterial activity during wort fermentation, *Pediococcus pentosaceus* - culture A was inoculated in wort at pH 5.0, until $O.D._{600nm}$ = 0.4 AbsU to obtain the final culture B. The same procedure was applied for the yeast strain, culture A was inoculated in wort and incubated for 2.5 h, until the optical density $O.D._{600nm}$ = 0.7 AbsU.

*Hop acid compounds*

To evaluate the antibacterial activity of hops, a clear yellow aqueous solution of potassium salts of hop-derived α-acids (30% m/v) and a clear brown aqueous solution of potassium salts of hop-derived β-acids (10% m/v), derived from a pure $CO_2$ supercritical extracted resin of hop, belonging to the Collection of Gembloux Agro Bio-Tech./Liège University, Belgium, were used.

*Antimicrobial activity of iso-α and β-acids from hops against Pediococcus pentosaceus*

The antibacterial activity of iso-α and β-acids from hops against *Pediococcus pentosaceus* in selective media, was performed according to the optimized method *(α,β-Inhibition Method)*. Samples were prepared by incubating *Pediococcus pentosaceus* culture in MRS liquid media with iso-α and β hop acids. The inoculation density was $O.D._{600nm}$ = 0.4 AbsU.

Five increasing concentrations of iso-α acids: 50 mg/l, 100 mg/l, 150 mg/l, 200 mg/l, 250 mg/l and β acids: 15 mg/l; 30 mg/l; 60 mg/l; 100 mg/l; 200 mg/l, were used. The control sample was prepared without hop extracts. Samples were incubated at 30 °C for 4.5 h under agitation on a rotary shaker at 100 rpm.

*Growth characteristics of Saccharomyces cerevisiae boulardii under iso-α acid stress*

The inhibitory effects of iso-α hop compounds in a mixed culture with *P. pentosaceus* and *Saccharomyces cerevisiae boulardii* were also studied. The experiments were carried out under optimal conditions for the *Saccharomyces cerevisiae boulardii* strain, both in wort and yeast selective media, (pH 5.0), at $O.D._{600nm}$ = 0.4 AbsU with five iso-α increasing concentrations: 20 mg/l; 50 mg/l; 100 mg/l; 150 mg/l; 300 mg/l. The contamination was performed with *Pediococcus pentosaceus* strain (culture B) and incubated at 30 °C for 4.5 h.

*Cell viability assay*

The analyses were carried out on a Genesys 20 Thermo Spectronic, Unicom model 4001/4 spectrofotometer using the optical density method. The cell incubation was monitored for 4.5 h in order to evaluate the antibacterial activity of hop acids. All samples were assessed spectrophotometrically at 600 nm after 30 min, 1.5 h, 2.5 h, 3.5 h, and 4.5 h.

*Flow cytometry analysis*

The analysis of GFP expression level was performed by Fluorescence Activated Cell Sorting (FACS) on a FACScan (Becton Dickinson) flow cytometer (Delvigne *et al*., 2011). The samples (1 ml) were transferred to microfuge tubes and cells were centrifugated at 14.000 rpm (16.000 xg) for 4 minutes. The supernatant was removed, cells were washed with cold phosphate-buffered saline (1 ml PBS) and after adding 10 µl propidium iodide (PI) the cells were gently vortexed and incubated at 30 °C for 15 min in the dark . Thereafter the cells were centrifugated at 14.000 rpm (16.000 x g) for 4 minutes. After removing the supernatant, cells were resuspended in 1ml PBS.

Analysis by flow cytometry was performed within 1 h. The results were analyzed by the FlowJo 7.6.1 software. For ethanol analysis by UV-method, an Enzymatic Bioanalysis Kit for Ethanol Assays, was used.

## Results and Discussion

*The inhibitory effects of iso-α and β-hop acids in MRS selective media*

The results for accessing the growth of the *Pediococcus pentosaceus* strain under iso-α and β hop acids stress are shown in Fig. 1. It was observed that *Pediococcus pentosaceus* was inhibited by iso-α and β acids in MRS media, during 4 h 30 min incubation at 30 °C/100 rpm and pH 6.0-7.0. The minimal inhibitory concentration of iso-α acids against *Pediococcus pentosaceus*, was 50 mg/l (13.2% inhibition ± 0.5%) and 15 mg/l (2.8% inhibition ± 0.3%) of β acids. The higher inhibitory concentration of iso-α-acids was 200 mg/l (96.3% ± 1.5% inhibition), while for β-acids at the same concentration a lower inhibition (89.6% ± 1.6%) was observed. The results showed that for 250 mg/l iso-α acids the maximal inhibition was achieved (100% after incubation at 30 °C/100 rpm for 4.5h).

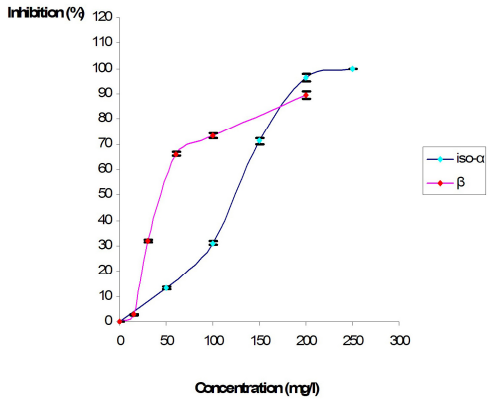


Fig. 1. The inhibitory effect of different concentration of iso-α and β hop acids against *Pediococcus pentosaceus* bacteria

According to the flow cytometry analysis, the profiles were divided in three distinct subpopulations (Fig. 2): "PI first stage" represents the exponential phase of the bacterial cells," PI - thermal stress" represents the cells exposed at 60 °C for 30 min and " PI - second stage" an intermediate subpopulation. The stressed fractions (PI - second stage and PI - thermal stress), were increased under iso-α acids activity after 4.5h incubation. The results of flow cytometry analysis (Fig. 2B and C), highlighted that *Pediococcus pentosaceus* exerted tolerance to the iso-α hop acids, cells (100 and 200 mg/l) showing a higher PI permeability after 4.5 h (Fig. 2A) and a lower PI permeability after 24 h (Fig. 2E and F) than the control sample. At a higher concentration, the bacterial cells were stronger both after 4.5 h and 24 h incubation time. Several studies have showed that this effect can be attributed to the bacteriostatic activity (Hrncic *et al*., 2019). The inhibitory effects of iso-α acids were stronger during the first stage of incubation (4.5 h), while after 24 h the viability of *Pediococcus pentosaceus* strain increased, both at 100 mg/l and 200 mg/l, beginning to develop resistance to the media. The resistance of certain strains of lactic acid bacteria to hop bitter acids is probably caused by a combination of mechanisms influencing the acidity inside the bacterial cell (Cermak *et al*., 2015).

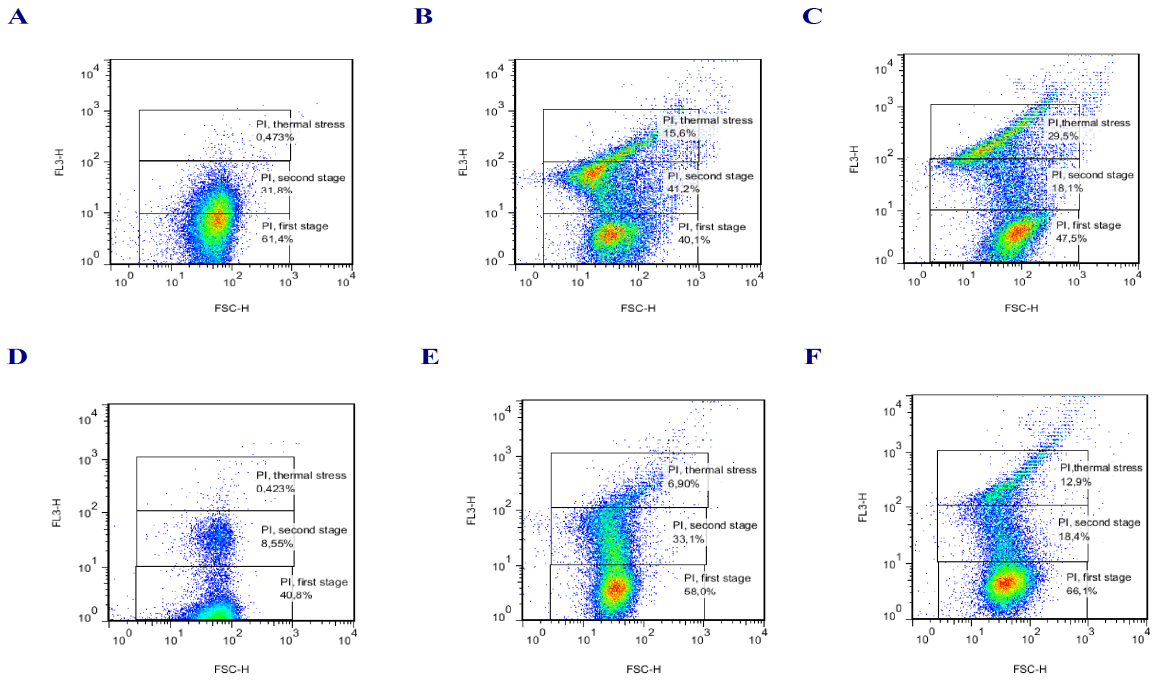


Fig. 2. Flow cytometry dot plots of *Pediococcus pentosaceus* incubated with iso-α hop acids A: control sample, B:100 mg/l iso-α acids, C:200 mg/l iso-α acids (after 4.5 h incubation) /D,E,F (after 24 h incubation)

The flow cytometry analysis provided a comparison between iso-α-acids and β-acids stress conditions on *Pediococcus Pentosaceus* cell viability, after which iso-α-acids were found to be less effective (Cermak *et al*., 2015) according to previous studies. In relation to the iso-α acids, *Pediococcus pentosaceus* cells cultivated under β-acid stress (Fig. 3B and C), exhibited a lower PI permeability after 4.5 h of incubation, compared to the control sample (Fig. 3A), while it also showed a lower PI permeability after 24 h for 100mg/l concentration, (Fig. 3F) (Gerhauser, 2005). It is known that iso-α-acids, as well as the products derived from α-acids act as ionophores, which alter the selective permeability of cytoplasmic membrane and cause intracellular acidification (Behr *et al*., 2007).

The inhibitory effects of β-hop acids were higher, but the viable cells were stronger during the first stage of incubation (4.5 h), indicating that *Pediococcus Pentosaceus* cells were resistant, However, after 24 h of incubation, cell viability decreased, and began to develop tolerance. There are several studies showing that tolerance may be developed when the microbes are exposed to a mild concentration of a weak acid, and rendering them resistant to a stronger dose (Jyoti Das *et al*., 2019).

*Effects of simultaneous cultivation of Pediococcus pentosaceus and S. cerevisiae boulardii in beer wort and selective media*

In the present study, the bacteriotatic activity of iso-α acids against *Pediococcus pentosaceus* strain in beer wort fermentation with *S. cerevisiae boulardii* strain was also evaluated. The analyses were performed to test whether the iso-α hop compounds change the activity of *S. cerevisiae boulardii* yeast and ethanol production. Many studies showed that *P. pentosaceus* have never been reported to cause any defect in finished beer (Zhao *et al*., 2017). Also in our study, the beer wort was an unfavourable medium for *P. Pentosaceus* cell growth, due to the lower pH (initial pH 5.0 and final pH 3.8-4.2 after 24h of incubation), lower nutrient value, hop-acid stress and alcohol (Pinto *et al*., 2004). The *Pediococcus pentosaceus* strain has slowly grown after 4.5 h, while in relation to the MRS selective medium (61.4%), the cell viability of the control sample (Fig. 4A) was lower (20.7%). In relation to the control samples, at lower pH, the bacteria were more tolerant both to 100mg/l and 300 mg/l iso-α acid concentration. After 24 h at pH 4.0, the viability of the cells was reduced to 4.5% and 5.4% (Fig. 4E and F), while in the MRS medium, the viability increased to 58.0% and 66.1% (Fig. 2E and F). As a result of the iso-α acids addition, a decrease of cell viability was induced at higher concentration, which was more pronounced after a short incubation period. Regarding iso-α acids activity on the *S. cerevisiae boulardii* strain, the results showed that the yeast was not affected by iso-α acids hop extract (30%) and the ethanol concentration was not influenced either (Fig. 5). This may be due to the slow development of *Pediococcus pentosaceus*, which could have competed with the yeast strain for micro and macro-nutrients (Blanco *et al*., 2007).

Because iso-β-acids in beer are in very small amounts due to their degradation during the boiling process, it could be concluded that iso-α acids present higher antibacterial properties in beer wort fermentation, rather than in a selective medium.

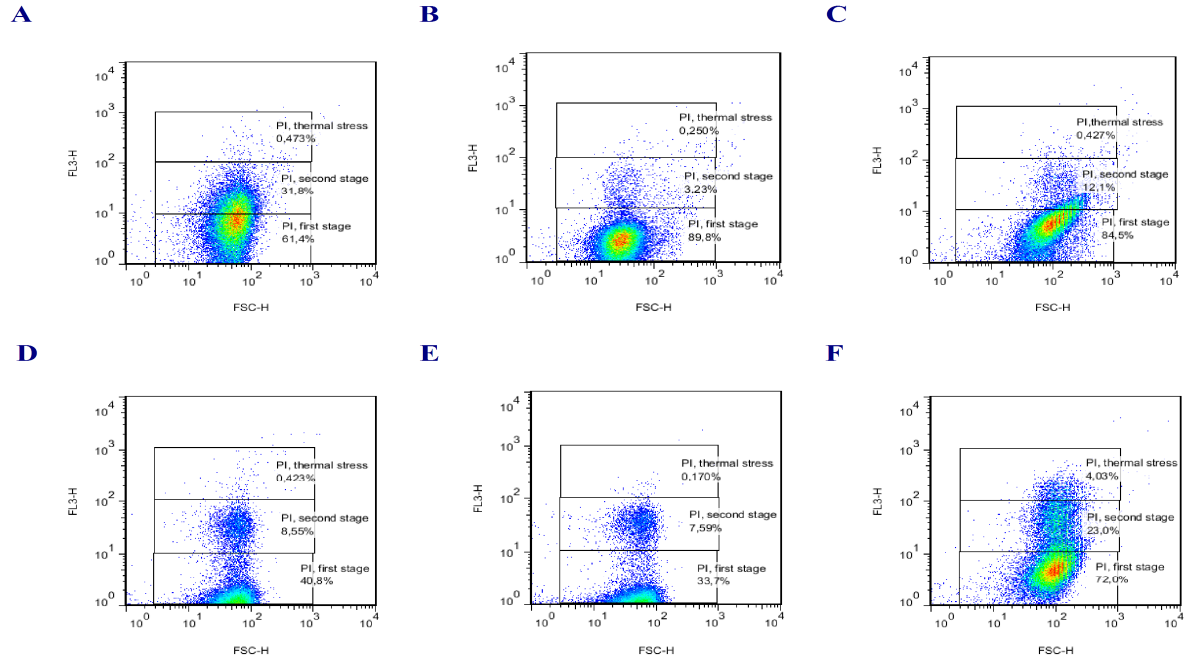


Fig. 3. Flow cytometry dot plots of *Pediococcus pentosaceus* incubated with β hop acids A: control sample, B: 30 mg/l β hop acids, C: 100 mg/l β hop acids (4.5 h); D,E,F (24 h)

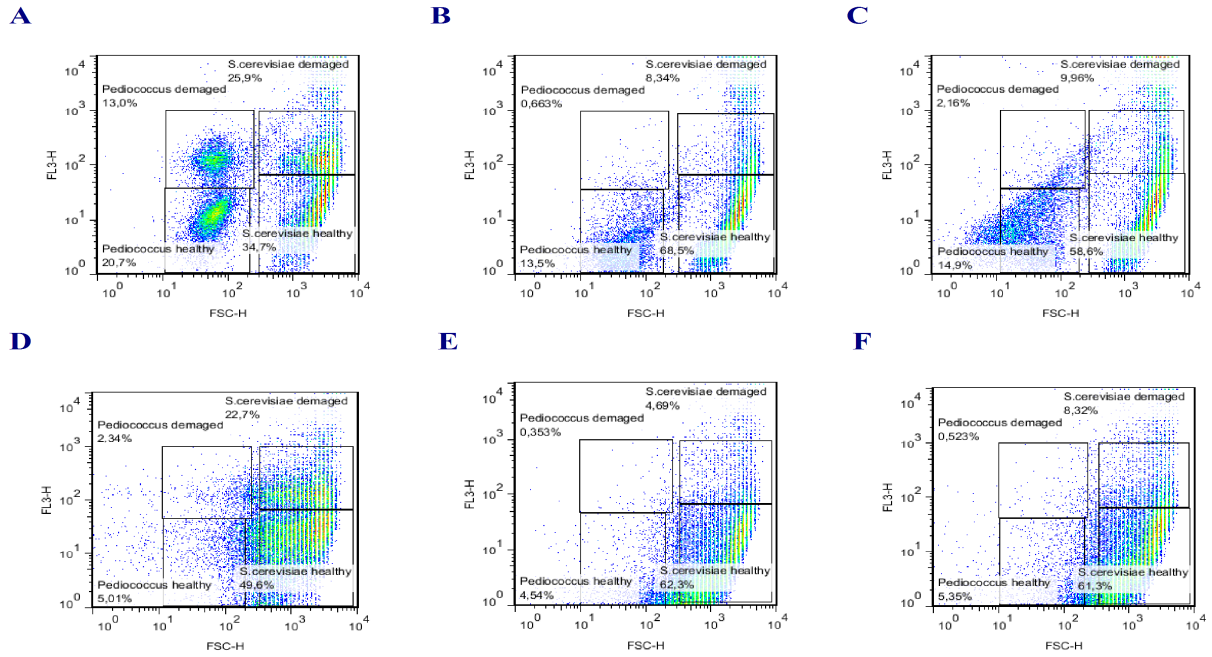


Fig. 4. Effects of iso-α hop acids against *S. cerevisiae* and *P. pentosaceus* in beer wort fermentation by flow cytometry analysis, A: control sample, B: 100 mg/l iso-α acids, C: 300 mg/l iso-α acids (4.5 h); D,E,F- (24 h)

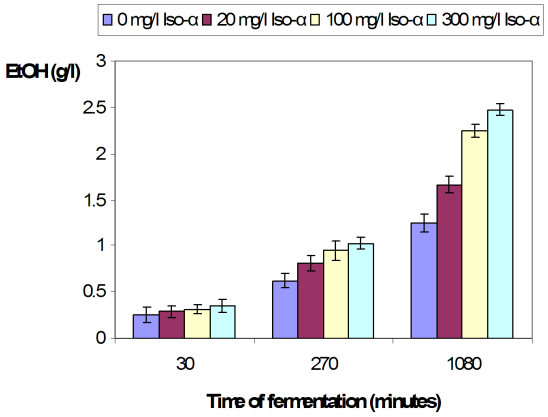


Fig. 5. Ethanol concentrations in beer wort fermentation with *S. cerevisiae boulardii* and iso-α acids hop extract (30%): control sample, 20 mg/l; 100 mg/l; 300 mg/l

Fig. 4C and F ilustrate the viability of *Pediococcus pentosaceus* cells in contact with 300 mg/l iso-α hop acids, which was higher than for 100 mg/l in the inhibition tests in selective medium (Fig. 2C and F). It was concluded that with the increase of hop extract concentration, the *Pediococcus pentosaceus* cells become stronger, revealing their bacteriostatic activity at pH 3.8-4.2.

The antibacterial activities of iso-α acids against the *Pediococcus pentosaceus* strain, during a similar fermentation in a selective medium are illustrated in Fig. 6. It was observed that cells viability was very low due to the unfavourable selective growth media, the antibacterial activity on *P. pentosaceus* being higher after 24h incubation when the yeast cells viability reached almost its maximum (Fig. 6E and F).

After 4.5 h incubation in selective media at pH 6.0-7.0, the iso-α and β-acids exhibits a bacteriostatic activity on *Pediococcus pentosaceus* bacteria. The results indicate that the *Pediococcus pentosaceus* strain was resistant to β-acids hop extract and tolerant to iso-α acids during the short incubation period. After 24 h incubation, under iso-α acid stress, the bacteria developed a resistance, thus reducing their sensitivity and consequently the kinetics of their destruction, while under β acids stress they were tolerant. Similarly, a study released by Garcia *et al.* (2016), showed that the *Pediococcus* bacteria were able to grow and adapt to increasing concentrations of hop compounds in culture media and beer, which shows that it could be considered a potential beer spoiler.

The bacteria were able to tolerate the iso-α acids in a stronger dose, showing to be stronger both after 4.5 h and 24 h incubation.

Regarding iso-α hop acids on the *S. cerevisiae boulardii* strain, the results showed that iso-α compounds do not change yeast activity but prevent the culture to be contaminated by *Pediococcus pentosaceus* bacteria. The yeast cell viability was slightly affected due to the *P. pentosaceus* strain competition, compared to the similar fermentation in a selective medium, where the viability of *P. pentosaceus* cells was very low and yeast cell viability reached almost the maximum (93%). At lower pH, during a short incubation period, the *Pediococcus pentosaceus* strain was more tolerant to the iso-α acids while for β-acids the tolerance started to develop after 24 h.

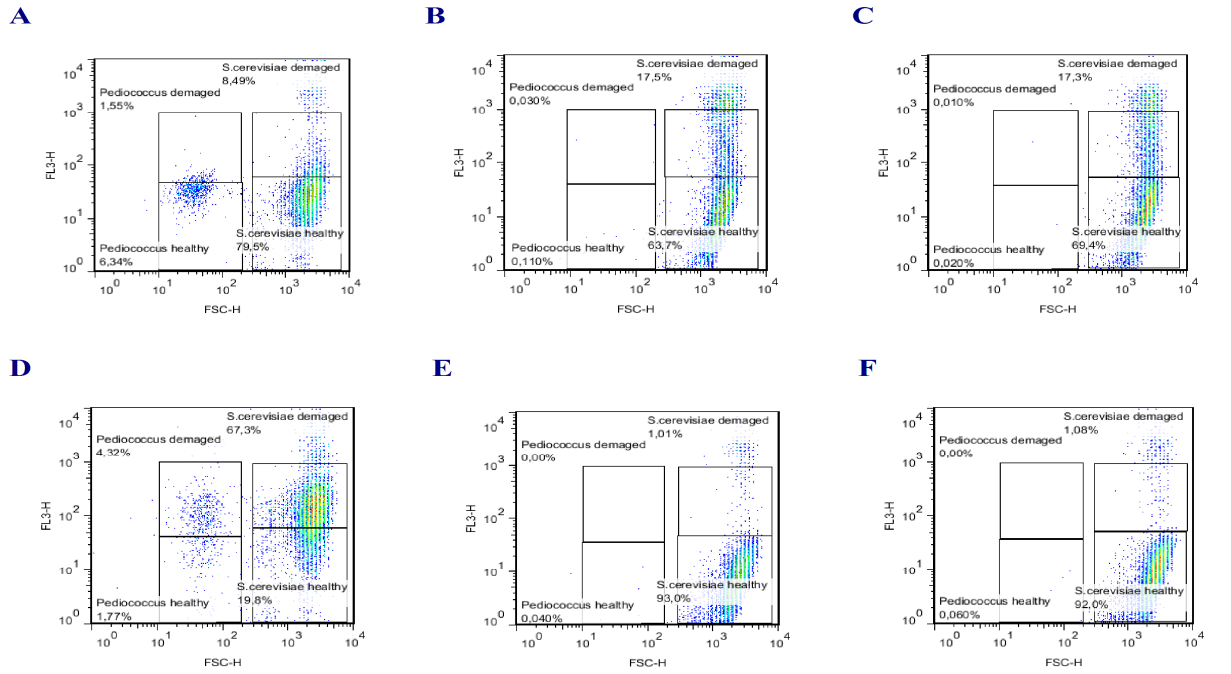


Fig. 6. Effects of iso-α hop acids against *S. cerevisiae* and *P. pentosaceus* in yeast selective media by flow cytometry analysis, A: control sample, B: 100 mg/l iso-α acids, C: 300 mg/l iso-α acids (4.5 h); D,E,F- (24 h)

## Conclusions

In conclusion, the results of this study strongly support the likelihood that hop extracts can successfully be used as a bacteriostatic agent against *Pediococcus Pentosaceus* and warrant further research on developing hop bactericidal activity against this strain by testing the inhibitory effects in MRS selective media at low pH (modified pH) under iso-α and β acid stress during a longer incubation period. The results obtained in this study collect information that enables the use of iso-α acids as antimicrobial agent during beer wort fermentation, exhibiting a stronger antimicrobial effect than in a yeast selective medium. The search for new hop compounds with bactericidal properties represents a possible solution to the global problem of resistant spoilage microorganisms. Thus, hops might present themselves as a useful source of potential antimicrobial agents applicable in food industry.

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## Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

## References

Bayrock DP, Ingledew WM (2004). Inhibition of yeast by lactic acid bacteria in continuous culture: nutrient depletion and/or acid toxici. Journal of Industrial Microbiology and Biotechnology 31(8):362-368.

Behr J, Israel L, Gänzle MG, Vogel RF (2007). Proteomic approach for characterization of hop-inducible proteins in *Lactobacillus brevis*. Applied and Environmental Microbiology 73(10):3300-3306.

Behr J, Vogel RF (2009). Mechanisms of hop inhibition: hop ionophores. Journal of Agricultural and Food Chemistry 57(14):6074-6075.

Blanco C, Rojas A, Nimubona D (2007). Effects of acidity and molecular size on bacteriostatic properties of beer hop derivates. Trends in Food Science & Technology 18(3):144-149.

Buggey LA, Price A, Stapely SJ (2001). The antibacterial activity of hop compounds. In: Proceedings of the 48th International Hop Growers Convention, Canterbury, UK.

Cermák P, Palečková V, Houška M, Strohalm J, Novotná P, Mikyška A,...Sikorová M (2015). Inhibitory effects of fresh hops on *Helicobacter pylori* strains. Czech Journal of Food Sciences 33(4):302-307.

Cermak P, Olsovska J, Mikyska A, Dusek M, Kadleckova Z, Vanicek J, ... Bostik P (2017). Strong antimicrobial activity of xanthohumol and other derivatives from hops (*Humulus lupulus* L.) on gut anaerobic bacteria. Apmis 125(11):1033-1038.

Cleemput V, Cattoor MKK, De Bosscher G, Haegeman D, De Keukeleire AH (2009). Hop (*Humulus lupulus*)-Derived bitter acids as multipotent bioactive compounds. Journal of Natural Products 72(6):1220-1230.

Delvigne F, Brognaux A, Gorretb N, Neubauerc P, Delafossed A, Collignond ML, ... Thonart P (2011). Characterization of the response of GFP microbial biosensors sensitive to substrate limitation in scale-down bioreactors. Biochemical Engineering Journal 55(2):131-139.

Garcia-Garcia JH, Damas-Buenrostro LC, Cabada-Amaya JC, Elias-Santos M, Pereyra-Alférez B (2016). Pediococcus damnosus strains isolated from a brewery environment carry the horA gene. Journal of the

Institute of Brewing 123(1):77-80.

Gerhauser C (2005). Broad spectrum antiinfective potential of xanthohumol from hop (*Humulus lupulus* L.) in comparison with activities of other hop constituents and xanthohumol metabolites. Molecular Nutrition and Food Research 49(9):827-831.

Haakensen M, Schubert A, Ziola B (2009). Broth and agar hop-gradient plates used to evaluate the beer-spoilage potential of *Lactobacillus* and *Pediococcus* isolates. International Journal of Food Microbiology 130(1):56-60.

Hrncic MK, Španinger E, Košir IJ, Knez Z, Bren U (2019). Hop compounds: extraction techniques, chemical analyses, antioxidative, antimicrobial, and anticarcinogenic effects. Nutrients 11(2):257-294.

Iijima K, Asano S, Suzuki K, Ogata T, Kitagawa Y (2008). Multiplex PCR to detect beer-spoilage bacteria. Bioscience, Biotechnology, and Biochemistry 72(10):2764-2766.

Jyoti Das A, Jyoti Das M, Miyaji T, Deka SC (2019). Growth and metabolic characterization of four lactic acid bacteria species isolated from rice beer prepared in Assam, India. Access Microbiology 1(4):1-14.

Maifreni M, Frigo F, Bartolomeoli I, Buiatti S, Picon S, Marino M (2015). Bacterial biofilm as a possible source of contamination in the microbrewery environment. Food Control 50:809-814.

Muthaiyan A, Limayem A, Ricke SC (2011). Antimicrobial strategies for limiting bacterial contaminants in fuel bioethanol fermentations. Progress in Energy and Combustion Science 37(3):4-13.

Obi CN (2017). Brewery contaminants, challenges and remedies - a review. Nigerian Journal of Microbiology 31(1):3926-3940.

Olsovska J, Bostikova V, Dusek M, Jandovska V, Bogdanova K, Cermak P, ... Kolar M (2016). *Humulus lupulus* L. (hops) – a valuable source of compounds with bioactive effects for future therapies. Military Medical Science Letters (Voj Zdrav Listy) 85(1):19-30.

Pinto MGV, Pasteris SE, Strasser de Saad AM (2004). Glycerol catabolism by *Pediococcus pentosaceus* isolated from beer. Food Microbiology 21(1):111-118.

Sakamoto K, Konings WN (2003). Beer spoilage bacteria and hop resistance. International Journal of Food Microbiology 89(2-3):112-114.

Skinner-Nemec KA, Nichols NN, Leathers TD (2007). Biofilm formation by bacterial contaminants of fuel ethanol production. Biotechnology Letters 29(3):379-383.

Takahashi M, Kita Y, Kusaka K, Mizuno A, Goto-Yamamoto N (2014). Evaluation of microbial diversity in the pilot-scale beer brewing process by culture-dependent and culture-independent method. Journal of Applied Microbiology 118(2):454-469.

Vaughan A, O'Sullivan T, Van Sinderen D (2005). Enhancing the Microbiological stability of malt and beer - a review. Journal of the Institute of Brewing 111(4):355-31.

Zanoli P, Zavatti M (2008). Pharmacognostic and pharmacological profile of *Humulus lupulus* L. Journal of Ethnopharmacology 116(3):383-396.

Zhao Y, Knøchel S, Siegumfeldt H (2017). Heterogeneity between and within strains of *Lactobacillus brevis* exposed to beer compounds. Frontiers in Microbiology 8(239):1-13.