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Proceeding Paper

Potential Antimicrobial Activity of Weak Acids in Combination with pH and Temperature on *Alicyclobacillus acidoterrestris* †

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Abstract: *Alicyclobacillus acidoterrestris* causes spoilage in fruit juices. This research investigated the effects of organic acids on the growth of two strains of *A. acidoterrestris* at different pH and temperatures over 2, 7 and 14 days. The results show different impacts of weak acids on *A. acidoterrestris* growth and survival. Ascorbic and malic acids have the highest antimicrobial activity; also, pH played a crucial role. Moreover, the results suggest a possible activity of acids on outgrowing spores rather than on spores. Understanding the organic acids interactions with *A. acidoterrestris* is crucial for implementing strategies to ensure quality and reduce spoilage incidents.

Keywords: *Alicyclobacillus acidoterrestris*; temperature; pH; weak acids; decrease

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1. Introduction

Alicyclobacillus is a group of Gram-positive, thermophilic, acidophilic, and non-pathogenic bacilli species, with optimal temperatures and pH at 42–53 °C, and 3.5–5.0 [1]. This spoiler is frequently associated with fruit juices and acidic drinks and the presence is often attributed to inefficient pasteurization processes. Among the various species in this genus, *A. acidoterrestris* is the major responsible for causing spoilage in juices [2]. The spores of *A. acidoterrestris* can survive pasteurization and subsequently germinate, leading to the production of compounds such as guaiacol and halophenols, resulting in undesirable sensory characteristics like sediment, cloudiness, or discoloration in the affected products [3]. For juices, the threshold level of *A. acidoterrestris* cells for spoilage to be evident is typically 4–5 log CFU/mL [4].

The acid resistance of *A. acidoterrestris* is well-documented, and it has been extensively studied for its ability to survive and grow in acidic conditions. However, less is known about how different weak acids, commonly present in fruits [5] and used as acidulants and preservatives in the food industry [6], impact the behaviour of *A. acidoterrestris*. Additionally, the response of *A. acidoterrestris* to different acids typically found in foods has not been thoroughly explored.

Organic acids, especially weak acids, are naturally present in fruits and contribute to their taste and suitability for juice processing. Furthermore, they exhibit antimicrobial activities, with various mechanisms such as pH reduction, disturbance of membrane transport and permeability, anion accumulation, enzyme inhibition, cytoplasm acidification, and specific antimicrobial effects of anionic species [7].

While some research has been conducted on the effects of different acids on *A. acidoterrestris*, the focus has mainly been on inactivation or resistance in combination with other factors. There is a need for a comprehensive study to investigate the impact of weak acids, commonly found in juices and used as preservatives, on soil strains of *A. acidoterrestris*. Thus, the aim of this research was to study the effects of specific weak acids present in juices (ascorbic, malic, citric, tartaric) in comparison to a weak acid used as an acidulant in food processing (lactic acid) and a strong acid (hydrochloric acid) on *A. acidoterrestris* soil strains, in combination with pH, contact time and storage temperature.

2. Materials and Methods

Two isolates of *A. acidoterrestris* (C1 and C13), genotypically and phenotypically characterized [8], from the Culture Collection of the Department of Agriculture, Food, Natural Resources, and Engineering (DAFNE), University of Foggia, were used in this study. Spores were produced and quantified through a standardized protocol.

For sample preparation, a full randomized design was used to combine the isolates, pH levels (2, 3, and 4), acids (ascorbic, malic, citric, tartaric, lactic, and hydrochloric acids), and temperatures (5 °C and 45 °C) in 72 combinations. Each combination was prepared in duplicate, and three technical replicates were performed for each batch. All experiments were done in Malt Extract broth, acidified to pH from 2 to 4 through a 10% solution of the different acids; then, samples were separately inoculated to 4–5 log CFU/mL of the spores of the two strains. After incubation either at 5 or 45 °C, viable count was assessed immediately after inoculation and after 2, 7, and 14 days.

For statistic, data were preliminary standardized as increase/decrease referred to inoculum and then modelled using a Multifactorial Analysis of Variance (MANOVA); kind of acid and strain, pH, temperature, and sampling time were used as categorical predictors.

3. Results and Discussion

The first output of MANOVA is the table of standardized effects, which gives statistical weight of the predictors through F-test. All single predictors have different statistical weight; the most significant predictor was pH (F-value, 1099.75), followed by temperature (F-value, 728.65), time (F-value, 72.09). The viable count of alicyclobacilli was also affected by some interactive terms, e.g., temperature*pH (F-value, 521.18), temperature*isolate (F-value, 223.17), acid*pH (F-value, 34.33).

The second output of MANOVA is the decomposition of statistical hypothesis. However, many predictors are used also the decomposition of the statistical hypothesis could give outputs difficult to understand; thus, the predictors were reduced, and Figure 1 shows the effects of pH and kind of acid on the strain C1 after 2 days.

Lactic and citric acid played a significant antimicrobial action only at pH 2, probably depending on their higher concentrations, and amount of undissociated (98.7% for lactic acid and 90.45%) compared to pH 3 or 4, with a mean reduction of viable count of 4 log CFU/mL. Malic, tartaric, and ascorbic acid play an antimicrobial action also at pH 3 (and pH 4 only for ascorbic acid).

Strain C13 showed a similar trend, although it appeared more sensitive in some combinations.

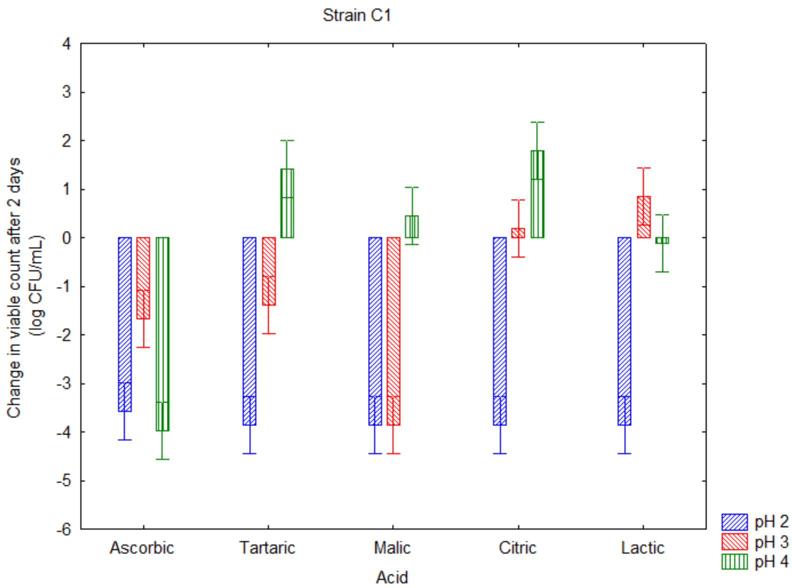

Figure 1. Change in the viable count of *A. acidoterrestris* strain C1 after 2 days, depending on pH and kind of acids used for acidification. Bars denote 95% confidence intervals.

Concerning the weight of the kind of acids, independently from the pH or time of sampling, statistic showed the strong quantitative effect of ascorbic acid, which caused a mean decrease of viable count of 1.6–1.7 log CFU/mL. The mechanism related to the antimicrobial activity of weak acids according to bibliography are mainly two. The first mechanism depends on the ability of entering the cell in the undissociated form with subsequent cytoplasm acidification, while the second one is related to the ability of reducing external pH [9]. In addition, the stronger effects at 45 °C suggest that weak acids probably act on outgrowing spores, as at this temperature alicyclobacilli spores germinate, while they do not at 5 °C.

In conclusion, the findings of this study demonstrate the potential of weak acids, especially ascorbic and malic acids, in effectively reducing the viable count of *A. acidoterrestris*. These acids can play a crucial role in controlling the spoilage of juices and concentrates when employed in proper combinations with low pH levels and appropriate temperatures.

However, further validation in real food products is necessary to develop predictive models for effective control measures against this spoiling bacterium. Understanding weak acid interactions with *A. acidoterrestris* in food systems is crucial for implementing preventive or corrective measures in the juice and concentrate industries to ensure product quality and reduce spoilage incidents.

This research provides valuable insights into the behavior of *A. acidoterrestris* in the presence of weak acids and contributes to the body of knowledge on controlling microbial spoilage in the food industry. Future studies could explore additional factors that might influence the antimicrobial efficacy of weak acids and focus on real juice products to assess their practical applicability in industrial settings. The combination of weak acids with other preservation methods could also be investigated to enhance their antimicrobial potential and provide an integrated approach to ensure the microbiological stability of fruit juices and concentrates.

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