



Influence of physicochemical characteristics on the growth and guaiacol production of *Alicyclobacillus acidoterrestris* in fruit juices

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ABSTRACT

Alicyclobacillus acidoterrestris is a bacterium known for causing spoilage in the taste and odour of fruit juices due to its thermoacidophilic nature. Its spoilage is attributed to the formation of guaiacol, which requires the presence of suitable precursors in the juices that *A. acidoterrestris* can metabolize. Therefore, *A. acidoterrestris* could exhibit different behaviour depending on the physicochemical characteristics the juice. In this study, we aimed to evaluate the behaviour of five *A. acidoterrestris* strains in seven different fruit juices by monitoring total cell and spore populations and quantifying guaiacol production. Also, physicochemical and phenolic profile, focusing on antimicrobials and guaiacol precursors, were analysed to better understand differences. Results showed growth in orange, apple, and plum juices for all the tested strains, with total cell populations reaching approximately 7 log cfu/mL, except for plum juice. In persimmon juice, growth was only observed in 3 out of 5 strains, for both total cells and spores. In contrast, all strains were inhibited in peach, black grape, and strawberry juices, maintaining a consistent population around 4 log cfu/mL. A strong negative correlation was observed between bacterial population and compounds such as kaempferol (for strains R3, R111, and P1), cyanidin chloride (for strains R111 and P1), and p-coumaric acid (for strain 7094 T). Regarding guaiacol production, orange and persimmon juices exhibited the highest guaiacol levels, with strain P1 (362.3 ± 12.6 ng/mL) and strain EC1 (325.1 ± 1.4 ng/mL) as the top producers, respectively. Plum, black grape, and strawberry juices showed similar guaiacol concentrations (16.9 ± 2.8 to 105.0 ± 33.7 ng/mL). Vanillin was showed positive correlations with guaiacol production in almost all strains (7094 T, R3, R111, and P1), with correlation coefficients of 0.97, 0.99, 0.82, and 0.87, respectively. We have reported different behaviour of *A. acidoterrestris* strains depending on juice type. Despite growth inhibition observed in some juices, enough guaiacol quantities to spoil the juice can be produced. This highlights the necessity of exploring strategies to prevent guaiacol production, even under growth restriction.

1. Introduction

In recent years, *Alicyclobacillus acidoterrestris* has emerged as one of the most important spoilage microorganisms in the fruit juice industry. The first spoilage incident was reported in Germany in 1984 involving spoiled apple juice (Cerny et al., 1984). Subsequently, *A. acidoterrestris* has been detected in soil and different fruit juices, and it has been increasingly studied given the characteristics that allow its growth in extreme environments (Neggazi et al., 2023; Steyn et al., 2011). *A. acidoterrestris* is a Gram-positive, non-pathogenic, thermoacidophile, spore-forming and rod-shaped bacteria. Its resistance to high

temperatures, acidic environments, and spore-forming ability are the main characteristics contributing to its persistence in fruit juices. When orange juice is contaminated by *A. acidoterrestris* spores, stored in favourable conditions, and reaches a population of 10^4 cfu/mL, the juice can become spoiled by the production of unpleasant compounds (Spinelli et al., 2009). Spoilage by *A. acidoterrestris* is difficult to notice since no gas production, cloudiness or other evident signs can be detected until the consumer complains about the off flavour (Huang et al., 2015; Sourri et al., 2022). Several authors have described the off-flavour in episodes of fruit juice spoilage as “medicinal”, “phenolic”, “smoky”, and “antiseptic” (Ciuffreda et al., 2015; Siegmund and

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Pöllinger-Zierler, 2006). Compounds identified as responsible for this off-flavour are 2-methoxyphenol (guaiacol), 2,6-dibromophenol and 2,6-dichlorophenol. Guaiacol is the main product produced by *A. acidoterrestris* and the direct precursor is vanillic acid. However, additional studies suggested alternative precursors for guaiacol production, such as vanillin or ferulic acid (Bahçeci et al., 2005; Cai et al., 2015; Ciuffreda et al., 2015; Witthuhn et al., 2012).

The approximate values of guaiacol concentration from which the unpleasant flavour begins to be noticed have been reported. However, a great variability has been observed in best-estimated thresholds (BET). Huang et al. (2015) reported that the guaiacol aroma could be detected at a concentration of 1.5 µg/mL in apple juice. Eisele and Semon (2005) described lower thresholds for aroma (0.91 ng/mL) and taste (0.24 ng/mL) in apple juice. In orange juice, Pérez-Cacho et al. (2011) reported values of 0.70 ng/mL and 0.53 ng/mL for odour and aroma, respectively. For water, the corresponding values of BET were 0.021 and 0.013 µg/mL for odour and taste, respectively (Pornpukdeewattana et al., 2019).

Despite *A. acidoterrestris*'s ability to spoil different juices, its behaviour is significantly influenced by the physicochemical characteristics of the juice. Pettipher et al. (1997) showed that *A. acidoterrestris* grew and produced guaiacol in orange and apple juice. The population reached 10^6 and 10^7 cfu/mL, with guaiacol production of 33 and 1 ng/mL, respectively. In contrast, Splittstoesser et al. (1994) reported that red grape, plum, cranberry and blends of these juices with apple juice, inhibited *A. acidoterrestris* growth. The authors attributed this growth inhibition to the presence of phenolic compounds or anthocyanins.

Due to the persistent spoilage incidents, it is essential to identify the fruit juice characteristics that affect the behaviour of *A. acidoterrestris* spores and its guaiacol production. This knowledge will aid in the development of effective strategies to prevent fruit juice spoilage caused by this microorganism. Thus, our objective was to evaluate the growth and guaiacol production of five strains of *A. acidoterrestris* in seven different fruit juices and identify the physicochemical characteristics that may influence to *A. acidoterrestris* behaviour.

2. Material and methods

2.1. Samples

Three pasteurized fruit juices (orange, black grape, and strawberry juice) and four reconstituted concentrate fruit juices (persimmon, plum, peach and apple) were evaluated for *A. acidoterrestris* growth and guaiacol production. Before the experiment, concentrated juices were reconstituted to soluble solids content (SSC) indicated in the Spanish regulation (Real Decreto 781/2013). All fruit juice samples were assessed for *A. acidoterrestris* detection according to IFUMM12 and no presence of *A. acidoterrestris* was found (IFU, 2019).

2.2. Physicochemical characterization of fruit juices

For each sample pH, SSC, water activity (a_w), titratable acidity (TA), total phenolic content (TPC), total anthocyanins content (TAC) and antioxidant activity (AA) methods were determined in triplicate.

The pH was measured using a pH-meter (XS Instrument, Carpi, Italy). SSC was measured directly using a digital refractometer (PAL-1, Atago, Japan) and the results were expressed as °Brix. Water activity was measured in a water activity-meter (Aqualab, Portugal).

For TA, 10 mL of each juice was titrated with 0.1 N NaOH to a pH of 8.1. The results were expressed as mmol of H⁺ per litre of juice, using Eq. (1) (UNE-EN 12147).

$$C_{H^+} = \left(\frac{1000 \cdot V_1 \cdot c}{V_0} \right) \quad (1)$$

where C_{H^+} is the titratable acidity, V_1 is the volume of NaOH 0.1 N, c is

the concentration of NaOH (0.1 N) and V_0 is the sample volume.

The TPC determination was carried out by the Folin-Ciocalteu method as following: 0.7 mL of each sample were taken and then 4.3 mL of deionised water and 0.5 mL of the Folin-Ciocalteu reagent were added. Then, the mixture was shaken and incubated for 5 min in the dark. After the incubation, 2.0 mL of sodium carbonate (10 %) solution was added. Afterwards, the mixture was shaken and incubated for 1 h in the dark. Finally, the absorbance was measured at 760 nm using a spectrophotometer (P-selecta, SP-200 V, Spain). A calibration curve of gallic acid was made to calculate the concentration of TPC. Results were expressed as mg of gallic acid/L of juice.

For TAC analyses, an aliquot of 0.5 mL was taken and mixed with potassic chloride buffer 0.025 M (pH 1.0) to a final volume of 5 mL. Another aliquot of 0.5 mL was mixed with sodium acetate buffer 0.400 M (pH 4.5) to a final volume of 5 mL. For each juice, both solutions were shaken and incubated for 30 min at room temperature in the dark. Then, the absorbance of both solutions was measured at 510 and 700 nm using a spectrophotometer. The results were expressed as mg of cyanidin-3-glucoside/L of juice using Eq. (2):

$$TAC = \frac{\Delta A \cdot MW \cdot DF \cdot 1000}{\epsilon \cdot L} \quad (2)$$

where TAC is the total anthocyanin content, ΔA is the absorbance differential = $(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$, MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), ϵ is the molar extinction coefficient and L the cuvette length (1 cm).

The AA was determined using two methods: 2,2-Diphenyl-picrylhydrazyl (DPPH·) radical scavenging and the ferric reducing antioxidant power (FRAP) described as following. For DPPH, 0.2 mL of the sample were taken and mixed with 2.8 mL of DPPH solution (0.1 mM). Then, the mixture was incubated at room temperature in the dark for 1 h. Later, the absorbance was measured at 515 nm and the results were expressed as mg of ascorbic acid/L of juice. For the FRAP method, first, the FRAP reagent was prepared by mixing acetate buffer 0.3 M (pH 3.6), TPTZ (2,4,6-tripiryridyl-s-triazine) 10 mM in 40 mM HCl and FeCl₃ 6H₂O 20 mM in a proportion 10:1:1 (v/v/v), respectively. Then, 0.2 mL of each sample were taken and mixed with 2.8 mL of FRAP reagent and incubated at 37 °C for 20 min in the dark. After this period, the absorbance was measured in a spectrophotometer at 593 nm. The results were expressed as mg of ascorbic acid/L of juice.

2.3. Phenolic profile

Fruit juices (persimmon, plum, peach, apple, orange, black grape, and strawberry) were filtered through a 0.22 µm hydrophilic membrane filter PTFE 13 mm (Phenomenex, CLARIFY Syringe Filters, Spain). Their phenolic profiles were characterised using an ACQUITY UPLC (Ultra Performance Liquid) binary system coupled to a Xevo TQS triple quadrupole mass spectrometer detector (Waters MS Technologies, UK). The filtered extracts were analysed using an ACQUITY UPLC HSS T3 silica C18 column (Waters MS Technologies, UK) with dimensions of 1.8 µm and 150 × 2.1 mm. The mobile phase was a mixture of water and methanol (98 and 2 %, respectively) (99 %), formic acid (1 %) (solvent A) and a mixture of methanol (99 %) and formic acid (1 %) (solvent B). The elution was at a flow rate between 0.3 and 0.4 mL/min by a linear gradient at 30 °C. The injection volume was 2.5 µL at 5 °C. In total 19 compounds were analysed, from which 15 were phenols (protocatechuic, p-coumaric, gallic, caffeic, ferulic, vanillic and sinapic acids, luteolin, myricetin, epigallocatechin gallate, vanillin, resveratrol, epicatechin, quercetin, and kaempferol) and 4 were anthocyanins (pelargonidin-3-rutinoside chloride, delphinidin-3-o-rutinoside chloride, cyanidin chloride, and pelargonidin chloride). Three of the 15 phenols are acknowledged as direct (vanillic acid) and indirect (vanillin and ferulic acid) guaiacol precursors. Results were expressed as ng/mL.

Table 1
Physicochemical characterization of fruit juice samples.

Juice	pH	Soluble solids content (°Brix)	Water activity (a_w)	TA (mmol/L juice)	TPC (mg gallic acid/L juice)	TAC (mg Cy-3-g/L juice)	Antioxidant activity	
							DPPH (mg ascorbic acid/L juice)	FRAP (mg ascorbic acid/L juice)
Orange	3.70 ± 0.08 c	11.3 ± 0.1 e	0.965 ± 0.028 a	114.0 ± 4.6 a	598.3 ± 22.8 b	–	650.0 ± 3.7 c	1404.9 ± 2.3 b
Apple	3.87 ± 0.04 b	11.6 ± 0.0 d	0.987 ± 0.002 a	66.3 ± 1.5 b	500.2 ± 2.9 d	–	388.8 ± 14.8 d	743.3 ± 1.8 f
Persimmon	4.07 ± 0.08 a	18.1 ± 0.0 a	0.887 ± 0.002 b	67.0 ± 1.7 b	605.1 ± 5.5 b	–	653.5 ± 13.3 c	926.5 ± 2.8 d
Plum	3.60 ± 0.04 c	13.4 ± 0.0 c	0.968 ± 0.004 a	109.0 ± 4.4 a	740.8 ± 4.4 a	–	721.6 ± 9.7 b	907.7 ± 0.9 e
Peach	3.89 ± 0.07 b	11.6 ± 0.0 d	0.898 ± 0.007 b	72.0 ± 3.5 b	557.4 ± 3.3 c	–	305.9 ± 27.4 e	663.6 ± 0.9 g
Black grape	3.35 ± 0.03 e	15.6 ± 0.1 b	0.992 ± 0.003 a	63.3 ± 2.1 b	435.1 ± 6.0 e	5.2 ± 0.2 a	759.2 ± 11.7 ab	1364.3 ± 4.4 c
Strawberry	3.50 ± 0.07 de	2.2 ± 0.1 f	0.955 ± 0.021 a	69.0 ± 3.6 b	349.8 ± 9.5 f	3.5 ± 1.9 a	799.1 ± 24.3 a	1528.9 ± 2.2 a

Results are expressed as mean ± standard deviation of three replicates ($n = 3$) for each analysis. Values with different letters in the same column are significantly different according to Tukey test HSD ($p < 0.05$). (–): not analysed. TA: Titratable acidity; TPC: Total phenolic content; TAC: Total anthocyanin content; Cyanidin-3-glucoside: Cy-3-g; DPPH: 2,2-Diphenyl-picrylhydrazyl; FRAP: Ferric reducing antioxidant power.

2.4. Growth capacity of *A. acidoterrestris* strains

2.4.1. Bacterial strains and preparation of spore suspension

A total of five strains of *A. acidoterrestris* were evaluated in this study. Four of them were isolated in a previous study (Neggazi et al., 2023): R3 (isolated from pineapple juice), R111 (isolated from a blend of red grape, pomegranate, and currant juice), P1 (isolated from a plum puree) and EC1 (isolated from an apple concentrate). The isolated strains were identified by 16S rDNA gene sequence analysis. For the identification of the isolates, the sequences were entered into the NCBI (National Center of Biotechnology Information) Genbank database using BLAST (Basic Local Alignment Search Tool) with Megablast algorithm to confirm that all isolates belong to *A. acidoterrestris* species and not to the novel specie such as *A. suci*. The reference strain CECT (Colección Española de Cultivos Tipo) 7094 T (isolated from soil) was also included in the study. All the strains were stored at $-80\text{ }^{\circ}\text{C}$, until their use.

Sporulation of *A. acidoterrestris* was induced following a modified method based on Baysal et al. (2013). Firstly, *A. acidoterrestris* strains were streaked onto YSG agar (Yeast Starch Glucose Agar, Scharlau, Spain) at a pH 3.7 ± 0.2 and plates were incubated at $45\text{ }^{\circ}\text{C}$ for 24 h. Then, to obtain fresh vegetative cells of *A. acidoterrestris*, a single colony was inoculated into 10 mL of BAT broth (*Bacillus acidoterrestris* broth, Scharlau, Spain) and the tube was incubated at $45\text{ }^{\circ}\text{C}$ for 48 h. Then, sporulation of the strains was induced by plating them onto YSG agar and incubating at $45\text{ }^{\circ}\text{C}$ for seven days until at least, 80 % of cells sporulated. Afterwards, to harvest the spores, 5 mL of sterile water was added to YSG agar plates and cells were dislodged by rubbing with a sterile Digralsky loop. Next, each strain's suspension was centrifuged (5000 xg, 15 min at $20\text{ }^{\circ}\text{C}$), and the pellet was resuspended in sterile water and centrifuged again (5000 xg, 10 min at $20\text{ }^{\circ}\text{C}$). This washing procedure was repeated four times. The final pellets were resuspended in sterile water and stored at $4\text{ }^{\circ}\text{C}$ until use, with a maximum usage period of 14 days. The percentage of sporulation in the suspension was monitored by assessing the total cell and spore population weekly as described below in section 2.4.2.

2.4.2. Sample inoculation and population monitoring

A sterile plastic flask containing 30 mL of each juice, was separately inoculated in triplicate with 0.3 mL of *A. acidoterrestris* spores' suspension to achieve a population of approximately 10^5 cfu/mL. Then, the flasks were agitated, and the initial population (day 0) of total cells and spores was determined. For total cells, the samples were serially diluted in saline peptone (SP) and plated onto YSG agar. For spores, according to IFUMM12 (IFU, 2019), samples were subjected to a thermal shock ($80\text{ }^{\circ}\text{C}$ for 12 min) followed by ice-cooling. Then, the samples were plated as described for total cells. The plates were incubated at $45\text{ }^{\circ}\text{C}$ for 24 h. The

inoculated juice samples were stored at $45\text{ }^{\circ}\text{C}$ for 7 days. *A. acidoterrestris* total cell and spore population in the juices was monitored on days 1, 2, 3, 4 and 7.

2.4.3. Guaiacol production

Guaiacol concentration in juices on the seventh day of storage was determined following an adapted method based on Noestheden et al. (2017). The analyses were performed by gas chromatography–mass spectrometry (CG-MS) (Agilent Technologies, United States). For each sample, 1.5 g of saturated sodium chloride were mixed with 5 mL of juice. Next, 50 μL of an internal standard 2-methoxyphenol-3,4,5,6-d4 (CDN Isotopes, Canada) were added and agitated. Subsequently, 2 mL of the extracting solution composed by hexane (Sigma Aldrich, Germany) and ethyl acetate (Sigma Aldrich, Germany) in a 1:1 (v/v) ratio, were added to each sample and agitated for 30 s. Samples were further agitated at 700 rpm for 5 min. After that, the tubes were centrifugated (5000 xg, 10 min) and an aliquot of the organic layer was transferred to a 1.5 mL glass vial for CG-MS analysis. For the analyses, a DB5 MS IS capillary column with dimensions of $30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ mm}$ (Agilent Technologies, United States) was used, and helium was the carrier gas (flow: 1 mL/min). The temperature of the injector port was $250\text{ }^{\circ}\text{C}$ in splitless mode injection, with a purge time of 0.01 min. The ions used for guaiacol quantification were 109 and 113, corresponding to guaiacol and 2-methoxyphenol-3,4,5,6-d4 (internal standard), respectively. Qualifiers ions 81 and 124 (also corresponding to guaiacol) were also used. The quantification limit for guaiacol production was 4 ng/mL.

3. Statistical analysis

All data obtained were processed and graphically represented using Microsoft Excel software. An analysis of variance (ANOVA) with the Tukey test ($p < 0.05$) was applied to evaluate physicochemical analyses, the phenolic profile, the growth, and guaiacol production of *A. acidoterrestris*. A Pearson correlation analyses were conducted to assess the growth and guaiacol production of the different strains in response to all the analysed variables. The correlation value from which the correlation was considered significant was 0.5. All the analyses were performed using JMP Pro 16 statistical software.

4. Results

4.1. Physicochemical characterization

Table 1 presents the results of the physicochemical characterization of the fruit juices, highlighting their diversity. pH values ranged from

Table 2
Concentration (ng/mL) of phenols and anthocyanins of each fruit juice.

Group	Compound	Fruit juice							
		Orange	Apple	Persimmon	Plum	Peach	Black grape	Strawberry	
Phenols	Protocatechuic acid	85.2 ± 1.0 f	160.4 ± 0.9 e	442.5 ± 15.1 c	541.4 ± 14.9 b	386.9 ± 1.5 d	627.4 ± 15.3 a	178.0 ± 3.0 e	
	p-coumaric acid	164.8 ± 1.7 f	548.3 ± 13.1 de	1219.1 ± 43.6 b	894.0 ± 12.2 b	371.8 ± 2.0 ef	781.1 ± 13.6 cd	10,921.1 ± 156.8 a	
	Gallic acid	112.8 ± 0.0 e	116.5 ± 1.4 e	12,372.3 ± 245.2 a	595.3 ± 15.0 d	119.0 ± 1.2 e	5725.7 ± 62.7 b	5045.1 ± 27.1 c	
	Caffeic acid	63.0 ± 1.2 d	1302.6 ± 29.3 c	2438.7 ± 75.4 b	1265.1 ± 55.0 c	1311.7 ± 32.4 c	61.5 ± 0.7 d	2938.3 ± 35.0 a	
	Luteolin	<DL	<DL	<DL	<DL	<DL	73.9 ± 0.7	<DL	
	Myricetin	<DL	<DL	<DL	<DL	<DL	1301.4 ± 20.3	<DL	
	Epigallocatechin gallate	<DL	<DL	<DL	<DL	<DL	10.5 ± 0.5	<DL	
	Sinapic acid	580.5 ± 4.9	<DL	<DL	<DL	<DL	<DL	<DL	
	Resveratrol	<DL	<DL	<DL	<DL	<DL	518.5 ± 26.3	<DL	
	Epicatechin	<DL	400.5 ± 15.9 b	<DL	41.8 ± 0.3 c	<DL	1614.8 ± 33.4 a	14.4 ± 0.3 c	
	Quercetin	87.3 ± 0.6 b	<DL	76.6 ± 0.9 b	<DL	132.1 ± 2.4 b	2033.6 ± 64.6 a	70.0 ± 2.2 b	
Anthocyanins	Kaempferol	58.8 ± 1.6 e	58.7 ± 0.1 e	73.7 ± 2.1 c	57.6 ± 0.1 e	64.7 ± 0.4 d	333.2 ± 2.3 a	139.8 ± 0.8 b	
	Pelargonidin-3-rutinoside chloride	<DL	<DL	<DL	<DL	<DL	<DL	1498.4 ± 47.4	
	Cyanidin chloride	1132.9 ± 22.7 d	163.9 ± 3.8 e	1571.5 ± 9.8 c	117.5 ± 1.6 e	282.7 ± 2.4 e	9997.3 ± 125.8 b	11,636.3 ± 9.8 a	
	Pelargonidin chloride	<DL	<DL	<DL	<DL	<DL	909.7 ± 11.8 b	1071.9 ± 6.3 a	
	Delphinidin-3-o-rutinoside chloride	<DL	<DL	<DL	<DL	<DL	<DL	<DL	
	Guaiacol precursors	Ferulic acid	1372.5 ± 6.6 b	329.3 ± 5.2 d	306.4 ± 15.1 d	363.0 ± 21.5 d	104.2 ± 0.3 e	478.2 ± 13.4 c	1763.0 ± 43.7 a
	Vanillin	87.7 ± 2.2 a	8.7 ± 0.1 d	28.8 ± 0.6 b	11.4 ± 0.4 d	15.4 ± 0.5 c	<DL	12.1 ± 0.2 cd	
Vanillic acid	<DL	<DL	408.2 ± 0.5 b	153.9 ± 0.6 c	739.5 ± 28.4 a	434.8 ± 15.0 b	107.6 ± 1.9 c		

Results are expressed as mean ± standard deviation of two replicates (n = 2) for each analysis. Values with different letters in the same row are significantly different according to the Tukey test HSD or t-Student (p < 0.05). <DL: below detection limit (10 ng/mL).

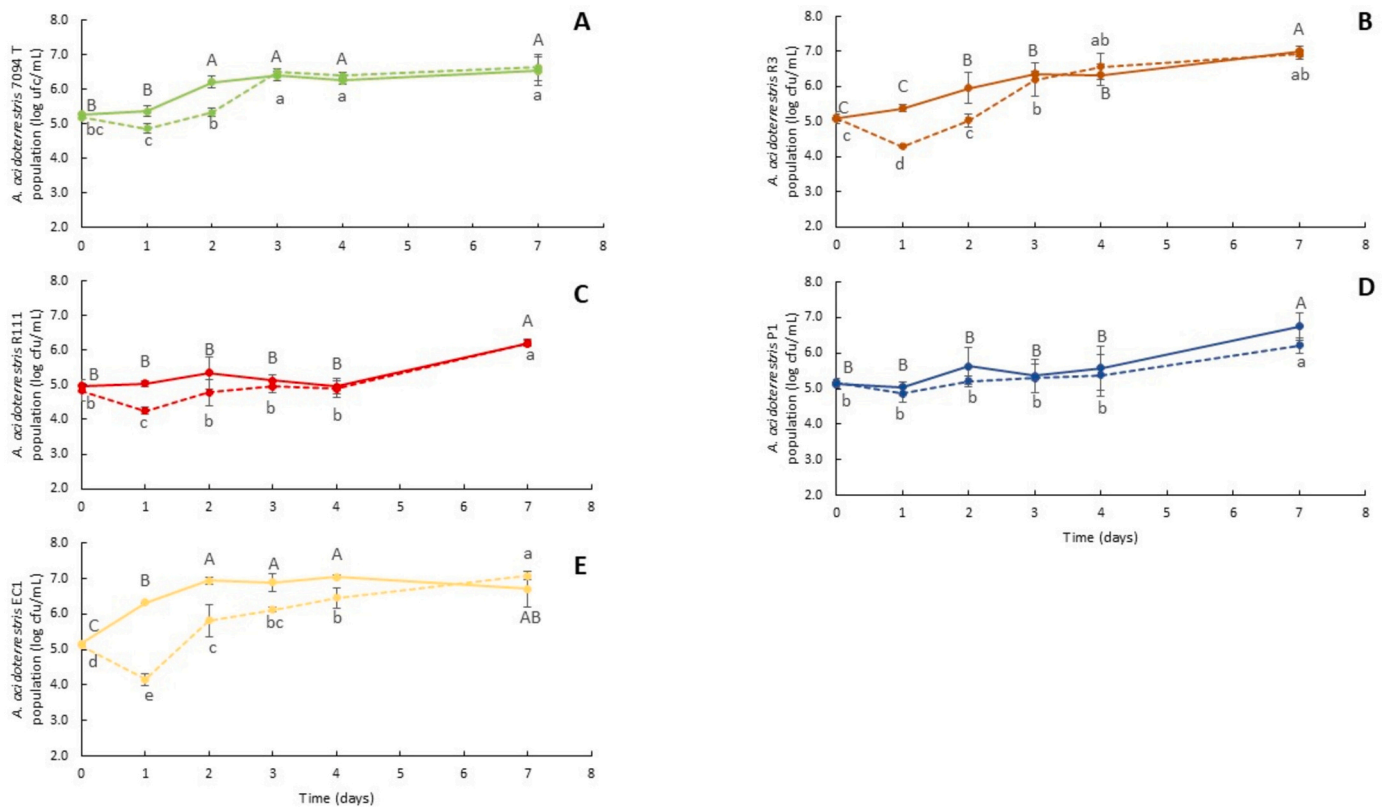


Fig. 1. Total cell populations (solid lines) and spore populations (dotted lines) of *A. acidoterrestris* strains 7094 T (A), R3 (B), R111 (C), P1 (D) and ECl (E) artificially inoculated in orange juice throughout storage for 7 days at 45 °C. Different capital letters (for total cells) and lowercase letters (for spores) indicate significant differences over time according to the analyses of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) test (p < 0.05).

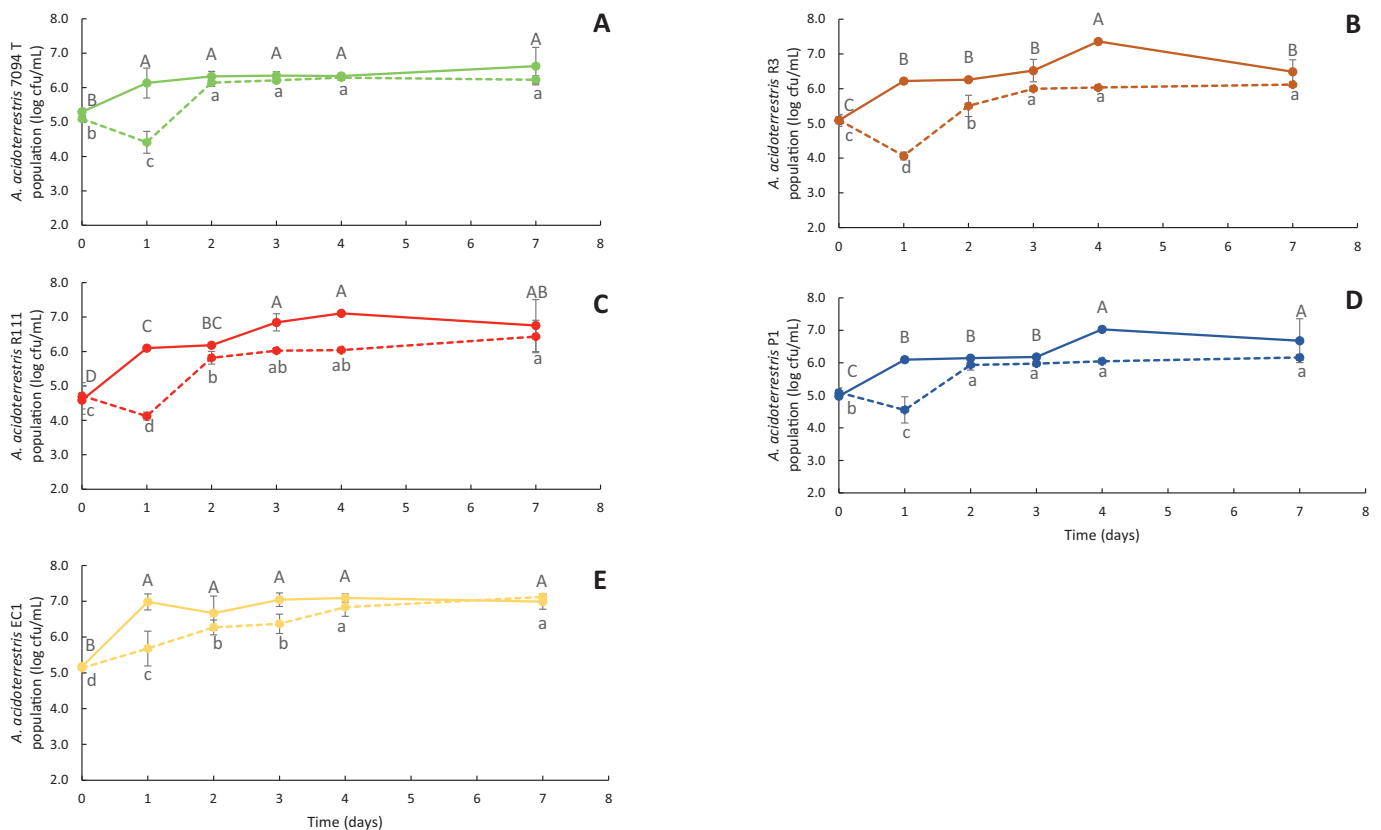


Fig. 2. Total cells populations (solid lines) and spore populations (dotted lines) of *A. acidoterrestris* strains 7094 T (A), R3 (B), R111 (C), P1 (D) and EC1 (E) artificially inoculated in apple juice throughout storage for 7 days at 45 °C. Different capital letters (for total cells) and lowercase letters (for spores) indicate significant differences over time according to the analyses of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) test ($p < 0.05$).

3.35 ± 0.03 (black grape juice) to 4.07 ± 0.08 (persimmon juice) indicating significant variability. SSC varied significantly, from 2.2 ± 0.1 (strawberry juice) to 18.1 ± 0.0°Brix (persimmon juice). Water activity is categorized into two distinct groups: below 0.900 (persimmon and peach juices) and above 0.900 for the rest, ranging from 0.887 ± 0.002 (persimmon juice) to 0.992 ± 0.003 (black grape juice). TA also divides the juices into two groups: one with values over 100 mmol/L (plum and orange juices) and another below 100 mmol/L (black grape apple, persimmon, strawberry, and peach juices). Regarding TPC, results showed great variability with significant differences between samples. The highest TPC content was observed in plum juice (740.8 ± 4.4 mg gallic acid/L of juice), meanwhile the lowest value was in strawberry juice (349.8 ± 9.5 mg gallic acid/L of juice). TAC was only analysed in black grape and strawberry juices due to the samples' characteristics, and there were no significant differences between the samples with 5.2 ± 0.2 mg cyanidin-3-glucoside/L in black grape juice, and 3.5 ± 1.9 mg cyanidin-3-glucoside/L in strawberry juice. Finally, AA ranged from 305.9 ± 27.4 to 799.1 ± 24.3 mg ascorbic acid/L of juice for DPPH method, and from 663.6 ± 0.9 to 1528.9 ± 2.2 mg ascorbic acid/L of juice for FRAP method. In both methods the samples with lower and higher antioxidant activity were peach and strawberry juice, respectively.

4.2. Phenolic profile

Table 2 shows the results of the concentration of phenolic compounds and anthocyanins of each fruit juice, with black grape and strawberry showing the highest diversity and concentrations.

Black grape was the only juice that contained luteolin (73.9 ± 0.7 ng/mL), myricetin (1301.4 ± 20.3 ng/mL), epigallocatechin gallate (10.5 ± 0.5 ng/mL), and resveratrol (518.5 ± 26.3 ng/mL). It also

presented the highest amounts of protocatechuic acid (627.4 ± 15.3 ng/mL), epicatechin (1614.8 ± 33.4 ng/mL), quercetin (2033.6 ± 64.6 ng/mL), and kaempferol (333.2 ± 2.3 ng/mL). Strawberry juice stood out for its significant concentrations of p-coumaric acid (10,921.1 ± 156.8 ng/mL), caffeic acid (2938.3 ± 35.0 ng/mL), and ferulic acid (1763.0 ± 43.7 ng/mL). Sinapic acid was only detected in orange juice (580.5 ± 4.9 ng/mL). The most abundant compound in plum, peach, and apple juices was caffeic acid, with concentrations of 1265.1 ± 55.0, 1311.7 ± 32.4, and 1302.6 ± 29.3 ng/mL, respectively. Persimmon juice stood out with significantly higher levels of gallic acid (12,372.3 ± 245.2 ng/mL) compared to the other juices. Regarding anthocyanins, black grape and strawberry juices showed the highest concentrations. In strawberry juice, pelargonidin-3-rutinoside chloride (1498.4 ± 47.4 ng/mL), cyanidin chloride (11,636.3 ± 9.8 ng/mL), and pelargonidin chloride (1071.9 ± 6.3 ng/mL) were detected. In black grape juice, only cyanidin and pelargonidin chloride (9997.3 ± 125.8 and 909.7 ± 11.8 ng/mL, respectively) were detected. Finally, delphinidin-3-rutinoside chloride was below the detection limit (< 10 ng/mL) in all samples.

Phenolic compounds recognized as guaiacol precursors (ferulic acid, vanillin, and vanillic acid) were also analysed in the fruit juices. Ferulic acid was detected in all the samples, with the highest content found in strawberry juice (1763.0 ± 43.7 ng/mL) and the lowest in peach juice (104.2 ± 0.3 ng/mL). Interestingly, ferulic acid was the most abundant compound in orange juice (1372.5 ± 6.6 ng/mL). Concerning vanillin, the highest quantity was observed in orange juice (87.7 ± 2.2 ng/mL), while a tenfold lower content was detected in apple juice (8.7 ± 0.1 ng/mL) and it was not detected in black grape. As for vanillic acid, the highest concentration was found in peach juice (739.5 ± 28.4 ng/mL), while it was not detected in apple and orange juices.

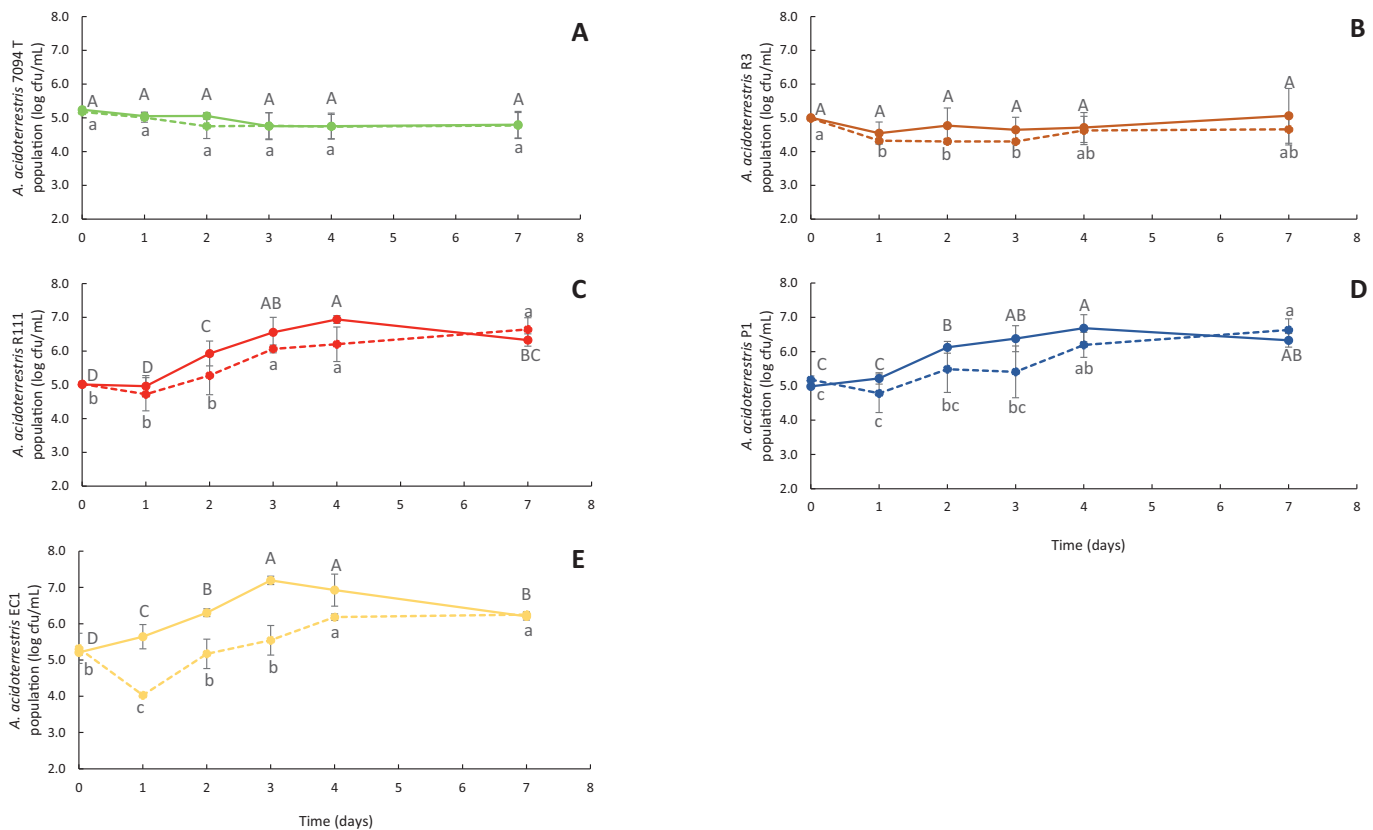


Fig. 3. Total cells populations (solid lines) and spore populations (dotted lines) of *A. acidoterrestris* strains 7094 T (A), R3 (B), R111 (C), P1 (D) and EC1 (E) artificially inoculated in persimmon juice throughout storage for 7 days at 45 °C. Different capital letters (for total cells) and lowercase letters (for spores) indicate significant differences over time according to the analyses of variance (ANOVA) and Tukey's Honestly Significant Differences (HSD) test ($p < 0.05$).

4.3. Growth capacity of *A. acidoterrestris* strains

Fig. 1–7 show the behaviour of *A. acidoterrestris* spores in the studied fruit juices. All fruit juices were inoculated with *A. acidoterrestris* spores at an initial population of 10^5 cfu/mL and stored at 45 °C for 7 days. The *A. acidoterrestris* total cell and spore populations were enumerated during the juice storage.

All the strains grew in orange juice (Fig. 1). After 7 days, total cells and spores' populations reached 6 log cfu/mL, with strains EC1 and R3 achieving 7 around log cfu/mL on days 4 and 7, respectively. Strains 7094 T and R3 showed a significant increase in total cell population after 2 days, reaching 6.2 ± 0.2 and 6.0 ± 0.4 log cfu/mL, respectively. Afterwards, the cell counts of 7094 T stabilized, whereas R3 increased significantly from day 2 to 7 reaching a final population of 7.0 ± 0.1 log cfu/mL. For R111 and P1, total cells and spore counts remained steady initially, however, both populations (total cells and spores) continued increasing notably after 7 days. The total cell counts of EC1 reached 6.3 ± 0.0 log cfu/mL by day 1, accompanied by a significant decrease in spore population to 4.2 ± 0.2 log cfu/mL, indicating spore germination. This spore reduction was similarly observed in strains R111 and R3.

The five strains showed growth in apple juice (see Fig. 2). Rapid total cell growth occurred from day 0 to 1, with total cell count between 6.1 ± 0.4 to 7.0 ± 0.2 log cfu/mL. From day 1 onwards, no significant differences were noted in total cells and spore population among the strains. Strains 7094 T and EC1 reached their maximum total cell population after 1 day, while strain R111 took 3 days, and strains R3 and P1 required 4 days. Regarding spores, in line with observations in orange juice, germination occurred on day 1 in all strains. Subsequently, the spore population increased, reaching values like those of total cells by the end of the storage period.

The five tested strains of *A. acidoterrestris* behaved differently in

persimmon juice (Fig. 3). Population of strains R111, P1 and EC1 reached 6.9 ± 0.1 , 6.7 ± 0.4 and 7.2 ± 0.1 log cfu/mL for total cells, respectively, by fourth day for R111 and P1 and third day for EC1 at 45 °C. After reaching their maximum total cell population, the populations of strains R111 and EC1 significantly reduced ($p < 0.05$) after 7 days of storage. On the seventh day, the spore populations for strains R111, P1, and EC1 were 6.6 ± 0.3 , 6.6 ± 0.3 , and 6.3 ± 0.1 log cfu/mL, respectively. The most significant reduction in spore population due to the germination process was observed in strain EC1 after 1 day, which reached 4.0 ± 0.0 log cfu/mL. In contrast, the growth of strains 7094 T and R3 in persimmon juice was inhibited, with their populations remaining constant (above 4 log cfu/mL) over time, for both total cells and spores.

Fig. 4 depicts growth patterns in plum juice for *A. acidoterrestris* strains 7094 T, R111, and P1, where significant increases ($p < 0.05$) in total cells were noted by the second day, reaching 5.8 ± 0.3 , 6.2 ± 0.4 , and 6.1 ± 0.1 log cfu/mL respectively. Following this, their total cell populations stabilized, while spore counts remained lower than total cells. Specifically, strain R111 spore population raised notably to 5.4 ± 0.6 log cfu/mL by day 7. Conversely, strains EC1 and R3 initially experienced significant total cells reductions, with decreases of 1.5 and 0.8 logarithmic units, respectively. Subsequently, from day 2 onwards, both strains exhibited exponential growth, achieving total cells population of 6.6 ± 0.3 and 6.5 ± 0.3 log cfu/mL, respectively.

In peach juice (Fig. 5), the growth of the tested strains was inhibited, with the populations of total cells and spores remaining stable around 5 log cfu/mL. No significant changes ($p < 0.05$) were observed over time for strains 7094 T, R3, R111, and P1, indicating that *A. acidoterrestris* predominantly existed in spore form in these samples, as evidenced by the consistent counts between total cells and spores for all evaluated strains. However, strain EC1 (Fig. 5, E) was the most sensitive,

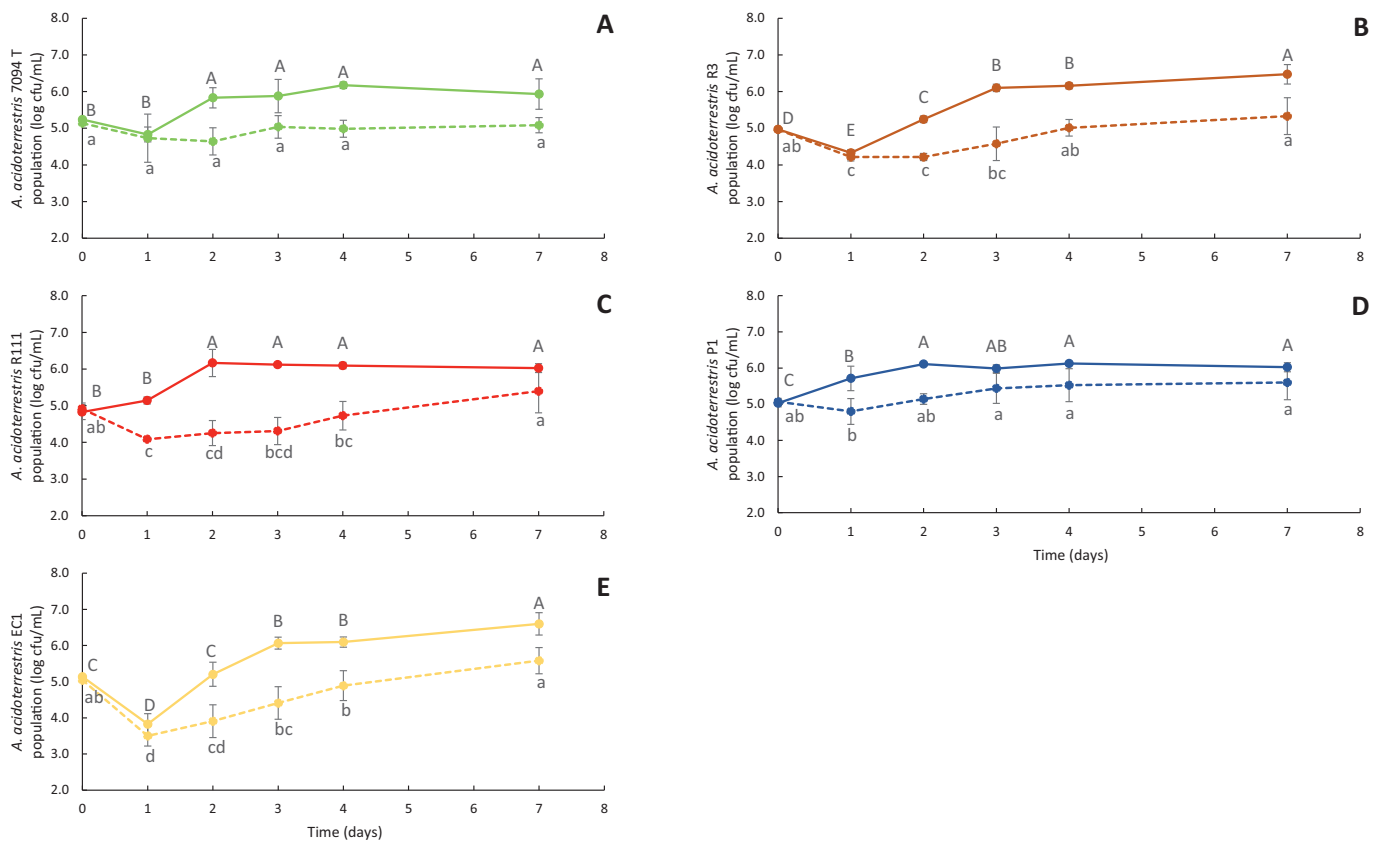


Fig. 4. Total cells populations (solid lines) and spore populations (dotted lines) population of *A. acidoterrestris* strains 7094 T (A), R3 (B), R111 (C), P1 (D) and EC1 (E) artificially inoculated in plum juice throughout storage for 7 days at 45 °C. Different capital letters (for total cells) and lowercase letters (for spores) indicate significant differences over time according to the analyses of variance (ANOVA) and Tukey's Honestly Significant Differences (HSD) test ($p < 0.05$).

experiencing a significant decline after just one day, with reductions of 1.1 log units for both total cells and spores. By the seventh day, the populations had decreased to 2.9 ± 0.1 and 3.1 ± 0.1 log cfu/mL for total cells and spores, respectively.

In black grape juice (Fig. 6), growth of all the tested strains was inhibited, with no significant differences observed between the total cell and spore populations. From day 1 to 7, the populations ranged from 4.9 ± 0.1 to 4.6 ± 0.3 log cfu/mL for strain 7094 T, from 4.2 ± 0.1 to 4.7 ± 0.6 log cfu/mL for strain R111, and from 4.2 ± 0.0 to 4.6 ± 0.5 log cfu/mL for strain P1. However, strains R3 and EC1 experienced a significant decrease in total cell population, reaching 4.2 ± 0.1 log cfu/mL and 3.7 ± 0.7 log cfu/mL after 7 days, respectively.

Fig. 7 shows that strawberry juice inhibited growth in all tested strains. For R3 and R111, total cell populations remained stable, ranging from 5.0 ± 0.1 to 5.3 ± 0.9 log cfu/mL for R3 and from 4.5 ± 0.4 to 4.6 ± 0.5 log cfu/mL for R111, from day 0 to day 7. Total cell population of the strain 7094 T decreased from 4.7 ± 0.3 log cfu/mL on day 3 to 4.3 ± 0.2 log cfu/mL by day 7. Strain EC1, dropped by 1.1 logarithmic units initially but stabilized from day 1 to 4 (4.1 ± 0.0 log to 4.2 ± 0.4 log cfu/mL), before rising to 5.4 ± 0.2 log cfu/mL on day 7, nearly returning to its initial level (5.2 ± 0.4 log cfu/mL). Total cell counts for strain P1 significantly decreased by 0.7 logarithmic units on the first day, then stabilized until day 7, ending at 4.9 ± 0.1 log cfu/mL.

4.4. Guaiacol production

Fig. 8 illustrates the guaiacol production produced by *A. acidoterrestris* strains in the different juices on day seven, revealing significant production differences. Orange and persimmon juices exhibited the highest guaiacol concentrations, with values of 362.3 ± 12.6 and 325.1 ± 1.4 ng/mL, respectively. In orange juice, R111 and P1

were the most productive strains (339.1 ± 12.3 and 362.3 ± 12.6 ng/mL, respectively), and EC1 the least (151.5 ± 7.1 ng/mL). In persimmon juice, R111, P1, and EC1 strains produced similar quantities (306.9 ± 11.4 , 286.6 ± 8.2 and 325.1 ± 1.4 ng/mL, respectively), but R3 produced significantly less (119.2 ± 65.1 ng/mL), and guaiacol produced by strain 7094 T was below the quantification limit (< 4 ng/mL). For plum, black grape and strawberry juice, guaiacol production was lower and more uniform among the strains. In plum juice, EC1 produced the highest amount of guaiacol (63.6 ± 1.8 ng/mL), while 7094 T was the less producer (23.4 ± 1.5 ng/mL). Black grape juice showed no significant variance in guaiacol levels (from 16.9 ± 2.8 to 49.9 ± 20.8 ng/mL). Strawberry juice revealed a similar pattern, except for strain 7094 T, which had no detectable guaiacol production. However, strains R111 and EC1 produced guaiacol within a range of 41.6 ± 36.5 to 105.0 ± 33.7 ng/mL, respectively. Apple juice, yielded low guaiacol levels, ranging from 5.4 ± 1.2 (strain EC1) to 8.4 ± 0.3 ng/mL (strain R3), while guaiacol produced by strain 7094 T was below the quantification limit. In peach juice, only the strain 7094 T produced guaiacol above the quantification limit (97.7 ± 8.4 ng/mL).

4.5. Pearson correlation

A Pearson correlation analysis (Fig. 9) was conducted to investigate the relationship between total cell and spore populations, and guaiacol production of *A. acidoterrestris* strains and juice's physicochemical characteristics. pH showed a strong positive correlation with the spore formation of strains R111 and P1, with coefficients of 0.63 and 0.71, respectively. Additionally, guaiacol production in strains R111 and EC1 was positively correlated, with coefficients of 0.54 and 0.63, respectively. TA was positively correlated with the total cell population of strain 7094 T (0.56) and with total cells, spores and guaiacol production

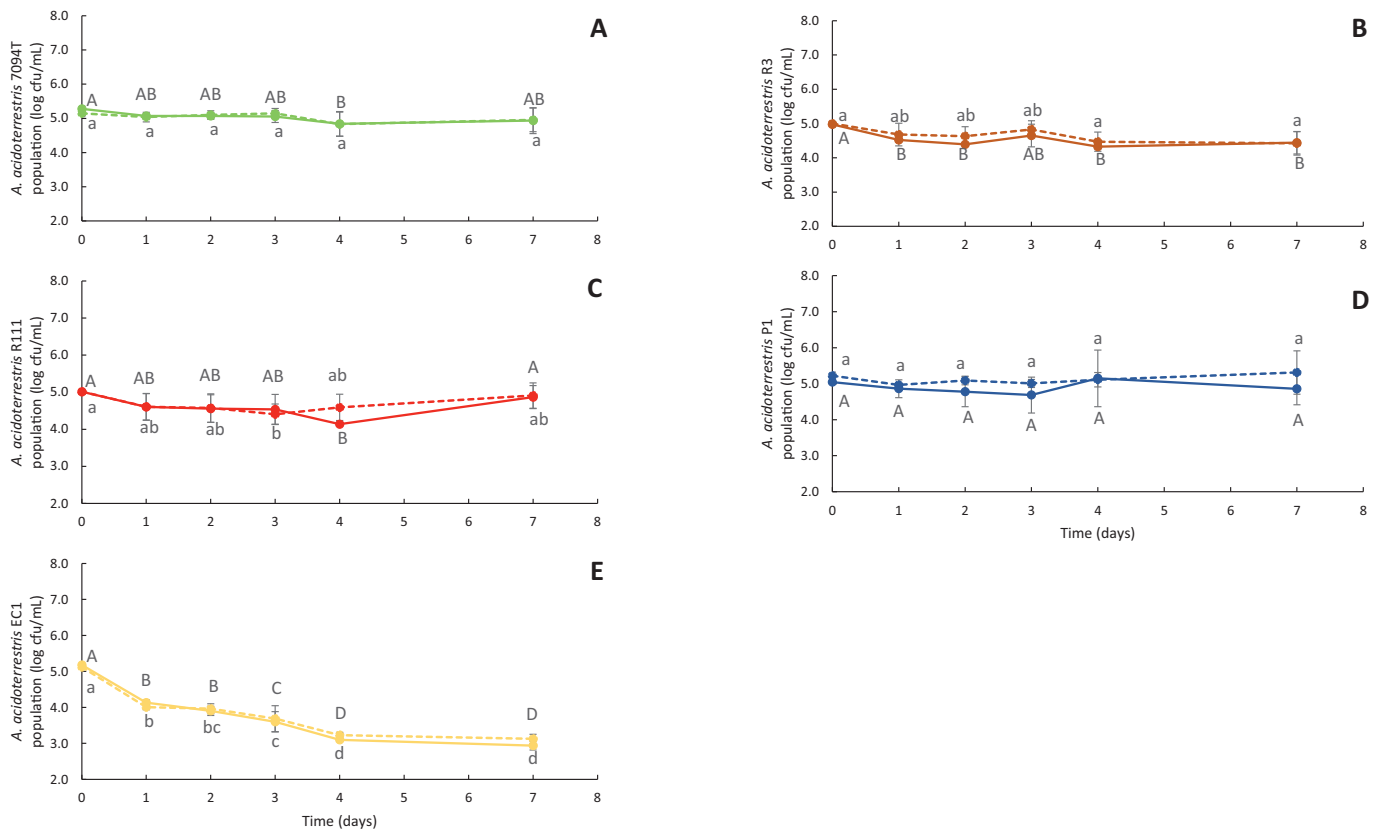


Fig. 5. Total cells populations (solid lines) and spore populations (dotted lines) population of *A. acidoterrestris* strains 7094 T (A), R3 (B), R111 (C), P1 (D) and EC1 (E) artificially inoculated in peach juice throughout storage for 7 days at 45 °C. Different capital letters (for total cells) and lowercase letters (for spores) indicate significant differences over time according to the analyses of variance (ANOVA) and Tukey's Honestly Significant Differences (HSD) test ($p < 0.05$).

of strain R3 (0.66, 0.62, and 0.61, respectively). Conversely, water activity was negatively correlated with the guaiacol production of strains R111, P1, and EC1 (−0.65, −0.56, and −0.96, respectively).

Phenolic compounds such as kaempferol and cyanidin chloride predominantly showed negative correlations with *A. acidoterrestris* growth. Kaempferol negatively correlated with the total cell counts of all tested strains (−0.50 to −0.60) and against spore population of strains R3 (−0.52), P1 (−0.63), and EC1 (−0.51). Cyanidin chloride also demonstrated negative correlations with the total cells of strain 7094 T (−0.65) and with both total cells and spores of strains R111 (−0.66 and −0.59) and P1 (−0.63 and −0.65). Furthermore, p-coumaric acid showed a strong negative correlation with guaiacol production of the strain 7094 T, with a correlation coefficient of −0.91. Quercetin was negatively correlated with guaiacol production across all the tested strains (−0.52 to −0.73).

Regarding guaiacol precursors, vanillin showed the highest correlation with guaiacol production across nearly all cases. Strains 7094 T, R3, R111, and P1 exhibited positive correlation coefficients of 0.97, 0.99, 0.82, and 0.87, respectively. Notably, strain 7094 T also demonstrated a strong correlation with both ferulic and vanillic acids (0.84 and 0.89, respectively). However, strain EC1 showed lower correlations between guaiacol and both vanillin and vanillic acid (0.32 and 0.33, respectively). Additionally, guaiacol production was positively correlated with compounds not traditionally recognized as precursors, including TAC, gallic acid, and epicatechin. Specifically, TAC showed a correlation coefficient of 1.0 with strains R3, R111, and EC1, indicating a perfect positive correlation. Gallic acid had a positive effect, particularly on strain EC1, with a correlation coefficient of 0.72. And epicatechin also had a positive correlation but only on strain 7094 T (1.0). Conversely, quercetin was associated with a negative effect on guaiacol production across all strains, with correlation coefficients of −0.73 for 7094 T,

−0.59 for R3, −0.61 for R111, −0.52 for P1, and −0.64 for EC1.

5. Discussion

The recurrent spoilage episodes in fruit juices caused by *A. acidoterrestris* concern fruit juice manufacturers. The formulation of strategies to avoid its growth and guaiacol production in fruit juices would reduce the number of spoilage incidents. For this, it is necessary to understand *A. acidoterrestris* growth and guaiacol production in fruit juices with different characteristics. In this study, the behaviour of five strains of *A. acidoterrestris*, four of them isolated from commercial juices (Neggazi et al., 2023) and one reference strain (CECT 7094 T) in seven fruit juices has been evaluated. To this day, this is the first study of five different *A. acidoterrestris* spores' behaviour in several fruit juices under the same conditions.

In the present study, the five tested *A. acidoterrestris* strains grew in orange juice, achieving populations of 7.0 log cfu/mL (total cells) and 7.1 log cfu/mL (spores) on the seventh day of incubation, with guaiacol production around 360 ng/mL. Similarly, Gocmen et al. (2005) reported that *A. acidoterrestris* ATCC 49025 reached population values close to 8.0 log cfu/mL in a commercial orange juice stored at 45 °C for 28 days.

Regarding apple juice, *A. acidoterrestris* grew reaching populations from 6.3 to 7.4 log cfu/mL for total cells and populations from 6.0 to 7.1 log cfu/mL for spores, with guaiacol productions ranging from 5.4 ± 1.2 (EC1) to 8.4 ± 0.3 (R3) ng/mL on the seventh day of storage. Bahçeci and Acar (2007) reported lower *A. acidoterrestris* DSM 2498 populations (10^5 to 10^6 cfu/mL) at the end of the lag phase (at 28.4 to 37.6 h, depending on the initial inoculum) in apple fruit juice stored at 46 °C. However, guaiacol production was higher than the reported in this study, reaching 70 µg/mL. Orr et al. (2000) also observed lower growth of a cocktail of *A. acidoterrestris* spores inoculated at approximately 3 log

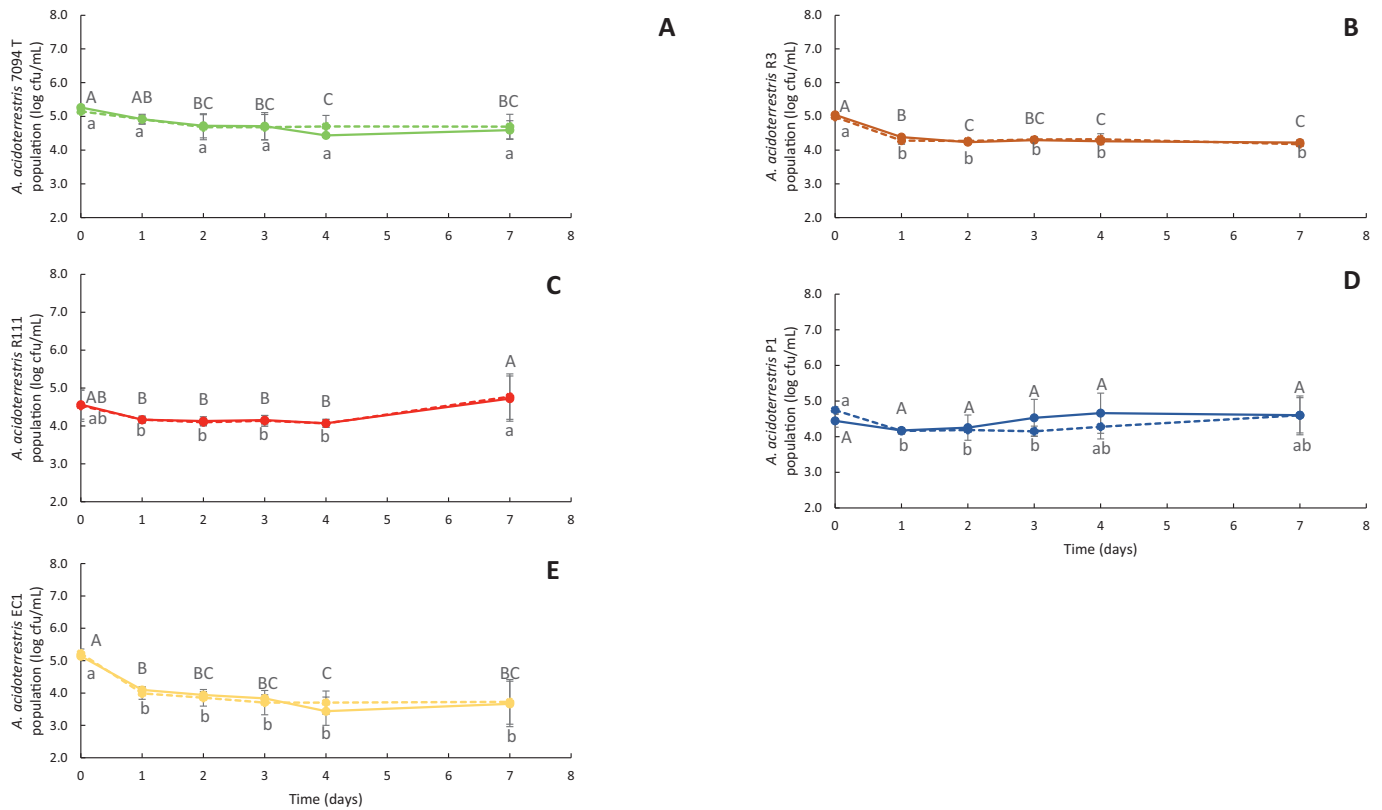


Fig. 6. Total cells populations (solid lines) and spore populations (dotted lines) population of *A. acidoterrestris* strains 7094 T (A), R3 (B), R111 (C), P1 (D) and EC1 (E) artificially inoculated in black grape juice throughout storage for 7 days at 45 °C. Different capital letters (for total cells) and lowercase letters (for spores) indicate significant differences over time according to the analyses of variance (ANOVA) and Tukey's Honestly Significant Differences (HSD) test ($p < 0.05$).

cfu/mL in apple juice and stored at 37 °C for sixty-one days, reaching 4.5 log cfu/mL of spores. Nonetheless, the guaiacol concentration detected by a sensory panel was similar to the concentration we have detected (between of 8.1 to 11.4 ng/mL). Variations between our findings and previous studies are attributable to multiple factors. The *A. acidoterrestris* strains used in our study differ from those in prior research, possibly leading to varied behaviours and responses. Methodological differences in measuring guaiacol production could also influence outcomes. Our results also showed distinct guaiacol production among strains, highlighting their variability. In persimmon and plum juices, certain strains showed growth, but only R111, P1, and EC1 managed to grow in persimmon juice. Growth was restricted in peach, black grape, and strawberry juices, with no existing data on *A. acidoterrestris* growth and guaiacol production in these specific juices.

Physicochemical factors influencing *A. acidoterrestris* growth and guaiacol production in juices include pH, SSC, water activity, and titratable acidity. Yokota et al. (2008) described optimal growth conditions as a pH range of 3.5 to 4.0, SSC between 10 and 18.0°Brix, and water activity above 0.900. Furthermore, Yokota et al. (2008), highlighted those factors such as the nutritional state of cells, types of organic acids, polyphenols, and precursor presence also play crucial roles in growth and guaiacol production. Bevilacqua et al. (2023) demonstrated that blends of organic acids, particularly ascorbic acid, significantly impact *A. acidoterrestris* spore activation, with ascorbic acid providing most effective in reducing viable counts in Malt Extract Broth.

All the juices tested in this study fell within the optimal ranges for *A. acidoterrestris* growth regarding physicochemical characteristics, except for SSC (18.1 ± 0.0) and water activity (0.887 ± 0.002 and 0.898 ± 0.007) in persimmon and peach juices, respectively. While the analysed juices had different types of main organic acid, attributing the growth or non-growth to a specific organic acid is challenging due to the complexity of food matrices. Although, in general, the values of pH, SSC,

and water activity were within the favourable range of growth of *A. acidoterrestris*, growth inhibition was observed in persimmon (strains 7094 T and R3), peach, black grape, and strawberry juices. However, in our correlation analyses factors such as pH, or TA influenced positively on *A. acidoterrestris* spore formation.

As mentioned above, phenolic compounds are another important factor that can influence *A. acidoterrestris* growth and guaiacol production. Some of these compounds are recognized for their antimicrobial properties, playing a crucial role in influencing microbial behaviour in foods containing these substances. Some authors have described the bacteriostatic or bactericidal effect of individual compounds, such as resveratrol, quercetin, vanillic acid, gallic acid, coumaric acid, synapic acid, catechins, myricetin, kaempferol, and luteolin (Lima et al., 2019; Oita and Kohyama, 2002; Puupponen-Pimiä et al., 2001; Sanhueza et al., 2017). Although anthocyanins, found in berries and including pelargonidin, delphinidin, and cyanidin exhibit antimicrobial effects against Gram-positive bacteria, suggesting they may control *A. acidoterrestris*, their specific interactions with this microorganism are yet to be explored (Cisowska et al., 2011; Ma et al., 2019; Puupponen-Pimiä et al., 2001; Naz et al., 2007). However, *A. acidoterrestris* ability to produce guaiacol, leading to juice spoilage, depends on the availability of specific precursors. Identifying these precursors in juices is essential for managing and mitigating spoilage risks.

Notably, gallic acid, recognized for its antimicrobial effects (Lima et al., 2019; Sanhueza et al., 2017), was abundant in persimmon juice, where the growth of the strains 7094 T and R3 was inhibited. This inhibition aligns with correlation analyses indicating that strains 7094 T (total cells-gallic acid = -0.58) and R3 (total cells-gallic acid = -0.44) were particularly affected by gallic acid. In peach juice, the main phenolic compounds identified were caffeic acid and vanillic acid. The growth inhibition of *A. acidoterrestris* in this juice could be attributed to the presence of vanillic acid, as it showed a negative correlation with the

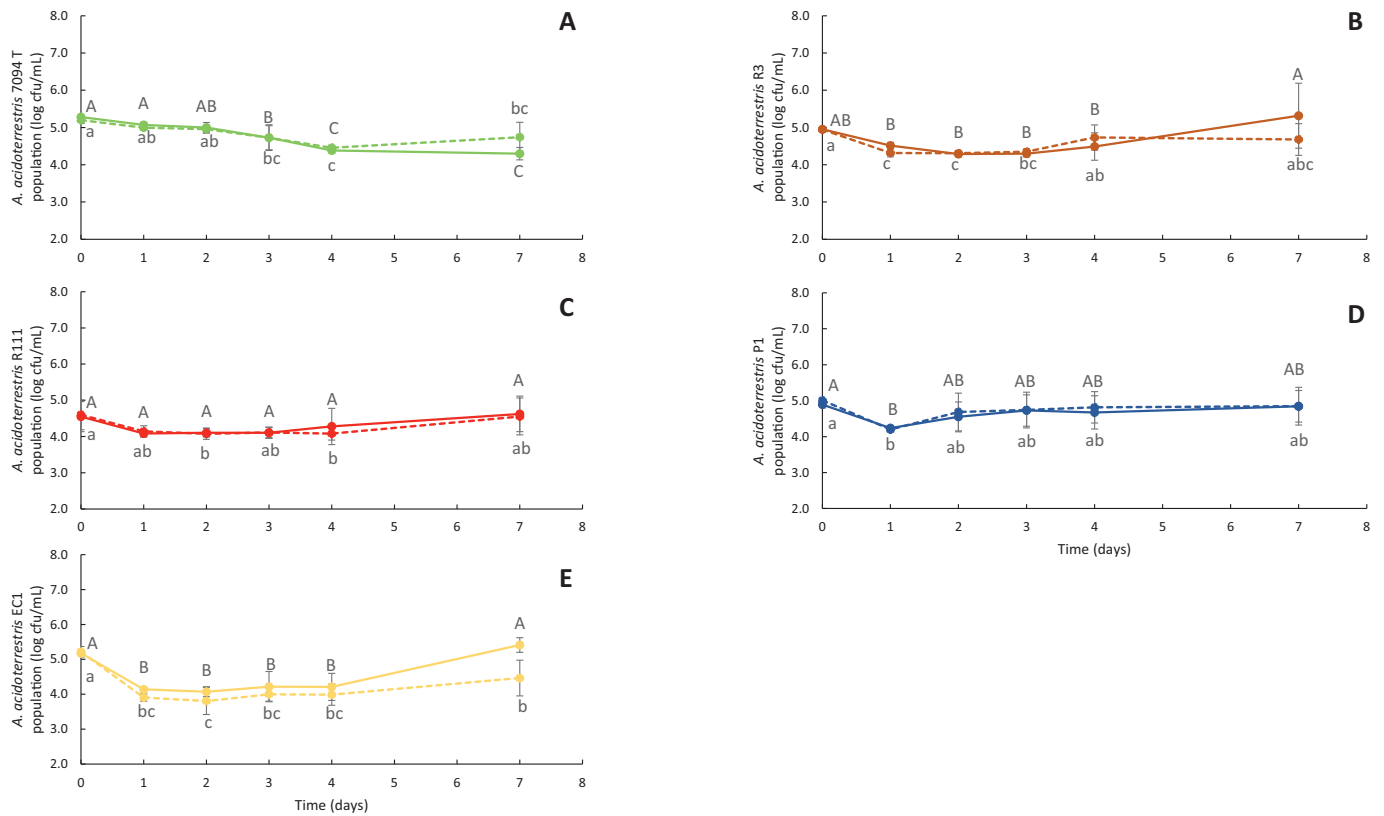


Fig. 7. Total cells populations (solid lines) and spore populations (dotted lines) population of *A. acidoterrestris* strains 7094 T (A), R3 (B), R111 (C), P1 (D) and EC1 (E) artificially inoculated in strawberry juice throughout storage for 7 days at 45 °C. Different capital letters (for total cells) and lowercase letters (for spores) indicate significant differences over time according to the analyses of variance (ANOVA) and Tukey’s Honestly Significant Differences (HSD) test ($p < 0.05$).

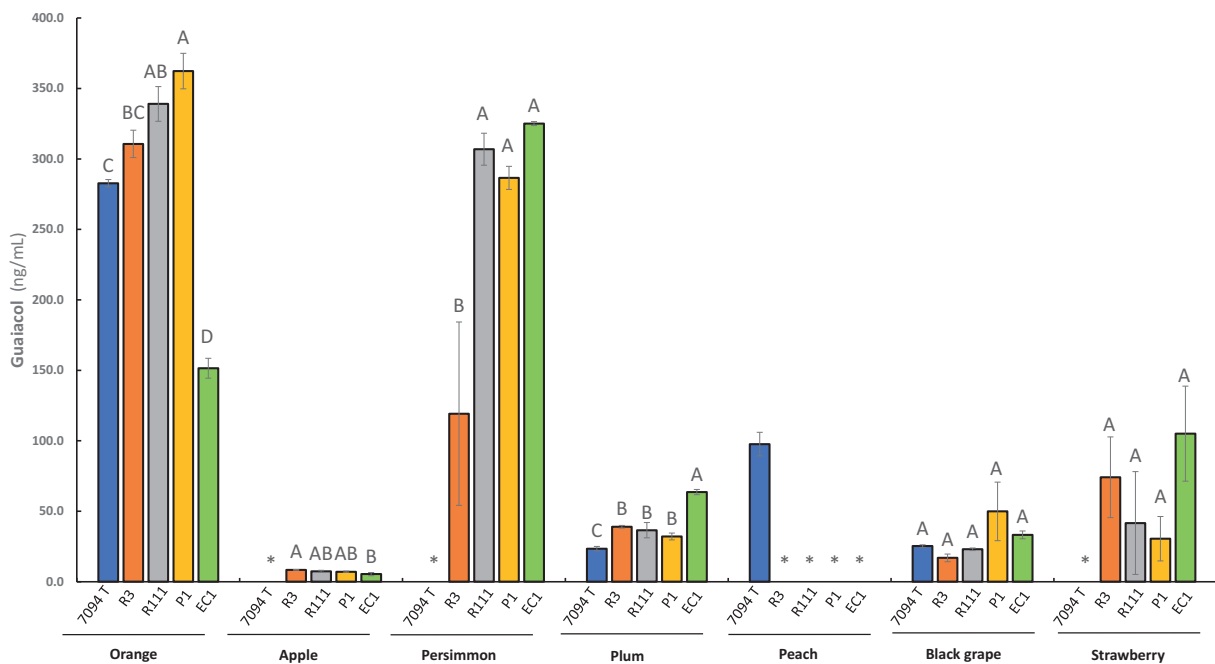


Fig. 8. Guaiacol concentration in fruit juices artificially inoculated with *A. acidoterrestris* strains after 7 days of incubation at 45 °C. The asterisk indicates that the concentration was below quantification limit (< 4 ng/mL). Values are the mean \pm standard deviation ($n = 2$). Different letters indicate significant differences in the guaiacol concentration between strains for each juice according to the analyses of variance (ANOVA) and the Tukey’s Honestly Significant Differences (HSD) test ($p < 0.05$).

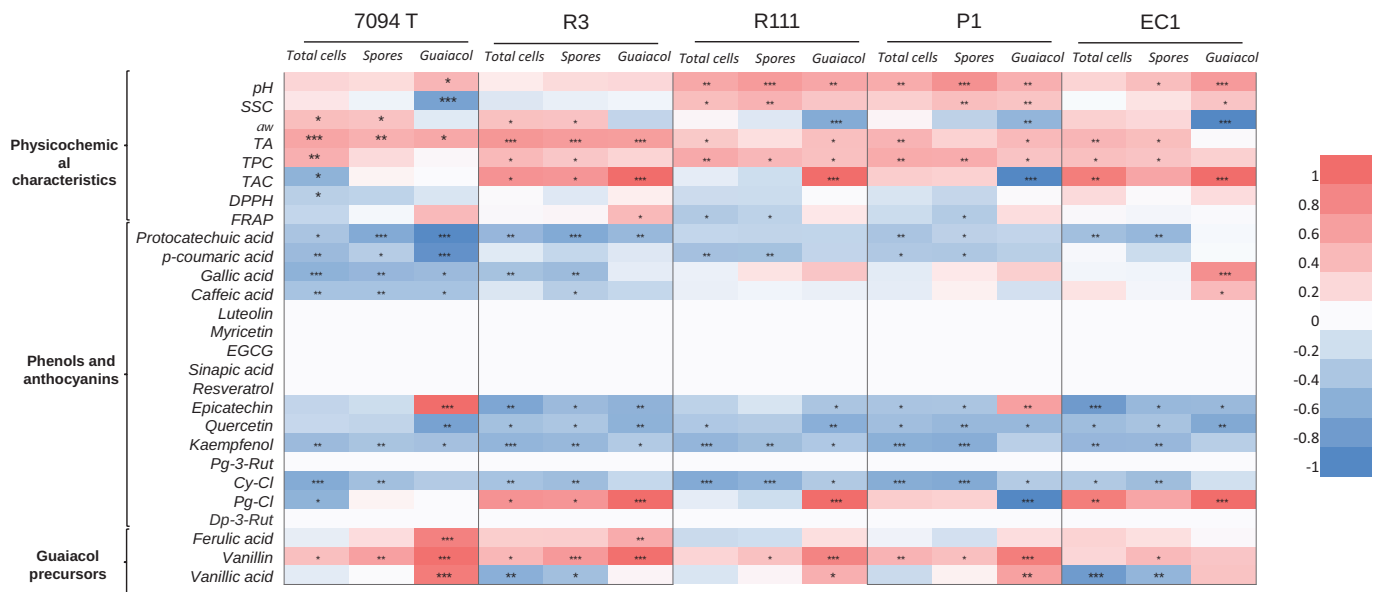


Fig. 9. Heat map showing the Pearson correlation between total cell population, spore population, and guaiacol production for each strain (7094 T, R3, R111, P1, and EC1) against the different variables analysed in fruit juices. SSC: Soluble solids content; a_w : water activity; TA: Titratable acidity; TPC: Total phenolic content; TAC: Total anthocyanins content; DPPH: 2,2-Diphenyl-picrylhydrazyl; FRAP: Ferric reducing antioxidant power; EGCG: Epigallocatechin gallate; Pg-3-Rut: Pelargonidin-3-rutinoside chloride; Cy-Cl: Cyanidin chloride; Pg-Cl: Pelargonidin chloride; Dp-3-Rut: Delphinidin-3-o-rutinoside chloride. (***) = $p < 0.0001$; ** = $p < 0.005$; * = $p < 0.05$)

total cell populations of strains R3 (−0.61) and EC1 (−0.76). The growth inhibition observed in black grape and strawberry juices may be linked to the wide variety of phenolic compounds present in their compositions. For example, kaempferol, found in higher concentrations in these juices, showed a negative correlation with the total cell populations of all strains (7094 T (−0.51), R3 (−0.56), R111 (−0.55), P1 (−0.60) and EC1 (−0.51)). P-coumaric acid, also observed in high quantities in these juices, negatively affected the total cell population of strains 7094 T (−0.50) and R111 (−0.45). Epicatechin, found in higher concentrations in black grape juice, showed a strong negative correlation with total cell population of the strains R3 (−0.70) and EC1 (−0.78). However, in the juices where *A. acidoterrestris* growth was observed (orange, apple, and plum juices), the detected compounds were present in lower amounts and did not significantly influence its development.

Regarding anthocyanins, black grape and strawberry juices were particularly rich in three of the four analysed anthocyanins, with cyanidin chloride being the most abundant in both samples. Significantly, cyanidin chloride was the anthocyanin most negatively correlated with the growth of *A. acidoterrestris* strains 7094 T, R111, and P1, indicating its high antimicrobial effect against these strains. It is crucial to recognize that the effectiveness of these compounds, including both anthocyanins and phenols, is enhanced through synergistic interactions, thereby inhibiting microbial growth more effectively (Salaheen et al., 2016; Sanhueza et al., 2017; Singh et al., 2016). Nevertheless, we have observed differences in resistance to certain phenolic compounds with antimicrobial properties among strains. This variability underscores the need of considering the specific characteristics and origin of each strain in understanding microbial resistance. Yokota et al. (2008) also reported that some strains might exhibit resistance to these antimicrobial factors, a finding corroborated by our study, which showed that only three of the five evaluated strains were able to grow in persimmon juice after 7 days at 45 °C.

Concerning guaiacol production, the highest concentrations were found in orange juice, which supported the highest growth of all *A. acidoterrestris* strains and contained vanillin and ferulic acid, but not vanillic acid. However, the most accepted precursor of guaiacol is vanillic acid (Chang and Kang, 2004; Witthuhn et al., 2013). Apple juice supported growth but yielded quite lower guaiacol levels, with detected

levels of vanillin (8.7 ng/mL) and ferulic acid (329.3 ng/mL), but vanillic acid was not detected. Despite the presence of all three guaiacol precursors in plum and persimmon juices, a marked difference in guaiacol production was noted in persimmon juice, guaiacol reached up to 325.1 ng/mL, whereas in plum juice the highest production was only 63.6 ng/mL. Both juices contained vanillin and vanillic acid, but plum juice had nearly half amounts found in persimmon juice, with similar levels of ferulic acid. Although R3 strain did not grow in persimmon juice, it did produce guaiacol, in contrast to 7094 T strain. In the case of peach juice, which presented the highest quantity of vanillic acid (739.5 ± 28.4 ng/mL), the growth of all *A. acidoterrestris* strains was inhibited and only the strain 7094 T produced guaiacol. Witthuhn et al. (2012) found that 1000 µg/mL of vanillic acid in BAT broth inhibited *A. acidoterrestris* growth and that guaiacol production. Therefore, growth and guaiacol inhibition in peach juice could be related to its vanillic acid content. In addition, Witthuhn et al. (2012) also reported that guaiacol production of its tested strain was faster from vanillin than from vanillic acid. Our Pearson correlation analysis study showed that vanillin strongly correlated with guaiacol production in four out of the five evaluated strains (7094 T, R3, R111, and P1) with coefficients ranging from 0.82 to 0.99 while the presence of vanillic acid only positively correlated with guaiacol production in strains 7094 T, R111, and P1, with values ranging from 0.47 to 0.89. However, for strain EC1, the correlation with vanillin and vanillic acid was similar and not significant. Given that orange juice lacked vanillic acid, it can be inferred that its guaiacol production primarily stemmed from ferulic acid and vanillin. Although Bahçeci and Acar (2007) identified ferulic acid as a potential guaiacol precursor, Cai et al. (2015) found that thirty *A. acidoterrestris* strains could convert vanillic acid and vanillin into guaiacol, but not from ferulic acid. Considering the findings reported by the authors, the production of guaiacol can be attributed to the presence of vanillic and vanillin. However, our study found a significant positive correlation between guaiacol production and ferulic acid content for strains 7094 T (0.84) and R3 (0.51). This highlights the variability in guaiacol production capabilities among strains, emphasizing the importance of considering strain-specific responses to both guaiacol precursors and antimicrobial compounds.

A. acidoterrestris growth was inhibited in black grape and strawberry

juices, stabilizing around 4 log cfu/mL, yet guaiacol was produced, facilitated by guaiacol precursors like ferulic and vanillic acids in black grape, and additionally vanillin in strawberry juice. A population of 4 log cfu/mL is enough for guaiacol production, potentially spoiling these juices as the threshold for odour and taste detection is very low, at 0.021 and 0.013 µg/mL, respectively (Pornpukdeewattana et al., 2019; Sourri et al., 2022).

6. Conclusion

To our knowledge, this is the first investigation into *A. acidoterrestris* behaviour and guaiacol production in less commonly consumed juices, addressing a growing interest in diversifying fruit juice offerings beyond traditional choices like apple and orange juice. All tested strains grew in apple, orange, and plum juices, but growth was limited to three strains in persimmon juice. Notably, orange and persimmon juices were distinguished by their guaiacol production levels. Interestingly, kaempferol, p-coumaric acid, and cyanidin chloride showed negative correlations with growth in nearly all tested strains. Moreover, vanillin was predominantly associated with guaiacol production in almost all tested strains. The presence of guaiacol above sensory detection thresholds poses significant concerns for juice producers, underscoring the necessity of devising strategies to curtail *A. acidoterrestris* growth and its guaiacol production. Such strategies could include the implementation of good production practices and the application of barrier technologies, like the addition of antimicrobial agents combined with final treatments such as high hydrostatic pressure or pasteurization. This is particularly critical for orange juice, given its global production, as evidenced by the growth and guaiacol output of all strains examined in our study. These insights underscore the complexity of managing *A. acidoterrestris* in fruit juices and the need for strategies specifically designed to address the unique physicochemical environments and microbial dynamics of these beverages.

CRedit authorship contribution statement

Isma Neggazi: Writing – original draft, Software, Methodology, Investigation, Data curation, Conceptualization. **Pilar Colás-Medà:** Writing – review & editing, Validation, Supervision, Software, Methodology, Investigation, Data curation, Conceptualization. **Inmaculada Viñas:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Maria Belén Bainotti:** Methodology, Investigation, Data curation. **Isabel Alegre:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Data availability

Data will be made available on request.

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