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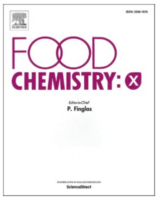

Exploring the growth characteristics of *Alicyclobacillus acidoterrestris* for controlling juice spoilage with zero additives

Congdi Shang[1], Tong Zhang[1], Junnan Xu, Ning Zhao, Wentao Zhang[*], Mingtao Fan[*]

College of Food Science and Engineering, Northwest A&F University, Shaanxi, Yangling 712100, China

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ABSTRACT

Fruit juice spoilage that caused by contaminated *Alicyclobacillus* has brought huge losses to beverage industry worldwide. Thus, it is very essential to understand the growth and metabolism processing of *Alicyclobacillus acidoterrestris* (*A. acidoterrestris*) in controlling juice spoilage caused by *Alicyclobacillus*. In this work, simulative models for the growth and metabolism of *A. acidoterrestris* were systematically conducted in the medium and fruit juice. The results showed that low temperature (4 ℃) and strong acidic environment (pH 3.0–2.0) of medium inhibited the growth and reproduction of *A. acidoterrestris*. In addition, with decreasing temperature, the color, smell and turbidity of commercially available juice supplemented with *A. acidoterrestris* significantly improved. This work provided a clear exploration of growth characteristics of *A. acidoterrestris* by applying theory (medium) to reality (fruit juices), and pave fundamental for exploring the zero additives of controlling juice spoilage.

Introduction

Fruit juice is popular for consumers due to its ability to preserve most nutrients of fruits and good taste (Zhang et al., 2021). The flavor of fruit juices plays an important role in beverage industry, but once the unpleasant flavors are present, it will dramatically reduce consumer acceptance and bring economic losses for juice industry (Bianchi et al., 2010). Microbiological contamination is one of the possible pathways to produce off-flavors. Species of *Alicyclobacillus,* one of the common sources of contamination, can multiply in fruit juice and lead to juice spoilage along with the production of medicinal and disinfectant-like off-flavor, phenolic, and visible sediment when cell concentration is high enough (Huang et al., 2014; Witthuhn et al., 2011). Due to its acid tolerance and wide survival temperature, spores of *Alicyclobacillus* can survive pasteurization of fruit juices. The residual spores germinate and produce guaiacol (off-flavor) when the bacterial counts reach 10^5 CFU/mL (Ju et al., 2020). Thus, it is very important to control and even prevent *Alicyclobacillus* growth at early stage for juice manufacture.

Many approaches have been reported to prevent or control *Alicyclobacillus* contamination including physical treatments, chemical treatments, adding natural antibacterial products, and controlling biofilm (Ju et al., 2020). Nevertheless, traditional physical methods may not kill all spores, thus generating negative organoleptic effects on fruit juice when used alone. Adding natural antibacterial products (e.g., bacteriocins, lysozyme, chitosan, and some plant extracts etc.) combined with or without physical methods is an effective alternative and has become a research hotspot for controlling *Alicyclobacillus* contamination in fruit juices (Roig-Sagués et al., 2015; Song et al., 2019). Despite the excellent antimicrobial properties of natural antibacterial products, the high cost of extraction and purification processing limits their utilization. Moreover, consumers prefer the natural and free of additive fruit juice (Pornpukdeewattana et al., 2020). Thus, it is imperative to have a clear understanding for the growth characteristics of *Alicyclobacillus* in juice under different storage conditions, which is necessary in developing new methods for controlling *Alicyclobacillus* contamination in the absence of additives and provide fundamental theory for exploring the zero additives for controlling juice spoilage.

In response to these demands, herein, the growth and metabolism conditions of *Alicyclobacillus acidoterrestris* (*A. acidoterrestris*) were simulated in the medium and juice environment. As previous study has been reported, pH and temperature are important for growth and metabolism of *A. acidoterrestris* due to its thermophilic and acidophilic properties (Bianchi et al., 2010), thus, the simulative models for contaminated *A. acidoterrestris* at different pH and temperature were

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[*] Corresponding authors.
E-mail addresses: zhangwt@nwsuaf.edu.cn (W. Zhang), fanmt@nwsuaf.edu.cn (M. Fan).

[1] Congdi Shang and Tong Zhang contributed equally to this work.

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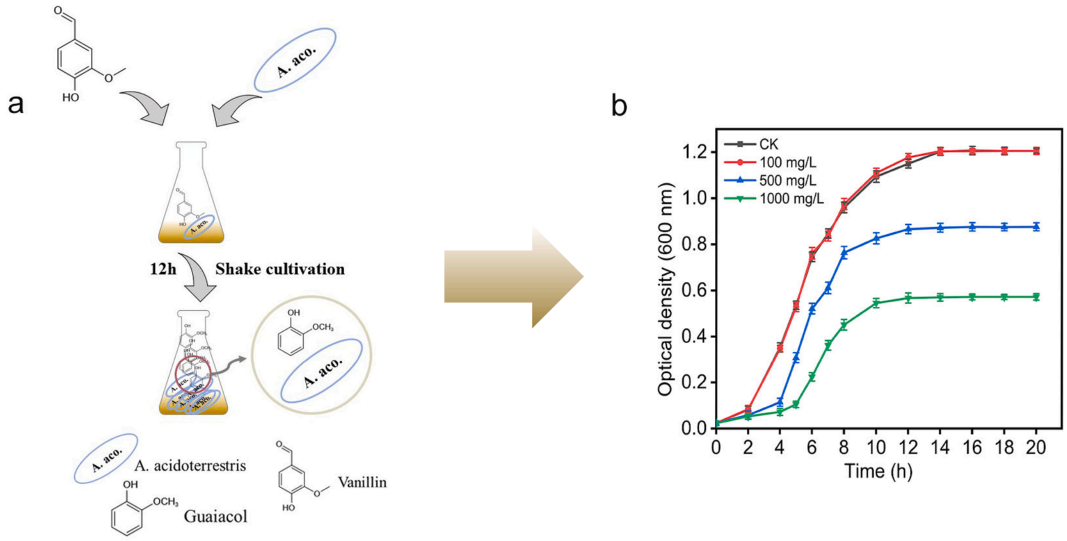

Fig. 1. The schematic diagram of guaiacol production for *A. acidoterrestris* (a). Effect of different concentration of vanillin for the growth of *A. acidoterrestris* (CK: normal culture; 100 mg /L: normal culture with 100 mg /L vanillin; 500 mg /L: normal culture with 500 mg /L vanillin; 1000 mg /L: normal culture with 1000 mg /L vanillin) (b).

systematically investigated using guaiacol as a representative in fluid nutrient medium. Furthermore, the simulative conditions were verified in juice to simulate actual contamination situations including viable count and analysis of the volatile components in the study.

Materials and methods

Materials

Vanillin and guaiacol were purchased from Shanghai Yuanye Co. Ltd. (Shanghai China). Both pure apple (pH 3.6) and orange (pH 3.6) juices are pasteurized concentrate and were purchased from local supermarket. *Alicyclobacillus acidoterrestris* (*A. acidoterrestris*) was stored at the Lab of Food Microbiology and Food Biotechnology in Northwest A&F University (Yangling, Shaanxi).

Effect of vanillin on a. Acidoterrestris

Strain activation was referenced to the method of Zhao et al. (2021). Vanillin was selected as a direct precursor to guaiacol, and vanillin stock solution (10 mg/mL vanillin dissolved in 50% ethanol (v/v) and filtered through a 0.22 μm membrane to remove bacteria) was added to the liquid medium to achieve the following final concentrations of vanillin: 100, 500 and 1000 mg/L. *A. acidoterrestris* was inoculated at 2% (*v*/*v*) into the AAM medium containing different concentrations of vanillin. The culture was incubated at 45 °C for 24 h at 150 r/min. Samples were measured every 2 h at OD_{600}.

The establishment of growth and metabolism model at different condition

Two common three-parameter mathematical models (Gompertz model and Logistic model) were chosen to fit the growth curve and metabolic guaiacol production curve of *A. acidoterrestris* in this study. Non-linear curve fitting in Origin software was used to complete the fitting of the *A. acidoterrestris* growth curve and the metabolic guaiacol production curve (Zhao et al., 2021).

Effect of different pH on growth and metabolism of guaiacol production by a. Acidoterrestris

Activated *A. acidoterrestris* were inoculated in liquid medium at different pH (2.5, 2.7, 3.0, 3.2, 3.5, 3.8 and 4.0). The liquid medium was incubated for 24 h at 45 °C and 150 r/min with shaking, and OD_{600} were measured every 2 h. The concentration of guaiacol was determined by high performance liquid chromatography (HPLC) based on Xu et al. (2019). Samples were taken at the following time points: 0, 4, 6, 8, 10, 12 and 16 h.

Effect of different temperature on growth and metabolism of guaiacol production by A. Acidoterrestris

Activated *A. acidoterrestris* were cultured in liquid medium (pH 4) at different temperature (45 °C, 25 °C and 4 °C) for 7 d (Samples were taken daily). Samples were used as standard plate counting to determine the number of viable bacteria. The samples were centrifuged and the supernatant was passed through a 0.22 μm membrane for guaiacol concentration determination using HPLC.

Effect of storage temperature on growth and metabolism of a. Acidoterrestris in fruit juice

The activated bacteria were inoculated into sterile apple and orange juice at a concentration of about 10^4 CFU/mL and stored at 45 °C (optimum growth temperature), 25 °C (room temperature) and 4 °C (refrigerated temperature) for 30 d. The juice without *A. acidoterrestris* was used as control, during which samples were taken at regular intervals and the viable bacteria were counted by standard plate counting. The metabolism of *A. acidoterrestris* was measured by analyzing the volatile components in fruit juice with the method of Xu et al. (2019) at the end of storage. Samples were taken after 30 days respectively, centrifuged at 5000 rpm for 10 min, and 5 mL of supernatant was added to a 20 mL headspace flask with 6 μL of 2-Octanol as internal standard, followed by 1.5 g of NaCl, and the volatile components in juice were determined using SPME-GC–MS. The volatile compounds were identified with reference to the retention indices of the standards, characterized by comparing the full scan mass spectra with those stored at NIST and quantified using the internal standard method.

Sensory analysis

Sensory analysis was completed according to the literature with some modifications (Piskernik et al., 2016). 10 experienced experts in the field of juice made up the evaluation team and carried out the sensory properties of labelled juices in a standard sensory laboratory. All

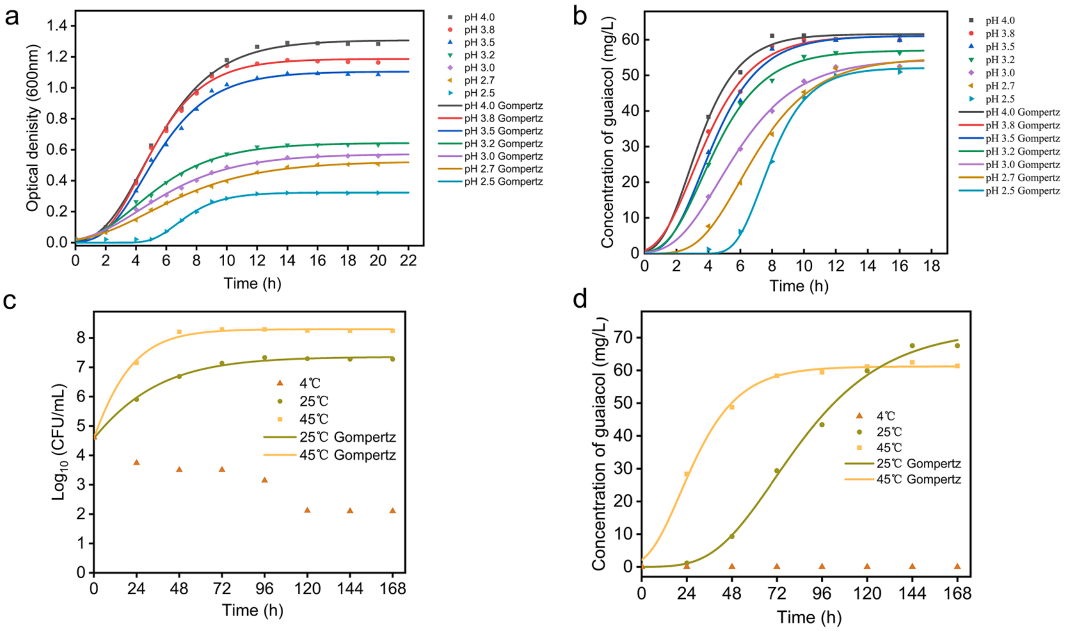

Fig. 2. The growth curves of *A.acidoterrestris* in medium with different pH (a); the curves of guaiacol production metabolism by *A.acidoterrestris* in medium with different pH (b); the growth curves of *A.acidoterrestris* in medium with different temperatures (c); Curves of guaiacol production metabolism by *A.acidoterrestris* in medium with different temperatures (d). At 4℃, the growth and metabolism of *A. acidoterrestris* did not conform to the S-shaped curve, so only the changes in data were shown in Fig. 2c and d.

samples were evaluated by the same team. For purpose of the evaluation, three parameters (color, smell, and turbidity) were introduced applied with analytical-descriptive test. In the test, sensory attributes of color and smell in juices were scored by non-structured scale from 1-4-7, and point 4 was considered to be the best, while scores $\geq$4.5 indicated the greater description of the property and scores $\leq$3.5 stand for the lesser description of the property. For turbidity, the structured scale score was 1–7, in which higher score means greater description of the property.

The samples for sensory analysis were prepared by storage uncontaminated and contaminated juice in 4, 25 and 45℃ respectively for 30 days. As presented in Table S7, S8, all samples labelled as A-G and offered to experts in separate glasses for analysis.

Statistical analysis

The data were represented as the mean ± standard deviation (SD). All experiments were triplicated. The data was analyzed with ANOVA using IBM SPSS 22 statistical software. Significant differences ($P < 0.05$) were evaluated by Tukey's significant difference test.

Result and discussion

The growth and metabolism of A. Acidoterrestris in medium

Effect of vanillin on A. Acidoterrestris

Guaiacol as a representative of metabolite for *A. acidoterrestris* requires the substrate vanillin to synthesize (Bahçeci and Acar, 2006; Corli Witthuhn et al., 2013). As a commonly used approach (Witthuhn et al., 2012; Cai et al., 2015a; Wang et al., 2021a), vanillin-guaiacol mode has been adopted in much research to explore the growth metabolism of *A. acidoterrestris* by adding vanillin to the studied system (Cai et al., 2015b; Cai et al., 2019; Wang et al., 2021b). To explore the ability of *A. acidoterrestris* to produce guaiacol, different concentration of substrate vanillin was added to the fluid nutrient medium, Fig. 1a, b shows the effect of different vanillin concentration on growth of *A. acidoterrestris*. Compared with the normal culture, the addition of 100 mg/L vanillin had not markedly inhibitory influence, but the addition of 500 mg/L and 1000 mg/L vanillin had remarkably negative effect on the growth of *A. acidoterrestris*. Considering the produce of guaiacol requires certain concentration of *A. acidoterrestris* at least 4–5 Log CFU/mL (Witthuhn et al., 2012), 100 mg/L vanillin addition kept *A. acidoterrestris* at low concentration with very weak ability to produce guaiacol.

The establishment of growth and metabolism model in medium

The three-parameter models, and the estimated value having one more degree of freedom, were used to fit the S-shaped curve which is necessary for curves with a small amount of test points. Therefore, the growth and metabolism of *A. acidoterrestrisit* could be predicted by the curve (Bahçeci and Acar, 2006). Two mathematical models, Gompertz and Logistic, were used to fit the growth curves and guaiacol production metabolism of *A. acidoterrestris* at different pH (Table S1 and S2). The criteria used to check the accuracy of the model are the coefficient of determination of the fit (R^2) and the standard error of prediction (S), and higher R^2 and smaller standard error S indicate more accuracy of the model. The results showed that there were larger determination coefficients in Gompertz model and smaller standard error than that in Logistic model. So Gompertz model was chosen to fit the growth curves of *A. acidoterrestris* in medium at different conditions.

As is known to all, pH and temperature are important factors affecting the growth of *A. acidoterrestris* comparing to other environmental stresses (Zhao et al., 2021). As pH decreases, the maximum bacterial density (OD_{max}) and the maximum growth rate (μ) was decreased, and the hysteresis period (λ) was also prolonged (Fig. 2a, Table S3). Although the growth of *A. acidoterrestris* was inhibited, it still grew at extreme pH environment (e.g., $OD_{max} = 0.323$, pH 2.5), illustrating that as an acidophilic bacterium, *A. acidoterrestris* was not completely inhibited by extreme acidic environments. The prolonged hysteresis period indicated *A. acidoterrestris* took longer time to acclimate and resume growth when dealt with acid stress at low pH (Estilo and Nakano, 2019). At different temperature, it can be found that the maximum bacterial count at 45 ℃ was higher about 1 Log10 CFU/mL than that at 25 ℃, while the bacterial count further decreased at 4 ℃,

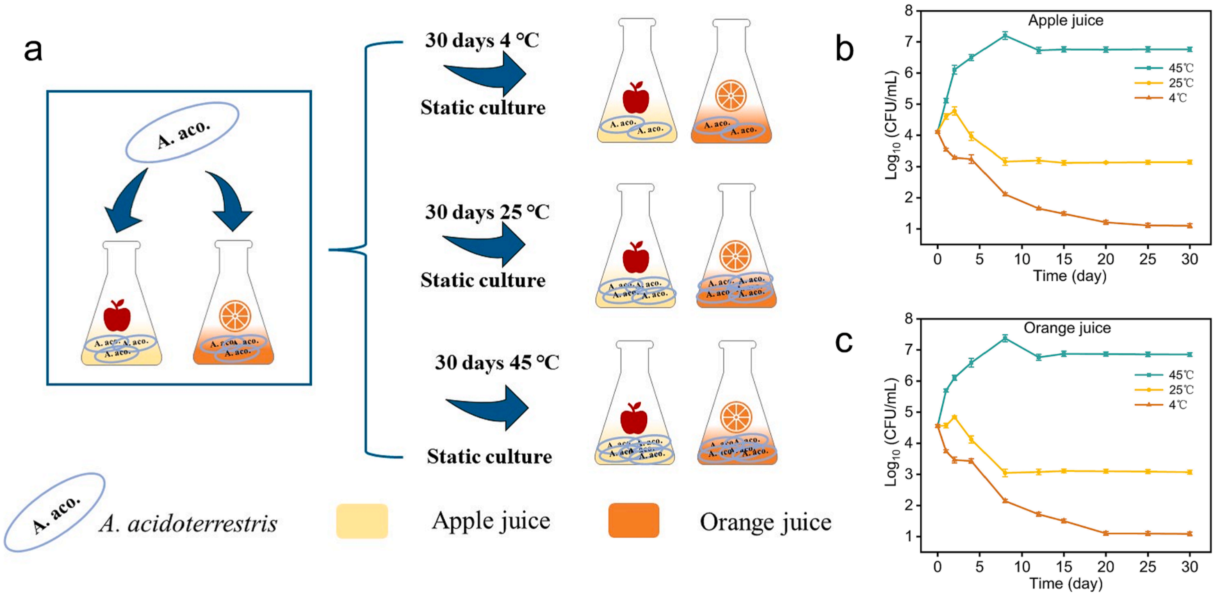

Fig. 3. The schematic diagram of the growth of *A. acidoterrestris* at different temperature in fruit juice (a). Effect of different temperature on growth of *A. acidoterrestris* in apple juice (b) and in orange juice (c). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

indicating that low temperature inhibited the growth of *A. acidoterrestris* (Fig. 3c, Table S4). Moreover, the maximum growth rate of *A. acidoterrestris* at 45 ℃ was significantly higher than that at 25 ℃. Compared with *A. acidoterrestris* at 45 ℃, the hysteresis period at 25 ℃ was significantly prolonged, indicating that *A. acidoterrestris* needed longer time to adapt to new environment at lower temperature. It was reported that *A. acidoterrestris* was classified as a mild to highly thermophilic bacterium and grew slowly at lower temperatures (Estilo and Nakano, 2019). Corli Witthuhn et al. (2013) also found that the growth rate of *A. acidoterrestris* at 25 ℃ was slower than that at 45 ℃. Under the non-optimal growth temperature (25 ℃), the maximum number of *A. acidoterrestris* was less than the maximum number under the optimal growth temperature (45 ℃). At low temperature (4 ℃), the bacterial counts further decreased (Fig. 2c).

In order to determine the effect of different conditions on metabolism of *A. acidoterrestris*, the metabolism of guaiacol production was chosen as representative indicator. Fig. 2b shows that low pH value has an inhibitory effect on guaiacol production by *A. acidoterrestris*, but not suppressed completely. As pH decreased, the rate of guaiacol formation (μ) and hysteresis period (λ) were also influenced (Table S5). The results also showed that low pH ($pH < 3.2$) retarded the growth of the bacteria and also delayed the production of guaiacol, because *A. acidoterrestris* needed more time to adapt to the new environment. The lag time was about 2 h at pH 3.5 to 3.0, and about 4 h at pH lower than 3.0, the same as the results obtained from the growth curve in Fig. 2a. For example, at pH 2.5, guaiacol was still produced by *A. acidoterrestris*, and the final concentration was only 52.105 mg/L. *A. acidoterrestris* in pH 3.5 needed longer time to produce guaiacol than in pH 4.0 and 4.5, which was consistent with the results in previous study (Chang et al., 2015). Temperature also affected the formation rate of guaiacol , more guaiacol was produced at 45 °C than at 25 °C (Table S6). It has been reported that *A. acidoterrestris* could produce guaiacol at both 25 °C and 45 °C, but the time required to produce guaiacol at 25 °C was longer than at 45 °C (Hu et al., 2020). Fig. 2d shows that the final concentration of guaiacol at 45 °C was almost the same as at 25 °C, suggesting that the potential for spoilage of juice products stored at ambient temperatures (25 °C) should not be underestimated. Guaiacol was not detected at 4 °C , it may be due to low number of *A. acidoterrestris*.

The growth and metabolism of a. Acidoterrestris in fruit juice

In the process of fruit juice production, juice was easily contaminated with microorganisms, and *A. acidoterrestris* was the main source of contamination in fruit juice spoilage (Kakagianni et al., 2020). As a heat-tolerant bacterium, temperature plays an important role in affecting the growth of *A. acidoterrestris*. This work aimed at exploring the effect of temperature on the growth and metabolism of *A. acidoterrestris* in juice, so as to provide fundamental theory for fruit juice manufacturers in controlling *A. acidoterrestris*.

The growth of a. Acidoterrestris in fruit juice

Based on our preliminary experiments and literatures reports (Bianchi et al., 2010; Wang et al., 2021), concentrate juice was used for the latter experiment. The growth diagram of *A. acidoterrestris* in fruit juice at different temperature was shown in Fig. 3a. The results showed that, at optimum growth temperature (45 °C), the number of bacterial counts increased continuously during the first 8 days, with a maximum count reaching 7.21 Log10 CFU/mL in apple juice (Fig. 3a) and 7.38 Log10 CFU/mL in orange juice (Fig. 3b), and then leveled off after 15 days until the end of storage. At 25 °C, the bacterial counts increased in the first 2 days, then declined from 3 to 8 days and levelled off after 8 days. At 4 °C, the counts declined continuously from beginning to 8 days and levelled off after 12 days. The counts in both apple and orange juice remained at around 1.10 Log10 CFU/mL until the end of the storage period.

In contrast to the situation in the medium, room temperature (25 °C) was not suitable for growth of *A. acidoterrestris* in juice, with a gradual decrease of the count to 1.0–1.5 Log10 CFU/mL compared to the initial inoculation, it may be attributed that in nutrient-rich medium, room temperature (25 °C) storage only retarded bacterial growth, while in nutrient-limited and more acidic juice, the growth of bacteria was further inhibited. Moreover, refrigerated temperatures (4 °C) has greater inhibitory effect on bacterial growth, with bacterial counts decreased by 3.0–3.5 Log10 CFU/mL compared to the initial inoculation.

The metabolism of a. Acidoterrestris in fruit juice

Since the aim of our work was to investigate the effect of *A. acidoterrestris* for zero additives juice other than vanillin-guaiacol mode, therefor, no additional vanillin added in juice system. Limited

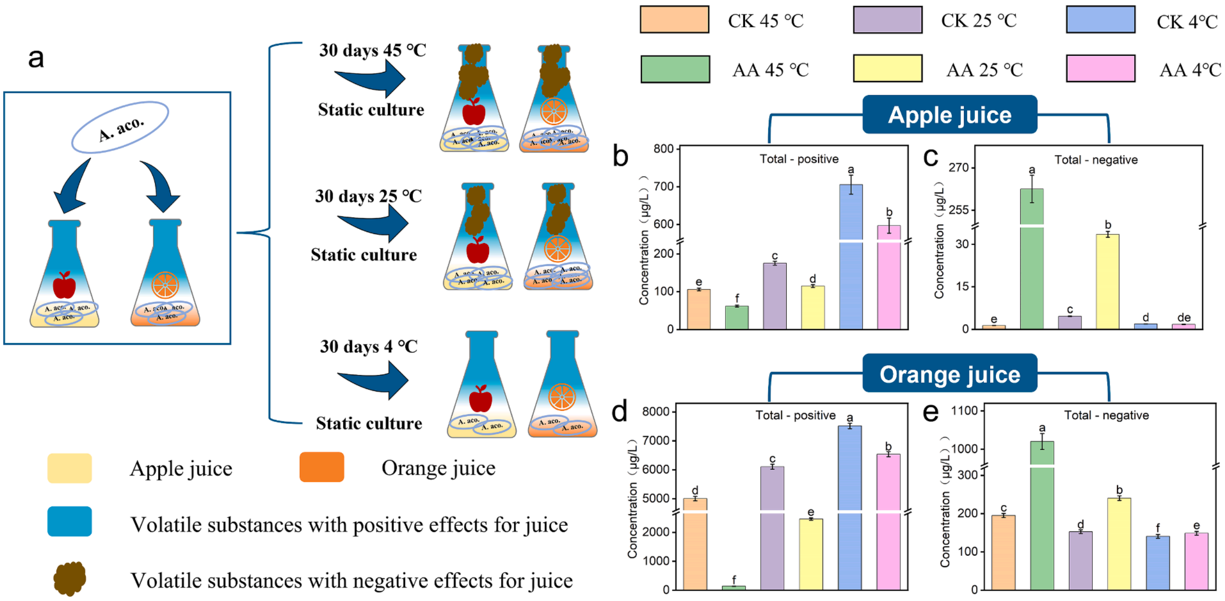

Fig. 4. The schematic diagram of the metabolism for *A. acidoterrestris* with different temperature in fruit juice (a). Total positive (b, d) and negative (c, e) contents of volatile components in different treatments for *A. acidoterrestris* in apple juice (b, c) and orange juice (d, e) after storage (CK: normal juice, AA: normal juice with *A. acidoterrestris*). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

by the used methods or the low content of guaiacol or vanillin (precursor of guaiacol), guaiacol was not detected in fruit juice. Thus, the effect of temperature on the metabolism of *A. acidoterrestris* in fruit juice was studied by sensory analysis and measuring the total volatile components.

As shown in Table S7 and S8, the evaluated color, smell and turbidity of contaminated juice (apple juice and orange juice) treated with 45 °C showed the darkest color, worst smell and the highest turbidity compared with other treatments. At 4 °C, compared with untreated juice, the appearance and smell of uncontaminated juice had no significant difference, while the contaminated juice had slight changes. It can be assumed that the sensory property of the juice gradually deteriorates with the increase of temperature, which, combined with the previous results of the effect of temperature on the growth of *A. acidoterrestris*, suggests that the growth and metabolic activity of *A. acidoterrestris* in the juice increased with raising temperature.

In order to deeply investigate the effect of temperature on the metabolism of *A. acidoterrestris* in fruit juice, volatile components were measured with different treatments. To clearly analyze the variations of different volatiles, the volatile components into were divided into three categories according to literatures (Bianchi et al., 2010; Liu et al., 2019; Schmutzer et al., 2014; Zhang et al., 2022; Reznik et al., 2021; Zhu et al., 2022; Sauceda-Gálvez et al., 2021): volatile components with positive, negative effect on the flavor of fruit juice and other volatile components with uncertain contributions. This work mainly focused on the first two broad categories in detail. The research schematic diagram and effect of storage temperature on metabolism of *A. acidoterrestris* in fruit juices was shown in Fig. 4a and the volatile components of the juices were determined. The total concentration of positive volatile flavor components of the contaminated juice was higher than that of uncontaminated juice (Fig. 4 b, d), and negative effects on the flavor of contaminated juice were lower than that of uncontaminated fruit juice under three temperatures tested (Fig. 4 c, e). Even at low temperatures, the metabolic activity of the bacterium was still going, resulting in juice spoilage.

For apple juice, a total of 54 volatile compounds was detected at different temperatures, including 12 alcohols, 12 aldehydes, 14 ketones, 5 acids, 9 esters and 2 phenols (Supplementary Material: Table S9), in which alcohols and esters are considered to contribute to the aroma. Compared with esters, alcohol, a degradation product of unsaturated fatty acids, was considered to be the second important contributor to aroma of apple juice (Han et al., 2021). As shown in Table 1, the alcohols that positively influenced the flavor of apple juice were significantly higher in the uncontaminated juice than in the contaminated juice at 45 and 25 °C, while at 4 °C, the alcohols that positively affect the flavor in

Table 1
Volatile compound total contents of uncontaminated and contaminated apple juice treated at different temperatures.

Volatile compounds (μg/L)	Indicators detected					
	45 CK	45AAT	25CK	25AAT	4 CK	4AAT
Alcohols						
Positive effect alcohols	85.41c ± 3.14	31.48d ± 0.92	140.53a ± 4.55	93.89b ± 3.26	140.94a ± 4.78	140.47a ± 4.07
Other alcohols	6.41c ± 0.16	12.92b ± 0.21	14.56a ± 0.25	12.97b ± 0.31	7.14d ± 0.19	3.35e ± 0.11
Esters						
Positive effect esters	0.75c ± 0.05	0.55c ± 0.04	4.14c ± 0.14	1.41c ± 0.12	369.98a ± 12.81	278.38b ± 8.14
Ketones						1.17b ± 0.05
Positive effect ketones	9.41d ± 0.14	12.30ab ± 0.36	8.52e ± 0.13	10.39c ± 0.27	11.99b ± 0.31	nd
Negative effect ketones	nd	48.39a ± 1.54	nd	16.77b ± 0.42	nd	nd
Other Ketones	2.17c ± 0.11	21.33a ± 0.45	1.11d ± 0.06	17.34b ± 0.44	1.02d ± 0.06	1.22d ± 0.06
Acids						
Negative effect Acids	1.37c ± 0.07	214.17a ± 6.90	4.62c ± 0.16	16.83b ± 0.78	1.90c ± 0.06	1.76c ± 0.07

All data were expressed as mean ± standard deviation (n = 3). Different letters in the same line indicate significant differences at 95% confidence according to Tukey test; CK: normal juice, AA: normal juice with *A. acidoterrestris*.

Table 2
Volatile compound total contents of uncontaminated and contaminated orange juice treated at different temperatures.

Volatile compounds (μg/L)	**Indicators detected**					
	45 CK	45AAT	25CK	25 AAT	4 CK	4 AAT
Alcohols						
Positive effect alcohols	87.04d ± 2.13	19.25e ± 0.37	155.84b ± 3.15	97.92c ± 2.54	167.74a ± 4.22	164.37a ± 4.31
Other alcohols	18.47b ± 0.33	19.11a ± 0.34	17.02c ± 0.36	18.66ab ± 0.41	nd	2.54d ± 0.14
Esters						nd
Positive effect esters	64.17c ± 0.94	15.22e ± 0.35	104.66b ± 2.40	21.81d ± 0.64	183.73a ± 5.39	107.35b ± 1.79
Terpenes						
Positive effect terpenes	4791.53d ± 68.79	97.10f ± 3.23	5805.63c ± 83.56	2304.78e ± 41.15	6995.33a ± 92.75	6153.25b ± 89.47
Negative affect terpenes	195.24c ± 4.54	425.34a ± 9.73	151.28d ± 3.79	226.51b ± 6.94	140.63f ± 4.02	148.75e ± 4.23
Other Terpenes	427.80d ± 7.88	107.98f ± 2.54	583.96b ± 8.27	253.60e ± 4.36	644.45a ± 7.14	**Other Terpenes**
Aldehydes						
Positive effect aldehydes	53.52c ± 1.28	nd	32.67d ± 0.66	4.27e ± 0.06	156.56a ± 4.21	101.67b ± 3.84
Other Aldehydes	nd	nd	31.09c ± 1.17	33.35a ± 1.23	31.98b ± 0.94	nd
Ketones						
Positive effect ketones	7.68d ± 0.13	6.34e ± 0.14	8.33c ± 0.14	6.63e ± 0.12	13.01a ± 0.25	10.84b ± 0.23
Negative effect ketones	nd	95.19a ± 2.89	nd	9.83b ± 0.14	nd	nd
Other ketones	12.27a ± 0.17	7.01d ± 0.08	4.13e ± 0.02	10.56b ± 0.14	8.50c ± 0.19	8.23c ± 0.06
Acids						
Negative effect acids	nd	499.60a ± 9.49	1.40c ± 0.02	4.20b ± 0.09	nd	nd

All data were expressed as mean ± standard deviation (n = 3). Different letters in the same line indicate significant differences at 95% confidence according to Tukey test; CK: normal juice, AA: normal juice with *A. acidoterrestris*.

uncontaminated apple juice were not significantly different with that in contaminated juice. The results indicated that the metabolism of alcohol production for *A. acidoterrestris* increased with temperature rising. In contrast, at 4 °C, the activity of *A. acidoterrestris* was very low, so the ability to produce alcohol was greatly diminished. Similar results were also observed for esters which were important contributors to apple juice flavor due to their fruity and sweet flavor (Li et al., 2021), and compared with 4 °C for normal juice having *A. acidoterrestris*, the total content of positive effective esters at 45 °C were greatly decreased (from 278.38 to 0.55 μg/L). Similarly, ketone, also important contributors for apple juice, was divided into positive effect ketones, negative effect ketones and other ketones, of which, *β*-Damascenone had a rose-like aroma and was used as a representative of positive effect ketones. The results showed that the content of *β*-Damascenone was high overall at 4 °C, and the content for uncontaminated juice was higher than contaminated juice. Meanwhile, as temperature increased, the content of *β*-Damascenone decreased and was completely eliminated at 45 °C (Supplementary Material: Table S9), which the probable reasons were volatilization of flavor placed at high temperature for long time (45 °C, 30 days) and metabolic exhaustion of *A. acidoterrestris*. Negative effect ketones including 2,3-Butandione, 3-Hydroxy-2-butanone, 2,3-Heptanedione had cream flavor which is off-odor for fruit juice (Zhu et al., 2022), and were only detected at 45 AAT (normal juice with *A. acidoterrestris* at 45 °C) and 25 AAT (normal juice with *A. acidoterrestris* at 25 °C), indicating that negative effect ketones could be synthesized in the process of growth and metabolism for *A. acidoterrestris*. Volatile acids, mainly fatty and cheesy odor, were assigned to negative effect acids in this work, which gave apple juice an unpleasant odor in high concentrations (Sauceda-Gálvez et al., 2021). The content of volatile acids was significantly higher in the contaminated juice than in the uncontaminated juice at 45 °C and 25 °C, in which the highest content was 45 AAT (214.17 μg/L). However, the negative effect volatile acids in apple juice were not significantly different from 45 CK (normal juice at 45 °C), 25 CK (normal juice at 25 °C), 4 CK (normal juice at 4 °C)and 4 AAT (normal juice with *A. acidoterrestris* at 4 °C), the reason may be that *A. acidoterrestris* could produce the volatile acids during its growth and metabolism. To sum up, it was mostly contaminated at 45 °C, the possible reason was that 45 °C is the optimum temperature for *A. acidoterrestris* (Zhao et al., 2021). The result (Fig. 4b, c) showed that the negative volatile components of apple juice almost the same as original at 4 °C, which means the activity of *A. acidoterrestris* was inhibited at 4 °C.

As a typical volatile compound of citrus fruits, terpenes were investigated in orange juice (Li et al., 2022) and the result was presented in Table 2 and Table S10. The concentration of positive effect terpenes was increased rapidly with temperature decreased from 45 ℃ to 4 ℃ in both contaminated and uncontaminated juice. Moreover, the concentration of positive effect terpenes in 45 CK was far higher than that in 45 AAT. Meanwhile, we also detected off-odor for terpenes including α-terpineol and carvone which was the degradation product of limonene and linalool (Reznik et al., 2021). Considering that terpenes can be used as carbon source (Bianchi et al., 2010), We believe that off-odors can be produced not only through self-deterioration but also from *A. acidoterrestris* using the positive flavor. In addition, the variation of alcohols, esters, ketones and acids in orange juice were almost the same as in apple juice.

Conclusion

In summary, we simulated two ways to probe the growth and metabolism of *A. acidoterrestris* for providing fundamental theory in exploring the zero addition to control juice spoilage. The results showed that guaiacol was not produced by *A. acidoterrestris* in absence of precusor substance (vanillin). Undesirable flavors produced by *A. acidoterrestris* in fruit juices not only originated from guaiacol, but also through the metabolism of other substances such as ketones and terpenes, which can be a direction for monitoring juice spoilage.

CRediT authorship contribution statement

Congdi Shang: Conceptualization, Validation, Writing – original draft. **Tong Zhang:** Conceptualization, Investigation, Writing – review & editing, Writing – original draft. **Junnan Xu:** Visualization. **Ning Zhao:** Software. **Wentao Zhang:** Validation, Supervision. **Mingtao Fan:** Writing – review & editing, Project administration, Resources.

Declaration of Competing Interest

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

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100790.

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