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Low soil pH enhances fruit acidity by inhibiting citric acid degradation in lemon (*Citrus lemon* L.)

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Abstract

Fruit acidity significantly influences fruit flavor, but the specific impact of soil pH on fruit acidity remains unclear. This study investigated the effects of various soil pH levels on fruit acidity and citric acid (CA) metabolism in lemon (*Citrus limon* L.). High soil pH (pH 8) decreased total soluble solids concentrations in lemon fruits, while low soil pH (pH 4) increased titratable acid and CA concentrations. Although low soil pH reduced the synthesis of CA due to the decreased citrate synthase and phosphoenolpyruvate carboxylase activities, the elevated fruit acidity under low soil pH conditions is not directly related to CA synthesis. Instead, low soil pH was found to suppress the activity of cytosolic aconitase (Cyt-ACO), an iron-dependent enzyme, indicating a potential role for CA degradation inhibition in low soil pH-induced CA accumulation. Furthermore, low soil pH significantly reduced cytosolic iron (Cyt-Fe) concentration, which was positively correlated with Cyt-ACO activity. In conclusion, low soil pH contributes to increasing fruit acidity in lemon, partially by inhibiting CA degradation due to the reduced Cyt-Fe concentrations. Our work unravels the influence of soil pH on CA accumulation and provides important clues for modulating CA levels through microelement fertilization in citrus.

Keywords Soil acidification, Fruit acidity, Citric acid, Citric acid degradation, Cytosolic nutrients

Introduction

Soil acidification, a significant cause of soil quality decline, is exacerbated by unreasonable fertilization (Chen et al. 2022; Zeng et al. 2017). Between the 1980s and 2000s, the soil pH of farmland topsoil in China

decreased by 0.5 units (Guo et al. 2010). This acidification accelerates CO₂ release, leaches calcium (Ca) and magnesium (Mg), and increases the dissolution of insoluble iron (Fe), manganese (Mn), and aluminum, ultimately reduces crop yield and quality (Yu et al. 2020; Chen et al. 2022; Muhammad et al. 2019; Li et al. 2015). Severe soil acidification is particularly prevalent in citrus orchards, such as the pomelo orchards in Fujian province, China (Chen et al. 2022). Despite this, the impact of soil pH on fruit acidity in citrus remains unknown.

Citrus is a crucial economic crop in China, with its quality determining market competitiveness. Sugars, organic acids, flavonoids, and vitamins are the primary constituents of citrus fruits, with sugars and acids being the main indicators of fruit flavor quality, while flavonoids and vitamins serve as nutritional indicators (Wu et al. 2021a; Li et al. 2022; Sheng et al. 2017). The decline

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in fruit quality may be linked to unreasonable fertilization, resulting in soil acidification. Over 80% of China's citrus orchards have acidic soils, with 50% having a $\text{pH} < 4.8$ (Wu et al. 2022). Various cultivation measures have been employed to enhance fruit quality, including the use of organic fertilizers, green manure planting, and lime application to improve acidic soils (Liang et al. 2021; Wu et al. 2020). Previous studies have indicated that Ca and Mg applications improved fruit mastication in Nanfeng tangerine, while boron and zinc applications enhanced the fruit flavor quality of Satsuma Mandarin (Zheng et al. 2015; Zhang et al. 2015).

Citrus fruits primarily contain organic acids, with citric acid (CA), malic acid, and quinic acid being the primary constituents. CA constituted over 70% of the total organic acids (Zhou et al. 2018). The accumulation of CA in fruits is regulated by its synthesis in mitochondria, degradation in cytoplasm, and storage in vacuoles (Wu et al. 2023; Wu et al. 2021b; Etienne et al. 2013). Several enzymes are crucial roles in these processes, shaping CA accumulation dynamics. For instance, enzymes such as citrate synthase (CS) and phosphoenolpyruvate carboxylase (PEPC) facilitate CA synthesis within mitochondria (Zhou et al. 2018; Wu et al. 2021a). A portion of the synthesized CA is degraded in cytoplasm by cytosolic aconitase (Cyt-ACO) and isocitrate dehydrogenase (Wu et al. 2021a; Guo et al. 2016) while the majority is stored in vacuoles (Wu et al. 2023). However, the influence of soil pH on fruit acidity through modulation of CA synthesis and degradation in citrus remains unexplored.

Tropical and subtropical regions extensively cultivate lemons, which are appreciated for their unique flavor and valued for their nutritional and medicinal benefits. This study focused on lemons to investigate how soil pH influences CA concentration and its synthesis and degradation processes. Furthermore, we analyzed the impact of soil pH on nutrient concentrations in both mitochondria and cytoplasm.

Materials and methods

Plant material and growth conditions

A pot experiment was initiated on 10th December, 2018, at the Yunnan Academy of Agricultural Sciences to investigate the effect of soil pH on fruit quality using 2-year-old lemon plants of the 'Yunning 1' cultivar. Five soil pH levels, pH 4, pH 5, pH 6, pH 7, and pH 8, were established as treatments by using citrate and carbonate buffers. The citrate buffer solution, composed of 0.10 M citric acid and sodium citrate (19:1), achieved a pH of 2.8, while the carbonate buffer solution, prepared with 0.10 M sodium carbonate and sodium bicarbonate (9:1), resulted in a pH of 10.3. Eight lemon trees per treatment were subjected

to the respective soil pH conditions. Uniform two-year-old lemon plants were cultivated in ceramic pots (45 cm × 35 cm) filled with 30 kg of yellow-brown soil (initial pH 5.86). These pots were placed in a transparent rainproof shed under natural conditions, watered with distilled water as needed, and fertilized monthly with 20 g of lemon-specific fertilizer (N: P_2O_5 : K_2O = 22:5:8) per pot.

Fruit sampling

In June and September 2019, winter and spring flower fruit samples were collected, respectively. Four representative fruits were selected randomly from each tree. The fruit samples were promptly divided into pulp and further portioned into three parts: (i) One fresh pulp portion was immediately frozen in liquid nitrogen and stored at -80°C for subsequent organic acid and organelle nutrient analysis; (ii) the second portion was juiced to evaluate fruit quality parameters, including total soluble solids (TSS), titratable acidity (TA), and vitamin C (Vc) (iii) the remaining pulp portion underwent enzyme deactivation at 105°C for 30 min, followed by drying at 65°C until a constant weight was achieved for mineral nutrient analysis.

Fruit quality analysis

TA concentration was determined by titrating fresh juice with 0.10 M NaOH, while TSS concentration was measured using an Abbe refractometer. The Vc content was ascertained via 2,6-dichloroindophenol titration as described previously (Zhou et al. 2018). Edible rate (ER) and juice yield (JY) were also calculated, representing the ratios of fruit pulp to total fruit weight and fruit juice to fruit pulp weight, respectively.

CA extraction and gas chromatography (GC) analysis

GC was used to determine CA concentrations, as described in our previous study (Wu et al. 2023). Fruit samples underwent extraction with 80% methyl alcohol at 75°C for 15 min. Upon centrifugation at 4000 g for 10 min, 0.5 mL of the supernatant was dried using an Eppendorf Concentrator 5301 vacuum centrifuge (Germany). The dried samples were then derivatized using hydroxylamine hydrochloride, hexamethyl, and trimethylchlorosilane disilazane. Finally, the derivatized samples were analyzed using an Agilent 6890 N GC system (Agilent, USA).

Measurement of soil and fruit nutrient concentration

The air-dried soil was ground through a 1.0 mm sieve and thoroughly mixed to analyze soil nutrients. A ~2.5 g soil sample was extracted with 25 mL of 1 M $\text{CH}_3\text{COONH}_4$ solution at 180 r min^{-1} and 25°C for

30 min. After filtering using Whatman filter paper, the filtrates were used to determine available K concentrations using a flame photometer, and available Ca and Mg concentrations using an atomic absorption spectrophotometer. Additionally, 12.5 g of soil was extracted with 25 mL of 0.005 M diethylene triamine pentaacetic acid solution, containing 0.01 M CaCl_2 and 0.10 M TEA, at 180 r min^{-1} and 25°C for 2 h. Following filtration using Whatman filter paper, the filtrates were used to measure available Fe and Mn concentrations using an atomic absorption spectrophotometer (Wu et al. 2019).

The samples, dried and ground, underwent digestion with a 5 mL $\text{H}_2\text{SO}_4\text{-H}_2\text{O}_2$ solution. A flame photometer (AP1200, AUPO, Shanghai, China) was used to measure the K concentrations in the resulting digestion solution. For Ca, Mg, Fe, and Mn concentrations, fruit samples were digested using a 10 mL $\text{HNO}_3\text{-HClO}_4$ solution (v/v, 4:1), followed by analysis with an atomic absorption spectrophotometer (Z2000, HITACHI, Tokyo, Japan). Enzyme solutions of Cyt-ACO and Mit-ACO (1 mL each) were concentrated and digested with 5 mL $\text{HNO}_3\text{-HClO}_4$ (v/v, 4:1) to determine cytosolic and mitochondrial nutrient concentrations.

Measurement of enzyme activities

The activities of CS, Cyt-ACO, mitochondrial aconitase (Mit-ACO), PEPC, and isocitrate dehydrogenase (IDH) were assessed according to our previously established protocols (Wu et al. 2021a; Wu et al. 2021b). Homogenized fruit samples (~2.0 g) were extracted with 2 mL of 0.20 M Tris-HCl buffer (pH 8.2, containing 0.60 M sucrose and 10 mM erythorbic acid) under ice-bath conditions. After centrifugation (4°C , 4000 g, 20 min), the supernatant was diluted to 5 mL, and 2 mL was

further centrifuged (4°C , 15,000 g, 15 min). The resulting supernatant was diluted to 4 mL with 0.20 M Tris-HCl buffer for Cyt-ACO activity determination, while the residue was diluted to 4 mL with the same buffer for Mit-ACO and nicotinamide adenine dinucleotid-IDH (NAD-IDH) analysis. Additionally, 3 mL of the initial 5 mL supernatant was diluted to 8 mL with 0.20 M Tris-HCl buffer, then divided into two parts. One part was dialyzed in 0.20 M Tris-HCl buffer at 4°C , and the obtained dialysate was used to analyze PEPC and CS activities.

Statistical analysis

Using SPSS 20.0 software, analysis of variance was conducted with ANOVA and Duncan multiple comparisons ($P<0.05$). Figures were prepared by using Origin 2017 software, while R software was used to generate the correlation matrix.

Results

Fruit quality of lemon grown on soil with different pH

Compared with pH 6 treatment, treatments with pH 4, pH 5, and pH 8 significantly increased TA concentration in winter flower fruit (WFF) by 25.27%, 19.61%, and 17.65%, respectively. In contrast, TSS concentration was decreased by 6.75% and 8.38% under pH 7 and pH 8 conditions in WFF (Table 1). Similarly, pH 4 treatment increased TA concentration in spring flower fruit (SFF) by 16.57%, while pH 8 treatment decreased TSS concentration by 4.29%. Furthermore, pH 4 and pH 5 treatments reduced ER and JY but increased Vc concentration in SFF (Table 1). These results indicate

Table 1 Effects of soil pH on fruit quality of lemon

Fruit types	Soil pH	SFW (g)	ER (%)	JY (%)	TSS (%)	TA (%)	V _c (mg/100g)
Winter flower fruit	4	158.77a	63.71a	48.12a	9.32a	5.75a	23.12a
	5	159.47a	61.62a	52.23a	9.33a	5.49b	21.84a
	6	175.08a	62.77a	49.12a	9.19a	4.59c	20.12a
	7	172.93a	63.24a	55.23a	8.57b	4.74c	20.28a
	8	151.74a	64.14a	48.89a	8.42b	5.40b	21.00a
Spring flower fruit	4	161.05a	64.59c	40.16b	10.28ab	5.91a	30.83a
	5	149.93a	65.97bc	41.56b	10.58a	5.68ab	27.46ab
	6	151.11a	67.58ab	48.20a	10.25ab	5.07b	25.49ab
	7	163.73a	67.60ab	46.53a	10.05bc	5.60ab	22.31b
	8	162.46a	68.54a	46.26a	9.81c	5.35ab	26.25ab

Note: 4, 5, 6, 7 and 8 represent the lemon plant was cultivated in different soil pH. SFW, ER, JY, TSS, TA and Vc represent the indexes of single fruit weight, edible rate, juice yield, soluble solid, titratable acid and vitamin C, respectively. Different lowercase letters indicate significant differences among the treatments by Duncan-test ($P<0.05$, $n=4$)

that soil pH impacts fruit quality, primarily by increasing TA concentrations.

Citric acid concentration and its metabolism-related enzymes activities at different soil pH

Our previous study revealed a decrease in fruit TA concentrations with lower soil pH levels. Since CA constitutes over 70% of fruit organic acids (Sheng et al. 2017), we examined the effect of soil pH on CA metabolism. CA concentrations were significantly increased under pH 4, pH 5, and pH 8 treatments in WFF, and under pH 4 treatment in SFF (Table 2). These changes in CA concentrations corresponded with the observed TA concentration patterns (Table 1).

To investigate the impact of soil pH on CA accumulation in lemon, we measured the activities of enzymes related to CA metabolism. PEPC activity was decreased by 0.07%, 11.52%, 15.23%, and 11.55% under pH 5, pH 4, pH 7, and pH 8 treatments, respectively, compared with the pH 6 treatment. Cyt-ACO activity was also decreased by 24.36%, 27.86%, 28.32%, and 33.69% under the same conditions. Mit-ACO activity was significantly lowered under pH 8 treatment but increased under pH 4 treatment. No significant differences were observed in CS and NAD-IDH activities among treatments in WFF (Fig. 1).

Enzyme activities of PEPC, CS, and Cyt-ACO were generally reduced under pH 4, pH 5, pH 7, and pH 8 treatments, compared with the pH 6 treatment. Furthermore, significant decreases were observed in PEPC activity under pH 7 and pH 8, CS activity under pH 4, pH 7, and pH 8, and Cyt-ACO activity under pH 4, pH 5, pH 7, and pH 8. However, in SFF, the NAD-IDH activity showed no significant differences among the treatments (Fig. 2).

Table 2 Effects of soil pH on citric acid concentrations (mg/g) in lemon fruit

Soil pH	Fruit types	
	Winter flower fruit	Spring flower fruit
4	52.76 ± 1.01a	52.36 ± 2.39a
5	49.62 ± 1.42a	50.13 ± 2.39ab
6	39.94 ± 1.85b	44.68 ± 2.50b
7	40.35 ± 2.84b	50.91 ± 1.00ab
8	48.98 ± 1.59a	45.75 ± 1.53b

Note: 4, 5, 6, 7 and 8 represent the lemon plant was cultivated in different soil pH. Different lowercase letters indicate significant differences among the treatments by Duncan-test ($P < 0.05$, $n = 4$)

Soil nutrient concentrations at different soil pH

To evaluate the influence of soil pH on nutrient availability, we measured the concentrations of various elements. Compared to the pH 6 treatment, K concentrations were higher under pH 4, pH 5, pH 7, and pH 8, with significant increases at pH 4 and pH 5. Conversely, Ca and Mg concentrations were lower under pH 4, pH 5, and pH 8, with substantial decreases in Ca at pH 4, pH 7, and pH 8, and in Mg at pH 4 and pH 5. Furthermore, Fe and Mn concentrations were reduced under pH 7 and pH 8, with striking decreases in Mn at both pH levels. These observations demonstrate that soil pH affects nutrient availability.

Fruit nutrient concentrations at different soil pH

To investigate the impact of soil pH on mineral nutrient accumulation in pulp, we measured concentrations in SFF and WFF. Compared to pH 6, the pH 4 treatment increased Ca, Fe, and Mn concentrations in WFF (Table 4). For SFF, K concentrations were rose under pH 4 and pH 7, Ca concentrations were significantly increased under pH 8, and Fe concentrations were significantly rose under pH 4 (Table 5). However, Mg and Mn concentrations in SFF showed no significant differences among treatments (Table 5).

To investigate the impact of soil pH on enzyme activity, mitochondrial and cytosolic ion concentrations were examined. Compared to the pH 6 treatment, pH 4 increased the Mit-K, Mit-Fe, and Mit-Mn concentrations in the mitochondria, while the pH 8 treatment reduced them. Notably, the pH 8 treatment resulted in significant decreases in K concentrations in SFF (Fig. 3). Similarly, compared to pH 6 treatment, pH 4 and pH 5 treatments elevated the cytosolic Cyt-K and Cyt-Mn concentrations, whereas pH 7 and pH 8 treatments decreased them. Conversely, Cyt-Fe and Cyt-Mg concentrations were reduced by pH 4, pH 5, pH 7, and pH 8 treatments, with significant decreases in Cyt-Fe under pH 4 conditions and in Cyt-Mg under pH 7 and pH 8 conditions in WFF (Figs. 4 and 5).

Correlations between organelle nutrient and enzyme activity in spring flower fruit

Cyt-Mg and Cyt-Fe showed significant positive correlations with Cyt-ACO activity, while Cyt-Mg and Cyt-K correlated positively with PEPC activity (Fig. 5a). Mit-Mg exhibited significant positive correlations with CS activity, and Mit-Fe and Mit-K correlated positively with Mit-ACO activity (Fig. 5b). These results showed that changes in the activity of CA metabolizing-enzymes may be associated with subcellular Mg, Fe, and K concentrations.

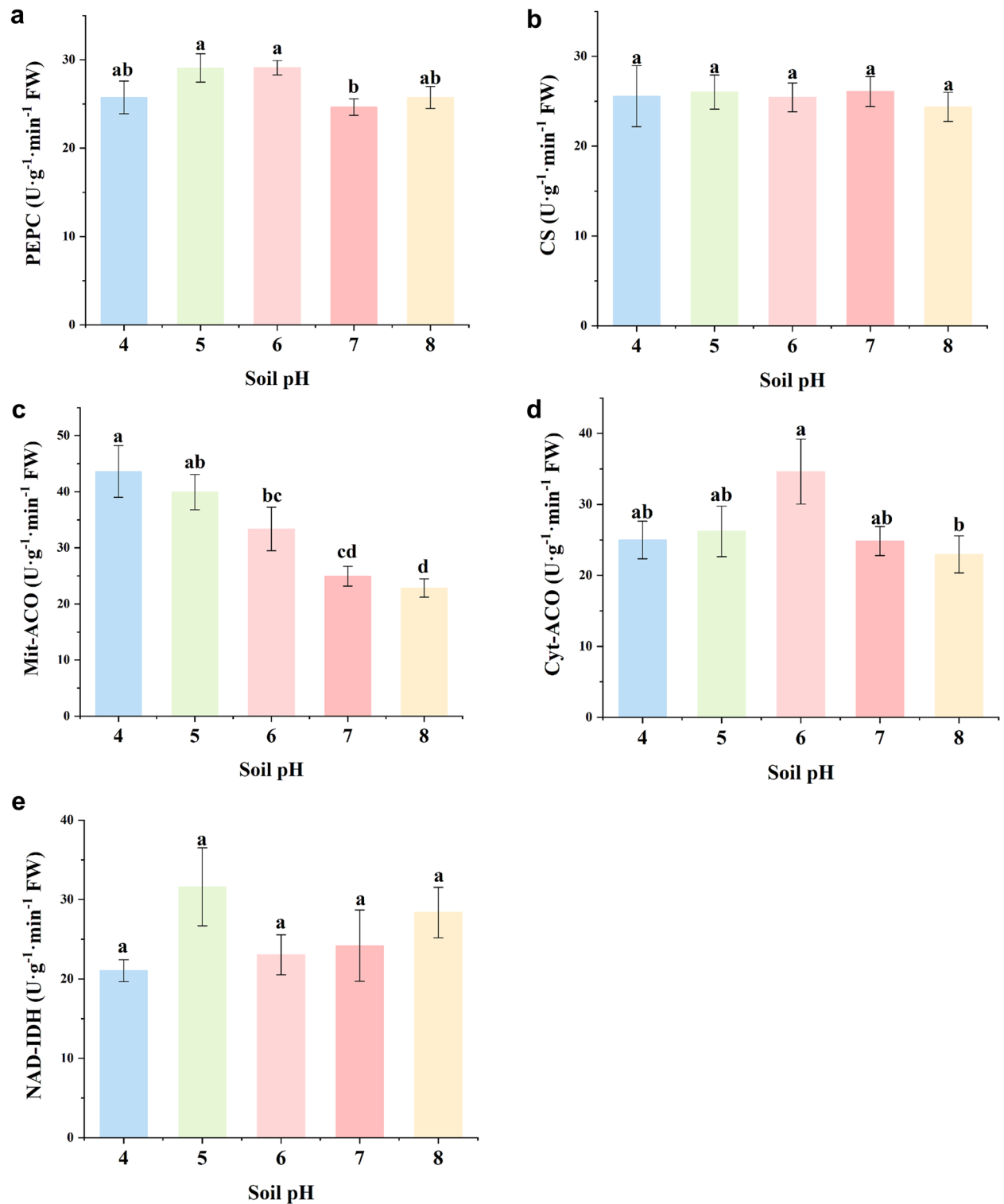


Fig. 1 Activities of enzyme related to citric acid metabolism in winter flower fruit under different soil pH. pH 4, pH 5, pH 6, pH 7, and pH 8 are the different soil pH conditions under which lemon plant was cultivated. **a** PEPC activity under different soil pH conditions. **b** CS activity under different soil pH conditions. **c** Mit-ACO activity under different soil pH conditions. **d** Cyt-ACO activity under different soil pH conditions. **e** NAD-IDH activity under different soil pH conditions. PEPC, Mit-ACO, CS, Cyt-ACO and NAD-IDH represent phosphoenolpyruvate carboxylase, mitochondrial aconitase, citrate synthase, cytosolic aconitase and nicotinamide adenine dinucleotide-dependent isocitrate dehydrogenase. Fresh weight FW. Different lowercase letters indicate the significant differences among the treatments by Duncan-test ($P < 0.05$, $n = 4$)

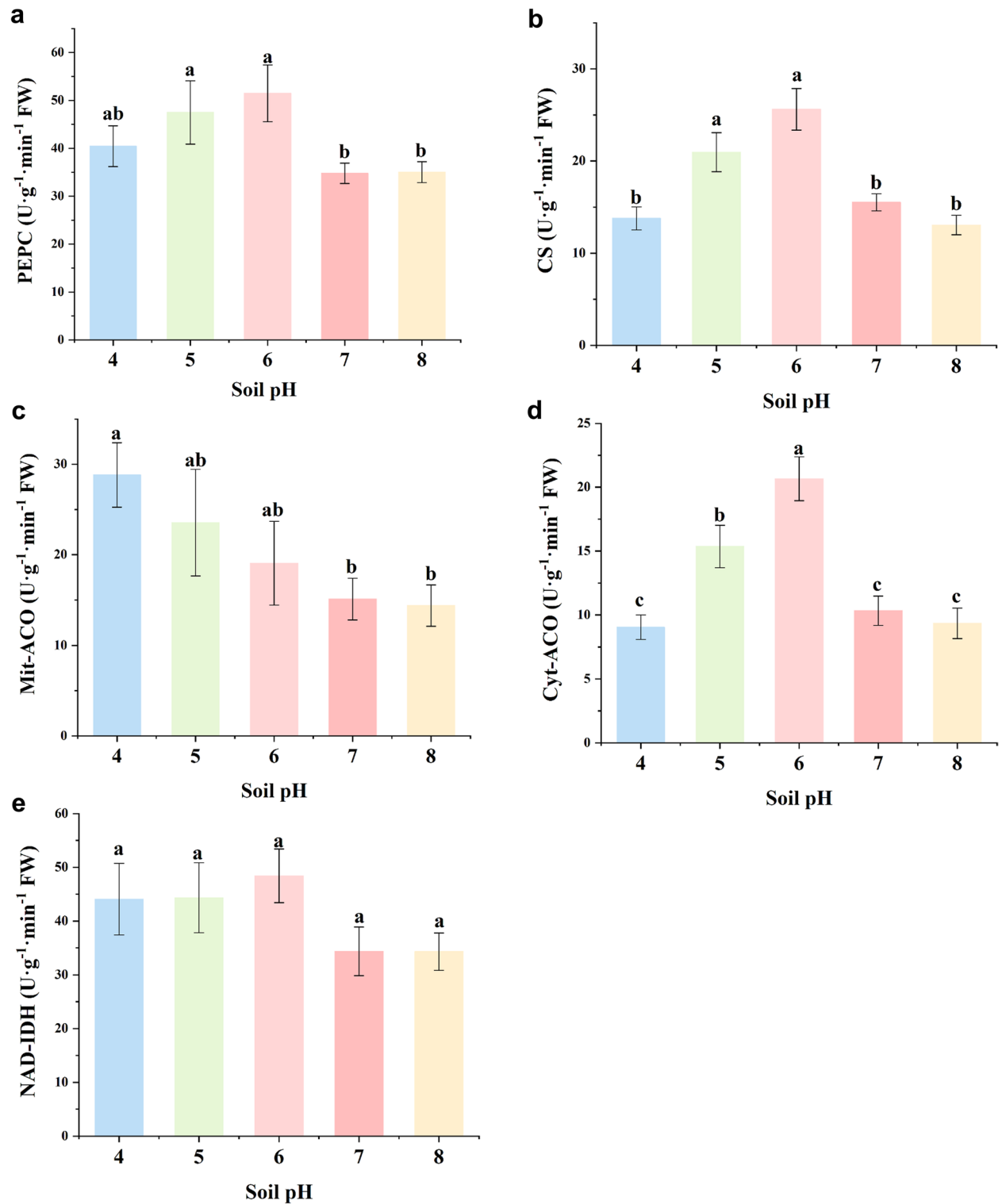


Fig. 2 Activities of enzyme related to citric acid metabolism in spring flower fruit under different soil pH. pH 4, pH 5, pH 6, pH 7, and pH 8 are the different soil pH conditions under which the lemon plant was cultivated. **a** PEPC activity under different soil pH conditions. **b** CS activity under different soil pH conditions. **c** Mit-ACO activity under different soil pH conditions. **d** Cyt-ACO activity under different soil pH conditions. **e** NAD-IDH activity under different soil pH conditions. PEPC, Mit-ACO, CS, Cyt-ACO and NAD-IDH represent phosphoenolpyruvate carboxylase, mitochondrial aconitase, citrate synthetase, cytosolic aconitase and nicotinamide adenine dinucleotide-dependent isocitrate dehydrogenase. Fresh weight FW. Different lowercase letters indicate the significant differences among the treatments by Duncan-test ($P < 0.05$, $n = 4$)

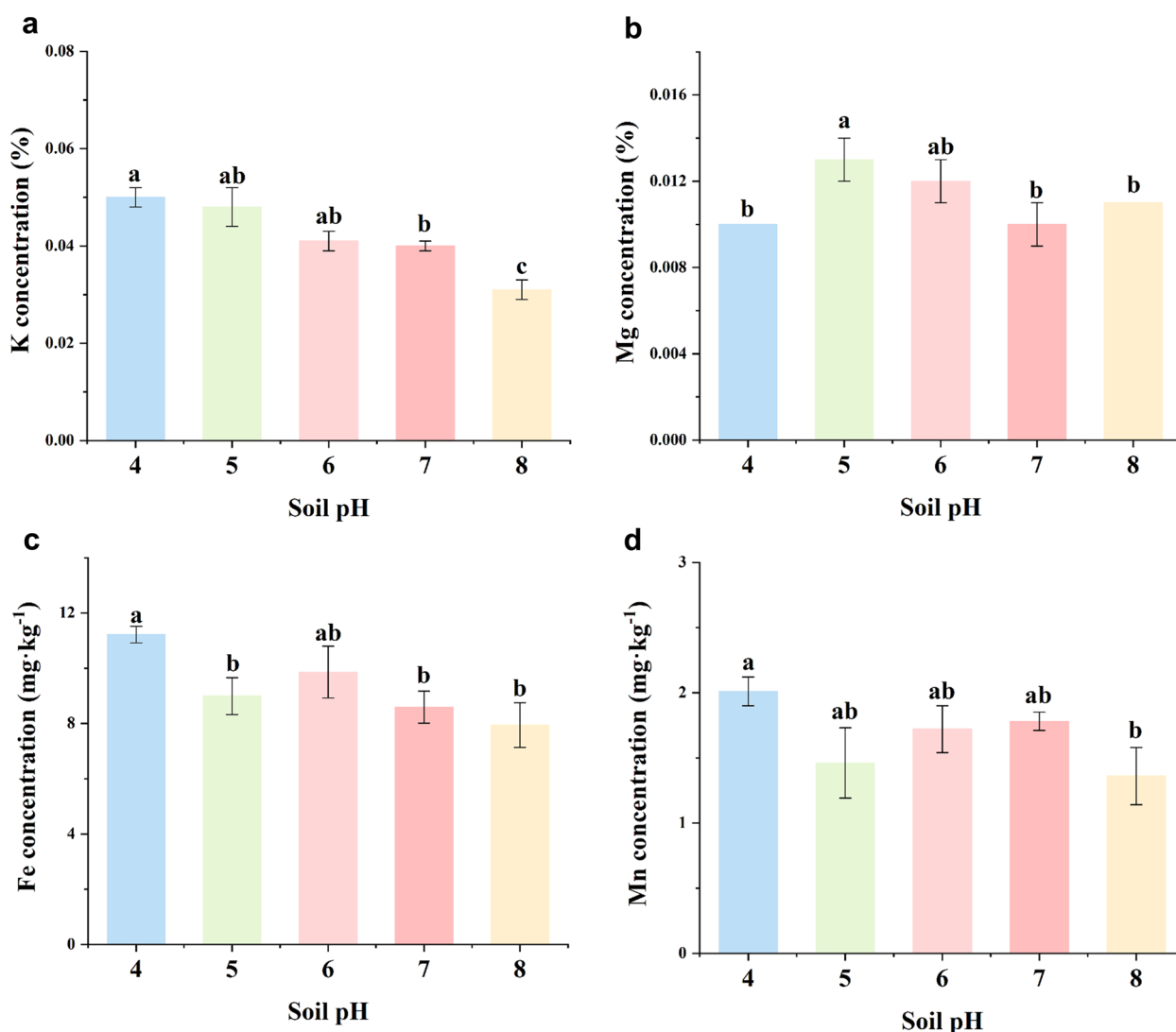


Fig. 3 Concentrations of mitochondrial K (a), Mg (b), Fe (c) and Mn (d) in spring flower fruit under different soil pH. pH 4, pH 5, pH 6, pH 7, and pH 8 are the different soil pH conditions for lemon plant. K, Mg, Fe, and Mn represent potassium, magnesium, iron, and manganese, respectively. Different lowercase letters indicate the significant differences among the treatments by Duncan-test ($P < 0.05$, $n = 4$)

Discussion

Low soil pH improves the lemon fruit acidity

Fertilization, a critical aspect of cultivation practices, significantly influences fruit quality but can lead to soil acidification, negatively affecting yield and fruit quality (Guo et al. 2010; Ge et al. 2018). Prior studies have shown that lime and biochar application can decrease TA concentrations in Satsuma mandarin under acidic soil conditions, indicating that low soil pH enhances fruit acidity (Wu et al. 2020). Our findings align with these observations, as both low (pH 4) and high (pH 8) soil pH significantly increased fruit TA concentrations

in lemon, with a more pronounced effect under low pH conditions (Table 1). In contrast, blueberries exhibited increased TA concentrations with soil pH levels from 4.5 to 6.0 (Jiang et al. 2019). Moreover, high soil pH resulted in a decrease of TSS concentrations in lemon, corroborating previous findings in blueberry fruit (Jiang et al. 2019). Further analysis revealed that low and high soil pH increased CA concentrations in lemon, with a greater increase under low pH conditions (Table 2). These results collectively suggest that low soil pH contributes to increasing fruit acidity in lemon.

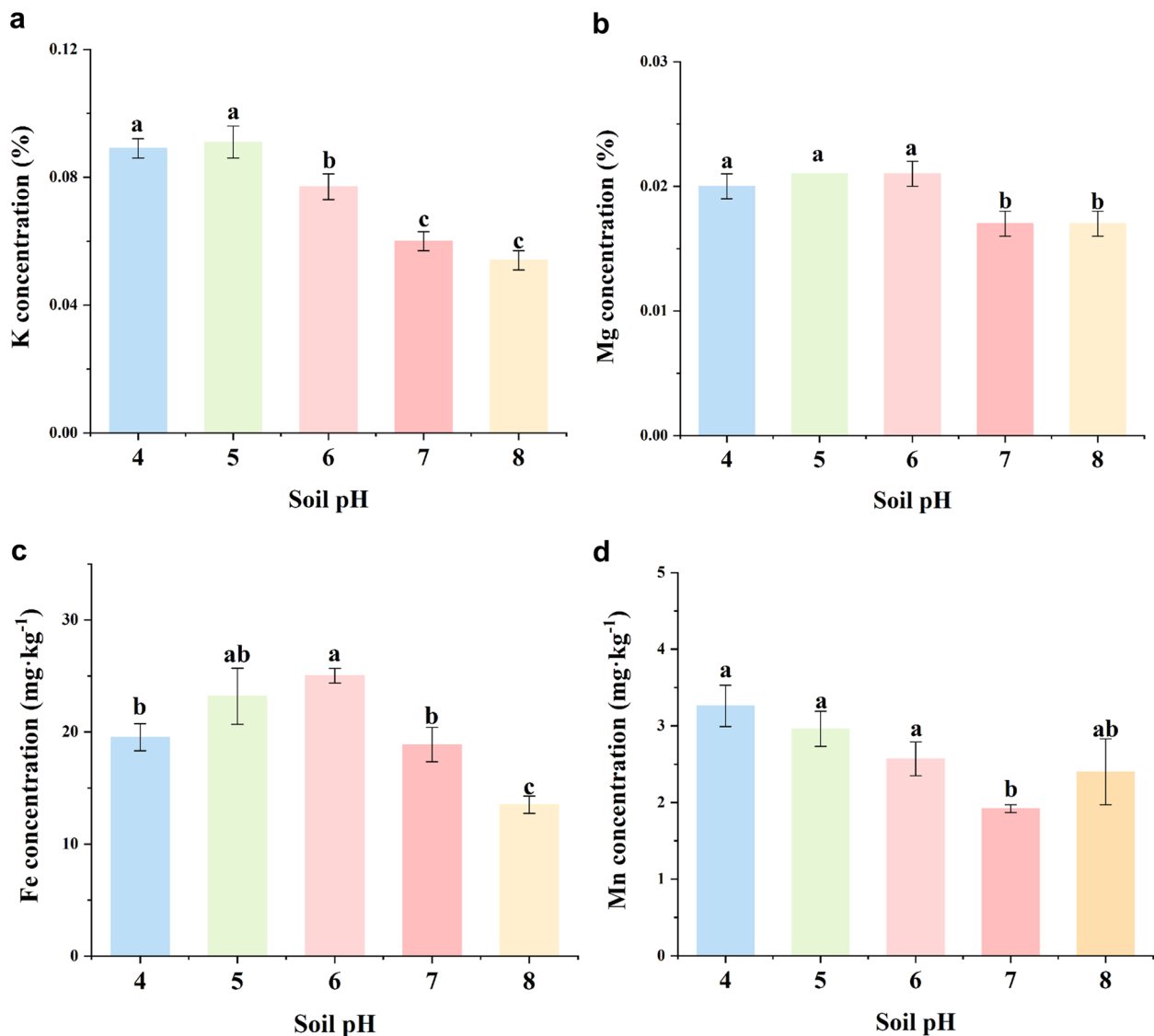


Fig. 4 Concentrations of cytosolic K (**a**), Mg (**b**), Fe (**c**) and Mn (**d**) in spring flower fruit under different soil pH. pH 4, pH 5, pH 6, pH 7, and pH 8 are the different soil pH conditions for lemon plant. K, Mg, Fe and Mn represent potassium, magnesium, iron, and manganese, respectively. Different lowercase letters indicate the significant differences among the treatments by Duncan-test ($P < 0.05$, $n = 4$)

Low soil pH affects soil nutrient availability and nutrient accumulation in lemon

Soil pH significantly influences nutrient availability, and regulating it through practices like biochar and lime application affects nutrient availability (Zhang et al. 2014; Wu et al. 2020). In alkaline soils, elements such as Fe, Mn, Cu, and Zn are typically fixed, while acidic conditions release these fixed metal elements due to free hydrogen ions (Marschner 2011). Our study corroborated previous findings (Guo et al. 2016; Wu et al. 2020), showing that high soil pH significantly

decreased Ca and Mn concentrations (Table 3). Notably, Fe and Mn concentrations increased in both WFF and SFF of lemon under pH 4 treatment, indicating a larger pool of Fe and Mn in low pH soils. These results emphasize the crucial role of low soil pH in activating soil nutrients.

Soil pH significantly affects the availability of most soil nutrients, with the Ca, Fe, and Mn concentrations in WFF, and K and Fe in SFF being notably influenced in lemon pulps (Tables 4 and 5). Soil nutrient concentrations are crucial in determining K, Fe, Mn, and Ca

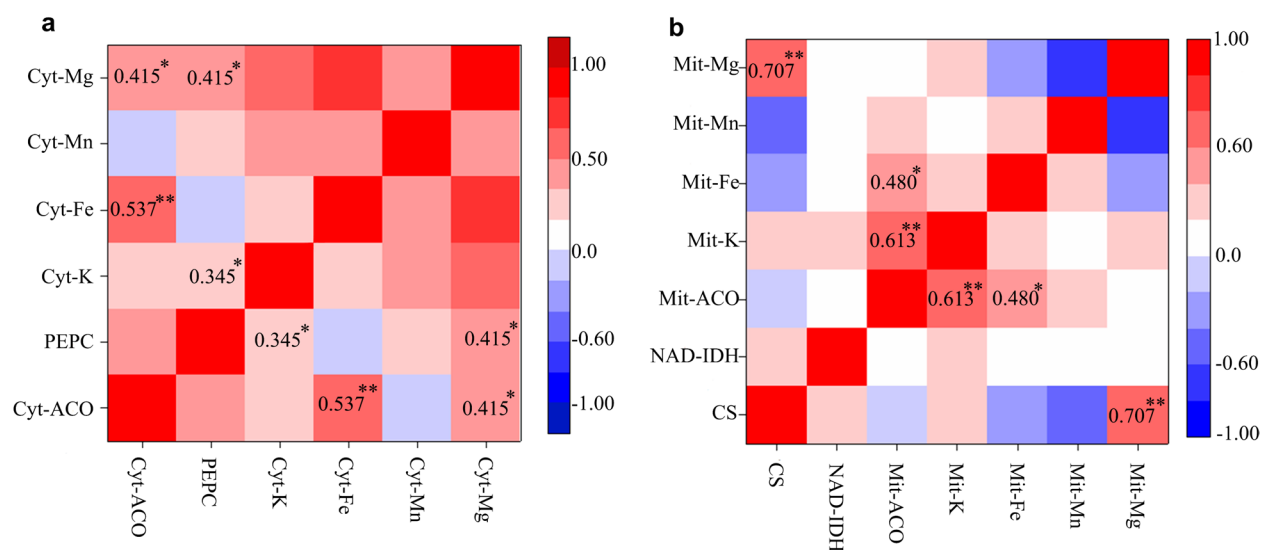


Fig. 5 Correlations of cytosolic (a) and mitochondrial (b) mineral nutrients and activity of citric acid metabolism enzyme. Numbers represent correlation coefficients; * $P < 0.05$, ** $P < 0.01$. PEPC, Mit-ACO, CS, Cyt-ACO, and NAD-IDH represent phosphoenolpyruvate carboxylase, mitochondrial aconitase, citrate synthase, cytosolic aconitase and nicotinamide adenine dinucleotide-dependent isocitrate dehydrogenase. Cyt-K, Cyt-Mg, Cyt-Fe and Cyt-Mn represent the cytosolic concentrations of potassium, magnesium, iron, and manganese in lemon fruits, respectively. Mit-K, Mit-Mg, Mit-Fe and Mit-Mn represent the mitochondrial concentrations of potassium, magnesium, iron, and manganese in lemon fruits, respectively

concentrations in lemon pulps. Additionally, nutrient accumulation in citrus may be influenced by the mycorrhizal uptake pathway, due to the scarcity of root hairs (An et al. 2018; Ji et al. 2023). Notably, the abundance of arbuscules, a structure formed by mycorrhizae, were found to increase in *Poncirus* roots under low soil pH

conditions (Guo et al. 2016). Therefore, the increased accumulation of K, Fe, Mn, and Ca in lemon may be regulated by both activated soil nutrients and the mycorrhizal uptake pathway under low soil pH conditions. Further studies are needed to fully elucidate the underlying mechanisms.

Table 3 Effects of soil pH on soil nutrient concentrations (mg/kg)

Soil pH	Available K	Available Ca	Available Mg	Available Fe	Available Mn
4	204.17 ± 5.70a	467.30 ± 14.40bc	115.16 ± 3.33b	19.99 ± 0.93a	12.55 ± 0.95a
5	169.50 ± 15.48ab	526.49 ± 23.58ab	116.65 ± 6.42b	19.61 ± 1.56a	11.88 ± 0.63a
6	127.03 ± 12.34c	548.27 ± 32.58a	140.44 ± 3.71a	19.24 ± 1.48a	10.42 ± 0.94a
7	144.15 ± 12.83bc	435.85 ± 21.74c	134.95 ± 11.30ab	16.91 ± 3.81a	4.57 ± 0.59b
8	149.70 ± 11.54bc	401.12 ± 23.24c	130.91 ± 6.40ab	17.96 ± 2.26a	3.69 ± 0.42b

Note: 4, 5, 6, 7 and 8 represent the lemon plant was cultivated in different soil pH. K, Ca, Mg, Fe, and Mn represent potassium, calcium, magnesium, iron, and manganese, respectively. Different lowercase letters indicate significant differences among the treatments by Duncan-test ($P < 0.05$, $n = 4$)

Table 4 Effects of soil pH on the K, Ca, Mg, Fe, and Mn concentrations in the winter flower fruit pulp

Soil pH	K (%)	Ca (%)	Mg (%)	Fe (mg/kg)	Mn (mg/kg)
4	1.18 ± 0.03a	0.93 ± 0.03a	0.13 ± 0.00a	76.74 ± 2.13a	25.00 ± 0.85a
5	1.16 ± 0.04a	0.77 ± 0.02b	0.13 ± 0.00a	48.86 ± 2.22bc	18.30 ± 0.64b
6	1.10 ± 0.0a	0.77 ± 0.03b	0.14 ± 0.00a	41.00 ± 2.25c	17.47 ± 0.74b
7	1.13 ± 0.04a	0.71 ± 0.06b	0.13 ± 0.00a	52.07 ± 4.05b	17.61 ± 0.37b
8	1.10 ± 0.03a	0.72 ± 0.01b	0.14 ± 0.00a	48.63 ± 3.29bc	19.21 ± 0.51b

Note: 4, 5, 6, 7 and 8 represent the lemon plant was cultivated in different soil pH. K, Ca, Mg, Fe, and Mn represent potassium, calcium, magnesium, iron, and manganese, respectively. Different lowercase letters indicate significant differences among the treatments by Duncan-test ($P < 0.05$, $n = 4$)

Table 5 Effects of soil pH on the K, Ca, Mg, Fe and Mn concentrations in the spring flower fruit pulp

Soil pH	K (%)	Ca (%)	Mg (%)	Fe (mg/kg)	Mn (mg/kg)
4	1.16 ± 0.05a	0.65 ± 0.04b	0.13 ± 0.00a	69.08 ± 4.03a	20.90 ± 0.76a
5	0.82 ± 0.04bc	0.71 ± 0.02ab	0.13 ± 0.00a	54.73 ± 4.82bc	19.94 ± 0.51a
6	0.76 ± 0.04c	0.69 ± 0.04b	0.13 ± 0.00a	45.27 ± 3.10c	18.99 ± 0.88a
7	0.90 ± 0.04b	0.71 ± 0.02ab	0.14 ± 0.00a	56.14 ± 2.77b	21.71 ± 0.62a
8	0.84 ± 0.03bc	0.81 ± 0.05a	0.14 ± 0.00a	48.73 ± 1.96bc	19.91 ± 1.76a

Note: 4, 5, 6, 7 and 8 represent the lemon plant was cultivated in different soil pH. K, Ca, Mg, Fe, and Mn represent potassium, calcium, magnesium, iron, and manganese, respectively. Different lowercase letters indicate significant differences among the treatments by Duncan-test ($P < 0.05$, $n = 4$)

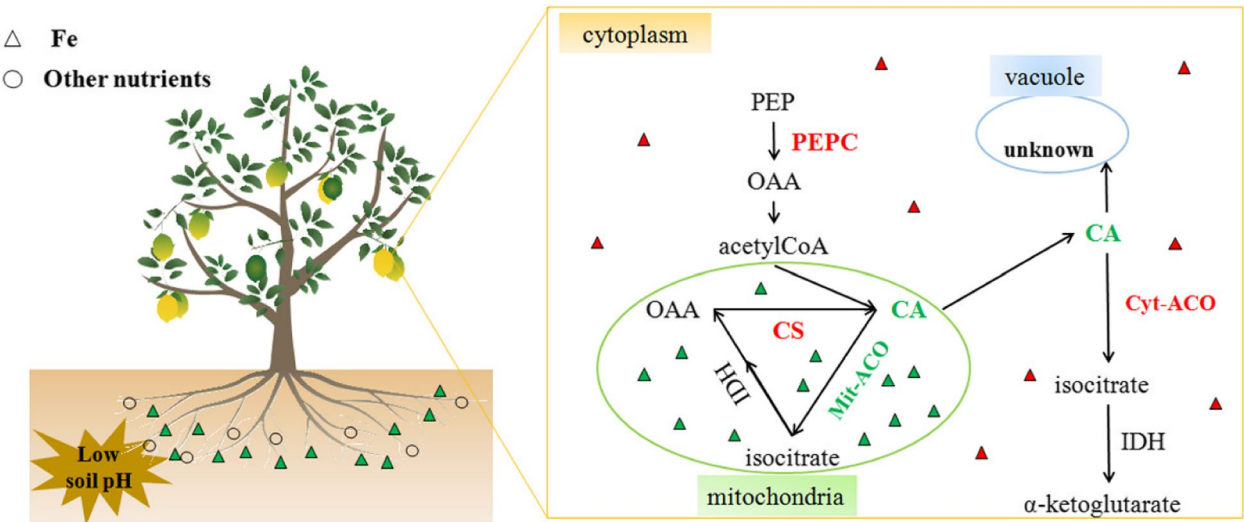


Fig. 6 A working model elucidating the effect of low soil pH on CA accumulations in lemon. PEPC, phosphoenolpyruvate carboxylase; Mit-ACO, mitochondrial aconitase; CS, citrate synthase; Cyt-ACO, cytosolic aconitase; IDH, isocitrate dehydrogenase; OAA, oxaloacetate; CA, citric acid; Fe, iron. The green triangles and words indicate the stimulatory effects under low soil pH condition, while the red ones indicate the repressive effects

Low soil pH enhances fruit acidity by inhibiting the citric acid degradation

Citrus fruit contains CA as over 70% of its organic acid content, significantly affecting flavor quality and fruit acidity (Sheng et al. 2017; Guo et al. 2016). CA accumulates gradually during early fruit development due to increased synthesis, but declines later as decomposition increases (Wu et al. 2021a; Zhou et al. 2018). The synthesis of CA involves the carboxylation of phosphoenolpyruvate (PEP) to oxaloacetate (OAA) by PEPC in the cytoplasm, followed by the production of CA in mitochondria using OAA as a substrate in the tricarboxylic acid (TCA) cycle under CS action (Etienne et al. 2013; Wu et al. 2021b). Higher PEPC and CS enzyme activities have been linked to the increased CA concentrations in citrus fruit (Lin et al. 2016; Wu et al. 2021a). However, our results show that low soil

pH (pH 4) decreased PEPC and CS activities in both WFF and SFF of lemon, despite the observed increase in CA concentrations (Table 2, Fig. 1 and 2). This implies that the enhanced fruit acidity under low soil pH conditions is not due to CA synthesis. CA accumulation is primarily regulated by its degradation, as highlighted in previous studies (Guo et al. 2016; Hussain et al. 2017). Synthesized in the mitochondria, CA translocates to the cytoplasm for breakdown and partial storage in vacuoles (Etienne et al. 2013). Low soil pH inhibits the activities of Cyt-ACO and NAD-IDH in both WFF and SFF of lemon fruits, resulting in the increased CA concentrations (Table 2, Fig. 1 and 2). This inhibition of CA degradation, consequently, leads to the elevated fruit acidity. Notably, vacuole storage is crucial for CA accumulation (Etienne et al. 2013), and further research is needed to elucidate the

role of low soil pH in this process. In summary, low soil pH enhances lemon fruit acidity by impeding CA degradation.

To better understand how low soil pH affects CA synthesis and degradation, we analyzed the mitochondrial and cytosolic K, Fe, Mn, and Mg concentrations in lemon fruits under varying pH conditions, as these elements are enzyme components and activators (Marschner 2011). Low soil pH increased Mit-K and Mit-Fe concentrations, which positively correlated with Mit-ACO activity. This aligns with Fe and K's roles as aconitase components and activators, respectively (Shlizerman et al. 2007; Marschner 2011). Additionally, low soil pH elevated Cyt-K, which positively correlated with PEPC activity, thereby supporting previous findings that K application increases PEPC activity in citrus fruit (Wu et al. 2021a). These results indicate that low soil pH influences CA synthesis through K and Fe activation in lemon fruits. However, this synthesis activation does not explain the increase in fruit acidity. Notably, low soil pH substantially decreased Cyt-Fe concentrations, with Cyt-ACO activity positively correlating with Cyt-Fe. Given that iron is a component of aconitase (Shlizerman et al. 2007), these findings suggest that low soil pH inhibits CA degradation by reducing Cyt-Fe concentrations, ultimately promoting CA accumulation in lemon fruits.

Conclusions

In conclusion, this study elucidates the multifaceted effects of soil pH on lemon fruit quality. High soil pH (pH 8) decreased TSS concentrations, while low soil pH (pH 4) increased TA and CA concentrations. Low soil pH not only enhanced fruit acidity but also affected soil nutrient availability and essential element accumulation in lemon pulp. It increased K, Fe, and Mn concentrations in the soil and raised Fe and Mn levels in lemon pulps. Surprisingly, low soil pH inhibited CA synthesis by reducing CS and PEPC activities, yet CA accumulation rose, indicating that the increased fruit acidity under low pH conditions was not due to CA synthesis. Instead, low soil pH suppressed Cyt-ACO and NAD-IDH activities, thereby hindering CA degradation and leading to higher CA concentrations. This inhibition coincided with a significant decrease in Cyt-Fe concentrations, implicating iron plays a role in CA degradation (Fig. 6). Our findings demonstrated that low soil pH enhances fruit acidity partly by inhibiting CA degradation in lemon fruits, highlighting the complex relationships among soil pH, nutrient availability, enzyme activities, and fruit quality. Therefore, these findings underscore the importance of considering these factors in agricultural practices to optimize fruit production.

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Authors' contributions

CH and SW conceived and coordinated this project; GG performed the experiments with the help of YD, XM, ZD; SW analyzed data and wrote the manuscript; CH, QT and XS revised the manuscript. All authors have read and approved the final version of the manuscript.

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Availability of data and materials

The datasets generated and/or analyzed are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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