



Physical and Physiological Responses of Cucumber (*Cucumis sativus* L.) Seeds to Citric Acid Treatment

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ABSTRACT

The physical appearance and physiological quality of seeds are critical indicators of cucumber seed quality in horticultural production systems. Improving seed quality requires seed treatments that are both effective and economical, while also ensuring seed viability remains safe. Citric acid has potential as a seed treatment due to its chemical properties and affordability. This research aimed to evaluate the effects of citric acid on cucumber seed quality, focusing on seed lightness, germination performance, and seed vigor. The experiment was conducted from August to November 2023 at PT East West Seed Indonesia, Jember, using a completely randomized design (CRD) with two factors: soaking duration (5, 10, and 15 minutes) and citric acid concentration (1.5%, 2.0%, and 2.5%). The results showed that the highest seed lightness (76.58%) was obtained after 5 minutes of soaking in 1.5% citric acid. The highest germination performance (100%) was achieved with 10 minutes of soaking in 2.0% citric acid, while the highest seed vigor (vigor index 98.67%) was observed at 10 minutes of soaking in 1.5% citric acid. These findings demonstrate that citric acid can improve cucumber seed quality by enhancing physical appearance without compromising physiological performance. Overall, soaking cucumber seeds in 1.5–2.0% citric acid for approximately 10 minutes is recommended as an optimal seed treatment to balance improvements in seed lightness, germination performance, and seed vigor. This research provides an applicable and economical seed treatment strategy for the horticultural seed industry.

INTRODUCTION

Seed quality is a critical determinant of successful cucumber (*Cucumis sativus* L.) cultivation, particularly in commercial horticultural production systems where uniform emergence, rapid establishment, and attractive seed appearance are required. In addition to physiological attributes such as germination performance and seed vigor, the physical appearance of cucumber seeds, including seed lightness and surface cleanliness, plays an important role in market acceptance and seed lot grading within the seed industry (Mubarok et al., 2018; Wahyuni et al., 2021). Consequently, post-harvest seed treatments that can improve cucumber seed quality without compromising physiological performance are increasingly needed, especially under competitive commercial seed markets (Copeland & McDonald, 2001; Bewley et al., 2013).

Over the past two decades, studies have shown that organic acids, particularly citric acid, can positively impact seed and seedling performance by enhancing metabolic activation, increasing enzymatic activity, and improving stress tolerance. Citric acid has been widely reported as an effective seed treatment for improving germination performance and early seedling growth in several crops,

including maize, soybean, and chickpea, primarily through its role in regulating antioxidant defense systems, cellular respiration, and reserve mobilization (Ghazijahani et al., 2018; Imran et al., 2023; Farooq et al., 2019). Furthermore, citric acid has been recognized for its antimicrobial properties associated with pH reduction, which suppresses microbial growth on biological surfaces and contributes to improved post-harvest quality (Mbulu et al., 2018; Sapers, 2001). Studies on horticultural seeds further emphasize that seed quality evaluation should integrate physical and physiological parameters to ensure high field performance and marketability (Mubarok et al., 2018; Maharani et al., 2023; ISTA, 2023).

Despite these advances, most existing studies have primarily focused on the physiological responses of seeds and seedlings following citric acid application, with limited attention given to changes in seed physical appearance, particularly seed lightness and surface cleanliness. Research addressing the interaction between citric acid concentration and soaking duration on cucumber seed quality remains scarce, and the chemical mechanisms underlying surface improvement of seeds are rarely discussed in the context of commercial seed processing (Maharani et al., 2016; Mbulu et al., 2018). Moreover, information on optimal treatment conditions that balance improvements in physical appearance and physiological performance under industrial seed production standards is still limited, particularly for vegetable seeds intended for high-value markets (Finch-Savage & Bassel, 2016).

The scientific novelty of this research lies in the integrated evaluation of citric acid as a dual-function seed treatment targeting both physical and physiological components of cucumber seed quality. This research explicitly elucidates the role of citric acid as a surface-cleaning agent through its chelating ability to bind metal ions and surface impurities, its capacity to dissolve surface pigments, and its pH-lowering effect that suppresses microbial activity on the seed coat, while simultaneously maintaining or enhancing germination performance and seed vigor (Mbulu et al., 2018; Imran et al., 2023; Farooq et al., 2019). Furthermore, this research systematically examines the interaction between soaking duration and citric acid concentration, providing quantitative evidence for optimal treatment conditions relevant to commercial seed processing. A commercially cultivated cucumber variety commonly used in industrial seed production was selected to ensure that the findings are directly applicable to standard industry practices (Nuraini et al., 2020).

Therefore, this research aimed to evaluate the effects of citric acid seed treatment on cucumber seed quality by analyzing changes in seed lightness, germination performance, and seed vigor, to determine the optimal citric acid concentration and soaking duration, and to assess the interaction between these factors in improving cucumber seed quality for horticultural seed production systems.

METHODS

Research Time and Place

This research is part of a collaborative project with seed-producing companies and was carried out from August to November 2023. The location of the research is located at PT. East West Seed Indonesia, which is located at Jalan Basuki Rachmad No. 019, Muktisari Village, Tegal Besar District, Jember Regency, East Java Province. All laboratory analyses, including physical and physiological seed quality assessments, were performed under controlled laboratory conditions following standard seed testing procedures.

Research Tools and Materials

The materials used in this study consisted of cucumber (*Cucumis sativus* L.) seeds obtained from a single commercially cultivated variety commonly used in industrial seed production to ensure uniformity and relevance to standard seed processing practices. Citric acid (analytical grade) was used to prepare treatment solutions at designated concentrations. Germination tests were conducted using

rice straw paper as the germination medium. Additional materials included distilled water, 70% alcohol, labels, and germination equipment.

Experimental Design

The experiment was arranged in a Completely Randomized Design (CRD) with two treatment factors and three replications. The first factor was soaking duration, consisting of 5, 10, and 15 minutes. The second factor was citric acid concentration, consisting of 1.5%, 2.0%, and 2.5%. The combination of the two factors resulted in 9 treatment combinations (3×3), each of which was repeated three times, making a total of 27 trial units. Each treatment combination consisted of 50 seeds per replication, resulting in a total of 27 experimental units.

The selection of the concentration range (1.5–2.5%) and soaking duration (5–15 minutes) was based on previous studies reporting positive physiological responses of seeds treated with citric acid at comparable concentration levels, while avoiding phytotoxic effects at higher concentrations (Maharani et al., 2016; Ghazijahani et al., 2018; Imran et al., 2023). In addition, preliminary observations conducted during routine seed processing activities at the company indicated that soaking durations longer than 15 minutes or concentrations exceeding 2.5% could potentially reduce seed surface integrity. Therefore, the selected treatment levels were considered appropriate to evaluate both physical and physiological responses without compromising seed viability.

Sampling and Observation Techniques

The observed parameters included seed lightness, germination performance, and seed vigor. Seed lightness was measured using a chromameter and expressed as L^* values based on the CIELAB color system. Germination performance was determined as the percentage of normal seedlings produced under standard laboratory conditions using rice straw paper as the germination medium. Seed vigor was expressed as a vigor index, calculated based on the percentage and speed of seed germination, following standard seed testing principles commonly applied in vigor assessment.

Data Analysis

All data were subjected to analysis of variance (ANOVA) according to the factorial CRD. When significant differences among treatments were detected, Duncan's Multiple Range Test (DMRT) was applied at a 5% significance level to compare treatment means. Statistical analyses were performed using SPSS software.

RESULTS AND DISCUSSION

Seed Lightness (L^*)

Seed lightness (L^*) of cucumber seeds was significantly affected by the interaction between soaking duration and citric acid concentration, indicating that the response of seed surface appearance was determined by the combined effects of both factors rather than by a single treatment variable. As illustrated in Figure 1 and supported by the numerical data in Table 1, the highest seed lightness value (76.58) was obtained from seeds soaked for 5 minutes in 1.5% citric acid, clearly demonstrating an optimal treatment peak. This pattern indicates that a short soaking duration combined with a low citric acid concentration is the most effective strategy for improving the physical appearance of cucumber seeds.

The improvement in seed lightness under mild citric acid treatment can be explained by the chemical cleaning mechanism of citric acid on the seed coat surface. Citric acid acts as a chelating agent capable of binding metal ions and surface-associated impurities, while simultaneously dissolving surface pigments and residues that contribute to dull seed appearance. In addition, the acidic environment lowers surface pH, suppressing microbial growth that may cause discoloration during post-harvest handling. These mechanisms collectively increase surface reflectance, resulting in higher L^* values, as visually confirmed by the distinct peak shown in Figure 1 and statistically supported by the significant differences presented in Table 1. Similar surface-cleaning and color-improving effects of

organic acids on seed and horticultural materials have been reported in national seed quality studies, particularly in relation to improvements in visual quality and market grading of seed lots (Maharani et al., 2016; Mbulu et al., 2018; Ningsih et al., 2018).

Table 1. Results of Advanced Test of Interaction of Long Soaking and Citric Acid Concentration on Seed Brightness Level (%) (*Lightness*, symbol L)

Treatment	Lightness (%)	
L2K3	73,64	a
L3K2	74,25	b
L1K3	74,34	b
L1K2	74,53	c
L2K2	74,93	d
L3K1	74,97	e
L3K3	75,16	f
L2K1	75,57	g
L1K1	76,58	h

Remarks: The number followed by the same letter shows no significant difference in the DMRT test at 1%.

In contrast, increasing the citric acid concentration to 2.5% or extending the soaking duration beyond 5 minutes led to a decline in seed lightness, as indicated by both the downward trend in Figure 1 and the lower mean values in Table 1. This decreasing response suggests that excessive acidity or prolonged exposure may disrupt the structural integrity of the seed coat, cause excessive pigment dissolution, or alter surface microstructure, thereby reducing optical reflectance. Similar responses have been reported in studies describing pigment degradation and surface erosion under exposure to high organic acids in biological tissues. Furthermore, previous studies have also described physical damage to seed surfaces and visual quality degradation following chemical treatments or excessive soaking (Maharani et al., 2016; Pathare et al., 2013). From a seed technology perspective, excessive chemical exposure may increase the risk of microstructural damage to the seed coat, which could negatively influence subsequent physiological performance if not properly controlled (Bewley et al., 2013). Overall, the combined interpretation of Figure 1 and Table 1 highlights the importance of optimizing treatment intensity to enhance seed brightness while avoiding surface damage.

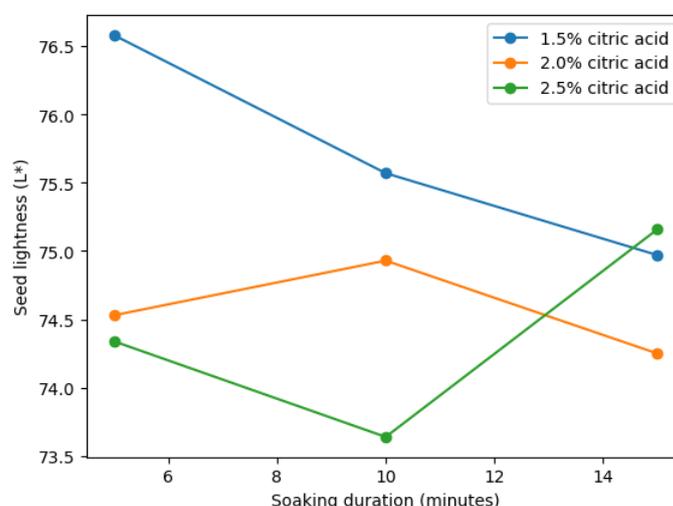


Figure 1. Interaction between soaking duration and citric acid concentration on seed lightness (L*) of cucumber seeds.

More clearly, Figure 1 demonstrates a significant interaction between soaking duration and citric acid concentration on seed brightness (L^*). The highest L^* value was obtained after 5 minutes of soaking with 1.5% citric acid, indicating that exposure to mild acid effectively cleaned the seed surface without inducing surface damage. Longer soaking duration or higher citric acid concentration resulted in a gradual decrease in seed brightness, reflecting excessive pigment dissolution or changes in surface microstructure under stronger acidic conditions. These findings emphasize that the interaction between soaking time and citric acid concentration must be precisely controlled to achieve optimal seed brightness while maintaining seed structural integrity, in line with recommendations for post-harvest seed handling and quality preservation in commercial seed systems (Copeland & McDonald, 2001; ISTA, 2023).

Germination Power (%)

Germination performance (%) of cucumber seeds was not significantly different among treatments; however, clear response trends were observed across soaking durations and citric acid concentrations. As illustrated in Figure 2 and supported by the numerical values presented in Table 2, the highest germination performance (100%) was achieved by seeds soaked for 10 minutes in 2.0% citric acid. This response indicates that moderate soaking duration combined with an intermediate citric acid concentration provided the most favorable conditions for physiological activation during the germination process.

The observed trend suggests that citric acid seed treatment at moderate levels enhances membrane permeability during imbibition, thereby facilitating water uptake and oxygen diffusion into seed tissues. Improved membrane permeability supports the activation of hydrolytic enzymes involved in reserve mobilization, resulting in more uniform and complete germination. Similar physiological mechanisms have been reported in studies demonstrating that citric acid seed treatment enhances germination performance through improved antioxidant activity, membrane stability, and metabolic regulation during early germination stages (Ghazijahani et al., 2018; Imran et al., 2023). These findings indicate that citric acid functions not only as a surface-modifying agent but also as a metabolic modulator during seed imbibition.

Table 2. Results of Advanced Test of Interaction of Soaking Length and Concentration of Citric Acid on Germination Power (%)

Treatment	Germination Power (%)	
L1K3	92,67 a	a
L3K1	94,00 from	off
L1K1	96,67 abc	abc
L1K2	97,33 abc	abc
L2K2	97,33 abc	abc
L2K3	98,00 bc	bc
L3K3	98,00 bc	bc
L3K2	98,67 bc	bc
L2K1	100,00 c	c

Remarks: The number followed by the same letter shows no significant difference in the DMRT test at 5%.

Consistent with this interpretation, studies on seed priming and invigoration have shown that appropriate soaking duration combined with mild chemical or organic priming agents can significantly improve germination percentage and early seedling performance. Alimuddin et al. (2023) reported that organic priming treatments combined with optimal soaking duration enhanced germination traits of sweet corn seeds, emphasizing the importance of balancing treatment intensity and exposure time. Likewise, organic priming using plant-based extracts was shown to improve germination performance

in chili seeds, supporting the role of pre-imbibition treatments in enhancing physiological readiness for germination (Putri et al., 2025). Similar trends were also observed in mung bean seeds, where moderate priming conditions resulted in more consistent germination responses compared with extreme treatments (Sagala et al., 2025).

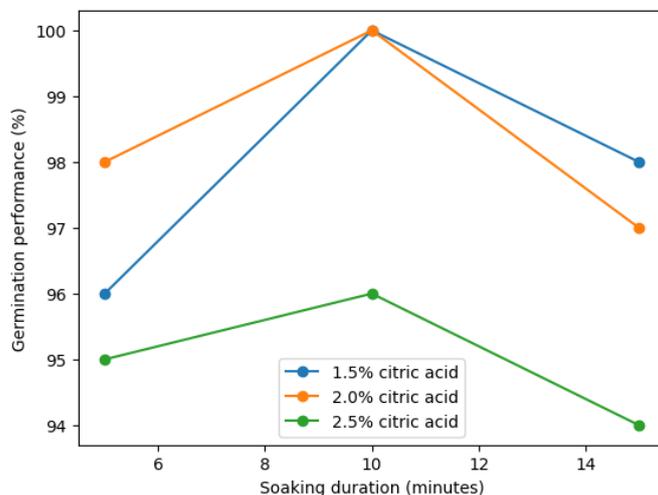


Figure 2. Interaction between soaking duration and citric acid concentration on germination performance (%) of cucumber seeds.

In contrast, shorter soaking duration combined with higher citric acid concentration tended to reduce germination performance, as reflected by the lower values shown in Figure 2 and Table 2. This response suggests that insufficient imbibition time may limit metabolic activation, while excessive acidity may interfere with membrane stability and disrupt early germination processes. Wahyuni et al. (2021) reported that inappropriate priming duration or excessive treatment intensity could negatively affect germination uniformity, reinforcing the importance of optimizing priming conditions. Too long soaking or too high a concentration does not always give better results. This can be caused by phytotoxic effects or tissue damage due to too strong a solution. In line with the opinion of Rahmawati et al. (2022), the length or short soaking time does not directly change the viability of the seeds, as viability is greatly influenced by the genetic factors of the seeds. In addition, Moiwend and Madauna (2015) stated that the success of germination is determined by two main factors, namely internal factors (physiological/genetic seeds) and external factors (environment and treatment). According to Paramita et al. (2018), germination performance reflects the ability of seeds to initiate metabolic growth, and values above 80% have been categorized as high-grade seeds.

Seed vigor

Seed vigor, expressed as vigor index, was significantly influenced by the interaction between soaking duration and citric acid concentration. As illustrated in Figure 3 and supported by the numerical values presented in Table 3, seed vigor tended to increase with longer soaking duration, particularly at lower citric acid concentrations. The highest vigor index was recorded in seeds soaked for 15 minutes with 1.5% citric acid, indicating that extended imbibition under mild acidic conditions enhanced early seedling establishment potential.

Table 3. Results of Follow-up Test of Interaction of Soaking Length and Citric Acid Concentration on Vigor Index (%)

Treatment	Vigor Index (%)	
L2K2	86.00 A	a
L1K3	89,33 from	off

Treatment	Vigor Index (%)	
L3K1	91,33 from	off
L1K2	94,00 from	off
L1K1	95,33 from	off
L2K3	96,00 from	off
L3K3	96.67 b	b
L2K1	97.33 b	b
L3K2	98.67 b	b

Remarks: The number followed by the same letter shows no significant difference in the DMRT test at 1%.

The improvement in seed vigor under moderate citric acid treatment can be explained by enhanced membrane permeability and membrane repair processes during imbibition, which facilitate metabolic reactivation and efficient mobilization of seed reserves. Mild acidic conditions may stabilize cell membranes, reduce solute leakage, and promote enzymatic activity associated with energy production and reserve utilization. Comparable physiological mechanisms have been reported in seed invigoration and priming studies, where controlled hydration treatments improved vigor index by accelerating germination speed and strengthening early seedling growth (Bewley et al., 2013; Finch-Savage & Bassel, 2016). Similar findings were also reported by Alimuddin et al. (2023), who showed that appropriate soaking duration combined with organic priming significantly enhanced seed vigor and early seedling performance.

Consistent with these findings, studies on seed priming using organic or chemical agents have demonstrated that moderate priming conditions effectively improve seed vigor across various crop species. Organic priming treatments were shown to increase vigor and uniformity of seedling growth by enhancing reserve mobilization and reducing physiological stress during early germination stages (Putri et al., 2025). Likewise, priming treatments applied under controlled soaking duration produced more stable and consistent vigor responses compared with extreme treatments in mung bean seeds, emphasizing the importance of optimizing treatment intensity (Sagala et al., 2025). Similar trends have been documented in vegetable and field crops, where excessive priming intensity led to diminishing vigor gains due to membrane destabilization and metabolic imbalance (Farooq et al., 2019).

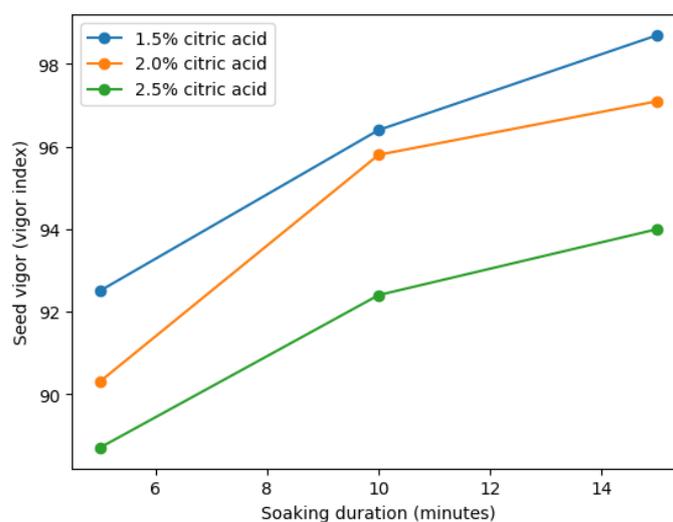


Figure 3. Interaction between soaking duration and citric acid concentration on seed vigor (vigor index)

In contrast, increasing citric acid concentration or applying prolonged soaking under stronger acidic conditions did not further enhance seed vigor, as indicated by the declining or plateauing trends shown in Figure 3 and Table 3. This response suggests the presence of a physiological threshold beyond which excessive acid exposure may disrupt membrane stability, induce metabolic imbalance, or impose mild stress on seed tissues. Such threshold effects have been widely reported in priming studies, highlighting that optimal vigor enhancement depends on precise regulation of both concentration and exposure duration (Copeland & McDonald, 2001; ISTA, 2023).

Overall, the combined interpretation of Figure 3 and Table 3 indicates that immersion times of 10–15 minutes combined with 1.5–2.0% citric acid provide the most favorable physiological responses in terms of germination performance and seed vigor. These findings support the potential application of citric acid as an effective, economical, and scalable post-harvest seed treatment for horticultural seed producers, particularly when treatment parameters are carefully optimized to avoid physiological stress.

CONCLUSIONS

This study demonstrates that citric acid is an effective post-harvest seed treatment whose impact on cucumber seed quality is governed by the interaction between soaking duration and citric acid concentration rather than by a single treatment factor. Citric acid acts through complementary mechanisms by improving seed surface cleanliness and modulating early physiological activation during imbibition, resulting in coordinated responses in seed lightness, germination performance, and seed vigor.

The results indicate that mild citric acid treatments provide the most efficient balance between physical and physiological seed quality attributes. Optimal conditions for improving seed lightness, germination performance, and seed vigor were not identical, underscoring that treatment parameters should be adjusted according to the specific quality objective rather than applied uniformly. This finding highlights the importance of precision in post-harvest seed treatment strategies to maximize benefits while avoiding adverse effects associated with excessive treatment intensity.

From a scientific standpoint, this study contributes to seed technology research by clarifying the dual functional role of citric acid as both a surface-modifying agent and a physiological regulator during early seed hydration. The integrated evaluation of physical and physiological responses advances current understanding of how organic acid-based treatments can be optimized to enhance seed quality without inducing phytotoxic stress.

From an application perspective, citric acid offers a practical, economical, and environmentally benign alternative for post-harvest treatment in horticultural seed production. The identified treatment ranges are readily applicable within commercial seed processing systems to improve visual seed quality while maintaining high physiological performance. Further studies are recommended to assess the robustness of these responses across different cucumber varieties, storage environments, and operational scales, thereby strengthening the potential for wider industrial adoption of citric acid-based seed treatment technology.

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