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THESIS OF THE DOCTORAL DISSERTATION

INVESTIGATION OF POSTHARVEST FACTORS AFFECTING THE RIPENING AND STORAGE BEHAVIOR OF FRESH TOMATOES

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1. BACKGROUND AND OBJECTIVES

Tomato (*Solanum lycopersicum L.*) is one of the most important and most widely produced fresh vegetable crops worldwide, possessing significant gastronomic, nutritional, and economic value. Its consumption and processing are both substantial, as its high water, vitamin, and antioxidant content — especially lycopene — make it an essential component of a healthy diet. This importance is further reinforced by the continuous increase in global production and by the fact that roughly one-third of the fruits and vegetables produced globally are lost before reaching consumers. Due to the perishability and sensitivity of fresh produce, combined with the complexity of the supply chain, reducing postharvest losses is of particular importance.

Tomato is a climacteric fruit that exhibits intense ethylene production and increased respiration during ripening. Postharvest physiological processes lead to rapid quality deterioration: softening, color changes, modifications in taste and aroma compounds, and increased susceptibility to mechanical damage. Meanwhile, the consumer market consistently demands high quality, making the development of effective postharvest technologies essential.

One of the most widely used ripening inhibitors is 1-methylcyclopropene (1-MCP), which slows ripening by blocking ethylene receptors. While 1-MCP has proven effective for several climacteric fruits, there is no unified understanding regarding optimal dosage, treatment conditions, and the crucial role of maturity stage in tomatoes. In addition, the inherent chilling sensitivity of tomatoes further complicates the extension of shelf life, as storage below 10 °C may lead to quality defects (e.g., color and flavor deterioration).

New directions in postharvest research include microbubble-based treatments, alternative methods for delivering 1-MCP, and non-destructive quality assessment techniques — such as chlorophyll fluorescence, the DA-index®, and acoustic firmness testing — providing more accurate monitoring of physiological status.

These factors justified a detailed experimental investigation of postharvest influences affecting tomato ripening, storability, and sensory–physiological responses. Accordingly, I formulated the following specific objectives:

1. To determine the optimal 1-MCP dose at different maturity stages, identifying which treatment levels most effectively slow the postharvest physiological behavior of tomatoes — specifically ripening and respiration.
2. To examine the role of maturity stage in 1-MCP efficacy, establishing the phenological phase in which treatment is most advisable.
3. To evaluate the applicability of the Polar Qualification System (PQS) for determining tomato maturity, by comparing digital image–derived color parameters with conventional methods (a* value, DA-index®).
4. To investigate the effects of low temperature at different storage levels (15 and 2 °C), with particular emphasis on respiration intensity, ethylene production, firmness (texture) changes, and chlorophyll fluorescence.
5. To assess the effectiveness of microbubble-based 1-MCP delivery and its ultrasonic enhancement, comparing these to the traditional gaseous treatment method.
6. To examine the effects of mechanical damage (drop height, maturity stage) on respiration, ethylene production, firmness parameters, and fluorescence values, with the aim of identifying early indicators suitable for damage detection.
7. To determine the primary sites of gas exchange on the tomato surface using various coating and sealing treatments, in order to clarify the respective roles of the calyx, stem scar, and fruit surface.

2. MATERIALS AND METHODS

The tomato samples of the Pitenza F1 variety used in the experiments were obtained from domestic production, sourced from a farm with uniform cultivation technology. For each study, fruit at the appropriate stage of maturity was selected from homogeneous lots, based on visual assessment and the internationally accepted CTIFL color chart (ranging from mature green to breaker and fully ripe stages). The experiments encompassed seven major topics, from investigating the dose-dependent effects of 1-MCP treatments to conducting coating experiments aimed at determining the sites of gas exchange.

For the ripening-inhibiting treatments, I used SmartFresh™ ProTabs containing 2% 1-MCP as the active ingredient, applying conventional gaseous treatment in sealed containers as well as alternative methods based on microbubble-assisted and ultrasound-assisted aqueous application. Conventional treatments were carried out at 15 °C (and in some cases at 20 °C) for 24 hours, while microbubble treatments were conducted for 5-, 10-, and 15-minute exposure times in a 20 °C water bath. The ultrasonic treatments were performed using a 72 W device operating at 20 kHz, producing diffuse ultrasound in the water.

To determine the optimal 1-MCP dose, conventional gaseous treatments were carried out at concentrations of 625 ppb and 1250 ppb. To identify the optimal maturity stage, six different maturity categories were treated and analyzed during storage at 15 °C and 20 °C.

For chilling injury studies, tomatoes were stored at 2 °C and 15 °C, each combined with untreated and 1-MCP–treated groups.

The effects of mechanical damage were studied using drop tests. Fruits were dropped from various heights, and—where possible—measurements were recorded separately on the damaged and undamaged sides, including ethylene production, respiration intensity, firmness, and chlorophyll fluorescence parameters.

To determine the primary sites of gas exchange, the surface regions of the fruit (peel, calyx, stem scar) were isolated using waxing and various masking techniques. In a preliminary experiment, only respiration intensity was measured on ripe tomatoes; subsequently, in a larger-scale study, green-mature fruit were monitored for 7 weeks following 1-MCP treatment. The purpose of these coverings was to reveal which surfaces were responsible for the majority of gas exchange.

A wide range of non-destructive and conventional analytical methods was applied:

Color measurement: CIELab color parameters (L*, a*, b*) were determined with a Konica-Minolta CR-400 tristimulus chromameter (Minolta Europe GmbH, Langenhagen, Germany).

DA-index® measurement: The state of chlorophyll degradation was monitored using a Sintéleia Vis/NIR DA-meter® FRM01-F (Sintéleia s.r.l., Bologna, Italy). Maturity determination was based on chlorophyll content, calculated from absorbance properties using the following equation [1]:

$$\text{DA-index}^{®} = I_{AD} = A_{670nm} - A_{720nm} \qquad [1]$$

where:
A_{670nm} = the absorption peak of chlorophyll-a
A_{720nm} = absorbance of the background

Acoustic and impact firmness measurement: Acoustic (S) [2] and impact (D) [3]firmness parameters were measured using an AWETA AFS desktop firmness analyzer (AWETA AFS Desktop System, DTF V0.0.0.105, AWETA BV., Pijnacker, The Netherlands).

$$S = f^2 \times m^{2/3} \times 10^{-6} \qquad [2]$$

$$D = \frac{1}{\Delta T^2} \qquad [3]$$

where:
S – acoustic firmness factor ($Hz^2g^{2/3}\times10^{-6}$);
f – resonance frequency (Hz);
m – weight (g).

where:
D – impact firmness factor (ms^{-2})
ΔT^2 – time difference between the start and peak of the first sine wave

Chlorophyll fluorescence: Changes in chlorophyll activity during storage were monitored using a Modular Multi Channel Chlorophyll Fluorometer (MONI-PAM) controlled by WinControl-3 software (v3.12, dev-rev. 396; Heinz Walz GmbH, Effeltrich, Germany) and an Open FluorCam imaging system (PSI – Photon Systems Instruments, Brno, Czech Republic). The parameters F_0 (minimal fluorescence), F_m (maximal fluorescence), and F_v (variable fluorescence = $F_m - F_0$) were determined.

Respiration intensity: CO_2 concentration changes were measured using an Ahlborn FY A600-CO2H sensor and ALMEMO3290-8 data logger (Ahlborn Mess- und Regelungstechnik GmbH, Holzkirchen, Germany), and respiration intensity was calculated as [4]:

$$Li = \frac{V_{sz} \cdot \Delta CO_{2(t_2-t_1)} \cdot 10^{-6}}{m \cdot (t_2 - t_1)} \quad [4]$$

where:
Li = respiration intensity [ml CO_2/(kg*h)]
Vsz = free volume of the container [ml]
$\Delta CO_{2(t2-t1)}$ = change in CO_2 concentration [ppm]
m = fruit weight [kg]
t_1 and t_2 = measurement time [hour]

Ethylene production: Measured using an SCS-56 handheld ethylene analyzer (Storage Control Systems Ltd., Paddock, UK). Results were expressed as µl ethylene$\cdot$kg$^{-1}\cdot$h^{-1}.

Digital image analysis: Images captured during the experiments were processed to calculate hue spectra, with saturation values scaled to 0–100%. The spectra were compressed using the Polar Qualification System (PQS) surface method, converting the spectrum into a polar dataset and calculating the centroid. Red, green, and blue color components and their normalized values were computed as follows [5]:

$$R_N = \frac{R}{R+G+B}, G_N = \frac{G}{R+G+B}, B_N = \frac{B}{R+G+B} \quad [5]$$

Statistical analysis: Conducted with SPSS software (version 29.0.1.0, Armonk, NY, USA, 2022). Two-way ANOVA was used to detect significant effects. Homogeneity of variances was assessed with Levene's test. Homogeneous variances were analyzed using Tukey HSD, while heterogeneous variances were analyzed using the non-parametric Games–Howell test. Significant differences were defined at $p < 0.05$. For digital image validation, Pearson and Spearman correlations were applied due to the expected nonlinear behavior of pigment concentration.

Sigmoid curve fitting: To model changes in tomato color (a* value) during ripening, nonlinear regression was applied using a logistic sigmoid function. Curve fitting and data processing were performed in Python 3.13.5 (Python Software Foundation, Beaverton, USA), using a function that applied nonlinear least squares to optimize the parameters (L, k, x_0, B) for best fit.

3. RESULTS

My results show that tomato ripening and quality are influenced by multiple interacting factors: temperature, 1-methylcyclopropene (SmartFresh™) treatment, mechanical and chilling injury, as well as changes in respiration and ethylene production all play important roles. Based on the results of Experiment I, it can be concluded that a concentration of 625 ppb was sufficient to effectively inhibit ripening. This confirms that with an appropriate dose, the ripening process can be significantly delayed, especially in maturity stages prior to the climacteric. The findings of Experiment II further refined this picture, highlighting that the choice of maturity stage is crucial; truly effective ripening inhibition can only be achieved in tomatoes that are still before the climacteric. Furthermore, the Polar Qualification System (PQS) and digital image analysis proved to be reliable tools for objectively determining maturity stages.

The chilling injury study (Experiment III) demonstrated that although storage at 2 °C slows metabolic processes, it leads to clear chilling injury—particularly in earlier maturity stages. The extent of chilling damage was strongly influenced by both temperature and duration of exposure. This process was accompanied by cell wall and membrane damage, stress-induced ethylene production, and characteristic surface symptoms. SmartFresh™ treatment was not effective at 2 °C; in fact, in some cases it appeared to accelerate the development of chilling injury, therefore the combined use of these technologies is not recommended. In samples stored at 15 °C, however, SmartFresh™ treatment significantly slowed ripening, as evidenced by changes in respiration intensity, ethylene production, and firmness measurements.

The microbubble-based 1-MCP treatment examined in Experiment IV did not produce striking results., and its effectiveness did not reach that of the traditional gaseous treatment. Although some treatments temporarily reduced respiration intensity, they did not ensure lasting ripening inhibition. Microbubble treatments combined with ultrasound appeared promising, as they initially reduced respiration intensity more effectively, but the effect did not persist. These findings indicate that further development and optimization of the technology are necessary before it can serve as a competitive alternative to conventional 1-MCP application.

Experiment V (mechanical damage) confirmed that impacts clearly affect ripening and quality, especially at more advanced maturity stages. Increased respiration intensity and ethylene production occurred as stress responses, consistent with previous literature. Impact firmness measurements proved more sensitive for detecting localized injuries than acoustic firmness, which reflects the differing sensitivities of the two methods and the distinction between global (acoustic) and point-specific (impact) measurement approaches. Chlorophyll fluorescence analysis also showed promise for early detection of damage, suggesting that further refinement of this method could offer significant practical benefits.

Experiments VI and VII, which used masking techniques to study respiration intensity, revealed that the tomato fruit epidermis has virtually no stomata, meaning that gas exchange occurs primarily through the pedicel and its attachment point to the fruit. This explains why waxing or covering the stem scar dramatically reduced (50-75%) respiration, while covering the fruit surface had little effect. These findings align with the literature and provide new evidence regarding the main gas-exchange surfaces of tomato fruit. Combining stem-scar waxing with SmartFresh™ treatment proved to be the most effective method for ripening inhibition, opening new possibilities for the development of postharvest technologies.

4. CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

- 1-MCP (SmartFresh™) treatment can be effectively applied to tomatoes, but its impact is maturity-dependent.
- Storage at 2 °C clearly results in chilling injury, particularly in fruit at earlier maturity stages, and 1-MCP treatment further exacerbates this effect.
- The current form of the microbubble technology does not replace traditional 1-MCP treatment, but it has development potential.

- In tomatoes, the primary site of gas exchange is the pedicel and stem-scar region, while transpiration occurs mainly through the fruit surface, offering new possibilities for targeted postharvest interventions..

Recommendations:

- 1-MCP (SmartFresh™) treatment should be applied under cooled conditions (10–15 °C) and at pre-climacteric maturity to achieve the most effective ripening inhibition.
- Storage below 10 °C should be avoided long-term, especially for less mature tomatoes, as it may induce chilling injury. However, in cases of limited processing capacity, storage at low temperature may be used as a compromise, since symptoms of chilling injury at 2 °C appeared only after approximately two weeks, and at higher temperatures this time interval may be even longer. In the future, it would be advisable to examine additional temperatures within the 2–10 °C range to determine the threshold for chilling injury, as there may be a temperature at which chilling injury does not occur yet metabolic activity is noticeably slowed. Investigating the phase transition temperature of the tomato lipid fraction using differential scanning calorimetry (DSC) would also be beneficial.
- Further research is required for the microbubble technology to determine optimal 1-MCP dosage, exposure time, and ultrasound parameters.
- Chlorophyll fluorescence measurements show strong potential for early damage detection. The method may be suitable for assessing the mechanical impact of handling equipment used in tomato processing or evaluating the suitability of different packaging methods.
- Targeted oxygen-impermeable sealing of the tomato stem scar, combined with 1-MCP treatment, may become an efficient and economical future ripening-inhibition technology, substantially improving the shelf life of fresh-market tomatoes.
- The PQS method may serve as a good alternative to traditional ripening-assessment techniques, as it is simpler, cheaper, and in some cases faster.

NEW SCIENTIFIC RESULTS

1. I found that the 24-hour 1-methylcyclopropene (SmartFresh™) treatment at a concentration of 625 ppb effectively increased the storability of tomatoes at 15 °C across all examined ripeness stages during the 14-day storage period. Complete ripening inhibition was observed in tomatoes at the pre-climacteric stage (CTIFL 1–2), while in samples that had already entered ripening (CTIFL 3–9), the treatment exerted only partial ripening inhibition. In fully ripe tomatoes (CTIFL 10–12), the treatment primarily slowed down the deterioration processes.
2. Using the Polar Qualification System (PQS), I demonstrated that the color parameters obtained through digital image processing show a strong correlation with traditional color-measurement methods (a* value; $r = 0.958$) and with the DA-index® related to chlorophyll content ($r = 0.904$). Therefore, the system is suitable for objectively determining tomato ripeness.
3. I found that decreases in Fm values were detectable as early as the first day after dropping tomatoes from a height of 40 cm—on both the intact and the impacted sides. Based on this, chlorophyll-fluorescence measurements proved suitable for the early detection of mechanical damage in tomatoes.
4. I found that in tomatoes stored at 2 °C, the 24-hour 1-methylcyclopropene (SmartFresh™) treatment at 625 ppb increased both the incidence and severity of chilling-injury symptoms compared to the control group stored at 2 °C.
5. I found that the main gas-exchange surface of the tomato is the pedicel and locule attachment area, as sealing these parts resulted in more than a 50% reduction in respiration rate, while covering the fruit surface had no significant effect.
6. I demonstrated that the color development of tomatoes stored at 15 °C for 14 days can be described by a sigmoid model, which enables prediction of the a* color parameter for any specific time point (day). This approach is critically important for yield estimation and planning the timing of harvest.

RELEVANT PUBLICATIONS

Horváth-Mezőfi Zsuzsanna; Baranyai László; Nguyen Lien Le Phuong; Dam Mai Sao; Ha Nga Thi Thanh; Göb Mónika; Sasvár Zoltán; Csurka Tamás; Zsom Tamás; Hitka Géza (2024) Evaluation of Color and Pigment Changes in Tomato after 1-Methylcyclopropene (1-MCP) Treatment SENSORS 24: 8 p. 2426 **Q1**

Horváth-Mezőfi Zsuzsanna; Bátor Emese; Szabó Gergő; Göb Mónika; Sasvár Zoltán; Nguyen Lien Le Phuong; Majzinger Koppány; Hidas Karina Ilona; Visy Anna; Hitka Géza; Zsom Tamás (2023) Effect of 1-MCP treatment on tomato photosynthetic chlorophyll activity during storage PROGRESS IN AGRICULTURAL ENGINEERING SCIENCES 19: S1 pp. 17-25., 9 p. **Q3**

Horváth-Mezőfi Zsuzsanna; Szabó Gergő; Göb Mónika; Bátor Emese; Nguyen Le Phuong Lien; Visy Anna; Hidas Karina; Nagy Zsófia; Hitka Géza; Zsom Tamás (2021) 1-MCP-VEL TÖRTÉNŐ ÉRÉSGÁTLÓ KEZELÉS HATÁSA KÜLÖNBÖZŐ ÉRETTSÉGI ÁLLAPOTÚ PARADICSOM MINŐSÉGÉRE ACTA AGRONOMICA ÓVÁRIENSIS 62: Különszám 3. pp. 62-75., 14 p.

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