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ORIGINAL ARTICLE

Pre-Harvest Calcium and Biostimulant Applications Mitigate Fruit Cracking and Improve Metabolome in Sweet Cherry (*Prunus Avium* L.)

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Abstract

Fruit cracking in sweet cherries (*Prunus avium* L.), significantly compromise fruit quality and reduces commercial yield. This study assessed the effects of foliar sprays of 0.5% calcium chloride in combination with glycine betaine, chitosan, silicon, thyme oil, and extract from *Ascophyllum nodosum* on the cracking incidence, antioxidant capacity, fruit biochemical composition, in the sweet cherry cultivar '0900 Ziraat'. Among treatments, $CaCl_2$ + glycine betaine was the most effective, reducing the cracking index to 1.6%, followed by $CaCl_2$ + chitosan (2.4%) and $CaCl_2$ + silicon (3.6%). Chitosan enhanced the amount of total glucose, fructose, malic and succinic acids, and vitamin C, while $CaCl_2$ + glycine betaine and $CaCl_2$ + *Ascophyllum nodosum* significantly increased antioxidant activity. According to Principal Component Analysis and correlation analysis, the majority of the metabolites under study had inverse relationships with the cracking index, demonstrating their protective role against cracking. Calcium chloride and natural biostimulants applied together shown definite synergistic effects, improving fruit quality and stress tolerance more than calcium alone. In order to prevent cracking and enhance the nutritional and antioxidant qualities of fruit, $CaCl_2$ + glycine betaine and $CaCl_2$ + chitosan are suggested as promising pre-harvest treatments. Foliar application targets fruit tissues precisely, is rapidly absorbed, and has no effect on soil microbiota, therefore, it is recommended over soil treatments. Future research should examine the effectiveness of treatments in various cultivars and environmental settings, as well as evaluations of consumer acceptability.

Keywords Foliar biostimulants · Fruit cracking · Nutraceutical qualities · Prunus avium · Sustainable horticulture

Introduction

Sweet cherries (*Prunus avium*) are highly valued for their nutritional and nutraceutical properties, primarily due to their rich antioxidant profile, which is attributable to ascorbic acid, carotenoids, and phenolic compounds (Usenik et al. 2008). These phenolic compounds play a crucial role in mitigatig damage caused by UV rays, free radical damage, and oxidative stress (Ferretti et al. 2010). Türkiye is the world's leading producer of sweet cherry because of its ideal soil and climate, as well as its ability to meet the quality expectations of discerning consumers (Yılmaz et al. 2023). However, the fruit's market presence is limited to just a few weeks due to its delicate nature and short harvest window (Correia et al. 2018). Rapid softening, fungal infections, fruit cracking and mechanical damage are the main causes of postharvest losses. The primary cause of fruit cracking is elevated turgor pressure resulting from excessive water uptake, particularly after rainfall or soil saturation (Xu et al. 2025). A fruit's vulnerability to cracking is primarily determined by anatomical characteristics such as skin flexibility, cuticle thickness, hypodermal cell layers, and peel mechanical properties (which are influenced by calcium content, pectin concentration, and volume of intercellular spaces) (Khadivi-Khub 2015). Morphological traits, such as fruit size and shape, also play a role in cultivar-specific sensitivity to cracking (Brüggenwirth and Knoche 2016). Various preharvest techniques have been investigated to reduce fruit cracking, such as rain covers, growth reg-

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ulators (gibberellic acid, and methyl jasmonate), biostimulants (calcium, glycine betaine, and seaweed extracts), and polyamines (putrescine) (Ruiz-Aracil et al. 2023; Correia et al. 2020). Due to their dual function of improving cell wall integrity and decreasing water permeability through the fruit cuticle, calcium-based treatments are among the most extensively studied. Additionally, calcium sprays have been shown to preserve important fruit quality characteristics while reducing postharvest diseases like rot and spotting (Erbaş and Koyuncu 2022). Glycine betaine (GB), a naturally occurring quaternary ammonium compound, plays an important role in osmoregulation, membrane stabilization, and cellular structural protection against abiotic stressors. Preharvest foliar application of GB in sweet cherry cultivation has been shown to enhance photosynthetic efficiency and plant water status, which in turn improves fruit firmness, soluble solids content (SSC), and titratable acidity (TA) (Serapicos et al. 2022). Additionally, GB has been effective in improving cuticular integrity and reducing excessive water uptake during rainstorm events, which has helped to mitigate rain-induced fruit cracking-a significant concern in cherry production. Similarly, seaweed extracts obtained from the brown alga *Ascophyllum nodosum* are rich in bioactive substances such as polysaccharides, phytohormones, and polyphenols that promote growth and metabolic activity (Afonso et al. 2022). Additionally, essential oils, such as thyme oil, can control transpiration and water uptake during rain by creating a semi-permeable protective layer on the fruit's surface, which may help reducing fruit cracking. This barrier decreases sudden water absorption through the skin, thereby lowering the internal pressure accumulation that causes cracking. Essential oils also have potent antibacterial and antioxidant properties that help protect biochemical quality characteristics like sugars, acids, and phenolics, and maintain fruit firmness while reducing decay during postharvest handling and storage (Christensen 1972; Sivakumar and Bautista-Baños, 2014). The objective of this research was to identify the most effective synergistic foliar formulations combining '$CaCl_2$', with 'Chitosan' (Ch), 'Glycine betaine'(GB), 'Silicon' (Si), 'Thyme Oil' (TO), and '*Ascophyllum nodosum*' (AN) to reduce fruit cracking and improve the metabolomics profile.

Materials and Methods

Plant Material

This study was conducted during the 2022–2023 growing season at the Çukurova University Pozantı Agricultural Research and Application Center, located in Alpu Village at an altitude of 1200 m. The trial was established on five-year-old *P. avium* cultivar '0900 Ziraat' sweet cherry trees grafted onto MaxMa 60 rootstock, planted at a spacing of 5 × 5 m, and trained under the central leader system. The treatments applied in the study were as follows: 0.5% $CaCl_2$, 400 ppm GB, 100 ppm Ch, 400 ppm TO, and water (control).

Applications for Reducing Fruit Cracking and Improving Fruit Quality

Plant growth regulators applied during the study included the following combinations:

- Calcium chloride ($CaCl_2$) + Glycine betaine
- Calcium chloride ($CaCl_2$) + Chitosan
- Calcium chloride ($CaCl_2$) + Thyme oil
- Calcium chloride ($CaCl_2$) + *Ascophyllum nodosum*

Water was used as the control treatment.

Experimental Design

The experiment was arranged in a randomized complete block design with three replications, each consisting of 10 trees. Treatments were applied approximately 30 days before harvest, during the "straw color" or "color break" stage when fruits began transitioning from yellow to pink. Applications were carried out using a 15-liter backpack sprayer, ensuring thorough wetting of the entire tree canopy. The treatments and their respective concentrations are presented in detail in Table 1.

Cracking Index (%)

Fruit cracking assessment was conducted between 08:00 and 10:00 in the morning. Immediately after harvest, fruits were brought to the laboratory for cracking test. The fruits were placed in 2-liter beakers and fully immersed in distilled water. Samples were kept at 20 ± 1 °C for 2, 4, and 6 h, as described by (Sivakumar and Bautista-Baños 2014). Cracked fruits were counted and removed every 2 h. The cracked fruit were discarded from the water at each time interval. The cracking index was determined with the Eq. 1,

Table 1 Preharvest treatment combinations and their application rates

Treatments	Application Rate
Control	100 LWater
$CaCl_2$	%0.5
$CaCl_2$ + Chitosan	%0.5 $CaCl_2$ + 100 ppm Chitosan
$CaCl_2$ + Thyme Oil	%0.5 $CaCl_2$ + 400 ppm Thyme Oil
$CaCl_2$ + Glycine Betaine	%0.5 $CaCl_2$ + 400 ppm Glycine Betaine
$CaCl_2$ + *Ascophyllum nodosum*	%0.5 $CaCl_2$ + 400 ppm *Ascophyllum nodosum*

where a, b, and c represent the number of fruits that cracked fruit after 2, 4, and 6 h, respectively.

$$\text{Cracking index} = (5a \pm 3b \pm c) \times 100/250 \quad (1)$$

Determination of Total Antioxidant Activity

The radical scavenging activity of DPPH (2, 2-Diphenyl-1-picrylhydrazyl) was determined with minor adjustments (Ganhão et al. 2019). A fresh solution of 0.06 µM ethanol DPPH was prepared. A 50 µL aliquot of juice sample was mixed with 1950 µL of DPPH, followed by one minute shaking. A MultiskanTM GO microplate spectrophotometer was used to determine the absorbance value at a wavelength of 515 nm. The following formula was used to determine the radical scavenging activity (%DPPH inhibition):

$$\%\text{Inhibition} = 100 \times \frac{\text{Abs}_{\text{control}} - (\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}})}{\text{Abs}_{\text{control}}}$$

$\text{Abs}_{\text{sample}}$_ absorbance of the reaction in the presence of the sample (sample dilution ± DPPH solution), $\text{Abs}_{\text{blank}}$ _ absorbance of the blank for each sample dilution (sample dilution ± DPPH solvent), and $\text{Abs}_{\text{control}}$ _ absorbance of the control reaction (sample solvent ± DPPH solution).

Determination of Total Phenol Content

The total phenolic content was determined using the Folin-Ciocalteau technique (Krawitzky et al. 2014) with minor modifications. 0.5 g sample of cherry puree was homogenized with 2.5 mL of 80% methanol and vortexed for one minute. The homogenate was centrifuged at 4000 rpm for four minutes. Separately, 5 mL of 80% methanol was mixed with 1 mL of the juice sample. The mixture was centrifuged at 5500 rpm for 10 min. 50 µL of resultant supernatant was transferred to tube containing 250 µL of Folin-Ciocalteu reagent. Subsequently, 750 µL of 20% (w/v) Na_2CO_3 was added. The reaction mix was incubated at room temperature for two hours in dark. The absorbance was recorded at 760 nm using a UV/VIS spectrophotometer (Thermo Fisher Scientific, FI-01620 Vantaa, Finland) against blank. Results were calculated based on calibration curve prepared daily using known concentrations of gallic acid (GA) standards and are expressed as mg GAE per 100 g fresh weight (FW).

Determination of Total Anthocyanin Content (TA)

The modified pH differential approach was used to determine the concentration of total anthocyanins (Cheng and Breen 1991).

Sugar and Organic Component Analyses

Homogenized samples were prepared for sugar and organic acid components analysis as described by Bozan et al. (1997). Briefly, one gram of sample was combined with 4 mL of ultrapure water, and the mixture was filtered through Whatman No. 1 before chromatographic analysis. HPLC was used to measure the amounts of sugar and organic acid (Shimadzu, Prominence LC-20A). Organic acids were separated on a Transgenomic 87H (7.8 × 300 mm), and detected by a UV detector. For sugar analysis, a Coregel-87C (7.8 × 300 mm) column with a RID (Refractive Index) was used. The reference standards' calibration curves were created, and the content was determined using these curves.

Statistical Analyses

For descriptive statistics, means ± standard errors were used. To assess variations in treatment means, a one-way analysis of variance (ANOVA) was used. The Duncan's multiple range test was used for pairwise comparisons when significant differences ($p < 0.05$) were found. IBM SPSS Statistics version 22 was used for all statistical analyses (Düzgüneş et al. 1987). R software version 4.4.2 was used for Pearson correlation matrices, principal component analysis (PCA) heatmaps, and bar plots.

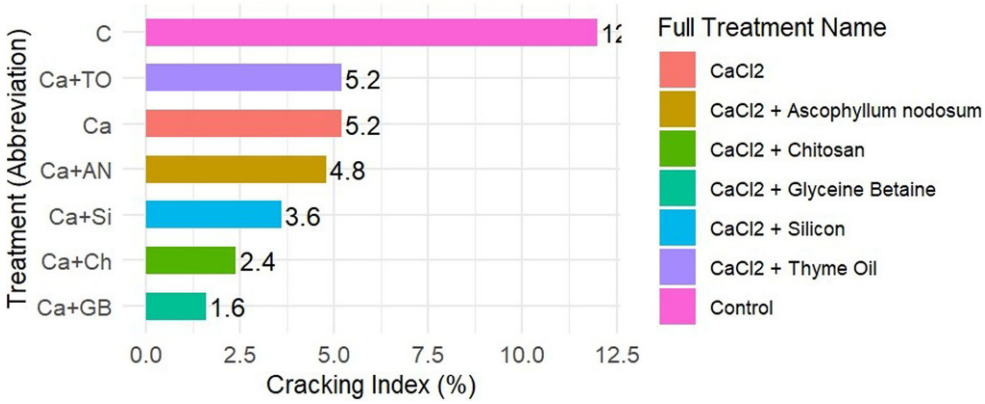


Fig. 1 The bar graph shows the effect of various preharvest foliar treatments on the sweet cherry fruit cracking index (%). All treatments were applied in combination with 0.5% $CaCl_2$. In comparison to the untreated control (12%), the cracking index was decreased by all treatments. Glycine betaine (1.6%) showed the highest reduction, followed by chitosan (2.4%), Silicon (3.6%), AN 4.8%, $CaCl_2$ (5.2%), and thyme oil (5.2%)

Results

Cracking Index

The cracking index (CI) of sweet cherries was assessed after immersion in distilled water for 2, 4, and 6 h. The mean CI values are presented in Fig. 1. Statistically significant differences were observed among the treatments ($p < 0.05$). The lowest CI was recorded in the $CaCl_2$ + glycine betaine treatment (1.6%), representing substantial reduction compared to the untreated control (12.0%). Other treatments including the application of $CaCl_2$ + thyme oil and $CaCl_2$ produced moderate CI values (both at 5.2%). The application of $CaCl_2$ combined with silicone, chitosan and *Ascophyllum nodosum* further reduced the CI to 2.4%, 3.6%, and 4.8% respectively. All treatments significantly reduced fruit cracking compared to the control.

Antioxidant Capacity (DPPH, Total Phenolic, and Anthocyanins)

As determined by DPPH radical scavenging activity, total phenolic content, and anthocyanin concentration, the different treatments had a significant effect on the antioxidant capacity and bioactive component content of sweet cherry fruits as shown in Table 2. The treatments with the highest DPPH values were '$CaCl_2$ + *Ascophyllum nodosum*' (17.14 ± 0.29%), and $CaCl_2$ + glycine betaine (16.19 ± 0.37%). These were considerably higher ($p < 0.05$) than the control (14.13 ± 1.91%). '$CaCl_2$ + *Ascophyllum nodosum*' treated fruits had the highest total phenolic content (122.16 ± 1.15 mg GAE 100 g^{-1} FW), followed by '$CaCl_2$ + silicon' (120.03 ± 1.43 mg GAE 100 g^{-1} FW), and '$CaCl_2$ + thyme oil' (118.95 ± 1.99 mg GAE 100 g^{-1} FW) foliar treatments. The anthocyanin content in each treatment varied significantly. Silicon had the highest anthocyanin value, while $CaCl_2$ (0.05 ± 0.00 mg C3G 100 g^{-1} FW) had the lowest.

Sugar (Sucrose, Glucose, Fructose, and Xylose)

The sugar composition of sweet cherry fruits, including sucrose, glucose, fructose, and xylose, was greatly influenced by the preharvest treatments. As shown in Table 3, the amount of sucrose in each treatment varied. Compared to the control (216.11 ± 0.29 mg 100 g^{-1}), highest sucrose level was recorded in '$CaCl_2$ + *Ascophyllum nodosum*' (270.45 ± 0.07 mg 100 g^{-1}), followed by '$CaCl_2$ + Silicon' (254.68 ± 0.28), and '$CaCl_2$ + Glycine betaine' (224.25 ± 1.15). Fruits treated with '$CaCl_2$ + chitosan' had the highest glucose content (7128.02 ± 1.05 mg100 g^{-1}), suggesting enhanced sucrose hydrolysis or increased carbohydrate retention. In contrast, the lowest glucose content was observed in '$CaCl_2$ + *Ascophyllum nodosum*' (3721.88 ± 9.20) and '$CaCl_2$ + Silicon' (3980.38 ± 16.87), whereas '$CaCl_2$' and '$CaCl_2$ + thyme oil' treatments also exhibited lower glucose content.

Table 2 Impact of various foliar treatments on sweet cherry fruit's total phenolic content, total anthocyanin concentration, and antioxidant activity (DPPH). The biochemical effects of the treatments differed significantly. Statistically significant differences ($p < 0.05$) are indicated by superscript letters.

Treatments	DPPH	Total Phenol	Anthocyanin
$CaCl_2$ + Silicon	9.65 ± 0.35d	120.03 ± 1.43ab	0.51 ± 0.00a
$CaCl_2$ + Thyme Oil	10.36 ± 0.33d	118.95 ± 1.99b	0.17 ± 0.00e
$CaCI_2$	13.43 ± 0.51c	96.31 ± 0.53d	0.05 ± 0.00ğ
$CaCl_2$ + Chitosan	13.56 ± 0.38c	101.91 ± 1.65c	0.13 ± 0.00fg
$CaCl_2$ + Glycine Betaine	16.19 ± 0.37b	96.31 ± 0.88e	0.14 ± 0.00f
$CaCl_2$ + *Ascophyllum nodosum*	17.14 ± 0.29b	122.16 ± 1.15a	0.25 ± 0.01d
Control	14.13 ± 1.91c	102.33 ± 2.65c	0.28 ± 0.00c

Table 3 Impact of different foliar treatments on the sugar content (concentrations of sucrose, glucose, fructose, and xylose) in sweet cherry fruit. Significant differences among treatments ($p < 0.05$) are shown by superscript letters.

Treatments	Sucrose	Glucose	Fructose	Xylose
$CaCl_2$ + Chitosan	211.15 ± 0.07ğ	7128.02 ± 1.05a	5768.43 ± 18.01a	236.23 ± 0.40a
$CaCI_2$	213.87 ± 0.51g	6225.68 ± 4.86b	4866.61 ± 8.18f	186.65 ± 0.89d
$CaCl_2$ + Thyme Oil	223.79 ± 0.11e	5753.55 ± 5.54c	5478.36 ± 6.98c	154.15 ± 1.21f
$CaCl_2$ + Glycine Betaine	224.25 ± 1.15e	5669.86 ± 10.74d	5669.28 ± 19.16b	186.44 ± 0.52d
$CaCl_2$ + Silicon	254.68 ± 0.28b	3980.38 ± 16.87f	3385.56 ± 1.54ğ	175.29 ± 1.20e
$CaCl_2$ + *Ascophyllum nodosum*	270.45 ± 0.07a	3721.88 ± 9.20g	3617.92 ± 3.15g	63.71 ± 1.35ğ
Control	216.11 ± 0.29f	5678.79 ± 8.02d	5390.99 ± 6.78d	97.78 ± 0.59g

The trend for fructose, which plays a major role in perceived sweetness, was comparable to that of glucose. The '$CaCl_2$ + Chitosan's role in enhancing sugar metabolism was further supported by the fact that it caused the largest fructose buildup (5768.43 ± 18.01 mg100 g^{-1}), followed by '$CaCl_2$ + Glycine betaine' (5669.28 ± 19.16 mg100 g^{-1}). Once more, '$CaCl_2$ + Silicon' and '$CaCl_2$ + *Ascophyllum nodosum*' treatment resulted in the lowest fructose concentration. Xylose, a pentose sugar typically found in lesser amounts, was lowest in '$CaCl_2$ + *Ascophyllum nodosum*' (63.71 ± 1.35 mg100 g^{-1}) and the Control (97.78 ± 0.59 mg100 g^{-1}), while the highest concentration was observed in '$CaCl_2$ + Chitosan' (236.23 ± 0.40 mg100 g^{-1}).

Organic Acid and Vitamin C

Ascorbic acid (vitamin C) and organic acids are essential indicator of nutritional value, flavor profile, and overall quality of sweet cherries. These compounds act as antioxidants, modulate postharvest physiology, and contribute to fruit acidity. The impact of different preharvest treatments on the concentration of citric acid, malic acid, succinic acid, and vitamin C revealed significant variations in metabolic activity and fruit quality (Table 4).

One of the main organic acids contributing to tartness and metabolic energy is citric acid. The highest citric acid content was observed in '$CaCl_2$' (14.92 ± 0.36 g/kg). On the other hand, the lowest citric acid concentrations were found in '$CaCl_2$ + *Ascophyllum nodosum*' (seaweed extract) and in the untreated control. The highest levels of malic acid, which is crucial for flavor and osmotic regulation, was found in '$CaCl_2$ + Chitosan' (110.68 ± 0.38 g/kg) treated cherries. In contrast, lower malic acid was observed in '$CaCl_2$ + *Ascophyllum nodosum*' (30.34 ± 0.44 g/kg) and '$CaCl_2$ + silicon' (39.09 ± 0.24 g/kg) treated cherries.

Succinic acid, a key intermediate in the TCA cycle is often associated with stress related metabolism and respiration. Highest concentration was observed in the '$CaCl_2$ + Chitosan' (1.44 ± 0.09 g/kg), indicating that these treatments may improve energy metabolism pathways or mitochondrial activity. In contrast, lowest levels were detected in control and '$CaCl_2$ + *Ascophyllum nodosum*' treated fruits, which may reflect reduced energy metabolism or ripening-associated decline in TCA intermediates. Vitamin C is key indicator of fruit nutritional quality and plays a critical antioxidant role in plants defense and physiology. Highest concentration was recorded in the '$CaCl_2$ + Chitosan' (18.65 ± 0.02 mg/100 g FW) treatments.

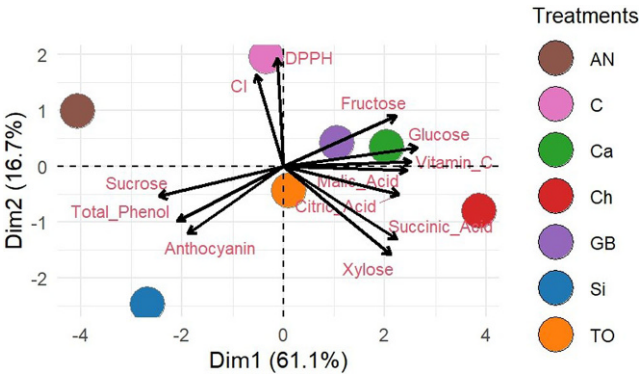


Fig. 2 The relationship between sweet cherry biochemical characteristics and preharvest interventions is shown in a Principal Component Analysis (PCA) biplot. The contribution of Dim1 and Dim2 to the overall variation is 61.1% and 16.7%, respectively. Cracking index (CI), antioxidant parameters (DPPH, total phenol, anthocyanin), sugars (sucrose, glucose, fructose, xylose), organic acids (citric acid, malic acid, succinic acid), and vitamin C are biological variables that are represented by vectors

Principle Component Analysis

Principle Component Analysis biplot (PCA) represented 77.8% of total variance in biochemical traits among treatments, with Dim1 and Dim2 representing 61.1% and 16.7% of the variability, respectively (Fig. 2). The cracking index (CI) vector was partially aligned with the DPPH vector and oriented mainly along the positive Dim 2 axis. This suggests that higher CI values were more strongly associated with increased antioxidant capacity. The treatments '$CaCl_2$ + *Ascophyllum nodosum*' (AN) and the control (C) were located close to the CI vector, indicating higher cracking index. In contrast, '$CaCl_2$ + Chitosan' (Ch), '$CaCl_2$ + calcium chloride' (Ca), and '$CaCl_2$ + glycine betaine' (GB) clustered on opposite side of the CI vector, demonstrating their corre-

Table 4 Effect of various foliar treatments on the vitamin C (mg/100g) content and organic acid composition (citric acid, malic acid, and succinic acid) of sweet cherry fruit.

Treatments	Citric Acid	Malic Acid	Succinic Acid	Vitamin C
$CaCl_2$ + *Ascophyllum nodosum*	1.70 ± 0.00ğ	30.34 ± 0.44ğ	0.41 ± 00.4g	9.69 ± 0.09d
$CaCl_2$ + Silicon	4.84 ± 0.02f	39.09 ± 0.24g	0.95 ± 0.05d	10.19 ± 0.35d
$CaCl_2$ + Glycine betaine	9.79 ± 0.32e	53.85 ± 0.74f	0.97 ± 0.01d	12.51 ± 0.48c
$CaCl_2$ + Thyme Oil	9.92 ± 0.44e	55.41 ± 0.32f	0.84 ± 0.02e	12.36 ± 0.56c
$CaCl_2$ + Chitosan	11.59 ± 0.08d	110.68 ± 0.38b	1.44 ± 0.09b	18.65 ± 0.02a
$CaCI_2$	14.92 ± 0.36c	69.29 ± 0.50d	0.95 ± 0.02d	15.07 ± 0.51b
Control	3.60 ± 0.08g	64.63 ± 0.48e	0.74 ± 0.04f	13.37 ± 1.12c

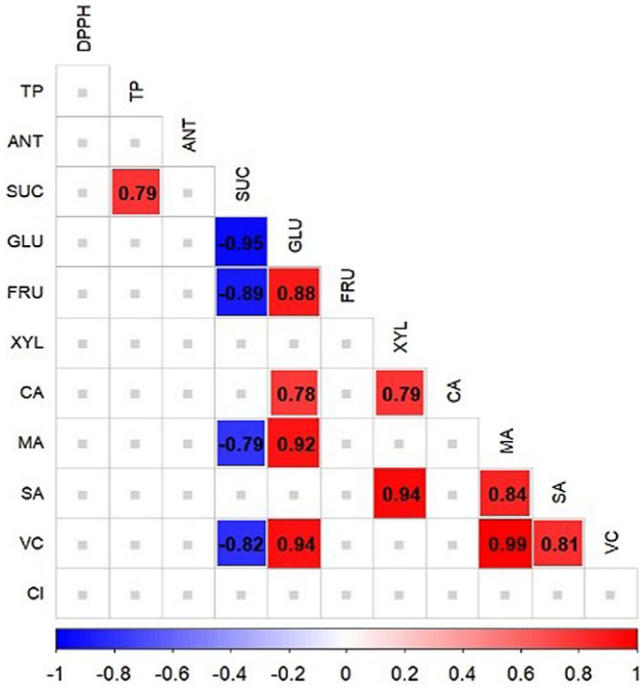


Fig. 3 Correlation heat map showing pairwise pearson correlation coefficient among CI and sweet cherry biochemical traits, red indicate positive and blue negative correlation

lation with a lower cracking incidence compared to the control (C) and '$CaCl_2$ + *Ascophyllum nodosum*' (AN). In contrast, treatments 'Gb', 'Ca', and 'Ch' were located in the positive Dim1 region, which was marked by high levels of succinic acid, glucose, fructose, and vitamin C, and were positioned opposite to CI vector along Dim2. This spatial distribution suggests that these treatments were linked to higher concentrations of primary metabolites involved in osmotic control and structural stability, as well as a decreased incidence of cracking. In comparison to the untreated control and 'AN', the overall separation of treatments along Dim2 highlights the effectiveness of 'Gb', 'Ca', and 'Ch' in reducing fruit cracking, hence supporting their role in enhancing the integrity of sweet cherry fruit.

Key biochemical components and the cracking index (CI) in sweet cherry fruit are shown in pairwise relationships in the correlation heat map (Fig. 3). The correlation coefficients range from + 1 (perfect positive correlation, red) to –1 (perfect negative correlation, blue). Interestingly, the CI showed substantial negative associations with a number of key metabolites, indicating that cracking susceptibility is decreased with increased concentrations of these chemicals. Specifically, CI was negatively correlated with Vitamin C (r = –0.82), Malic Acid (r = –0.79), Glucose (r = –0.95), Fructose (r = –0.89).

Discussion

A comprehensive strategy that combines pre-harvest interventions with in-depth metabolomic studies is required to address the complex problem of rain-induced cracking in sweet cherry. In this work, the effectiveness of various pre-harvest treatments in combination with '$CaCl_2$' with 'Chitosan', 'Calcium Chloride', 'Thyme oil', 'Glycine betaine', 'Silicon', and '*Ascophyllum nodosum*' in preventing rain-induced cracking in sweet cherry cultivars was examined, along with a thorough analysis of the metabolomic profiles. The results of this study shed light on the intricate interactions between biochemical pathways that affect fruit integrity and lay the foundations for developing targeted strategies to increase cherry yields in areas that frequently experience rainfall during the ripening season. It is necessary to integrate biophysical stress theories with biochemical defense mechanisms in order to comprehend rain-induced cracking in sweet cherries. There are two main scientific theories that explain cracking. The critical turgor hypothesis states that the intake of rainwater causes internal pressure and tension on fruit skins till tissue rupture. Zipper hypothesis hypothesis states that surface water infiltration causes pre-existing micro-cracks to enlarge, promoting the formation of new cracks. Our findings support these models and show how pre-harvest interventions that reinforce barriers and regulate osmotic pressure can effectively reduce cracking (Schumann and Knoche 2020).

In comparison to the untreated control (12.0%), the sweet cherry cracking index was significantly reduced by the treatments used in this investigation. However, the efficacy of combinations glycine betaine, chitosan, calcium chloride, silicon, and oil varied. The combination containing chitosan demonstrated notable effectiveness, as its film forming ability enhanced cuticular integrity and reduced water absorption, thereby lowering the CI to 2.4%. Recent research shows that chitosan-based coatings enhanced with nanoparticles (such as phosphorylated zein and cellulose nanocrystals) reduce cracking by enhancing their wettability and barrier qualities (Chen et al. 2024). Similarly, Lahdenperä (2006), demonstrated that preharvest application of 'GB' reduced fruit cracking in sweet cherry cultivar 'Garnet'. In 2020, Correia et al. (2020), found reduction in CI in GB treated 'Sweetheart' cherry. Calcium (Ca) sprays have demonstrated promising outcomes. Water-induced rupture is reduced when calcium binds to the cell wall's pectin, improving its structure and increasing the threshold tolerated strain. PaPIP1;4 was shown to be upregulated by $CaCl_2$ application indicating a molecular function in regulating water and ROS flux and reducing oxidative stress at the cellular level (Chen et al. 2024).

Michailidis et al. (2021), found that applying Ca to the 'Sweetheart' cultivar reduced the cracking index by approx-

imately fifty percent. Additionally, Correia et al. (2020), verified that 'Ca' treatments reducted in cracking in 'Sweetheart' and 'Skeena'. Erogul (2014), conducted a study, showed that foliar application of calcium compound significantly reduced fruit cracking in sweet cherry cultivar '0900 Ziraat'. $CaCl_2$ and Ca $(OH)_2$ were found to be most effective and reduced cracking 66% and 62% while calcium caseinate reduced cracking upto 50%. Calcium treatments enhanced fruit firmness without affecting fruit weight, soluble solids/titratable acidity.

İmrak (2025), evaluated the effects of GB, Ca, and Si on cracking and fruit quality in sweet cherry cultivar 'Royal Tiago'. In that study, Si, Ca, and GB significantly reduced cracking by 71.0%, 35.38%, and 26.0% respectively, whereas, AN (*Ascophyllum nodosum*) increased cracking (26.12%). The CI had negative correlation with acidity, phenolic content, antioxidant activity, and firmness. However, AN, GB, and Ca improved sugars, organic acids, anthocyanins, and overall fruit quality, while, Ca and Si increased malic acid levels. Rombolà et al. (2023), showed that preharvest foliar treatments can significantly reduce fruit cracking in sweet cherries. They conducted four field studies using the cultivars 'Van', 'New Star', and 'Emperor Francis', testing canopy-applied sodium silicate in addition to calcium chloride and water (control). It was found that sodium silicate reduced the percentage of cracked fruits to an equivalent or even higher degree than calcium chloride under conducive conditions. According to these findings, silicon-based treatments such as sodium silicate might be a promising substitute for calcium salts in preventing rain-induced cracking, especially in open-field orchards. Breia et al. (2020), evaluated *PaPIP1;4*, the most abundantly expressed aquaporin in sweet cherry (*Prunus avium* L.) fruit, providing evidence for the physiological basis of calcium's effectiveness in preventing fruit cracking. *PaPIP1;4* is localized in the plasma membrane and has the capacity to transport hydrogen peroxide and water. Interestingly, preharvest fruits treated with 0.5% $CaCl_2$ showed a substantial increase in its expression. This increase, especially in the fruit peduncle, suggests a possible involvementole for *PaPIP1;4* in regulating oxidative stress responses and water transport, which may help to lower the incidence of cracking. These findings provide molecular-level justification for the application of calcium-based foliar sprays in cherry orchards. Plant-derived oils are lipophilic in nature. Because of this characteristic, they are useful ingredients in edible coatings used to maintain fruit quality and protect against decay. For example, it has been demonstrated that composite coatings made of chitosan and olive and flaxseed oil significantly reduce weight loss and respiration rates while enhancing resistance to fungal degradation (Rob et al., 2024; Moghadas et al. 2024; Pereda et al. 2012; Gursoy et al. 2018). Considering previous studies that focused on the role of essential oils in preserving fruits quality parameters, we used $CaCl_2$ in combination with thyme oil to overcome sweet cherry fruit cracking. Our findings demonstrated moderate reduction in the CI.

Antioxidant Capacity (DPPH, Total Phenols, Anthocyanin)

AN and GB significantly increased antioxidant activity, increased total phenol. These findings align with İmrak (2025), who reported that seaweed biostimulants increased phenolics and antioxidants in cherries. Si was very effective in maintaining high levels of total phenols (120.03) and increasing anthocyanin (0.51). A recent investigation on GB and Si foliar treatments found a similar correlation between a lower cracking index and greater DPPH and phenolics (İmrak 2025). According to earlier research, calcium and chitosan can help retain but do not actively promote phenolics and anthocyanins accumulation. This is supported by the moderate antioxidant effects observed for $CaCl_2$, chitosan, and thyme oil (Correia et al. 2019).

Sugars (Sucrose, Glucose, Fructose, Xylose)

Significant increases in sucrose (270 and 255 mg/100 g FW compared to control 216 mg/100 g FW in the control) were seen in AN and Si. Due to mild stress induction and changed respiration, chitosan frequently upregulates the activity of enzymes that hydrolyze sucrose, such as invertase and sucrose synthase (El Amerany et al. 2022). This enzymatic activation could explain the enhanced sucrose hydrolysis observed. Chitosan produced the highest levels of fructose and glucose, indicating more active hydrolytic conversion of sucrose into its constituents monosaccharide. This is similar to the protective and enzyme-modulatory functions of chitosan observed in mangoes and strawberries. The intermediate sugar accumulation induced by $CaCl_2$ and thyme oil suggests a delayed metabolic turnover. Gonçalves et al. (2020), reported that sweet cherries treated with AN and GB exhibited higher total soluble solids, pH and higher acidity.

Organic Acid and Vitamin C

Malic, succinic acids, and vitamin C were all markedly elevated by chitosan; this was probably due to its effect on the activity of stress-response enzymes. This observation aligns with İmrak (2025), studies linking these applications to the stabilization of fruit acidity and ripening under stress have found that $CaCl_2$ and Si maintained moderate levels of organic acids and vitamin C, as reported by İmrak (2025). In contrast, AN showed decreased citric, malic, and succinic acids and had lower vitamin C, which may be due to

increased metabolic respiration during fruit development. Rishitha et al. (2023), applied seaweed extract in combination with humic acid on strawberries. Their results showed higher TTS, lower acidity and high amount of ascorbic acid. These foliar applications have a significant impact on fruit quality. Seaweeds consistently boosts TTS, vitamin C and phenolic contents, supporting the finding of present investigation. Sajid et al. (2020), sprayed different concentrations (0.5, 0.5 and 1.0%) of chitosan on peach trees at different time before harvest. Their results showed that 1% chitosan application significantly increased vitamin C content, titratable acidity, and TTS while decreasing disease incidence. Rahman et al. (2018), applied chitosan on strawberries. Interestingly, fruits from plants sprayed with chitosan had higher contents of carotenoids, anthocyanin, phenolics, and flavonoids compared to untreated control. Chitosan enhances organic acids, and antioxidants, aligning with the present investigation.

Conclusion

The commercial yield of sweet cherry is significantly limited by fruit cracking. According to this study, adding calcium chloride (0.5%) along with different biostimulants and natural substances can greatly increase fruit firmness, decrease cracking, and boost nutritional value. The studied combinations, especially $CaCl_2$ with glycine betaine and $CaCl_2$ with chitosan, shown remarkable efficacy in reducing the occurrence of cracking and enhancing the sugar and antioxidant profiles. These results imply that calcium applied topically in combination with osmoprotectants and bioactive substances strengthens cell walls and increases fruit resistance to environmental stressors that cause breaking. The practical implementation of these mixed foliar treatments offers growers a cost-effective and environment friendly solution to increase yield and marketable quality. In order to make more comprehensive recommendations, further investigation is suggested to optimize application timings and dosages as well as validate these tactics over a range of climatic conditions and cherry varieties.

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Declarations

Conflict of interest Y. Polat, S. Yasmin, B. İmrak, M.A. Gündeşli, M. Güney, S. Çömlekçioğlu and N. E. Kafkas declare that they have no competing interests.

Clinical Trial Not applicable.

Ethical standards For this article no studies with human participants or animals were performed by any of the authors.

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