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Effects of preharvest salicylic acid and calcium chloride applications on postharvest quality and storability of 'Barhi' date fruits

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ABSTRACT

Date palm (*Phoenix dactylifera* L.) is a fruit tree of great economic, cultural, and nutritional value in the Middle East and North Africa, with 'Barhi' being an important cultivar. At the Rutab stage, its fruits are highly susceptible to deterioration because of their high moisture and sugar content. This study evaluated the effects of preharvest foliar applications of salicylic acid (SA) and calcium chloride (CaCl_2) on the quality and storability of 'Barhi' dates during cold storage. SA (1 and 2 mM) and CaCl_2 (1.5% and 3%) were applied at the Khalal stage to 18-year-old palms under field conditions. Fruits were harvested at the Rutab stage and stored at 4 °C for six months, with quality attributes assessed monthly. Preharvest treatments affected several quality attributes during storage. CaCl_2 at 1.5% maintained higher moisture content but showed the highest weight loss after six months. SA treatments delayed acidity decline and promoted sugar stability. Notably, no souring was observed in fruits treated with 3% CaCl_2 throughout storage. Soluble solids content was not markedly affected by the treatments. Overall, CaCl_2 at 3% and SA at 1 mM showed favorable effects on fruit quality and reduced deterioration during refrigerated storage. These treatments may have potential as preharvest options for improving the storage performance of 'Barhi' Rutab dates at 4 °C.

Key Words: *Phoenix dactylifera*, preharvest treatment, fruit quality, cold storage, storage life.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is an ancient fruit tree that yields berry fruits (dates), which constitute a primary food staple in the Middle East and North Africa region, where hundreds of date cultivars are grown and play a significant role in the economy, history, and culture of the people. The development of date fruit goes through five maturity/ripening stages: Hababouk, Kimri, Khalal (Bisr), Rutab, and Tamer (Atia et al., 2020). Date fruit is edible in

the last three stages of development. The most desirable form of consumption of dates in most varieties is the Rutab stage, at which stage the fruit is soft, partially or completely brown, and its moisture content is 30-45 percent (Mortazavi et al., 2011).

One of the most important Iranian date varieties consumed in the Rutab stage is 'Barhi'. 'Barhi' is one of the most important high-yielding and late-maturing date cultivar and is one of the most important commercial cultivars in the world and in the southwestern regions of the country, which can be consumed in all three stages: khalal, Rutab and Tamar. Fruits in the Rutab stage have a short storage life and are quickly contaminated by microorganisms such as yeasts and molds due to their high moisture content, high sugar content and soft texture (Ahmed et al., 2021). The direct result of this is increased waste and reduced productivity in this sector, which in the long term could challenge the development of this popular and globally productive cultivar throughout the country.

Since harvested horticultural products are highly perishable, it is essential to use appropriate techniques and methods to increase their storage life for all-season use and export. Today, there is a global desire to find alternative methods for post-harvest waste control, with priority given to healthy methods to prevent the negative impact of chemical pesticides on human health, and also due to the existence of strict regulations on chemical pesticide residues, the possibility of using chemicals has been reduced (Johnson and Sanghchote, 1994). One of the important areas of organic agriculture that has attracted a lot of attention today is the use of natural compounds that are compatible with plants, nature, and humans in the production and preservation of crops. One of the most important compounds is the phenolic compound salicylic acid, which plays an important role in regulating plant growth and development (Wang et al., 2006). It has been reported that the application of salicylic acid activates the systemic acquired resistance system, synthesis of metabolites and antioxidant enzymes. Salicylic acid also plays a high potential role as a natural compound in preventing the production and effect of ethylene (Raskin, 1992;

Zhang et al., 2003). Recent studies have further demonstrated the potential of salicylic acid in maintaining the postharvest quality of horticultural crops. Exogenous salicylic acid can delay fruit ripening and senescence, reduce ethylene production and respiration, maintain cell membrane integrity and antioxidant capacity, and enhance resistance to postharvest pathogens. Importantly, both preharvest and postharvest applications of salicylic acid have been reported to influence subsequent fruit quality and storability (Wang et al., 2022; Chen et al., 2023). Kazemi et al. (2011) reported that the use of salicylic acid at a concentration of 3 mM in Janagold apple fruit significantly inhibited weight loss during storage.

Among the nutrients, calcium plays an important role in the strength and mechanical power of cells. Calcium stabilizes or strengthens the cell walls of fruits, making them resistant to decay by fungal enzymes. The activity of hydrolases that break down pectic acids is often reduced by changes that calcium makes to their precursors. It is thought that these changes in the cell wall caused by calcium make the wall more resistant to enzymes produced by fungal pathogens, which slows fungal penetration and reduces decay (Conway and Sams, 1984).

Previous studies have demonstrated the potential of salicylic acid and calcium chloride for maintaining the quality and storage performance of 'Barhi' dates. Ahmed et al. (2021) investigated preharvest applications of salicylic acid, calcium chloride, chitosan, and their combinations on 'Barhi' fruit harvested at the Bistr (Khalal) stage, while Atia et al. (2020) evaluated pre-storage treatments with calcium chloride and salicylic acid for maintaining the quality of freshly harvested Khalal 'Barhi' dates during cold and controlled-atmosphere storage. However, maintaining the quality of 'Barhi' fruit after reaching the Rutab stage remains a practical postharvest challenge because of its soft texture and high susceptibility to quality deterioration. Moreover, limited information is available on the use of simple preharvest treatments to extend the refrigerated shelf life of Rutab 'Barhi' fruit. We hypothesized that preharvest foliar application of salicylic acid and calcium chloride at the

Khalal stage could delay postharvest deterioration and maintain the quality of fruit harvested at the Rutab stage during cold storage. Therefore, the objective of this study was to evaluate the potential of these treatments for extending the shelf life of Rutab 'Barhi' fruit under refrigerated conditions while maintaining its physicochemical quality and reducing quality deterioration during storage.

MATERIAL AND METHODS

Plant materials

This research was conducted at Umm al-Tamir Station, located at the Date and Tropical Fruits Research Institute (near Ahvaz, Iran), at a longitude of 48°32'E and latitude of 31°15'N, at an altitude of 22.5 meters above sea level, on fifteen 18-year-old date palm trees of the 'Barhi' cultivar.

The experiment was conducted with 5 treatments and 3 replications in a randomized complete block design over a year. Each palm tree was considered as a replication for applying one foliar spray treatment.

Treatments

The experimental treatments were as follows: foliar sprays of salicylic acid at concentrations of 1 mM (SA1) and 2 mM (SA2), calcium chloride at concentrations of 1.5% (Ca1) and 3% (Ca2), and water spraying as the control (C0). The foliar spray treatments were applied at two stages, i.e., at the beginning of the khalal stage (12/8/2023) and two weeks later. Fruits from each cluster were harvested separately at the Rutab stage (11/9/2023) and transported to the laboratory. Abnormal and damaged Rutab fruits were removed, and healthy and uniform fruits were selected. The Rutabs were arranged and packaged in sterilized containers, and the packages were stored at 4°C (refrigerator temperature) for six months. Because a temperature of -18°C is desirable for long-term storage of Rutabs, some untreated Rutab fruits were stored at this temperature (freezer temperature) as a storage control. The field experiment consisted

of five preharvest foliar treatments arranged in a randomized complete block design with three biological replicates (15 palms). The five treatment groups, including the untreated control, were evaluated under refrigerated storage at 4 °C. In addition, a portion of the fruit from each untreated control replicate was stored at -18 °C as a separate reference storage condition representing the conventional method commonly used for long-term storage of Rutab dates. Thus, the -18 °C condition did not constitute an additional level of the foliar spray factor or an independent field treatment. Rather, it served as a storage-temperature reference derived from the same untreated control replicates. The -18 °C reference observations were included in the statistical comparisons to assess the extent to which preharvest foliar treatments followed by refrigerated storage could maintain fruit quality relative to the frozen reference condition. The samples were measured for physical and chemical properties immediately after harvest and during storage. The samples were examined for their quality characteristics every month for 6 months. At each stage, the measurement of these characteristics was performed on the samples after they were removed from the cold storage and their temperature reached room temperature (About one to two hours later) (Ahmed et al., 2021). The studied traits included measuring moisture content, SSC, acidity, pH, reducing sugars and total sugars, sourness percentage, and fruit weight loss percentage during storage periods.

To measure the percentage of weight loss immediately after harvesting and packaging and before placing the samples in the cold storage, the weight of each package was measured with a digital scale with an accuracy of 0.01 grams and immediately after each level of the storage period, they were re-weighed and the percentage of weight loss was calculated using the following formula:

$$\text{Percentage of weight loss} = [(\text{initial weight} - \text{secondary weight}) / \text{initial weight}] \times 100$$

In order to calculate the percentage of sourness after each storage period, fruits in each package were individually evaluated for souring based on sensory and visual characteristics. Fruits

exhibiting a characteristic fermented or sour odor and/or visible symptoms of souring were considered soured fruits. The number of soured fruits in each package was recorded, and the percentage of souring for each treatment was calculated using the following formula:

$$\text{Incidence of souring (\%)} = (\text{number of sour fruits} / \text{total number of fruits}) \times 100$$

Moisture content was determined in triplicate according to the AOAC method (AOAC, 2012). For each sample, a portion of the fruit was taken and the seed was removed. The edible pulp was weighed to record the initial fresh weight and then dried in an oven at 70 °C for 48 h. After drying, the sample was removed from the oven and weighed again. Moisture content was calculated from the difference between the initial fresh weight and the final dry weight and expressed as a percentage of the initial fresh weight.

To measure soluble solids concentration (SSC), titratable acidity (TA) and reducing sugars of dates, 25 grams of pitted date fruit (date flesh) was carefully weighed and 100 ml of distilled water was added to it. Then, it was thoroughly mixed with a mixer and became a uniform liquid and was passed through filter paper. The obtained extract was used to measure the said traits. Soluble solids concentration was determined with a hand refractometer (Model RX-5000; Atago, Japan), and results were expressed as percent of soluble solids concentration (Brix, %) in juice at 25°C. To measure the amount of TA, titration method with 0.1 normal NaOH and phenolphthalein indicator was used. The amount of TA was calculated and recorded as a percentage based on malic acid. To measure total and reducing sugars in the fruit, 25 g of fruit was mixed with four times its weight of water (about 100 milliliters) and thoroughly mixed and filtered using filter paper. Then according to the Eynon-Lane method (Fehling test) and the AOAC (2012) method, the amount of total and reducing sugars in this solution was measured.

Data Analysis

The data were statistically analyzed using SAS version 9.1 based on a split-plot-in-time arrangement within a randomized complete block design (RCBD), with three replicates. The

experimental treatment group was considered the main-plot factor, consisting of five preharvest foliar treatment groups stored at 4 °C and an additional untreated-control reference group stored at -18 °C. The -18 °C condition was derived from the same untreated control replicates and was included as a reference storage condition rather than as a level of the foliar spray factor. Sampling time was considered the subplot factor, and the experimental treatment group × sampling time interaction was included in the analysis. Means were compared using Duncan's multiple range test at the 5% significance level.

RESULTS AND DISCUSSION

Humidity percentage

According to the variance analysis results of the data (Table 1), the moisture content of the 'Barhi' cultivar during cold storage (temperature 4 °C) was affected by the simple effects of foliar spray treatments during storage at a probability level of one percent.

According to Figure 1, the highest moisture percentage was related to fruits treated with calcium chloride 1.5%, which was not significantly different from fruits stored at freezer temperature and fruits treated with salicylic acid of 1 and 2 mM, and the lowest moisture content was related to fruits treated with calcium chloride 3%, which was not significantly different from the freezer temperature treatment. These results indicate that the response of fruit moisture content to calcium chloride was concentration-dependent rather than uniformly positive across both calcium treatments.

The relatively higher moisture retention observed in some treated fruits may be associated with the role of calcium in maintaining cell wall and membrane integrity and with the ability of salicylic acid to delay senescence-related processes. Both mechanisms could contribute to reducing water loss during storage by helping to preserve tissue structure and limiting the physiological processes associated with dehydration. The contrasting effects of the two CaCl₂ concentrations on fruit moisture suggest a concentration-dependent response. The higher

moisture retention at 1.5% CaCl_2 may be associated with improved maintenance of cell wall and membrane integrity, thereby limiting water loss during storage. In contrast, the lower moisture content observed at 3% CaCl_2 may indicate that increasing the CaCl_2 concentration did not further enhance water retention under the conditions of the present study. This response may be related to concentration-dependent effects of calcium salts on fruit tissue and water balance. However, the precise physiological mechanism underlying the different responses to CaCl_2 concentrations remains unclear and requires further investigation.

According to Figure 2, the moisture content of the fruits decreased during cold storage, and they had the highest moisture content at the time of harvest and the lowest moisture content at the end of six months of storage. This decline is consistent with the gradual loss of water from fruit tissues during prolonged storage as a result of transpiration and other physiological processes.

Soluble solids concentration (SSC)

According to Table 1, the SSC value of the 'Barhi' samples was not affected by any of the studied factors. Contrary to the results of this project, there have been various reports about the increase in the amount of soluble sugars in various products such as cantaloupe or papaya (Azene et al., 2014) and yam (Ashurnejad and Ghasemnejad, 2012) during storage. The stability of SSC in the present study may be related to the low storage temperature, which can slow respiration and other metabolic processes associated with ripening and carbohydrate transformation. In addition, the preharvest applications of salicylic acid and calcium chloride did not significantly alter SSC, suggesting that these treatments did not substantially affect the overall soluble-solids concentration of 'Barhi' fruit under the storage conditions evaluated. The maintenance of SSC during six months of storage is therefore indicative of relatively stable soluble-solids characteristics despite prolonged refrigerated storage.

Titrateable acidity (TA)

According to the analysis of variance (Table 1), titratable acidity (TA) of 'Barhi' fruit was significantly affected by the interaction between foliar spray treatments and storage duration at the 1% probability level. As shown in Table 2, TA values varied among treatments and sampling times during storage, indicating that changes in acidity depended on both the preharvest treatment and storage duration. Although several treatment combinations showed lower TA values toward the end of storage, the changes were not uniform throughout the storage period. Similar changes in acidity during refrigerated storage have also been reported for fresh 'Barhi' dates. Ghafoor et al. (2022) observed changes in acidity and pH of fresh 'Barhi' dates during storage at 4 °C, with the direction and magnitude of the changes depending on the coating treatment.

Changes in TA during storage may be associated with ongoing respiratory and metabolic processes, including the utilization and transformation of organic acids. The low storage temperature used in the present study may have slowed these metabolic processes and contributed to the retention of measurable acidity during refrigerated storage. The significant treatment $\times$ storage-time interaction further indicates that the preharvest applications of salicylic acid and calcium chloride influenced the pattern of acidity changes during subsequent storage, although the magnitude and direction of the response varied among treatments and sampling times.

pH

According to the analysis of variance (Table 1), the pH of 'Barhi' fruit was significantly affected by the interaction between foliar spray treatment and storage duration at the 1% probability level. As shown in Table 2, pH values varied among treatments and sampling times during storage. The highest pH value was recorded in fruit treated with 3% calcium chloride at harvest (7.33), whereas the lowest value was observed in the same treatment after six months

of storage (6.41). Overall, pH decreased from harvest to the end of storage, although the changes were not uniform across all sampling times and treatments.

The decrease in pH during storage may be associated with changes in organic acid metabolism and other biochemical processes occurring during fruit ripening and senescence. A slight decrease in pH during refrigerated storage of fresh 'Barhi' dates has also been reported by Ghafoor et al. (2022), although the magnitude of the change depended on the preservation treatment and storage conditions. The significant treatment $\times$ storage-time interaction observed in the present study indicates that the preharvest applications of salicylic acid and calcium chloride influenced the pattern of pH changes during subsequent refrigerated storage. However, because the response varied among treatments and sampling times, the effect of these treatments on pH should be interpreted in relation to both treatment concentration and storage duration.

Reducing sugar

According to the analysis of variance (Table 1), the interaction between foliar spray treatment and storage duration at 4 °C had a significant effect on the reducing sugar content of 'Barhi' Rutab fruit at the 1% probability level. As shown in Table 3, reducing sugar values varied considerably among treatments and sampling times during storage. The highest value was recorded in fruit treated with 1 mM salicylic acid after six months of storage (35.55%), whereas the lowest value was observed in fruit treated with 1.5% calcium chloride after one month of storage (17.37%). At harvest, no significant differences were observed among the treatments. Although the reducing sugar content at the end of six months was numerically higher than at harvest for all treatments, the changes during storage did not follow a consistent increasing or decreasing pattern. Considerable month-to-month fluctuations were observed, particularly in fruits treated with calcium chloride. These fluctuations may reflect dynamic changes in carbohydrate metabolism during storage as the fruit continued to undergo biochemical changes

under refrigerated conditions. Therefore, the observed fluctuations should be considered part of the experimental variation rather than interpreted as a continuous trend in reducing sugar accumulation or depletion.

Overall, the significant treatment $\times$ storage-duration interaction indicates that the response of reducing sugars to preharvest salicylic acid and calcium chloride applications depended on the duration of storage. The relatively higher reducing sugar values observed after six months in all treatments, particularly with 1 mM salicylic acid, suggest that preharvest treatments may have influenced carbohydrate changes during prolonged refrigerated storage; however, the irregular pattern of changes indicates that this effect should be interpreted cautiously.

Total sugar

According to Table 1, the amount of total sugar in the Rutab of the 'Barhi' cultivar at 4 ° C was affected by the interaction of foliar spray treatments and storage time at a probability level of one percent.

The highest percentage of total sugar was related to the salicylic acid treatment 1 mM after six months of storage and the lowest was related to the calcium chloride 3% treatment during one month of storage. No significant difference was observed between the different treatments at the time of harvest. As can be seen, the amount of total sugar increased during storage in each treatment (Table 3). The changes in total sugar during storage did not follow a consistent increasing or decreasing pattern. Considerable fluctuations were observed among sampling times, with the magnitude and direction of changes varying according to the foliar spray treatment. Nevertheless, the total sugar content at the end of six months was numerically higher than at harvest in all treatments. These changes may be associated with ongoing biochemical changes in carbohydrate metabolism during storage as the fruit continues to undergo ripening and senescence. However, because the observed changes were not uniform throughout the storage period, the results should not be interpreted as evidence of a continuous increase in

total sugar. The significant interaction between foliar spray treatment and storage duration indicates that preharvest applications of salicylic acid and calcium chloride influenced the pattern of total sugar changes during storage. The relatively high total sugar content observed after six months, particularly in fruit treated with 1 mM salicylic acid, suggests that this treatment may have contributed to maintaining higher sugar levels during prolonged refrigerated storage. However, given the considerable fluctuations among sampling times, the effects should be interpreted in relation to the specific treatment and storage duration.

Weight losses percentage

The percentage of weight loss of the fruit was significantly affected by the interaction between foliar spray treatment and storage duration at the 5% probability level.

According to Table 4, weight loss generally increased with increasing storage duration across the treatments. The highest percentage of weight loss was recorded in fruit treated with 1.5% calcium chloride after six months of storage (0.66%), whereas the lowest values were observed in fruit stored at freezer temperature during the first three months (0%). However, no significant differences were observed among the foliar treatments within individual sampling months. The increase in weight loss during storage is a natural consequence of water loss through evaporation and transpiration, as well as respiratory activity, resulting from differences in water vapor pressure between fruit tissues and the surrounding atmosphere (Mostofi et al., 2013).

Previous studies have reported that salicylic acid may reduce weight loss in fruit during storage. For example, the application of 1 mM salicylic acid reduced juice loss in 'Granny Smith' apple (Babalar et al., 2014), while salicylic acid has also been suggested to influence respiration and water loss through its effects on physiological processes (Kazemi et al., 2011). However, under the conditions of the present study, the weight-loss response did not indicate a consistent

beneficial effect of either salicylic acid or calcium chloride. In particular, the highest numerical weight loss was observed with 1.5% calcium chloride after six months. Therefore, the effects of these preharvest treatments on weight loss should be interpreted cautiously.

Sourness percentage

According to Table 1, the percentage of sourness of 'Barhi' fruit during storage at 4 °C was affected only by the main effect of foliar spray treatment at the 1% probability level.

According to Figure 3, the highest percentage of sourness was observed in the untreated control fruit stored at 4 °C, whereas the lowest percentage was observed in the frozen fruit and in fruit treated with 3% calcium chloride.

No souring was observed in the frozen fruit throughout the storage period. Under refrigerated storage at 4 °C, the percentage of souring was generally lower in the treated fruit than in the untreated control. Notably, no souring was observed in Rutab fruit treated with 3% calcium chloride throughout the six-month storage period, similar to the frozen reference fruit.

Souring in date fruit has been associated with biochemical changes and fermentation processes that may involve the activity of microorganisms under favorable conditions, particularly when moisture and temperature conditions support their development (Hamad, 2008). However, no microbiological analysis was conducted in the present study; therefore, the observed absence or reduction of souring cannot be attributed to a specific microbial mechanism.

Previous studies have reported that preharvest salicylic acid treatments may reduce disease incidence in fruit. For example, Yao and Tian (2005) reported that preharvest salicylic acid treatment reduced disease incidence in cherry fruit. Similarly, calcium treatments have been reported to delay fruit deterioration and maintain fruit tissue integrity, potentially through interactions with cell-wall components and calcium pectate formation (Conway et al., 2002). Calcium chloride treatment has also been reported to delay biochemical and physiological processes associated with fruit ripening (Atia et al., 2020). Zhang et al. (2003) reported that

salicylic acid delayed fruit ripening, senescence, and decay during storage. These previous findings provide possible physiological explanations for the responses observed in the present study; however, the specific mechanisms responsible for the reduction in souring observed here require further investigation.

CONCLUSION

This study demonstrated that preharvest foliar application of salicylic acid and calcium chloride at the Khalal stage influenced several quality attributes of 'Barhi' dates harvested at the Rutab stage and stored at 4 °C. Calcium chloride at 1.5% was associated with higher moisture content, whereas the highest weight loss was recorded in this treatment after six months of refrigerated storage. Salicylic acid treatments, particularly 1 mM, were associated with relatively higher reducing sugar values at the end of the storage period. Calcium chloride at 3% was particularly notable for its effect on souring, with no souring observed in the treated fruit throughout the six-month storage period. Overall, the results indicate that preharvest foliar treatments can influence the quality changes of 'Barhi' Rutab dates during refrigerated storage, with 3% CaCl₂ showing a particularly favorable response with respect to the absence of observed souring.

The main contribution of the present study is the evaluation of a practical preharvest approach for maintaining the quality of Rutab 'Barhi' fruit during refrigerated storage, rather than maintaining the fruit at the Khalal stage. The results suggest that appropriate preharvest applications of salicylic acid and calcium chloride may have potential for improving quality retention under the storage conditions evaluated in this study. However, the present experiment did not directly evaluate microbial populations, commercial marketability, or export performance. Therefore, further studies are needed to clarify the physiological and biochemical mechanisms underlying the observed responses and to evaluate these treatments under commercial storage, marketing, and postharvest handling conditions.

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CRedit AUTHORSHIP CONTRIBUTION STATEMENT

Ensieh Ghorbani: Conceptualization, Methodology, Formal analysis, Writing – original draft.

Maryam Boroujerdnia: Investigation, Supervision.

Esmail Fallahi: Supervision, Writing – review & editing.

Mozhgan Moghadam: Investigation, Data curation.

Seyed Naser Mousavi: Investigation, Data curation.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no competing interests that could have influenced the work reported in this manuscript.

ETHICS AND PERMIT APPROVALS

The study was conducted under the institutional authorization of the Date Palm and Tropical Fruits Research Institute as part of the approved research project (Project Code: 3-38-3333-065-011167). All experimental activities were conducted at the authorized experimental site of the Institute in accordance with its research procedures and regulations.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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AI USE STATEMENT

ChatGPT (OpenAI) was used solely for language editing, including grammar correction and linguistic refinement of the manuscript. No AI tool was used to generate scientific content, interpret data, formulate conclusions, or replace the intellectual contributions of the authors. All scientific content, data analysis, interpretation, and conclusions remain the sole responsibility of the authors.

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Table 1. Results of variance analysis of the effect of foliar spraying of salicylic acid and calcium chloride on some quality characteristics of ‘Barhi’ Rutab at a temperature of 4°C during the year 2023.

Change sources	Degrees of freedom	Humidity	SSC	TA	pH	Reducing sugar	Total sugar	Weight losses	Sourness percentage
Block	2	33.13**	28.71	0.01	0.08**	12.41	0.88	0.13**	269.7*
experimental treatment group	5	42.92**	60.32**	0.02*	0.2**	9.16	70.26*	0.37**	309.9**
Block× experimental treatment group (Error 1)	10	50.91**	25.62**	0.04*	0.02**	14.36	25.98	0.33**	397.68**
Time	6	46.36	30.45**	0.97**	0.54**	170.96**	180.8**	0.19**	115.9
experimental treatment group×Sampling time	30	10.53	50.97**	0.16**	0.11**	34.75**	63.36**	0.01*	42.9
Block×Sampling time	12	8.05	22.83	0.03	0.009	14.33	10.86	0.03**	41.4
Total	125	---	---	---	---	---	---	---	---
Coefficient of variation (CV)	---	7.68	9.53	20.76	1.92	12.06	14.91	18.87	29.6

* and **, significant at the 5 and 1% probability levels, respectively.

Table 2. Mean Comparison for the interaction effect of salicylic acid and calcium chloride spraying × sampling time on the TA and pH value (%) of ‘Barhi’ Rutab at 4°C during the year 2023.

Treatment	TA							pH						
	At harvest	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	At harvest	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Freezer temperature	1.16 ^{b-e}	1.43 ^{a-d}	1.51 ^{abc}	1.58 ^{ab}	0.98 ^{def}	1.17 ^{b-e}	0.98 ^{def}	7.27 ^{ab}	6.67 ^{j-m}	7.14 ^{abc}	7.1 ^{a-e}	7.1 ^{a-e}	7.06 ^{b-f}	7.06 ^{b-f}
Control	1.16 ^{b-e}	0.8 ^{ef}	1.69 ^a	1.47 ^{a-d}	1.07 ^{c-f}	1.37 ^{a-d}	0.62 ^f	7.27 ^{ab}	6.98 ^{c-h}	6.93 ^{c-j}	6.86 ^{d-l}	6.82 ^{f-l}	6.8 ^{f-l}	6.75 ^{h-m}
Salicylic acid 1 mM	1.07 ^{c-f}	1.69 ^a	1.53 ^{abc}	1.31 ^{a-d}	1.63 ^{ab}	1.16 ^{b-e}	0.8 ^{ef}	7.16 ^{abc}	6.04 ^o	6.71 ^{h-m}	6.94 ^{c-i}	6.78 ^{g-l}	6.87 ^{d-l}	6.69 ^{i-m}
Salicylic acid 2 mM	1.25 ^{a-e}	1.16 ^{b-e}	1.3 ^{a-d}	1.38 ^{a-d}	1.7 ^a	1.25 ^{a-e}	1.16 ^{b-e}	7.27 ^{ab}	6.98 ^{c-h}	6.93 ^{c-j}	6.64 ^{k-n}	6.78 ^{g-l}	6.94 ^{c-i}	6.71 ^{i-l}
Calcium chloride 1.5%	1.6 ^{ab}	1.6 ^{ab}	1.51 ^{abc}	1.38 ^{a-d}	1.16 ^{b-e}	1.07 ^{c-f}	0.8 ^{ef}	7.12 ^{a-d}	6.83 ^{e-l}	7.02 ^{b-g}	6.95 ^{c-i}	6.9 ^{c-k}	6.65 ^{k-n}	6.65 ^{k-n}
Calcium chloride 3%	1.52 ^{abc}	1.25 ^{a-e}	1.63 ^{ab}	1.45 ^{a-d}	1.45 ^{a-d}	1.25 ^{a-e}	0.8 ^{ef}	7.33 ^a	6.95 ^{c-i}	6.81 ^{f-l}	6.96 ^{c-i}	6.61 ^{lmn}	6.51 ^{mn}	6.41 ⁿ

Values are means of three biological replicates (n=3). Means followed by at least one common letter are not significantly different at the 5% probability level according to Duncan's multiple range test.

Table 3. Mean Comparison for the interaction effect of salicylic acid and calcium chloride spraying × sampling time on the reducing and total sugar percentage of ‘Barhi’ Rutab at 4°C during the year 2023.

Treatments	Reducing sugar (%)							Total sugar (%)						
	At harvest	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	At harvest	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Freezer temperature	25.47 ^{d-k}	24.13 ^{f-k}	26.1 ^{c-k}	29.11 ^{a-j}	30.8 ^{a-g}	29.12 ^{a-j}	30.21 ^{a-h}	30.7 ^{a-k}	26.01 ^{h-l}	26.97 ^{f-l}	33.65 ^{a-h}	22.5 ^{klm}	36.17 ^{a-e}	34.45 ^{a-h}
Control	25.47 ^{d-k}	22.01 ^{kl}	32.26 ^{a-d}	23.35 ^{h-l}	33.34 ^{ab}	29.002 ^{a-j}	29.8 ^{a-i}	30.7 ^{a-k}	19.86 ^{lm}	31.45 ^{a-j}	32.68 ^{a-i}	37.92 ^{ab}	31.67 ^{a-j}	36.39 ^{a-d}
Salicylic acid 1 mM	26.16 ^{c-k}	24.9 ^{e-k}	32.62 ^{abc}	24.91 ^{e-k}	26.17 ^{c-k}	29.26 ^{a-j}	35.55 ^a	27.22 ^{e-l}	35.69 ^{a-f}	34.96 ^{a-h}	30.34 ^{b-k}	34.74 ^{a-h}	36.95 ^{abc}	39.58 ^a
Salicylic acid 2 mM	22.96 ^{i-l}	31.8 ^{a-e}	33.46 ^{ab}	30.98 ^{a-f}	23.37 ^{h-l}	23.83 ^{g-l}	30.02 ^{a-h}	22.96 ^{j-m}	34.3 ^{a-h}	28.2 ^{c-l}	34.96 ^{a-h}	26.47 ^{g-l}	32.69 ^{a-i}	34.16 ^{a-h}
Calcium chloride 1.5%	23.58 ^{h-l}	17.37 ^l	33.42 ^{ab}	23.79 ^{g-l}	32.86 ^{abc}	22.45 ^{jkl}	32.7 ^{abc}	33.01 ^{a-i}	22.37 ^{klm}	33.58 ^{a-h}	35.54 ^{a-g}	35.35 ^{a-g}	24.28 ^{i-m}	36.61 ^{a-d}
Calcium chloride 3%	22.63 ^{jkl}	24.9 ^{e-k}	30.05 ^{a-h}	29.23 ^{a-j}	30.22 ^{a-h}	28.17 ^{b-k}	32.23 ^{a-d}	28.4 ^{c-l}	16.4 ^m	30.73 ^{a-k}	32.93 ^{a-i}	32.32 ^{a-i}	27.79 ^{d-l}	33.04 ^{a-i}

Values are means of three biological replicates (n=3). Means followed by at least one common letter are not significantly different at the 5% probability level according to Duncan’s multiple range test.

Table 4. Mean Comparison for the interaction effect of salicylic acid and calcium chloride spraying $\times$ sampling time on the weight losses percentage of 'Barhi' Rutab at 4°C during the year 2023.

Treatment	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Freezer temperature	0 ^d	0 ^d	0 ^d	0.09 ^{cd}	0.23 ^{a-d}	0.33 ^{a-d}
Control	0.25 ^{a-d}	0.21 ^{a-d}	0.39 ^{a-d}	0.48 ^{abc}	0.53 ^{abc}	0.58 ^{ab}
Salicylic acid 1 mM	0.37 ^{a-d}	0.39 ^{a-d}	0.42 ^{a-d}	0.45 ^{a-d}	0.47 ^{a-d}	0.54 ^{abc}
Salicylic acid 2 mM	0.33 ^{a-d}	0.37 ^{a-d}	0.42 ^{a-d}	0.44 ^{a-d}	0.46 ^{a-d}	0.45 ^{a-d}
Calcium chloride 1.5%	0.42 ^{a-d}	0.45 ^{a-d}	0.5 ^{abc}	0.55 ^{abc}	0.59 ^{ab}	0.66 ^a
Calcium chloride 3%	0.17 ^{a-d}	0.28 ^{a-d}	0.37 ^{a-d}	0.47 ^{a-d}	0.49 ^{abc}	0.55 ^{abc}

Means that have at least one letter in common are not significantly different at the 5% probability level, based on Duncan's Multiple Range Test.

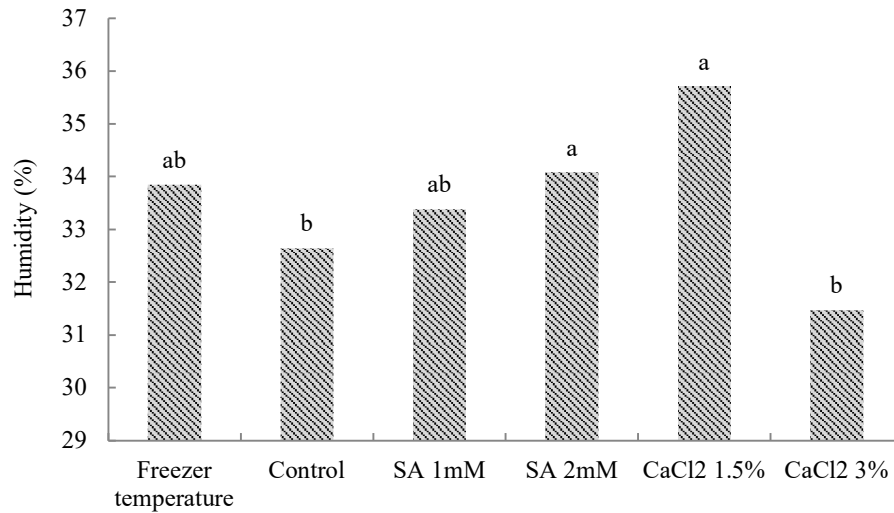


Figure 1- Mean comparison of the effects of foliar spray treatments and freezing temperature on the moisture content of ‘Barhi’ rutab. SA and CaCl₂ denote salicylic acid and calcium chloride, respectively. Foliar spray treatments included SA at 1 and 2 mM and CaCl₂ at 1.5% and 3%, along with the control; “Freezer temperature” represents untreated fruit stored at freezing temperature. Different letters above the bars indicate significant differences at the 5% probability level according to Duncan’s multiple range test.

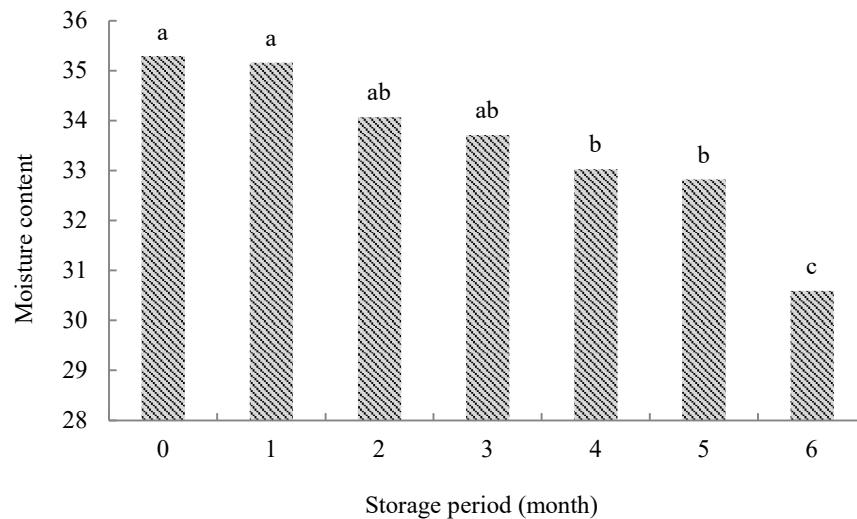


Figure 2- Mean comparison of the effect of storage period on the moisture content of ‘Barhi’ rutab during storage at 4 °C. Different letters above the bars indicate significant differences among storage periods at the 5% probability level according to Duncan’s multiple range test

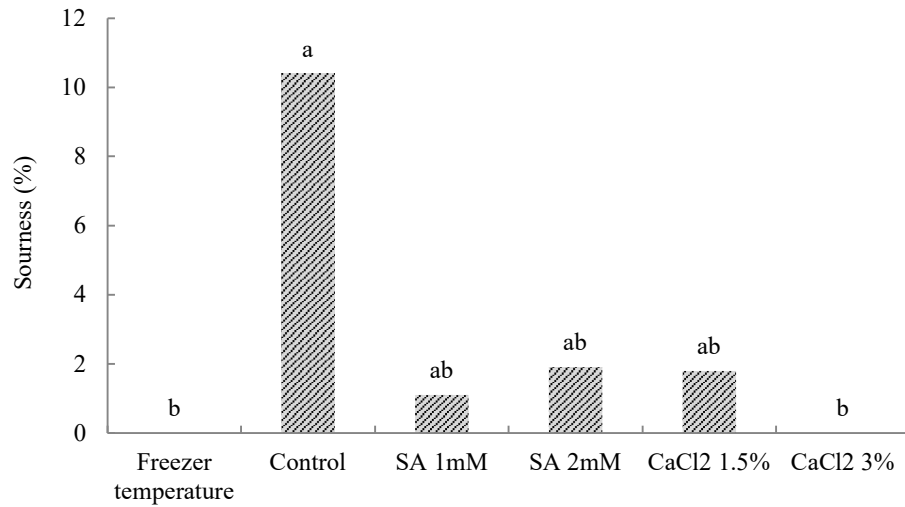


Figure 3- Mean comparison of the effects of foliar spray treatments and freezing temperature on the sourness (%) of 'Barhi' rutab. SA and CaCl₂ denote salicylic acid and calcium chloride, respectively. Foliar spray treatments included SA at 1 and 2 mM and CaCl₂ at 1.5% and 3%, along with the control; "Freezer temperature" represents untreated fruit stored at freezing temperature. Different letters above the bars indicate significant differences among treatments at the 5% probability level according to Duncan's multiple range test