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Optimization of Apple (*Malus domestica* Borkh.) Shelf-life by Calcium Chloride and *Aloe vera* Gel Using Response Surface Methodology (RSM)

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ARTICLE INFO	*Corresponding author's email: babashpour@gmail.com			
	ABSTRACT			
Article history:	Using organic and inorganic substances is a healthy and affordable way			
Received: 20 February 2023, Received in revised form: 4 February 2024, Accepted: 11 February 2024	to enhance fruit quality during storage. Apple is a popular fruit with high nutritional value and mainly experiences prolonged storage periods. The present study was conducted to optimize the shelf-life of apple fruits (<i>Malus domestica</i> Borkh. 'Golden Delicious') by <i>Aloe vera</i> gel and calcium chloride (CaCl ₂) and to evaluate apple characteristics			
Article type:	after 90 days of cold storage. Using response surface methodology			
Research paper	(RSM), measurements were aimed at fruit weight loss, pH, total soluble solids (TSS), total sugar (TS), fruit firmness, and juice content. As			
Keywords:	evidenced from RSM analysis, optimal conditions for apple shelf-life were achieved by 5.9% CaCl ₂ and 33.6% <i>Aloe vera</i> gel, which reduced			
Apple postharvest, Fruit firmness, Response surface methodology, Shelf-life, TSS	weight loss and maintained fruit firmness, pH, total soluble solids, total sugars, and juice content. <i>Aloe vera</i> gel and CaCl ₂ showed similar modes of action in each case of the measured traits, except for TSS where the <i>Aloe vera</i> gel increased apple TSS, whereas CaCl ₂ decreased it. Therefore, an optimized combination of <i>Aloe vera</i> gel and CaCl ₂ offered a promising approach to the maintenance of apple quality in storage.			

Introduction

Apple (*Malus domestica* Borkh.) is one of the most important fruit species in temperate regions and is grown commercially in different parts of the world (Prasad et al., 2021). Apples are prevalent among fruit growers due to their excellent nutritional value, high storage capacity, and suitability for fresh consumption (Lemmens et al., 2020). An apple fruit usually contains sugar (11%), fat (0.4%), protein (0.3%), carbohydrates (14.9%), vitamins, and minerals (Khan et al., 2020). Apples are usually stored for long durations at low temperatures. The quality and nutritional value of the fruits decrease during storage, and several factors affect the shelf-life of

apple fruits, such as growth time, harvesting operations, and storage conditions (Korićanac et al., 2020). Decreased fruit quality is often caused by relatively high metabolic activities during storage (Ranjbar et al., 2018; Nybom et al., 2020). Therefore, it is essential for economic and health reasons to use techniques that improve the quality of stored apple fruits.

Calcium (Ca²⁺) is a crucial mineral in cell walls and cellular membranes. It can maintain fruit quality and firmness while increasing fruit shelflife (Barzegar et al., 2018; Amiri et al., 2021; Habibi et al., 2023). Ca²⁺ can delay fruit ripening and prevent a fast decline in the nutritional status

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of the fruit while reducing its susceptibility to diseases associated with postharvest decay (Xiong et al., 2021). Ca²⁺ can improve antioxidant capacity by scavenging free radicals imposed by biotic stressors like pathogens (García-Martí et al., 2019). The positive impact of applying Ca²⁺ on fruits is notable in postharvest management. However, excessive Ca²⁺ concentrations can cause adverse effects on fruit quality, such as loss of taste (Barzegar et al., 2018). Meanwhile, there is a growing interest in plant-based materials to improve fruit quality in postharvest management. Aloe vera gel is a natural, eco-friendly, plantbased edible coating. It is a pulp-based gelatinous matrix extracted from the leaf tissues of Aloe vera plants. Aloe vera gel coating reportedly mitigated browning and preserved fruit quality by inhibiting phenolic oxidation, peroxidase, and polyphenol oxidase activity (Ali et al., 2019). It is a common practice to use Aloe vera gel as a natural, inexpensive, edible coating that can improve fruit quality and shelf life (Habibi et al., 2022). In previous research, Aloe vera gel and Ca²⁺ effectively enhanced fruit quality and increased the period of apple availability for consumer consumption. The beneficial role of Aloe vera gel in maintaining postharvest quality has been reported in various fruits like strawberries (Sogvar et al., 2016; Amiri et al., 2021), litchi (Ali et al., 2019), green chilies (Ul Hasan et al., 2021), 'Gola' Guava (Rehman et al., 2020), and sapodilla (Khaliq et al., 2019). Other studies confirmed the beneficial effects of Ca²⁺ on fruit storage in papaya (Gao et al., 2020), Ziziphus mauritiana Lamk. (Jain et al., 2019), and apple (Ranjbar et al., 2018). However, there is an absence of information on the effects of combining Aloe vera gel and Ca²⁺ on the postharvest quality of apple fruits. Therefore, the present study aimed to evaluate how Aloe vera gel and Ca²⁺ affect apple fruits during storage using response surface methodology (RSM).

Materials and Methods

Apple fruits were randomly harvested from apple trees (*Malus domestica* Borkh. 'Golden Delicious') at full maturity when the fruits had grown into a desirable color and firmness. The trees were located in an orchard in Maragheh, East Azerbaijan province, Iran (37° 40' N and 46° 41' E) in October 2020. The fruits were free from disease and mechanical injuries. They were uniform in size, shape, and color. After the harvest, they were immediately taken to a laboratory for different treatments.

Three-year-old *Aloe vera* plants were selected from a commercial greenhouse. The leaves were

washed with tap water to remove dirt and were dipped in 0.1% sodium hypochlorite for 3 min. After drying, the leaves were manually peeled with a stainless steel knife. The resulting mucilaginous gel was collected and mixed in a blender. After blending, the resultant gel was filtered with a sterile muslin cloth to remove any fibrous fraction. Raw gel pH was adjusted to 3.75 (Navarro et al., 2011) with citric acid, pasteurized at 65 °C for 30 min (Vieira et al., 2016), and cooled for further use. The gel solution was diluted with distilled water at a 1:1 (v/v) ratio, and glycerol (1%) was added as a plasticizer. Calcium chloride (CaCl₂, Sigma-Aldrich, US) was purchased as a powder from Petrokimia Company (Iran). CaCl₂ treatments were 1, 3, 5, 7, and 9%, so relevant amounts of CaCl₂ were precisely weighed and dissolved in distilled water. The fruits were immersed in the solutions for 5 min. After the treatments, the fruits were stored in cold storage (2 °C) for 90 days, and apple quality traits were measured thereafter.

Weight loss was calculated according to the following formula:

Fruit weight loss (%)

$$= \left[\frac{(initial weight - final weight)}{initial weight}\right] \times 100$$

Fruit firmness was measured on both sides of each fruit with a penetrometer (Effegi, Florence, Italy) fitted with an 11 mm diameter probe (Ranjbar et al., 2018). The fruit pH was determined by a pH meter when sampling the fruits. The TSS was measured using a digital hand refractometer (PAL, Atago, Tokyo, Japan) at room temperature and was expressed as Brix value (Ali et al., 2011). The total and reduced sugar contents in fruits were determined by a relevant method in the available literature (Chandraju et al., 2013). Response surface methodology (RSM) was applied to optimize Aloe vera and CaCl2 doses on the shelf-life of apple fruits. Data analysis and model construction were carried out using Design-Expert software (version 6.0.8, Stat-Ease Inc., Minneapolis, MN). The Central Composite Rotatable Design (CCRD) with a quadratic model was employed. Each independent variable had 5 levels, pertaining to *Aloe vera* gel and calcium chloride treatments. Six replicates of the central point were chosen in random order according to a CCRD configuration for three factors divided in three blocks. The α -values in the design outside the ranges were selected for rotatability of the design (Thompson et al., 1982). The central points for these designs were selected with the treatments at levels expected to yield satisfactory experimental results. The response functions were weight loss, fruit firmness, pH, total soluble solids, total sugars, and fruit juice. The response variable values are related to the coded variables by a second degree polynomial. Two treatment groups, each at 5 levels, were applied in three replicates. The CaCl₂ concentration at 3-9% and *Aloe vera* gel at 10-50% were independent variables that were applied to affect fruit weight loss, fruit firmness, pH, total soluble solids (TSS), total sugar, and juice content.

Results

Fruit weight loss

Fruit weight loss was significantly affected by the interaction of CaCl₂ and *Aloe vera* gel (Table 1). By the application of CaCl₂ and *Aloe vera* gel, fruit weight was better maintained during storage, compared to the control. The *Aloe vera* gel showed a significant ability to prevent fruit weight loss (at 30% concentration) and exhibited

maximal efficiency slightly beyond the 40% concentration ($P \le 0.01$) (Fig. 1a). The preventive effect of CaCl₂ on fruit weight loss occurred significantly by applying CaCl₂ concentrations higher than 5%, peaking at 7.07% that reduced the weight loss (2.1%) significantly ($P \le 0.01$) (Fig. 1b). Optimal concentrations of CaCl₂ and *Aloe vera* gel were 7.07 and 43.08%, respectively. According to Figure 1, the trends of changes in fruit weight loss that occurred in response to CaCl₂ and *Aloe vera* gel applications were almost similar. Based on R² and standard deviation, the predicted model confirmed the adequacy and accuracy of the tests (Equation 1).

Equation 1.

Weight loss = $3.7092 - 0.2080 \ CaCl^2$

- 0.0468 Aloe vera gel

- $+ 0.0007 CaCl^{2}$
- × Aloe vera gel

 $+ 0.0131 \times (CaCl^2)^2$

+ 0.0004 × (Aloe vera gel)²

Table 1. ANOVA for apple fruit traits in response to Aloe vera gel (AVG%) and calcium chloride (CaCl₂%) applications.

MS								
S.V.	Weight loss	Firmness	pН	TSS	TS	Juice		
Model	0.09**	0.17^{**}	0.016**	0.041**	0.15**	2.43**		
CaCl ₂ (%)	0.15**	0.17^{**}	0.034**	0.08^{**}	0.37^{**}	0.98^{**}		
AVG (%)	0.21**	0.55**	0.041^{**}	0.002 ^{ns}	0.068^{**}	8.81^{**}		
$CaCl_2 \times AVG$	0.0009 ^{ns}	0.00 ^{ns}	0.0004^{**}	-	-	0.026**		
$(CaCl_2)^2$	0.063**	0.062^{**}	0.0006^{**}	-	-	0.55^{**}		
$(AVG)^2$	0.057^{**}	0.075^{**}	0.0034**	-	-	2.31**		
Lack-of-fit	0.001^{**}	0.0006 ^{ns}	0.00004^{ns}	0.005 ^{ns}	0.0002^{**}	0.034**		
Error	0.00003	0.00013	0.00003^{**}	0.006^{**}	0.0006 ^{ns}	0.0002**		
CV	1.15	0.22	0.14	0.52	0.19	0.52		

^{ns} non-significant, * and ** represent significance at 5 and 1 percent probability levels, respectively.

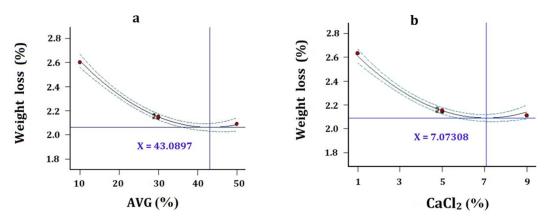


Fig. 1. Changes in fruit weight loss (%) in response to *Aloe vera* gel (AVG%) (a) and calcium chloride (CaCl₂%) (b) applications, showing a significant ability to control fruit weight loss in percentages.

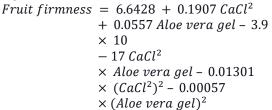
Fruit firmness

Interactions of CaCl₂ and *Aloe vera* gel significantly affected fruit firmness (Table 1). By the application of CaCl₂ and *Aloe vera* gel, fruit

firmness partly retained its original condition through storage, compared to the control. The *Aloe vera* gel showed a significant ability to prevent the loss of fruit firmness, and this was

evident from the 30% Aloe vera gel concentration onwards. Maximum efficiency occurred at 48% Aloe vera gel concentration that maintained fruit firmness at 8.61 kg cm⁻² (P \leq 0.01) (Fig. 2a). The supportive effect of CaCl₂ on maintaining fruit firmness occurred significantly by applying CaCl₂ concentrations higher than 5%, reaching maximum efficiency at 7.29% CaCl₂ that maintained fruit firmness at 8.5 kg cm⁻² ($P \le 0.01$) (Fig. 2b). Fruit firmness then decreased slightly when using CaCl₂ beyond the 7.29% concentration. Optimal concentrations of CaCl₂ and Aloe vera gel were 7.29 and 48%, respectively. According to Figure 2, the trends of changes in fruit firmness that occurred in

response to $CaCl_2$ and *Aloe vera* gel applications were almost similar. Based on R^2 and standard deviation, the predicted model confirmed the adequacy and accuracy of the tests (Equation 2). Equation 2.



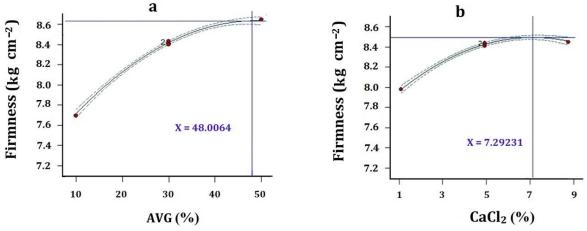


Fig. 2. Changes in fruit firmness (kg cm⁻²) in response to *Aloe vera* gel (AVG%) (a) and calcium chloride (CaCl₂%) (b) applications, showing a significant ability to maintain fruit firmness.

Fruit pH

Changes in fruit pH were observed during the storage period. Maintenance of fruit pH occurred in response to CaCl₂ and Aloe vera gel applications (Table 1), whereby the fruit pH did not increase markedly in storage, compared to the control. The Aloe vera gel showed a significant ability to prevent pH increase and exhibited maximal efficiency at 50% concentration $(P \le 0.01)$, causing a significant maintenance of the pH level (4.18) during storage (Fig. 3a). The preventive effect of CaCl₂ on the increase in pH occurred significantly by applying concentrations higher than 5%, peaking at 9% that maintained fruit pH (4.13) significantly (Fig. 3b). Optimal concentrations of CaCl2 and Aloe vera gel were 9 and 50%, respectively. According to Figure 3, the trends of changes in fruit pH that occurred in response to CaCl₂ and *Aloe vera* gel applications were almost similar. According to R² and standard deviation, the predicted model confirmed the adequacy and accuracy of the tests (Equation 3). Equation 3.

$$pH = 4.7053 - 0.05502 \ CaCl^2 \\ - 0.01454 \ Aloe \ vera \ gel \\ + 0.0005 \ CaCl^2 \\ \times \ Aloe \ vera \ gel \\ + 0.00133 \ \times \ (CaCl^2)^2 \\ + 0.00010 \ \times \ (Aloe \ vera \ gel)^2$$

Fruit total soluble solids (TSS)

CaCl₂ and *Aloe vera* gel affected changes in fruit TSS differently. The results showed that CaCl₂ and *Aloe vera* gel functioned dissimilarly on TSS accumulation (Fig. 4). CaCl₂ decreased the TSS, whereas the *Aloe vera* gel caused a negligible increase in TSS. The application of *Aloe vera* gel (50%) increased the TSS slightly (14.9 Brix) during storage (Fig. 4a). CaCl₂ (9%) had the most significant effect on reducing the TSS (from 15.17 to 14.72 Brix) (Fig. 4b). According to Figure 4, the trends of changes in fruit TSS were dissimilar when comparing the effects of CaCl₂ and *Aloe vera* gel applications. According to R², a model could not be predicted for the effects of CaCl₂ and *Aloe vera* gel on TSS (Table 1). Equation 4 describes CaCl₂ and *Aloe vera* gel affecting fruit TSS. Equation 4.

 $TSS = 15.04186 - 0.04083 CaCl^{2} + 0.00133 Aloe vera gel$

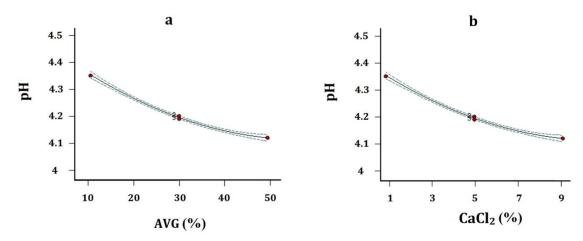


Fig. 3. Changes in fruit pH in response to *Aloe vera* gel (AVG%) (a) and calcium chloride (CaCl₂%) (b) applications, showing that the treatments prevented increases in fruit pH value.

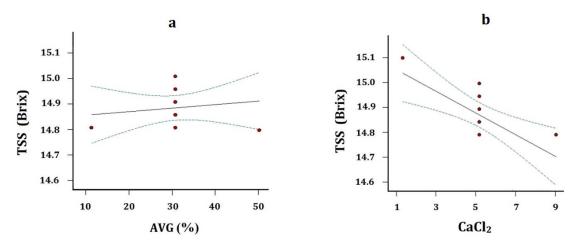


Fig. 4. Changes in fruit total soluble solids (Brix) in response to *Aloe vera* gel (AVG%) (a) and calcium chloride (CaCl₂%) (b) applications, showing that the *Aloe vera* gel slightly increased the total soluble solids (TSS), whereas calcium chloride (CaCl₂) decreased it.

Fruit total sugar (TS)

TS accumulation was significantly ($P \le 0.01$) affected by the interaction of CaCl₂ and *Aloe vera* gel application (Table 1). According to RSM results, CaCl₂ was more effective in altering the TS, compared to the effectiveness of *Aloe vera* gel (Fig. 5). Changes in fruit TS were observed during the storage period. Maintenance of fruit TS occurred in response to CaCl₂ and *Aloe vera* gel applications (Table 1), whereby the fruit TS did not increase markedly in storage, compared to the control. The *Aloe vera* gel showed a significant ability to prevent TS increase and exhibited maximal efficiency at 50% concentration ($P \le 0.01$), causing a significant maintenance of TS

(11.0) during storage (Fig. 5a). The preventive effect of CaCl₂ on the increase in TS occurred significantly by applying concentrations higher than 5%, peaking at 9% that maintained the fruit TS (11.0) significantly (Fig. 5b). Optimal concentrations of CaCl₂ and *Aloe vera* gel were 9 and 50%, respectively. According to Figure 5, the trends of changes in fruit TS that occurred in response to CaCl₂ and *Aloe vera* gel applications were almost similar. According to R² and standard deviation, the predicted model confirmed the adequacy and accuracy of the tests (Equation 5). Equation 5.

 $= 11.64038 CaCl^2 - 0.05 Aloe vera gel$

+ 0.00133 Aloe vera gel

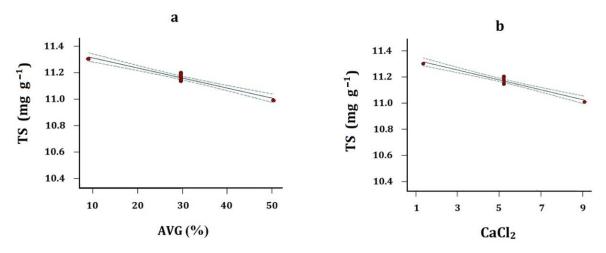


Fig. 5. Changes in total sugar content in response to *Aloe vera* gel (AVG%) (a) and calcium chloride (CaCl₂%) (b) applications, showing that the treatments prevented increases in the total sugar (TS) content.

Fruit juice (%)

Fruit juice content was significantly affected by the interaction of CaCl₂ and Aloe vera gel $(P \le 0.01)$. Juice content significantly increased in response to CaCl₂ and Aloe vera gel (Fig. 6). Specifically, 6.77 and 43.57% of CaCl₂ and Aloe vera gel, respectively, appeared to be optimal concentrations that led to maximum juice content. Interactions of CaCl₂ and Aloe vera gel significantly affected fruit juice content (Table 1). By the application of CaCl₂ and Aloe vera gel, fruit juice partly retained its original condition through storage, compared to the control. The Aloe vera gel showed a significant ability to prevent the loss of fruit juice, and this was evident from the 30% Aloe vera gel concentration onwards. Maximum efficiency occurred at 43.57% Aloe vera gel concentration that maintained the fruit juice at 24.2% ($P \le 0.01$) (Fig.

6a). The supportive effect of CaCl2 on maintaining fruit juice content occurred significantly by applying CaCl₂ concentrations higher than 5%, reaching maximum efficiency at 6.77% CaCl₂ that maintained the fruit juice at 23.92% (P \leq 0.01) (Fig. 6b). According to Figure 6, the trends of changes in fruit juice that occurred in response to CaCl₂ and Aloe vera gel applications were almost similar. Based on R² and standard deviation, the predicted model confirmed the adequacy and accuracy of the tests (Equation 6). Equation 6.

 $Juice \ content = 17.27343 + 0.40573 \ CaCl^2$ $+ 0.25614 \ Aloe \ vera \ gel$ $+ 0.004 \ CaCl^2$ $\times \ Aloe \ vera \ gel$ $+ 0.038739 \times (CaCl^2)^2$ + 0.0031734 $\times (Aloe \ vera \ gel)^2$

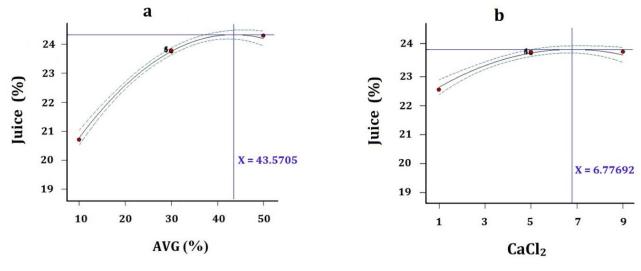


Fig. 6. Changes in fruit juice content (%) in response to *Aloe vera* gel (AVG%) (a) and calcium chloride (CaCl₂%) (b) applications, showing a significant ability to maintain fruit juice.

The treatment variables that led to different responses in stored apple fruits are summarized in Table 2. It is noteworthy that each trait responded differently to various treatment concentrations. Weight loss, pH, TSS, and TS were intended for minimum values, whereas fruit firmness and fruit juice content were intended for maximum values upon the application of *Aloe vera* gel and calcium chloride treatments in a dose-dependent manner.

Table 2. Ranges in variables for maintaining quality characteristics in apple fruits in response to <i>Aloe vera</i> gel (AVG%)
and calcium chloride ($CaCl_2\%$) applications.

Variable	Purpose	Minimum	Maximum
CaCl ₂ (%)	Dose-dependent	1	9
AVG (%)	Dose-dependent	10	50
Weight loss (%)	Minimum value	2.05	2.6
Fruit firmness (kg cm ⁻²)	Maximum value	7.75	8.65
pH	Minimum value	4.11	4.35
Total soluble solids (Brix)	Minimum value	14.72	15.1
Total sugar (mg g ⁻¹)	Minimum value	10.65	11.5
Juice content (%)	Maximum value	20.7	24.3

Discussion

A decrease in fruit weight can be related to respiration, metabolic activities, and transpiration (Barzegar et al., 2018; Rehman et al., 2020). Previous reports indicated various degrees of weight loss in fruits during the postharvest period (Barzegar et al., 2018). Coating apple fruits with Aloe vera gel created a physical barrier to moisture loss, thereby alleviating weight loss in fruits treated with Aloe vera gel. The RSM results indicated that Aloe vera gel at 43.08% optimally minimized fruit weight loss. Similar to this research, previous studies indicated doses and applications that mitigated fruit weight loss by Aloe vera gel on guava (Rehman et al., 2020), strawberry (Sogvar et al., 2016), and litchi (Ali et al., 2019).

CaCl₂ reduced weight loss in apple fruits. Since CaCl₂ maintained cellular membrane integrity and functionality, it led to a lower rate of moisture loss from the fruits. Ca can decrease fruit respiration rates and partly stabilize fruit weight during storage (Angeletti et al., 2010). In line with our results, Ranjbar et al. (2018) showed a significant mitigation of weight loss in apple fruits treated with calcium-based compounds.

Fruit firmness decreased during storage. However, the *Aloe vera* gel and CaCl₂ treatments alleviated the decrease in fruit firmness, primarily because these treatments stabilized cellular membranes and maintained their integrity (Ranjbar et al., 2018). Interactions between *Aloe vera* gel and CaCl₂ decreased enzymatic activities involved in fruit softening (Seyed et al., 2021). In previous research, nano-calcium treatments resulted in greater firmness values compared to calcium chloride treatments, possibly due to better absorption by plant cells. *Aloe vera* gel reportedly assisted in maintaining fruit firmness in Fagonia indica (Khaliq et al., 2019), strawberry (Sogvar et al., 2016), and papaya (Farina et al., 2020). Furthermore, CaCl₂ contributed to the maintenance of fruit firmness in Ziziphus mauritiana (Jain et al., 2019), papaya (Gao et al., 2021), and apple (Ranjbar et al., 2018) compared to their respective control groups.

Fruit pH increased during the storage period, but the interaction between *Aloe vera* gel and CaCl₂ mitigated the increase in pH value. The stability of pH under storage conditions is considered necessary for maintaining fruit quality. The increase in pH during storage results from the breakdown and decomposition of organic acids through respiratory processes (Sogvar et al., 2016). Similar to our results, Vieira et al. (2016) indicated that *Aloe vera* gel inhibited the increase of pH in blueberry juice during storage. *Aloe vera* gel and CaCl₂ increased the juice content in apple fruits. RSM results showed that 6.77% CaCl₂ and 43.8% *Aloe vera* gel were optimum treatment levels for achieving maximum juice content.

TSS decreased with CaCl₂ application but increased by *Aloe vera* gel. The increase in TSS during storage is usually a manifestation of polysaccharide hydrolysis and higher extract concentration. It is partly related to the decrease in fruit juice, which in turn enhances TSS. Also, respiration and aging in fruit tissues culminate in the breakdown of polysaccharides and their conversion to simpler compounds, thereby increasing TSS (Daisy et al., 2020). The TSS increased in response to *Aloe vera* gel because of starch hydrolysis into sugar compounds (Rehman et al., 2020). Previous findings reported a positive correlation between TSS and respiration rate (Khaliq et al., 2019). The decline in TSS could be due to the conversion of sugars into organic acids and a reduced respiration rate (Javed et al., 2018). Moreover, a lower TSS may cause carbohydrate hydrolysis into sugar compounds (Rehman et al., 2020). TSS and TS usually correlate with carbohydrate content. Increased levels of TSS and TS can lead to cell wall degradation. Similarly, Ranjbar et al. (2018) showed an increase in TSS and TS in apple fruits under Ca and nano-Ca applications.

Conclusion

CaCl₂ and *Aloe vera* gel caused improvements in the postharvest quality of apple fruits in storage. Since apple fruits are subjected to long durations of storage, finding appropriate doses of treatments that maintain fruit quality can be of importance from commercial prime а perspective. CaCl₂ and *Aloe vera* gel assisted in storage conditions for apple fruits and caused optimum shelf-life. Apple shelf life optimally increased by applying 5.9% CaCl₂ and 33.6% *Aloe* vera gel, as evidenced from RSM analysis. Thus, we can recommend these doses of CaCl₂ and Aloe vera gel as effective treatments for increasing apple shelf life during the storage time. Future research can consider the inclusion of other protective compounds that fortify the effects of CaCl₂ and *Aloe vera* gel on apple fruits for longer storage periods.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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