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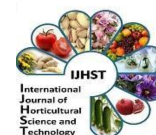

Supplementary Pollination Effect on the Postharvest Quality of Hayward Kiwifruit during Cold Storage

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ABSTRACT

The current research applied four methods of supplementary pollination from male flowers of kiwifruit (cv. Tomori). These methods were open pollination, hand pollination, spray pollination with pure pollen, and spray pollination with impure pollen, collected from the entrance of bee hives at the time of male kiwifruit flower opening and arranged in three replications in 2021. The fruits were harvestable after reaching the maturity stage (6.2 °Brix) and stored for 90 days at 0 °C. The results showed that kiwifruit decay (1.70%), weight loss (3.14%), total soluble solids (TSS) (149.62%), and TSS: titratable acidity (TA) ratio (TSS/TA) (333.04%) significantly ($P \leq 0.01$) increased during 90 days of cold storage. However, fruit firmness (38.24%), TA (42.10%), total chlorophyll (53.72%), carotenoid (18.65%), vitamin C (10.34%), flavonoid (12.87%), and antioxidant activity (13.29%) significantly decreased through storage time. Furthermore, total phenolic content (TPC) increased substantially after 30 days of cold storage but then decreased. Generally, fruits from vines sprayed with impure pollen had higher firmness, bioactive compounds, and sensory attributes. They had lower decay and less weight loss after 90 days of cold storage. Overall, spraying kiwifruit vines with impure pollen could be recommended as a practical supplementary pollination in kiwifruits, cv. Hayward, regarding postharvest quality maintenance.

Introduction

To optimize yield and fruit or seed quality, more than 70% of all crops rely on pollinators to varying degrees (Sáez et al., 2019). Kiwifruit (*Actinidia deliciosa*) produces male and female flowers on different plants, making it a prime candidate for supplemental pollination. As kiwifruit is primarily an insect-pollinated crop, the wind and insects often carry pollen from male to female flowers (Broussard et al., 2019; Broussard et al., 2021). Kiwifruit is one of the cornerstones of Iranian agriculture, rendering the country the fifth biggest kiwifruit producer worldwide. Due to its nutritional value, national kiwifruit production in Iran will probably increase in the coming years (FAO, 2021; Shiri et

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al., 2016 a, b).
Apis mellifera, or bees, are the primary pollinators of kiwifruit. However, due to factors like the absence of nectar in the pistillate flower of this species, their effectiveness is restricted (David et al., 2022; Broussard et al., 2022). Accordingly, several supplemental pollination techniques have been developed, including manual pollination, propelled fan systems, dusting pollen, and liquid pollen sprays (Gianni and Vania, 2018; Pathak, 2022).
Dry pollen and pollen suspension (wetter pollen) are two essential methods of supplementary pollination. Dry pollination helps the performance of honey bees because there is no need to visit male flowers when pollinating female flowers. The presence of pollen on female flowers stimulates bees, increases their activity, and pollen lands directly on the stigma (Gaspar et al., 2022; Wu et al., 2022). Moreover, when there are not enough active bees or male flowers in the orchard or when the weather is cold and wet, like rainy conditions, bees become limited in their activity during the pollination period. Thus, using more pollination methods or pollen suspension is possible. They provide a direct transfer of pollen to the stigma and are highly useful. At the same time, with this method, users can increase pollen viability and efficiency via diluting liquids and different types of auxiliary nutrients (Gianni and Vania, 2018; Eyles et al., 2022).
Previous research indicated that increasing artificial pollination in addition to insect pollinators improved fruit set and fruit quality. Artificial pollination has been the primary approach utilized globally, with many producers finding it a significant tool due to the additional pollination efficiency in increasing fruit output and quality (Abou Nader et al., 2022; Broussard et al., 2023).
Most recently, researchers have focused on evaluating supplementary pollination on kiwifruit. Many researchers have given limited attention to fruit yield (Abbate et al., 2021) or, on one occasion, discussed a robotic kiwifruit pollinator (Williams et al., 2020; Li et al., 2022 a, b). Generally, there is an informational gap about the impact of supplementary pollination on the postharvest behavior of kiwifruit. Thus, the current research introduced the most effective supplemental pollination techniques that help maintain the postharvest quality of kiwifruits, cv. Hayward.

Materials and Methods

Experimental site and plant materials

The Citrus and Subtropical Fruits Research Center in Ramsar, Iran, served as the research location, with coordinates of 36°54'11″ N 50°39'30″ E, an average annual temperature of 21 °C, and 1200 mm rainfall. Eighteen-year-old vines were trained on a T-bare system using male individuals of the 'Tomori' cultivar as the pollinizer at an 8:1 ratio, and they were spaced 6 × 4 m apart (417 vines ha^{-1}). Irrigation was done from mid-May to mid-October based on the plant water requirement using a micro-jet, with a water discharge rate of 1.5 L min^{-1}. Tensiometers in the root zone scheduled irrigations whenever soil tension reached 40 kPa. Most weeds were removed from the planting location by tilling and mowing. Before experimenting, soil samples (30 and 60 cm) were collected from the orchard under study, and their chemical and physical characteristics were determined (Table 1).

Table 1. Physicochemical characteristics of kiwifruit orchard soil.

Depth	pH	EC	OC	CCE	Clay	Silt	N	P	K	Fe	Zn	Cu
cm	-	(dS m^{-1})	%					Mg kg^{-1}				
0-30	7.2	0.19	1.9	1.0>	31.2	43.1	0.21	41.1	116.0	10.7	2.6	2.4
30-60	7.0	0.2	1.7	1.0>	35.0	43.3	0.16	23.0	94.4	9.4	0.6	1.5

EC: Electrical conductivity, OC: Organic carbon, CCE: Calcium carbonate equivalent.

Pollination treatments

Male flowers of the 'Tomori' cultivar were collected at the end of May, before the opening of the anthers, i.e., one day before flower opening. They were placed in envelopes and immediately transferred to the laboratory. Then, the flowers were gently stretched on the sieve to separate the stamens and placed in a desiccator at 30-35 °C for 24 h to dry. Ultimately, the pollen material was separated from anthers (Borghezan et al., 2011).

In the open pollination method, vines were pollinated naturally by bees and wind. In the hand pollination method, these pollens were transferred manually on the stigma of female flowers using a sterilized special brush. The Brubaker culture medium (Holman and Brubaker, 1926) enabled the testing of pollen viability. Foliar spraying with pure pollen involved using a suspension solution from the prepared pollen, followed by immediate foliar spraying on the female flowers with a hand sprayer. Furthermore, foliar spraying was associated with impure soluble pollen from a pollen collection device in front of bee hives when male flowers were opening. A suspension of the collected pollen was prepared and immediately sprayed on the flowers using a hand sprayer.

Measurements

Fruits were harvested in the second week of November 2021, at physiologic maturity, reaching 6.2–6.5 °Brix of total soluble solids (TSS) (Shiri et al., 2014). After being harvested, we transferred the fruits quickly to the lab and screened them for consistency in size, shape, and weight. Fruit quality was assessed at harvest time using thirty kiwifruits from each replication. Ninety kiwifruits from each replicate remained for 90 days at 0 °C and 95% relative humidity (RH). Three replicates underwent fruit quality assessment at 0, 30, 60, and 90 days of cold storage.

At the onset, we weighed the fruits immediately after harvest and again after 30, 60, and 90 days of cold storage. Fruit weight loss was assessed as a percentage loss of initial weight (Shiri et al., 2013a). Fruit decay (%) was measurable via two categories, i.e., pathogenic rots and skin appearance. Decayed fruits were discarded during the inspection process. Decay incidence (%) was measurable by counting the number of fruits in each replication (Shiri et al., 2013b). After removing the peel, fruit firmness was assessed on both sides using an 8-mm-tipped penetrometer (Effegi, model FTO11, Milan, Italy) (Ghasemnezhad et al., 2013).

TSS, titratable acidity (TA), and TSS/TA ratio were evaluated using juice samples derived from 10 fruits in each replication. The extraction of juice involved using an A2 104 extractor. TSS was measured using a digital refractometer (Atago-ATC-20, Japan) at ambient temperature and expressed as °Brix (Shiri et al., 2011a). TA value appeared as a percentage of citric acid, determined by titration of well-mixed juice with 0.1 M NaOH using a digital titrometer, described by Shiri et al. (2011b).

Using UV-vis spectrophotometry (UV-1800, Shimadzu, Japan), the amounts of total chlorophyll and total carotenoids were measured, according to Wellburn (1994). In summary, 10 milliliters of 80% acetone (distilled water: acetone, 80:20 v:v) were mixed with 0.05 g of freeze-dried pulp samples that had been homogenized. After separating the supernatant, absorbance values were measured at 470.0, 646.8, and 663.2 nm.

Vitamin C concentration was measured as mg 100 g^{-1} fresh weight (FW) by titrating 15 mL of filtrated juice with 2,6-dichlorophenol indophenols (DCIP) containing NaHCO3 (Shiri et al., 2011a).

The Folin-Ciocalteu method, first described by Singleton et al. (1999), enabled measuring the total phenolic content (TPC). At a wavelength of 765 nm, the measurement involved using a UV/Vis spectrophotometer. The calibration curve was produced using gallic acid as the standard. Milligrams of gallic acid equivalent (mg GAE) per 100 g of fruit fresh weight (FW) quantified the findings.

The total flavonoid content was determined using the aluminum chloride colorimetric method, as described by Du et al. (2009). In brief, 150 µL of extract, 1,700 µL of 30% ethanol, 75 µL of 0.5 mol L^{-1} NaNO2, and 75 µL of 0.3 mol L^{-1} AlCl3.6H2O were added and combined. After five min, 500 µL of 1 mol L^{-1} NaOH was added. A spectrophotometer facilitated the assessment of sample absorbance at 515 nm after 15 min. The total flavonoid content was measured using the quercetin standard curve and expressed as mg equivalents (QE) per 100 g of fruit FW.

The antioxidant activity was measured using the 2, 2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging technique, with a few minor adjustments from Brand-Williams et al. (1995). Two mL of a 0.15 mM DPPH solution in methanol and 1 mL of methanolic extract were vortexed and left to stand at room temperature in the dark. Sample absorbance values were measurable at 517 nm after 30 min, using a UV/Vis spectrophotometer. Measuring the difference in absorbance from the control group indicated the amount of DPPH scavenged, thus ascertaining antioxidant activity.

Sensory attributes were analyzed at harvest and the end of the fruit storage period. Five panelists were trained for the sensory assessment of kiwifruit. These panelists were selected based on their desire to eat kiwifruit, familiarity with sensory testing, availability, and lack of an allergic history. Before the test began, the fruits were left to stand at 25 °C for almost three hours. Based on a 10-point hedonic scale, ratings were assigned. To identify variations in the texture, flavor, and

taste of each treatment group, the sensory panel completed eight hours of training, during which they constructed and defined a descriptive vocabulary comprising nine qualities (Shiri et al., 2016b). Fruit sensory quality findings were presentable without any statistical analysis.

Experimental design and statistical analysis

We conducted a factorial experiment that comprised four supplementary pollination methods and four evaluated times. The experiment operated through a randomized complete design in three replicates. The PROC ANOVA approach involved using SAS software (ver. 9.1 2002-2003, SAS Institute, Cary, NC) to analyze the data. The Kolmogorov-Smirnov and Cochran tests determined data normality and homoscedasticity before variance analysis. While observing a significant ANOVA effect, we analyzed differences among mean values using Duncan's multiple-range test.

Results

The results showed that individual and combined effects of cold storage time and supplementary pollination method significantly (p≤0.01) affected fruit decay (Table 2). No decay was observed from day 30 to 60 of storage time. Symptoms of fruit decay were observed at the end of storage. The lowest fruit decay was found in kiwifruits sprayed with impure pollen (1.09%), whereas open-pollinated fruits had the highest (2.16%) decay (Table 2).

Table 2. Effect of storage time and different supplementary pollination methods on decay, weight loss, and firmness of kiwifruit, cv. Hayward, during 90 days of cold storage.

		Decay (%)	**Weight loss** (%)	**Firmness** (kg cm^{-2})
Storage (S)		**	**	**
Pollination (P)		**	**	**
S×P		**	**	**
Storage time	**Pollination method**			
0 (at harvest)	**Open pollination**	0.00[a]†	0.00[a]	7.36[a]
	Hand	0.00[a]	0.00[a]	7.31[a]
	Pure pollen	0.00[a]	0.00[a]	7.32[a]
	Impure pollen	0.00[a]	0.00[a]	7.30[a]
30 days	**Open pollination**	0.00[a]	0.76[a]	7.02[a]
	Hand	0.00[a]	0.76[a]	7.02[a]
	Pure pollen	0.00[a]	0.72[a]	6.98[a]
	Impure pollen	0.00[a]	0.68[a]	7.03[a]
60 days	**Open pollination**	0.00[a]	1.71[a]	5.67[a]
	Hand	0.00[a]	1.67[a]	5.65[a]
	Pure pollen	0.00[a]	1.66[a]	5.63[a]
	Impure pollen	0.00[a]	1.60[a]	5.97[a]
90 days	**Open pollination**	2.16[a]	3.35[a]	4.30[b]
	Hand	1.93[b]	3.36[a]	4.33[b]
	Pure pollen	1.62[c]	3.21[a]	4.29[b]
	Impure pollen	1.09[d]	2.63[b]	5.17[a]

** indicated significance at p≤0.01.
† At each evaluation time, means followed by the same letters are not significantly different at p≤ 0.01 according to Duncan's multiple range test. Slicing was performed based on storage time.

Fruit weight loss significantly ($p\leq0.01$) was affected by individual and combined effects of cold storage time and supplementary pollination method (Table 2). During 90 days of cold storage, kiwifruit weight loss significantly increased ($p\leq0.01$). While there was no significant difference between fruits pollinated by different methods during 30 and 60 days of storage, the lowest fruit weight loss occurred at the end of storage in kiwifruits sprayed with impure pollen (2.36%) (Table 2).

As shown in Table 2, individual and combined effects of cold storage time and supplementary pollination method significantly ($p\leq0.01$) affected fruit firmness. At the beginning of storage (day 0) and after 30 and 60 days of storage, there was no significant difference among fruits pollinated by different methods in terms of fruit firmness. After 90 days of cold storage, kiwifruits sprayed with impure pollen showed the lowest fruit firmness (5.17 kg cm^{-2}) compared to others (Table 2).

Kiwifruit TSS content was significantly ($p\leq0.01$) affected just by the individual effect of cold storage time (Table 3). TSS content was 6.65 °Brix at the harvest time and significantly increased ($p\leq0.01$) to 8.59, 10.64, and 16.60 °Brix at 30, 60, and 90 days of cold storage, respectively (Table 3). Moreover, TSS ranged from 10.64 to 11.01 °Brix in fruits pollinated by different methods, and no significant difference was observed between them (Table 3).

Table 3. Effect of storage time and different supplementary pollination methods on some physicochemical attributes of kiwifruit, cv. Hayward, during 90 days of cold storage.

	TSS (°Brix)	TA (%)	TSS/TA	Chlorophyll (mg 100^{-1} g FW)	Carotenoid (mg 100^{-1} g FW)
Storage (S)	**	**	**	**	**
Pollination (P)	NS	**	NS	**	NS
S×P	NS	NS	NS	NS	NS
			Storage time (day)		
0 (at harvest)	6.65^{d}†	1.14^{a}	5.78^{d}	2.68^{a}	0.445^{a}
10	8.59^{c}	0.97^{b}	8.92^{c}	2.27^{b}	0.418^{b}
20	10.64^{b}	0.86^{c}	12.26^{b}	1.65^{c}	0.388^{c}
30	16.60^{a}	0.66^{d}	25.03^{a}	1.24^{d}	0.362^{d}
			Pollination method		
Open pollination	10.48^{a}	0.89^{b}	13.11^{a}	1.77^{c}	0.397^{a}
Hand	10.46^{a}	0.91^{ab}	12.71^{a}	1.75^{c}	0.394^{a}
Pure pollen	10.54^{a}	0.91^{ab}	12.87^{a}	1.94^{b}	0.412^{a}
Impure pollen	11.01^{a}	0.93^{a}	13.30^{a}	2.38^{a}	0.405^{a}

NS and ** indicate non-significance and significance at $p\leq0.01$, respectively.
† Means followed by the same letters are not significantly different ($p\leq0.01$) according to Duncan's multiple range test.
Slicing was performed based on storage time.

Only the individual effects of cold storage time and supplementary pollination method significantly ($p\leq0.01$) affected kiwifruit TA content (Table 3). TA content was 1.14% at the harvest time, which significantly ($p\leq0.01$) decreased to 0.66% at the end of cold storage (Table 3). Among the different supplementary pollination methods, only open pollination (0.89%) and spraying with impure pollen (0.93%) showed a significant difference ($p\leq0.01$) in terms of fruit TA content during 90 days of cold storage (Table 3).

According to Table 3, the individual effect of cold storage time significantly ($p\leq0.01$) affected kiwifruit TSS/TA. TSS/TA significantly ($p\leq0.01$) increased from 5.78 to 25.03 during 90 days of cold storage (Table 3). TSS/TA ranged from 12.71 to 13.30 in fruits pollinated by different methods,

although without any significant difference (Table 3).
Only the individual effects of cold storage time and supplementary pollination method significantly ($p \leq 0.01$) affected kiwifruit total chlorophyll content (Table 3). Chlorophyll content was 2.68 mg 100^{-1} g FW at the harvest time, which significantly ($p \leq 0.01$) decreased to 1.24 mg 100^{-1} g FW after 90 days of cold storage (Table 3). While open-pollinated (1.77 mg 100^{-1} g FW) and hand-pollinated (1.75 mg 100^{-1} g FW) fruits had no significant difference with each other, fruits sprayed with pure pollen (1.94 mg 100−1 g FW) and impure pollen (2.38 mg 100^{-1} g FW) had higher total chlorophyll content (Table 3).
During 90 days of cold storage, kiwifruit carotenoid content was significantly ($p \leq 0.01$) affected just by the individual effect of cold storage time (Table 3). Kiwifruit carotenoid content was 0.445 mg 100^{-1} g FW at the harvest time and then significantly decreased ($p \leq 0.01$) to 0.418, 0.388, and 0.362 mg 100^{-1} g FW at 30, 60, and 90 days of cold storage, respectively (Table 3). Moreover, kiwifruit carotenoid content ranged from 0.394 to 0.412 mg 100^{-1} g FW in fruits pollinated by different methods, and no significant difference was observed between them (Table 3).
Only the individual effects of cold storage time and supplementary pollination method significantly affected ($p \leq 0.01$) kiwifruit vitamin C content. Vitamin C content was 57.09 mg 100^{-1} g FW at the harvest time, which significantly ($p \leq 0.01$) decreased to 51.19 mg 100^{-1} g FW at the end of storage time (Table 4). During 90 days of cold storage, open-pollinated (52.45 mg 100^{-1} g FW) and hand-pollinated (53.24 mg 100^{-1} g FW) fruits had no significant difference with each other in vitamin C content. Furthermore, fruits sprayed with pure pollen (54.40 mg 100^{-1} g FW) and impure pollen (57.70 mg 100^{-1} g FW) retained more vitamin C content during cold storage (Table 4).

Table 4. Effect of storage time and different supplementary pollination methods on some biochemical attributes of kiwifruit, cv. Hayward, during 90 days of cold storage.

	Vitamin C (mg 100^{-1} g FW)	**TPC** (mg GAE100^{-1} g FW)	**Flavonoid** (mg 100^{-1} g FW)	**Antioxidant** (%DPPHsc)
Storage (S)	**	**	**	**
Pollination (P)	**	**	NS	*
S×P	NS	NS	NS	NS
		Storage time (day)		
0 (at harvest)	57.09^{a}†	74.36^{b}	53.35^{a}	62.37^{a}
10	55.84^{b}	78.35^{a}	52.08^{ab}	61.10^{a}
20	53.67^{c}	71.04^{c}	50.73^{b}	58.89^{a}
30	51.19^{d}	65.61^{d}	46.48^{c}	54.08^{b}
		Pollination method		
Open pollination	52.45^{c}	70.45^{b}	50.45^{a}	56.68^{b}
Hand	53.24^{c}	70.63^{b}	51.43^{a}	58.57^{ab}
Pure pollen	54.40^{b}	71.87^{b}	49.58^{a}	59.58^{ab}
Impure pollen	57.70^{a}	76.42^{a}	51.20^{a}	61.56^{a}

NS, ** and * indicate non-significance and significance at $p \leq 0.01$ and $p \leq 0.05$, respectively.
† Means followed by the same letters are not significantly different according to the least significant difference test.

Kiwifruit TPC was significantly affected ($p \leq 0.01$) just by the individual effects of cold storage time and supplementary pollination (Table 4). At harvest, TPC was 74.36 mg GAE100^{-1} g FW and then significantly increased ($p \leq 0.01$) to 78.35 mg GAE100^{-1} g FW on day 30 of cold storage. Subsequently, kiwifruit TPC significantly decreased ($p \leq 0.01$) to 71.04 and 65.61 mg

GAE100^{-1} g FW on days 60 and 90 of cold storage, respectively. While open-pollinated (70.45 mg GAE100^{-1} g FW), hand-pollinated (70.63 mg GAE100^{-1} g FW), and pure pollen-sprayed fruits (71.87 mg GAE100^{-1} g FW) had no significant difference with each other in TPC, fruits sprayed with impure pollen (76.42 mg GAE100^{-1} g FW) had the highest TPC during the three months of cold storage (Table 4).

The individual effect of cold storage time significantly affected ($p \leq 0.01$) kiwifruit flavonoid content (Table 4). Fruit flavonoid content was 53.35 mg 100^{-1} g FW at harvest and significantly decreased ($p \leq 0.01$) to 52.08, 50.73, and 46.48 mg 100^{-1} g FW at 30, 60, and 90 days of cold storage, respectively (Table 4). Moreover, kiwifruit flavonoid content was ranked 49.58-51.43 mg 100^{-1} g FW in fruits pollinated by different methods, and no significant difference was observed among them (Table 4).

Kiwifruit antioxidant activity was significantly affected just by the individual effects of cold storage time ($p \leq 0.01$) and supplementary pollination method ($p \leq 0.05$) (Table 4). Kiwifruit antioxidant activity slightly decreased from 62.37% DPPHsc (at harvest) to 61.10 and 58.89% DPPHsc at 30 and 60 days of cold storage. However, at the end of cold storage, antioxidant activity significantly decreased ($p \leq 0.01$) to 54.08% DPPHsc (Table 4). Among the different supplementary pollination methods, only open pollination (56.68% DPPHsc) and spraying with impure pollen (61.56% DPPHsc) showed a significant difference ($p \leq 0.05$) in fruit antioxidant activity during 90 days of cold storage (Table 4).

The results showed that after 90 days of cold storage, the fruits of impure pollen-sprayed vines had better sensory attributes such as higher skin resistance, pulp consistency, sweetness, and better flavor than the others. Therefore, these fruits had better marketability (Fig. 1).

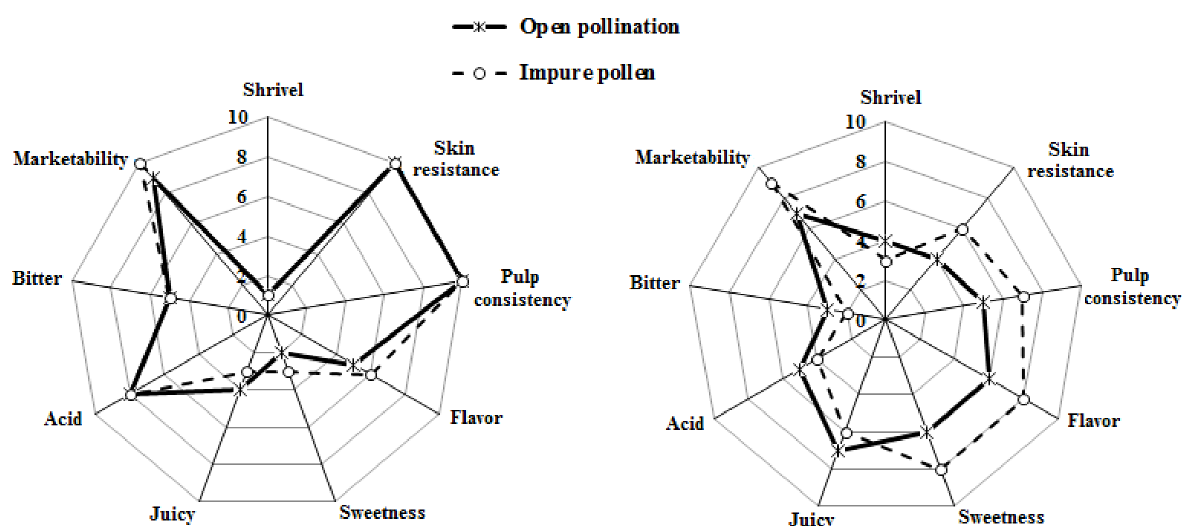
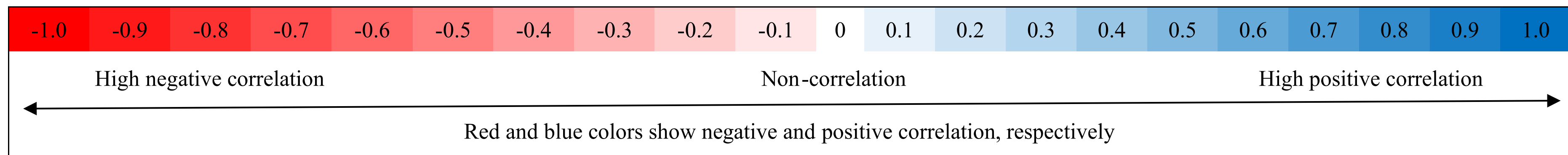


Fig. 1. Fruit sensory attributes of open pollination and impure pollen spray on kiwifruit, cv. Hayward, at harvest (left) and at the end of 90 days of cold storage (right). In the case of each variable, the possible value range was 0-10.

A positive and significant correlation was found between fruit decay and weight loss (Table 5). Moreover, fruit decay and weight loss correlated positively and significantly with TSS and TSS/TA. However, they correlated negatively and significantly with fruit firmness, TA, total chlorophyll, carotenoid, TPC, flavonoid, and antioxidant activity (Table 5). Kiwifruit firmness correlated negatively and significantly with TSS and TSS/TA. However, it correlated positively and significantly with TA, total chlorophyll, carotenoid, TPC, flavonoid, and antioxidant activity. TSS and TSS/TA correlated negatively and significantly with total chlorophyll, carotenoid, TPC, flavonoid, and antioxidant activity. Kiwifruit TA content showed a negative significant correlation with TSS/TA but a positive significant correlation with total chlorophyll, carotenoid, TPC, flavonoid, and antioxidant activity. Finally, positive and significant correlations occurred among total chlorophyll content, carotenoid, TPC, flavonoid, and antioxidant activity (Table 5).

Table 5. Correlation coefficients between some postharvest physicochemical attributes of kiwifruit, cv. Hayward, in response to different supplementary pollination methods.

	Decay	Weight loss	Firmness	TSS	TA	TSS/TA	Chlorophyll	Carotenoid	Vitamin C	TPC	Flavonoid
Weight loss	0.84**										
Firmness	-0.90**	-0.96**									
TSS	0.85**	0.97**	-0.94**								
TA	-0.76**	-0.91**	0.93**	-0.94**							
TSS/TA	0.89**	0.88**	-0.95**	0.99**	-0.94**						
Chlorophyll	-0.69**	-0.87**	0.88**	-0.81**	0.89**	-0.80**					
Carotenoid	-0.64**	-0.76**	0.78**	-0.74**	0.81**	-0.74**	0.74**				
Vitamin C	-0.65**	-0.78**	0.76**	-0.66**	0.73**	-0.68**	0.89**	0.64**			
TPC	-0.72**	-0.70**	0.77**	-0.68**	0.67**	-0.71**	0.80**	0.59**	0.81**		
Flavonoid	-0.70**	-0.73**	0.76**	-0.78**	0.77**	-0.78**	0.71**	0.58**	0.56**	0.59**	
Antioxidant	-0.63**	-0.64**	0.64**	-0.57**	0.59**	-0.58**	0.64**	0.74**	0.67**	0.55**	0.40**



** indicated significance at $p \leq 0.01$.

TSS: total soluble solids, TA: titratable acidity, TPC: total phenolic content.

Discussion

Effects of cold storage on kiwifruit quality

Our results mentioned that kiwifruit (cv. Hayward) fruit decay and weight loss occurred significantly during cold storage time, while firmness decreased significantly (Table 2). These results are consistent with Shiri et al. (2016a) in the cold storage of kiwifruit (cv. Hayward).

Losing weight reduces saleable fruit weight and may damage its texture and aesthetic appeal. Because of the moisture loss through the epidermis due to transpiration and respiration during storage, fruit weight loss often increases linearly in time (Yahaya and Mardiyya, 2019; Lufu et al., 2020).

Firmness is one of the most influential physical characteristics that determine the quality of fruits during maturation, storage, and marketing, marking it as an issue that lowers fruit quality via firmness loss (Sañudo-Barajas et al., 2019). The increased activity of polygalacturonase (PG) enzyme in fruits may cause fruit flesh softening during storage (Hossain et al., 2020; Shi et al., 2022a). This enzyme breaks down soluble pectin. Furthermore, the main reasons for softness include xyloglucan depolymerization, loss of galactose and arabinose, pectin solubilization, and the activities of cell wall-degrading enzymes during ripening and postharvest conditions (Farcuh and McPherson, 2021; Shi et al., 2023).

Generally, the increase in fruit weight loss and a decrease in fruit firmness creates favorable conditions for the growth of microorganisms that cause decay in the fruit and increase the decay rate (Chen et al., 2021).

TSS and TSS/TA significantly increased during storage, and TA content significantly decreased (Table 3). The climacteric fruits show an increasing trend of TSS during postharvest storage. Water loss, the conversion of polysaccharides and pectin materials into other sugars, the breakdown of starch, modifications to the juice content, and the enhancement of mono- and disaccharide quantities in response to degradation might all be contributing factors to the rising TSS over storage (Horvitz, 2017; Yahaya and Mardiyya, 2019).

The amount of organic acids in the fruit is crucial for preserving fruit quality and closely correlates with TA. Fruit metabolism and respiration lower the TA and convert acidic components into nonacidic molecules via biochemical events (Vallarino and Osorio, 2019).

Total fruit acceptability emanates from the balance between TSS and TA. Numerous metabolic alterations occur during storage, disrupting the TSS/TA and making the fruit unfit for consumption. The rise in TSS and reduction in fruit acidity during storage was the cause of the increase in TSS/TA that the current investigation observed. As a result, TSS/TA rose through the ongoing reduction in acidity and concurrent rise in SSC concentration. An advanced fruit ripening stage indicates a higher TSS/TA value, serving as a ripening indicator (Shiri et al., 2016a; Martínez-González et al., 2017).

Consistent with previous research by Shiri et al. (2016a), our investigation found that after 90 days of cold storage, the pigment level of kiwifruit flesh decreased dramatically (Table 3). Primary morphological alterations in kiwifruit resulted from enzymatic and nonenzymatic formations of brown pigments. Another factor was decolorization brought on by the breakdown of carotenoids and chlorophyll. A drop in color intensity may result from several factors leading to the loss of natural coloring pigments, such as chlorophylls (Solovchenko et al., 2019). Intercellular acids and enzymes are released when fruit is stored, and these substances may come into touch with chlorophyll–protein complexes. The physical injury to the tissue and its interaction would be the first stage of chlorophyll breakdown. Moreover, oxidative enzyme breakdown of carotenoids during storage and senescence processes may decrease the carotenoid concentration (Pott et al., 2020; Ebrahimi et al., 2023).

The vitamin C concentration significantly declined throughout cold storage (Table 4).

These findings on kiwifruit storage (cv. Hayward) were consistent with those by Shiri et al. (2016b). Ascorbic acid, or vitamin C, is a crucial indicator of nutritional quality. Compared to other nutrients, it is susceptible to oxidation during food preparation and storage, making it a water-soluble vitamin. Due to ascorbic acid oxidizing to dehydroascorbic acid and then further breaking down to 2,3-diketo-gluconic acid by the ascorbic acid oxidase enzyme, the content of ascorbic acid may have altered during storage (Mellidou et al., 2019; Hossain et al., 2020).

TPC of kiwifruit cv. Hayward was seen to rise after 30 days of cold storage, then decreased at 60 and 90 days (Table 4). The increase in TPC could be due to synthesizing phenolic compounds via the phenylpropanoid pathway during cold storage (Yahia, 2019). Furthermore, ethylene activity may increase polyphenol concentration. The phenylalanine ammonium lyase (PAL), a crucial enzyme in polyphenol biosynthesis, is activated by this phytohormone, which results in polyphenol production. On the other hand, the polymerization or degradation of phenolic compounds by polyphenol oxidase (PPO) enzyme

activity may decrease TPC during postharvest storage (Horvitz, 2017; De la Rosa et al., 2019).
Antioxidant component loss has links to the storage of fruits and vegetables. Shiri et al. (2016b) demonstrated that kiwifruit antioxidant activity peaked upon harvest but declined throughout extended storage. Reactive oxygen species (ROS) are produced in significant quantities while fruits and vegetables are stored, and their production increases with time. Increased ROS may lead to oxidative damage, oxidation of lipids and proteins, disruption of cell membranes, tissue damage, and ultimately worse fruit and vegetable marketability and storage quality (Madani et al., 2019). Antioxidant levels decrease during storage since they are essential for scavenging reactive oxygen species (ROS) produced during senescence. Moreover, since our study found a positive correlation among antioxidant activity, TPC, and vitamin C (Table 5), fruits' decreased antioxidant activity during long-term storage may be due to a decline in phenolic compounds and vitamin C (Martínez-González et al., 2017; Yun et al., 2022).
Prolonged storage lengthens deterioration, which lowers overall fruit attractiveness in sensory attributes. Furthermore, phenolic compounds define flavor, taste, and health-promoting properties in plant-derived meals. Reports indicated that fruit flavor and sensory qualities changed in response to alterations in some phenolic chemicals (Sánchez-Rodríguez et al., 2019; Titeli et al., 2023).
Shiri et al. (2016b) demonstrated a linear association between phenolic content and antioxidant activity in kiwifruit (cv. Hayward), which is consistent with our findings. The scavenging process depends heavily on the fundamental structural elements of compounds and other structural elements. The reactive centers are the aromatic OH groups, particularly the 3', 4′-dihydroxy catechol group. Other substituents with electron-donating properties might increase the activity of these groups (Liaudanskas et al., 2021; Shi et al., 2022b). Furthermore, flavonoid antioxidant activity varied according to the quantity and location of hydroxyl groups on their nucleus. However, it is generally consistent with differences in their antiradical activity (Hassanpour and Doroudi, 2023; Zeng et al., 2023).

Effect of supplementary pollination methods on the postharvest quality

Our results mentioned that supplementary pollination of kiwifruit flowers with spraying impure pollen led to better fruit postharvest quality. Kiwifruit plants are typically dioecious, meaning there are male and female plants, and not all female flowers receive adequate pollen for fertilization (Tacconi et al., 2016). Spraying pollen directly onto the flowers increases the chances of successful pollination due to more pollen grains provided for the flowers in fertilization. It can allocate more resources to fruit development and maturation, thus improving fruit quality. These fruits can maintain their quality optimally during storage. Adequate pollination can lead to healthier fruit with fewer physical defects, making them less susceptible to fungal and bacterial pathogens that can cause decay (Abrol, 2015; Oh et al., 2022).
Well-pollinated kiwifruits are more likely to develop a strong fruit structure with better skin integrity and a waxy cuticle layer, which provides more robust barriers to gas exchange during storage. These fruits are less prone to moisture loss and physical damage. The two can contribute to postharvest weight loss, decay incidence, and firmness decrease. Furthermore, less gas exchange during storage could slow respiration and oxidation processes. As a result of the reduction of oxidation, the degradation of bioactive compounds during storage decreases. Therefore, vitamin C, chlorophyll, and phenolic compounds are better preserved (Abrol, 2015; Broussard et al., 2021).
As mentioned above, supplementary pollination with spraying pollen can lead to better fruit set, development, and maturation. Higher-quality fruits are more likely to have higher concentrations of beneficial biochemical compounds and better retention of these compounds during storage. Pollinated flowers produce fruits that contain defense-related substances such as phenolic compounds, which may aid in shielding the fruit from biotic and abiotic challenges in storage. These compounds also contribute to the fruit's antioxidant activity, which can help preserve fruit quality during cold storage (Lim et al., 2014; Cacioppo et al., 2018; Dung et al., 2021).
Supplementary pollination ensures adequate pollen is available, which maximizes fertilization and seed development in each fruit. The seeds are a source of plant hormones that regulate fruit development and ripening. Hormonal changes can also influence the synthesis and accumulation of beneficial compounds, such as phenolic compounds, chlorophyll, and vitamin C, which can help preserve the fruit's quality during cold storage (Halder et al., 2019; Oh et al., 2022).
Fruits grown on vines sprayed with impure pollen (Fig. 1) seemed to have better sensory quality, indicating that this treatment was more effective

in postponing the loss of biochemical compounds and caused improved sensory attributes.

In this experiment, the foliar application of impure pollen was more effective than pure pollen in maintaining fruit postharvest quality. This point might be because preparing pure pollen grains takes time and requires drying, grinding, sieving, and sometimes keeping them at a low temperature, which can negatively affect the viability of pollen grains. Furthermore, dissolving the pollen in the auxiliary solution is a demanding task and requires detergents. On the other hand, impure pollen is prepared directly from the bee hive and is ready for foliar spraying without any manipulation or additional steps. This point has made this treatment more efficient compared to other treatments.

Conclusion

Fruit firmness, TA, total chlorophyll, carotenoid, vitamin C, flavonoid, and antioxidant activity significantly decreased in storage ($p \le 0.01$). However, kiwifruit decay, weight loss, TSS, and TSS/TA significantly increased ($p \le 0.01$) during 90 days of cold storage. Among the different supplementary pollination methods, impure pollen led to higher quality fruits with lower decay, less weight loss, and higher firmness, TA, total chlorophyll, vitamin C, TPC, and antioxidant activity during 90 days of cold storage. Therefore, spraying kiwifruit vines with impure pollen could be recommended as a practical approach to maintaining the postharvest quality of kiwifruit (cv. Hayward).

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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