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Postharvest quality of new quince cultivar and promising genotype (*Cydonia oblonga* Mill.) in response to harvesting time and length of the cold storage period

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ABSTRACT

Purpose: The aim of this study was to determine the most appropriate harvesting time and to evaluate the storage period of some new quince cultivar and the promising genotype. **Research method:** The fruits of 'Isfahan' and 'Behta' cultivars along with NB4 promising genotype were harvested on 6th, 14th, and 21st October from Isfahan Agricultural Research Station, Iran. The fruits were transferred to the storage and placed at a temperature of 0±1°C and relative humidity of 90±5%. Traits were evaluated at harvesting time and also at one-month intervals for five months after storage using a factorial experiment based on a completely randomized design. **Main findings:** The highest percentage of total soluble solids (TSS) was obtained in the third harvesting time and after five months of storage for 'Isfahan'. The highest firmness was obtained at the first harvesting time without storage for 'Behta'. 'Isfahan' at the time of the second and third harvest showed the highest total phenol content and 'Behta' at the first harvest and five months after storage showed the lowest value of this trait. The most weight loss was observed in 'Isfahan' in the third harvest and the fifth month of storage. Experimental treatments had no effect on pectin content. The highest surface browning was observed in the third harvest and the fifth month of storage. **Research limitations:** No limitations were found. **Originality/Value:** The best harvest time for 'Isfahan', 'Behta', and NB4 was similarly 193 days after flowering. As well as storage of these fruits for four months is recommended.

INTRODUCTION

Quince (*Cydonia oblonga* L.) belongs to the Rosaceae family. This species is known as a native of Iran and its distribution centers are the forests of northern Iran from Astara to Katoul Gorgan and the middle latitudes of northern Iran (Abdollahi, 2021). The first program to collect of native cultivars and genotypes in Iran was conducted by Ghasemi (2002) in Isfahan province. 'Behta' new cultivar is one of these cultivars that have been considered in recent years due to its desirable quality, high productivity, and relative tolerance to fire blight (Abdollahi, 2019). NB4 promising genotype is also one of the genotypes that are being introduced due to the high fruit quality and quantity (Tatari & Abdollahi, 2021).

The beneficial effects of quince fruit as a source of pectin (Moradi et al., 2016) and antioxidant compounds (Wojdylo et al., 2013) have been previously reported. Quince is a climacteric fruit that is usually harvested from October to November in the Northern hemisphere (Sharma et al., 2011). In climacteric fruits, an increase in respiration occurs at the ripening time, therefore if the temperature of storage is reduced, the respiration of the fruit will be slowed down and the fruit ripening will be delayed and the storage period of the fruit will be increased (Luengwilai & Beckles, 2013). Firmness and TSS are two important qualitative factors in determining the maturity and harvest time of fruit that change during storage. Water loss occurs during long-term storage and leads to reduced economic benefits due to reduced fruit weight and leads to increased fruit shrinkage (Veraverbeke et al., 2003). Pectin is a complex polysaccharide composed mainly of polygalacturonic acid and it is an important compound in the cell wall that is usually dissolved when the fruit ripens (Acikgoz, 2011). One of the major problems during the marketing of quince cultivars is the enzymatic browning, which leads to postharvest physiological disorders. This physiological disorder is caused by pre-harvest and harvest conditions as well as storage conditions (Kuzucu & Sakaldas, 2008). Browning occurs due to polyphenol oxidase activity (Holderbaum et al., 2010). During enzymatic browning, phenolic compounds such as chlorogenic acid are oxidized to the quinone by polyphenol oxidase. Then quinone is converted to melanin by a non-enzymatic polymerization process, which results in the destruction of the fruit and the formation of yellow or brown pigments. Phenolic compounds are substrates of polyphenol oxidases (Awad & De Jager, 2000).

The quince fruit has a shelf life of more than three months (Gunes et al., 2012). According to the results of a study, cold storage of fruit significantly reduced fruit waste. Also, with the delay in harvesting time and increasing the storage period in the cold storage, fruit firmness decreased and the surface browning increased (Gunes, 2008). Depending on the cultivar or genotype, the quince fruit can be stored at a temperature of 2±1°C and relative humidity of 80-90% (Moradi et al., 2017).

With increasing cold storage period, fruit weight loss of the 'Gorton' quince cultivar increased. Prolonged harvesting time and increased storage period reduced the fruit firmness and increased the surface browning. In 'Gorton', most TSS was obtained in the second harvest and after 135 days of cold storage (Gunes, 2008). Fruit weight loss of 'Esme' quince cultivar increased after six months of storage with prolonged harvesting time. TSS in the third harvest increased and the TA decreased after six months of storage (Kuzucu & Sakaldas, 2008).

Fruits harvesting at the suitable time is one of the most important factors before harvest for reducing storage rot and fruit waste in the postharvest period, so determining the correct harvesting time is very important. In production areas of quince, production density occurs in October; therefore, it is necessary to store additional products (Kuzucu & Sakaldas, 2008). Due to the lack of widespread cultivation of quince in the world, the qualitative traits of this

fruit in storage and postharvest damage have not been extensively studied (Moradi et al., 2017). Considering that 'Behta' and NB4 are new genetic materials of quince in Isfahan province, it is necessary to determine the most appropriate harvesting time and storage period for them.

MATERIALS AND METHODS

Plant materials

This research was conducted in Isfahan Agricultural Research Station, Iran in 2018 and 2019. The experiment was carried out on 'Behta' cultivar and NB4 promising genotype as well as 'Isfahan' (control) that were grafted on hawthorn seedling rootstock. In April, the flowering time of cultivars and genotype was recorded and when 85-90% of the flowers opened, the time of full bloom was recorded separately for each cultivar and genotype so that the harvesting time could be reported based on the number of days after full bloom. Harvesting was done on 6^{th}, 14^{th}, and 21^{st} October. The fruits of each cultivar and genotype were randomly harvested from three trees (three replications) and transferred to the cold storage with a temperature of 0±1°C and relative humidity of 95%. At the harvesting time and also at intervals of one month and for five months, some quantitative and qualitative characteristics (as follows) of stored fruits were examined.

Evaluated traits

Three days after removing the fruits from storage and storing them at 20°C, the fruit surface browning was recorded. So that without browning or very low browning, low browning, medium browning, high browning, and very high browning were considered 0-10%, 10-30%, 50-30%, 70-50%, and 90-70%, respectively. To evaluate the percentage of decay in each replication, the average decay of fruits was observationally recorded. Each test plot was weighed before transfer to the cold storage and weighed again after that. By calculating the difference between primary and secondary weight, weight loss percentage was calculated in each test plot. To determine the fruit firmness, a penetrometer (model EFFEGI, Italy, plunger diameter 11.1 mm, depth 7.9 mm) was used and the applied force was recorded as kg/cm^2. Total soluble solids (TSS) was measured using an ATAGO N-1α refractometer made in Japan. Titratable acidity (TA) was reported by titration of extracted juice with sodium hydroxide (0.1 N) up to pH 8.1 and expressed as a percent of malic acid (Roussos et al., 2011). The taste index (TSS/TA) was obtained by dividing TSS by TA. The pectin in the samples was measured by the weighting method and by determining calcium pectinate (Thakur et al., 1996). The total phenol content of fruit juices was measured using the Folin-Sikalcho method (Singleton & Rossi, 1965). The absorbance of the samples was determined at 765 nm wavelength with spectrophotometer model T80 UV/Visible, then compared with the standard of gallic acid and expressed as mg gallic acid per 100 grams of fresh weight.

Statistics design

Obtained results were analyzed using a factorial experiment with tree factors (tree cultivars and genotype, three harvesting time, and duration of storage in six levels) based on a completely randomized design with three replications and 10 samples per replicate during two years. Due to the non-significance of Bartlett's test, a combined analysis was performed for an average of two years. For two traits, surface browning and decay percentage, data normalization was performed with the ArcSin formula using Excel software. Analysis of data was performed by ANOVA method using statistical software SAS (version 9.1) and mean comparisons using LSD.

RESULTS

Flowering time

Results showed that similar to 'Isfahan', 'Behta' and NB4 were also late flowering and had a good flowering overlap with 'Isfahan' (Table 1). Due to higher temperatures in 2019, flowering occurred earlier than in 2018.

The effect of treatments on the evaluated traits

According to Table 2, none of the evaluated traits were affected by year × cultivar × storage time × harvesting time. The effect of cultivar × harvesting time × storage period on weight loss, TSS, taste index, firmness, and total phenol content was significant. TA was affected by harvest time × cultivar and storage period × cultivar. The effect of harvest time × duration of storage on surface browning was significant. Storage period had a significant effect on the percentage of decay. Pectin content was not affected by the applied treatments. The effect of year was significant on some traits.

Table 1. Flowering time of quince cultivars and promising genotype in 2018 and 2019.

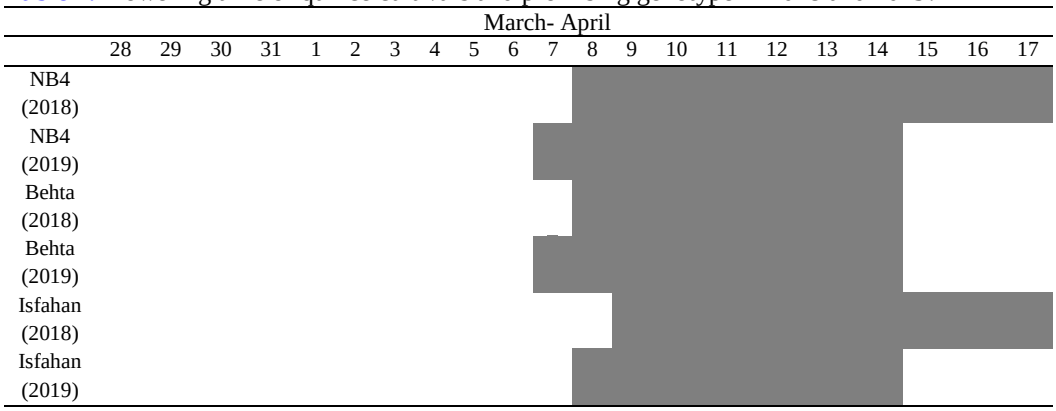

Table 2. Combined analysis of variance for effect of year, harvesting time, cultivar and duration of storage on measured characteristics.

Source of Variation	Degrees of freedom (df)	Traits				
		Weight loss (%)	TSS (%)	TA (%)	TSS/TA	Firmness (kg/cm^2)
Year	1	944.74**	705.19**	0.02*	1609.52**	0.31**
Replication (year)	4	30.06	28.24	0.04	174.96	1.17
Harvesting time	2	87.13**	287.90**	0.60**	4599.27**	4.61**
Cultivar	2	79.14**	188.12**	1.70**	6495.23**	23.48**
Storage period	5	2855.71**	511.85**	1.01**	4080.60**	27.94**
Harvesting time×Cultivar	4	1.85^{ns}	91.62**	0.02**	627.70**	0.44**
Harvesting time×Storage period	10	9.97*	70.12**	0.01^{ns}	168.63**	0.13**
Storage period×Cultivar	10	13.57**	97.46**	0.01**	48.55^{ns}	0.28**
Storage period× Harvesting time×Cultivar	20	6.82*	95.88**	0.006^{ns}	52.80*	0.09**
Harvesting time×Year	2	1.37^{ns}	0.37^{ns}	0.0001^{ns}	1.19^{ns}	0.0008^{ns}
Cultivar×Year	2	0.36^{ns}	0.19^{ns}	0.0001^{ns}	24.29^{ns}	0.0006^{ns}
Storage period×Year	5	118.46**	0.43^{ns}	0.0008^{ns}	16.00^{ns}	0.0006^{ns}
Cultivar× Harvesting time×Year	4	2.14^{ns}	0.56^{ns}	0.0001^{ns}	2.48^{ns}	0.0005^{ns}
Storage period× Harvesting time×Year	10	14.17**	2.10^{ns}	0.0007^{ns}	2.16^{ns}	0.0005^{ns}
Storage period×Cultivar×Year	10	3.53^{ns}	0.73^{ns}	0.0005^{ns}	3.06^{ns}	0.0006^{ns}
Storage ×Cultivar× Harvesting time×Year period	20	9.17^{ns}	2.06^{ns}	0.0002^{ns}	2.73^{ns}	0.0006^{ns}
Error	212	5.24	0.41	0.006	28.73	0.003
C.V.	-	23.89	4.30	13.02	19.35	1.77

Table 2. Continued. Combined analysis of variance for effect of year, harvesting time, cultivar and duration of storage on measured characteristics.

Source of Variation	Degrees of freedom (df)	Traits			
		Pectin (g/100 g)	Total phenol (mg/100 g (FW	Fruit decay (%)	Fruit browning (%)
Year	1	475.26^{ns}	16.46^{ns}	0.027^{ns}	0.00001^{ns}
Replication (year)	4	478.09	592.70	0.046	0.001
Harvesting time	2	492.96^{ns}	8368.48^{**}	0.026^{ns}	0.002^{*}
Cultivar	2	480.76^{ns}	25775.43^{**}	0.0008^{ns}	0.0006^{ns}
Storage period	5	502.60^{ns}	25301.50^{**}	0.13^{**}	0.004^{**}
Harvesting time×Cultivar	4	476.81^{ns}	439.83^{**}	0.0009^{ns}	0.00005^{ns}
Harvesting time×Storage period	10	480.58^{ns}	98.28^{**}	0.008^{ns}	0.009^{*}
Storage period×Cultivar	10	478.72^{ns}	1575.40^{**}	0.004^{ns}	0.0006^{ns}
Storage period× Harvesting time×Cultivar	20	479.28^{ns}	180.15^{**}	0.001^{ns}	0.0004^{ns}
Harvesting time×Year	2	479.67^{ns}	1.59^{ns}	0.009^{ns}	0.0000003^{ns}
Cultivar×Year	2	475.94^{ns}	1.37^{ns}	0.012^{ns}	0.00001^{ns}
Storage period×Year	5	478.38^{ns}	3.96^{ns}	0.012^{ns}	0.00005^{ns}
Cultivar× Harvesting time×Year	4	479.55^{ns}	2.69^{ns}	0.009^{ns}	0.00001^{ns}
Storage period× Harvesting time×Year	10	478.14^{ns}	2.33^{ns}	0.005^{ns}	0.00002^{ns}
Storage period×Cultivar×Year	10	478.82^{ns}	2.58^{ns}	0.007^{ns}	0.00003^{ns}
Storage period×Cultivar× Harvesting time×Year	20	477.84^{ns}	2.37^{ns}	0.007^{ns}	0.000009^{ns}
Error	212	478.18	14.43	0.007	0.0004
C.V.	-	18.93	8.87	15.82	4.21

Weight loss

In each cultivar and genotype, weight loss gradually increased with prolonged storage period and harvesting times (Table 3). The highest weight loss was related to the third harvest of 'Isfahan' in the fifth month of storage (21.71%). Under similar conditions, 'Behta' and NB4 had a weight loss of 20.54% and 20.2%, respectively. The value of this trait in 2018 was more than in 2019 (Table 4).

TSS and taste index

The highest percentage of TSS was obtained in the third harvest of 'Isfahan', so that TSS in this cultivar, four and five months after storage was 18.83% and 18.16%, respectively (Table 3). After that, 'Behta' in the third harvest and four months after storage had TSS equal to 17.83%. The lowest amount of TSS belonged to NB4. The amount of TSS in this genotype at the first harvest (October 6) was 10.16% and after one month of storage was 11.5%, which was equal to TSS in the first harvest of 'Behta'. In general, the third harvest and longer storage increased the percentage of TSS.

The highest taste index was 73.33, which belonged to 'Isfahan' that had been stored for five months in cold storage. Under similar conditions, the taste index in 'Behta' and NB4 were 48.19 and 41.25, respectively. NB4 at the first harvesting time and one month after storage showed the lowest taste index with averages of 9.5 and 12.2, respectively (Table 3). TSS and taste index in 2019 was significantly higher than in 2018 (Table 4).

Table 3. Mean comparison of cultivar, duration of storage and harvesting time on weight loss, TSS, firmness and total phenol ± SD in two years.

Cultivar	Harvesting time	Duration of storage (Month)	Weight loss (%)	TSS (%)	Taste index	Firmness (kg/cm^2)	Total phenol (mg/100 gFW)
NB4	1	At harvest	-	10.16±1.72	9.5±1.75	4.36±0.16	56.71±3.83
NB4	1	1	2.73±2.78	11.5±1.87	12.2±2.42	4.06±0.09	40.25±5.21
NB4	1	2	4.64±2.34	12.83±1.72	15.2±2.46	3.21±0.10	43.2±3.66
NB4	1	3	8.88±2.18	14.5±1.87	19.91±3.32	2.86±0.13	23.09±3.83
NB4	1	4	14.01±2.82	15.66±2.16	22.36±5.35	2.52±0.12	17.74±3.52
NB4	1	5	17.18±3.67	15.83±1.72	26.88±4.88	2.28±0.04	3.51±1.12
Behta	1	At harvest	-	11.5±1.87	12.84±2.22	4.95±0.11	33.99±3.69
Behta	1	1	4.23±2.59	11.83±1.72	14.76±3.22	4.69±0.09	35.82±4.82
Behta	1	2	6.42±2.05	13.16±1.72	19.35±2.50	4.47±0.09	25.52±11.67
Behta	1	3	12.81±4.32	15.16±1.72	23.26±4.71	4.06±0.16	21.57±4.93
Behta	1	4	16.06±4.43	15.83±1.72	27.54±4.98	3.69±0.11	11.53±4.58
Behta	1	5	16.65±4.99	16.16±1.72	30.5±3.98	3.27±0.14	2.68±0.66
Isfahan	1	At harvest	-	13.5±1.87	17.97±1.87	4.56±0.10	98.21±3.61
Isfahan	1	1	3.78±3.12	13.83±1.72	20.03±3.51	4.14±0.15	73.02±5.45
Isfahan	1	2	6.38±2.98	14.5±1.87	24.94±3.43	3.69±0.16	58.41±3.47
Isfahan	1	3	9.91±3.17	15.5±1.87	33.41±4.29	3.3l±0.16	32.44±5.27
Isfahan	1	4	12.84±3.50	15.83±1.72	33.47±6.86	2.89±0.12	11.64±3.91
Isfahan	1	5	18.77±5.54	16.33±1.86	45.27±8.24	2.46±0.14	5.37±2.14
NB4	2	At harvest	-	10.83±1.72	11.25±2.07	4.3±0.22	69.52±4.09
NB4	2	1	2.68±2.33	12.16±1.72	16.27±2.46	3.7±0.20	60.67±5.05
NB4	2	2	4.94±0.89	13.83±1.72	21.18±4.25	3.19±0.12	54.73±4.43
NB4	2	3	12.36±3.58	14.5±1.87	21.78±2.62	2.89±0.09	46.49±4.45
NB4	2	4	14.05±3.72	15.16±1.72	23.54±3.28	2.71±0.17	29.24±4.37
NB4	2	5	19.005±6.27	16.16±1.72	31.75±4.87	2.53±0.07	10.98±3.64
Behta	2	At harvest	-	12.83±1.72	14.88±2.45	4.87±0.18	46.76±4.59
Behta	2	1	4.51±2.17	14.16±1.72	19.08±3.21	4.47±0.20	42.5l±4.64
Behta	2	2	10.33±5.12	15.5±1.87	22.83±2.99	3.94±0.16	30.79±4.09
Behta	2	3	14.12±3.81	15.5±1.87	23.5±3.93	3.64±0.12	24.11±3.57
Behta	2	4	17.01±1.78	15.83±1.72	28.22±4.72	3.33±0.11	15.73±3.70
Behta	2	5	19.12±2.31	16.33±1.86	36.66±5.41	2.98±0.12	14.62±4.88
Isfahan	2	At harvest	-	13.16±1.72	21.53±3.19	4.51±0.09	103.32±5.38
Isfahan	2	1	4.06±2.03	13.5±1.64	24.75±2.94	4.09±0.20	80.61±3.84
Isfahan	2	2	6.64±3.27	15.16±1.72	28.72±4.70	3.55±0.18	73.01±5.48
Isfahan	2	3	11.94±3.08	16.16±1.72	37.66±5.40	2.88±0.20	59.37±4.05
Isfahan	2	4	15.64±5.35	16.83±1.72	37.83±5.76	2.35±0.12	40.59±6.16
Isfahan	2	5	18.09±4.73	10.83±1.72	33.19±7.53	2.11±0.18	12.69±3.51
NB4	3	At harvest	-	11.83±1.72	13.68±2.04	3.72±0.24	84.36±4.14
NB4	3	1	5.02±2.36	12.83±1.72	17.26±2.91	3.1±0.12	69.25±4.20
NB4	3	2	7.01±1.83	13.83±1.72	21.53±5.26	2.8±0.10	62±5.77
NB4	3	3	11.77±3.22	14.83±1.72	24.66±5.49	2.55±0.09	45.99±4.61
NB4	3	4	15.06±5.55	15.83±1.72	33.77±6.85	2.34±0.11	32.45±3.75
NB4	3	5	20.2±4.7	16.5±1.62	41.25±13.01	2.1±0.12	21.81±5.28
Behta	3	At harvest	-	15.83±1.72	20.77±3.05	4.57±0.10	47±4.71
Behta	3	1	5.36±1.81	16.16±1.72	22.76±3.29	4.39±0.13	45.97±4.84
Behta	3	2	9.63±2.30	16.83±1.72	25.43±3.81	4.005±0.08	32.24±4.39
Behta	3	3	14.49±3.63	17±1.54	28.37±5.79	3.73±0.15	25.76±3.96
Behta	3	4	18.22±5.38	17.83±1.72	38.74±9.72	3.34±0.20	19.98±4.55
Behta	3	5	20.54±3.47	17.5±1.64	48.19±4.92	2.88±0.15	15.54±3.80
Isfahan	3	At harvest	-	16.5±1.87	30.22±4.23	3.92±0.12	101.64±4.67
Isfahan	3	1	3.56±2.27	16.83±1.72	39.16±5.02	3.72±0.14	85.79±3.84
Isfahan	3	2	4.5±2.62	17.16±1.72	46.19±10.26	3.39±0.16	74.17±3.42
Isfahan	3	3	11.67±4.36	17.5±1.64	50.83±7.85	2.89±0.12	57.63±4.61
Isfahan	3	4	18.68±7.01	18.16±1.72	55.55±9.58	2.47±0.16	44.22±3.48
Isfahan	3	5	21.71±4.98	18.83±1.32	73.33±17.51	2.1±0.14	36.12±4.16
LSD (0.05)			0.85	0.09	0.46	0.007	0.43

Table 4. Mean comparison of year on weight loss, TSS, TA, taste index and firmness.

Year	Weight loss (%)	TSS (%)	TA (%)	Taste index	Firmness (Kg/cm^2)
2018	11.29a	13.41b	0.608b	25.47b	3.4b
2019	7.87b	16.36a	0.625a	29.93a	3.46a
LSD	1.69	0.81	0.06	4.08	0.33

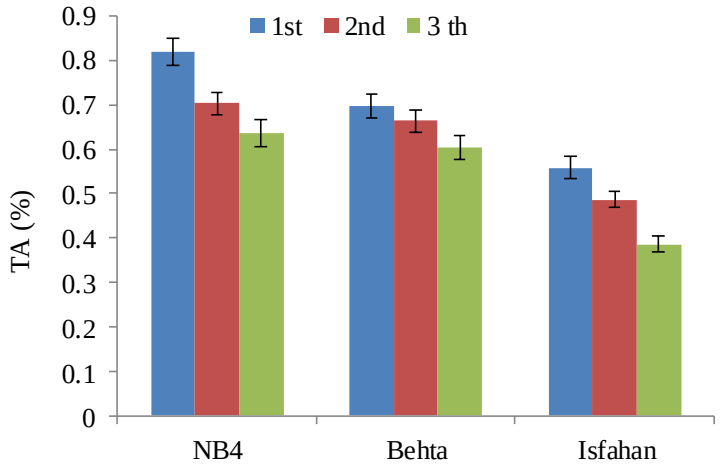

Fig. 1. Effects of cultivar and harvesting time on TA percentage.

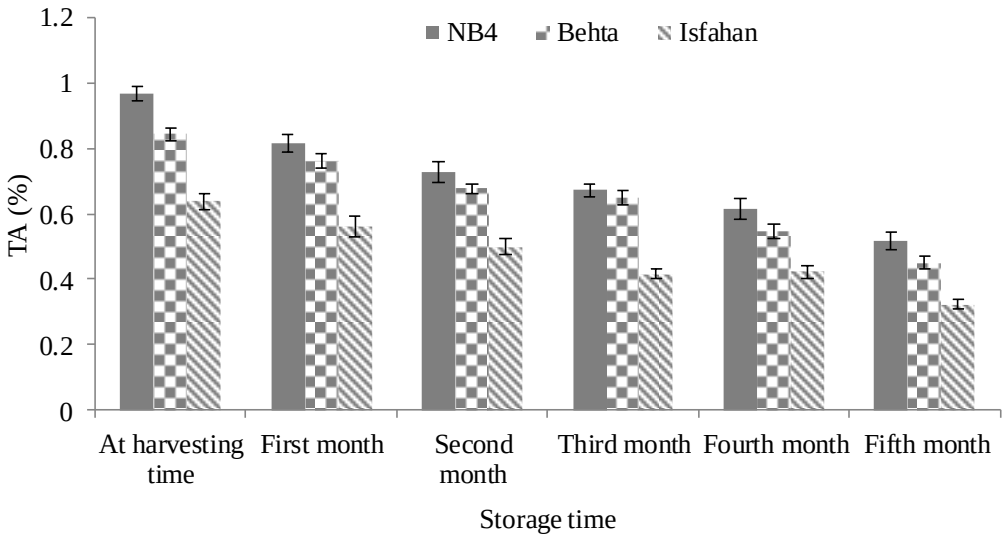

Fig. 2. Effects of cultivar and storage period on TA percentage.

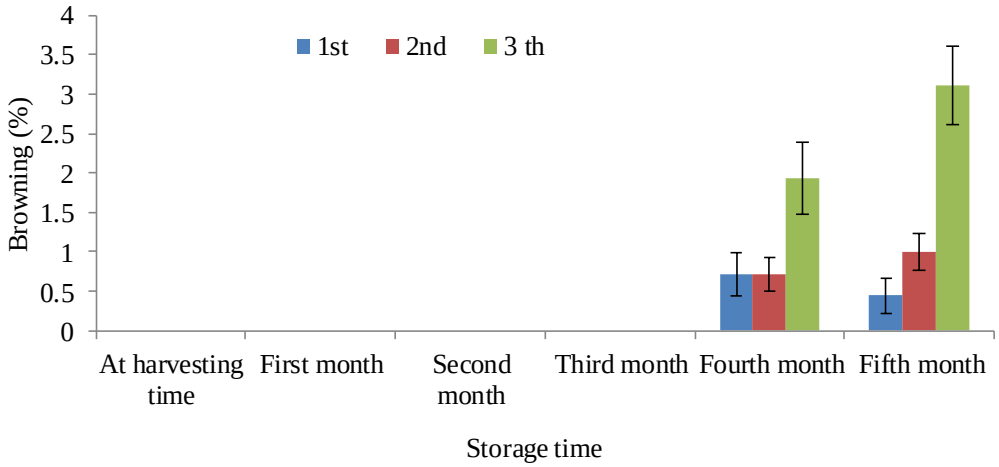

Fig. 3. Effects of storage period and harvesting time on browning percentage.

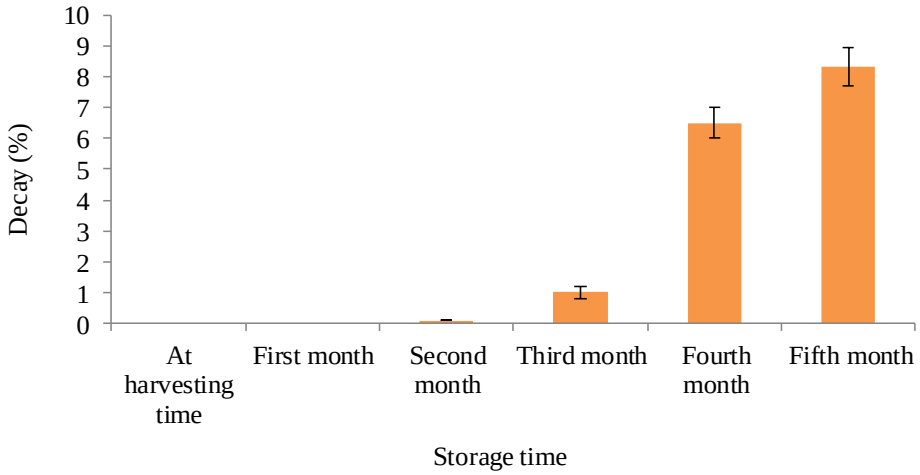

Fig. 4. Effects of storage period on decay percentage.

Firmness and total phenols

According to Table 3, the highest fruit firmness was at harvesting time. The highest firmness belonged to 'Behta' at the first and second harvesting time as well as one-month storage after the first harvest with averages of 4.95, 4.87, and 4.69 kg/cm^2, respectively. The lowest fruit firmness was related to 'Isfahan' and NB4 in the third harvest and after five months of storage (2.1 kg/cm^2). In general, delay in harvest and longer storage reduced the fruit firmness. Fruit firmness in the second year was significantly higher than the first year (Table 4).

Most of the total phenol content belonged to 'Isfahan'. The total phenol content in this cultivar at the second and third harvest was 103.32 and 101.64 mg/100g, respectively. The lowest total phenol belonged to 'Behta' in the first harvest and after five months of storage with an average of 2.68 mg/100gFW (Table 3).

The highest and lowest TA was observed in the first harvest of NB4 genotype (0.81%) and the third harvest of 'Isfahan' (0.38%), respectively. 'Behta' had a moderate level of TA in

all harvests compared to 'Isfahan' and NB4. In the studied two cultivars and one genotype, the amount of TA decreased with prolonged harvesting time (Fig. 1). The value of this trait in 2019 was more than in 2018 (Table 4).

The effect of cultivar and storage period on TA is shown in Figure 2. In all studied genetic material, TA decreased with increasing storage period. In all storage periods, NB4 and 'Isfahan' had the highest and the lowest TA, respectively and 'Behta' was in the middle.

Surface browning was affected by harvesting time and storage period (Fig. 3). No surface browning was observed until three months after storage. The amount of this trait started from the fourth month of storage. Harvested fruits in the first and second harvest and after the fourth and fifth months of storage did not have a significant difference in the percentage of fruit browning, but in the third harvest, the percentage of surface browning in fruits with 5 months storage was more than the amount of this trait in fruits with 4 months storage.

The effect of storage period on the percentage of fruit decay (Fig. 4) showed that for two months after storage, no decay was observed in the fruits. From the third month, decay occurred and reached its maximum in the fifth month.

DISCUSSION

Weight loss

Water loss during storage resulting in weight reduction that had a negative effect on fruit appearance (Pasquariello et al., 2013). In this research, weight loss increased with prolonged storage period and harvesting times. In agreement with the present study, other studies have also indicated an increase in water loss and a decrease in fruit weight with increasing shelf life. For example, with increasing cold storage period, fruit weight loss increased in 'Gorton' quince cultivar (Gunes, 2008). Also, the fruit weight loss of 'Esme' quince cultivar after six months of storage in the first, second and third harvest was 9%, 10.5%, and 11.5%, respectively (Kuzucu & Sakaldas, 2008). The rate of weight loss was different among studied genetic materials in this research (Table 3). Burdon and Klark (2001) stated that the difference in weight loss between different cultivars was due to differences in fruit storage conditions, the fruit minerals, and the ratio of fruit surface to fruit volume.

TSS and taste index

The soluble sugars (sucrose, fructose, and glucose) contents resulting from the hydrolysis of starch during ripening, are determined by evaluation of concentration of total soluble solids (Etienne et al., 2013). In general, the third harvest and longer storage increased TSS (Table 3). Similarly, Arzani and Mousavi (2008) showed that Asian pears had high levels of sucrose at harvest, which after storage, sucrose was converted to simpler sugars, and the percentage of TSS increased. It has been reported that the increase in TSS during storage is not only related to the accumulation of sugar but also the increase and decrease of other substances such as acids, soluble pectins, and phenolic compounds (Amodio et al., 2007). The quality characteristics of the fruit after harvest and during storage changes that are effectively affected by the cultivar (Moradi et al., 2017). In this study, TSS was significantly different among 'Behta', 'Isfahan', and NB4. Gorji Chakespari et al. (2010) also reported significant differences in TSS between 'Shafiabadi' and 'Golab Kohanz' apple cultivars (11.1% and 8.75%, respectively). The difference in TSS between these two apple cultivars was due to genetic differences and the effect of environmental conditions in which these cultivars grew. In the present study, the differences among 'Behta', 'Isfahan', and NB4 in addition to genetic differences can be due to the different origins of these plants. In another study, TSS in 'Isfahan' cultivar in the last harvest and after five months of storage was 16.20% and at the

first harvesting time was 14.75%, which is less than the values reported in the present study (Mosharraf & Ghasemi, 2004). Deficiency of water resources in recent years in Isfahan, Iran, which leads to an increase in the concentration of cell sap in tissues, can be the reason for the higher values of TSS in the current study.

In the genetic materials of this research, increasing the storage period and prolonging the harvesting time led to an increase in the taste index (Table 3). In another study, quality index and taste index increased with prolonging apples fruit storage (Ahmad et al., 2021).

Firmness and total phenols

Delay in harvesting time and prolonged storage reduced the fruit firmness (Table 3). Similarly, in the 'Yali' pear, the fruit firmness decreased during storage (Chen et al., 2006). In the 'Esme' quince, the fruit firmness in the third harvest decreased rapidly and after six months of storage reached 3 kg/cm^2. The highest firmness was observed in the first harvest, which was 12.5 kg/cm^2 (Kuzucu & Sakaldas, 2008). Fruit firmness depends on the structure and composition of the cell wall (Valero & Serrno, 2010). The fruit ripening and senescence leads to the dissolution of the middle septum and the loss of cell wall cohesion. The activity of hydrolyzing enzymes increases and the firmness of the fruit tissue reduces. Under these conditions, the susceptibility of the fruit to postharvest disorders depends on the degree of fruit maturity at harvesting time (Raese & Drake, 2000). On the other hand, the property of sucrose polysaccharides also causes firmness. During cold storage, climacteric fruits continue to ripen, and extensive changes are made by enzymes in the cell wall polysaccharides, and sucrose is converted to simpler sugars. Thus, with the ripening of the fruit, the amount of sucrose and firmness of the fruit is reduced (Jan & Rab, 2012). As mentioned, the studied fruits in this research had different firmness (Table 3). In other studies, differences in fruit firmness of apple cultivars have been reported. For example, the fruit tissue of 'Red Delicious' was much firmer than that of 'Golden Delicious'. The firmness of 'Gol Shahi' was higher than 'Red Delicious', 'Golden Delicious' and 'Abbasi' in the Khorasan region, Iran (Hoseini Farahi et al., 2008). The effect of harvesting time on fruit firmness after storage has also been reported by Konopacka and Plocharski (2002).

According to Table 3, the lowest total phenol was observed in the third harvest after five months of storage. Other researchers have shown that total phenol levels gradually decreased with the long-term storage of fruits (Gorji Chakespari et al., 2010; Castro-Lopez et al., 2016). The amount of phenol in fruits and vegetables after harvest can be reduced or increased, which depends on the harvesting time and storage conditions (Kalt, 2005). Phenylalanine aminolysis is one of the main enzymes in the production of phenolic compounds so that an increase or decrease in the activity of this enzyme can be associated with an increase or decrease in phenolic compounds (Lemoine et al., 2007).

TA, browning, and decay

With the prolongation of the harvesting time and storage period, TA decreased (Fig. 1 and 2). A large volume of fruit at the beginning of fruit development belongs to organic acids, so fruits have a high pH before ripening due to the presence of organic acids. With the fruit ripening, most of the organic acids are broken down or converted into other organic acids or sugars and increase the sweetness of the fruit (Hudina & Stampar, 2004). In the present study, there was no significant difference among the fruit of 'Behta', 'Isfahan' and NB4 in TA, but other researchers have reported differences in TA among different cultivars (Gorji Chakespari et al., 2010; Mosharaf & Ghasemi, 2004).

In this study, surface browning was affected by harvesting and storage period (Fig. 3). Browning started in the fourth month of storage and increased in the fifth month. Contrary to

the findings of this study, in the studied quince collection by Abdollahi (2012), surface browning was observed in some fruits after two months of storage. According to his report, 20 to 30% of the fruits after a few months turned brown. Surface browning in the third harvest of 'Esme' quince cultivar was higher than the previous harvests and eventually reached 70% (Kuzucu & Sakaldas, 2008). Arzani and Mousavi (2008) reported that increased levels of sugars and organic acids delay fruit browning. Therefore, higher TSS in the fruit of genetic materials can delay browning in this study.

The results of the present study did not show any difference in the percentage of browning among the fruit of 'Behta', 'Isfahan' and NB4, but in the studied cultivars and genotypes by Abdollahi (2012), there were significant differences in the rate and severity of browning in storage. So that SVS1 and SVS2 quince genotypes from Isfahan showed great sensitivity to browning. It seems that the significant difference in the results is due to differences in studied genetic materials in the present study compared to 40 cultivars and genotypes studied by Abdollahi (2012) as well as differences in the status of mother trees and storage conditions.

The effect of storage period on the decay percentage (Fig. 4) showed that decay occurred from the third month and reached its highest rate in the fifth month. Evaluation of quince genotypes in different parts of Iran showed that produced fruits in wetter regions have more fruit decay, while produced quince fruits in drier areas have smoother skin and are more marketable. This indicates that the quince tree is more compatible with lowland areas with semi-arid climates (Abdollahi, 2012).

More heat and less humidity in 2019 than in 2018 led to the production of smaller fruit and less fruit water content in the second year. Decreasing fruit water content in 2019 caused less weight loss and firmer fruit this year. An increase in the concentration of cell sap in tissues in the second year can be the reason for the higher values of TSS and taste index in 2019 (Table 4).

According to the mentioned results and the study of TSS, TA and other traits of 'Behta', 'Isfahan' and NB4 as well as considering that the number of days after full bloom is an important indicator to determine the fruit ripening, the best harvesting time for 'Isfahan', 'Behta' and NB4 is the third harvest (21th October). In this study, 193 days after full bloom is the best time to harvest for 'Isfahan', 'Behta', and NB4. Flowering time may change every year depending on environmental conditions, especially temperature, but the period of fruit growth (number of days from full bloom to ripening) is almost constant for each cultivar. Other researchers have used the number of days after full bloom for the determination of harvesting time of different quince cultivars in different areas. For example, Gunes (2008) reported that the appropriate harvesting time for 'Gorton' in Mashhad, Iran was 191 days after the full bloom stage. Mosharraf and Ghasemi (2004) also reported the best harvesting time for 'Isfahan' was 180 days after flowering and the most desirable period for storage of this cultivar was five months after storage.

Although all studied fruits with prolonged storage showed a higher taste index, in the last month of storage, they were soft and had an undesirable taste due to storage. So, storing these fruits is not recommended for more than four months. In this time antioxidant properties and total phenolic content will reduce as well as surface browning and decay will increase.

CONCLUSION

According to the results, the best harvesting time for 'Isfahan', 'Behta', and NB4 was 193 days after full bloom. Cold storage for four months is advisable for these fruits.

Conflict of interest

The author has no conflict of interest to report.

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Effect of moisture content on the engineering properties of African yam bean (*Sphenostylis stenocarpa*) seed

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ABSTRACT

Purpose: This work was carried out to investigate the effect of moisture content on the engineering properties of African Yam Bean (*Sphenostylis stenocarpa*) seeds. **Research method:** Physical and mechanical properties of African Yam Bean seeds were evaluated. The physical and mechanical properties were evaluated at five moisture content levels of 8, 12, 16, 20 and 24% dry basis (d.b). **Findings:** From the physical properties, the average length, width, thickness, arithmetic mean diameter, geometric mean diameter and equivalent diameter were found to increase significantly (P≤0.05) with an increase in moisture content. The surface areas, volume, sphericity, aspect ratio, flakiness ratio, porosity, static angle of repose, dynamic angle of repose and static coefficient of friction of the seeds were also found to increase significantly (P≤0.05) with increasing seed moisture content. The mean results of seed densities showed a decrease in bulk density (0.840±0.045 to 0.806±0.074 g/cm^3) and true density (1.268±0.083 to 1.238±0.079 g/cm^3) with the increase in seed moisture content from 8% to 24 % (d.b). From the mechanical properties, the mean force, deformation, strain and energy for African yam bean seeds decreased significantly (P≤0.05) with the increase in moisture content. The mechanical properties evaluated showed a decrease in the force at peak (176.564±20.137 to 104.860±9.814 N), force at yield (168.548±24.049 to 84.694±53.464 N) and force at break (172.880±19.506 to 96.986±14.536 N) with the increase in seed moisture content from 8% to 24 % (d.b). **Research limitations:** No limitation to the report, yet, the study cannot claim to have exhausted all factors that influence engineering properties of African Yam Seed; thus, effects of other factors such as accessions, varieties, cultivars and species is recommended for further studies. **Originality/Value:** The results provide relevant data on efficient process handling and equipment design of the seeds to the engineers and designers.

INTRODUCTION

African yam bean (*Sphenostylis stenocarpa*) is an annual grain legume and has a pattern of growth similar to those of other grain legumes (Ameh, 2003). African yam bean is cultivated mainly for home consumption and only about 30% of the dry grain produced is sold (Osuagwu & Nwofia, 2014). It is a good source of protein, energy and the most culturally and economically important of the seven species in the genus *Sphenostylis* (Ojukwu *et al*., 2012). It is cultivated in South-Eastern Nigeria for its edible seeds, but cultivated in Central African Republic, Zaire, East Africa and Ethiopia for its tubers. Nutritionally, the African yam beans seed contains 62.6% carbohydrates, 21-29% protein and 2.5% fat. The grain is also high in Sulphur-containing amino acids (Ojukwu et al., 2012). In areas where it is grown for its seeds, the African yam bean has become an important substitute for animal protein and more widely-eaten cowpea (Ameh, 2007). Like cowpea, the seeds are contained in a pod, with each pod containing between ten and thirty seeds. The seeds of the African yam bean have a brown, black, white, grey or speckled bean-shaped appearance (Asoiro & Ani, 2011). The crop helps agriculturally to enrich the soil by its ability to fix nitrogen from the atmosphere. Studies have shown that the underutilized legumes are highly nutritious and are used as food, cover crops, green manure and natural fertilizers (Klu et al., 2000).

In Nigeria, the consumption of African yam bean seeds is restricted to the harvesting period, after which their availability in the rural market becomes scarce, as they are not cultivated in large quantities and their uses are limited (Ojukwu et al., 2012). This trend is not unconnected with the fact that there is hardly any food processor of African yam bean that possesses mechanized equipment for its harvesting, handling or processing. Most subsistence and small-scale producers perform the key operations manually which yields a product with poor quality and low nutrients. The design and fabrication of mechanical systems and associated equipment for handling, harvesting, processing, moving, aeration and storing the African yam bean seeds will be predicated on the determination of the engineering properties of the seeds. Asoiro and Ani (2011) investigated and reported the various post-harvest physical properties of African yam beans (*Sphenostylis stenocarpa*). The major diameter, intermediate diameter, minor diameter, and geometric mean diameter were determined as 8.1778 cm, 6.712 cm, 6.3025 cm and 7.0128 cm, respectively. The sphericity indicated that the bean shape (0.85933) is close to a sphere. The surface area and specific surface area were 77.404 cm^2 and 169.709 cm^2/cm^3, respectively. The static coefficient of friction on three different material surfaces varied from 0.114 to 0.196 on asbestos, from 0.097 to 0.1997 on aluminum, and from 0.1534 to 0.2049 on plywood. The angle of repose which was by the emptying method was 23.7750. The solid volume, bulk volume, solid density, bulk density, seed mass and porosity were 0.2387 cm^3, 7.6552 cm^3, 1.0179 g/cm^3, 1.0036 g/cm^3, 0.2362 g and 1.6805%, respectively. The moisture content varies from the range of 2.84% wb to 3.13% wb or a range of 2.93% d.b to 3.23% d.b. The data so generated there, because few researchers have worked on this study area, would be handy to overcome some of the handicaps which may be faced in the fabrication process of processing equipment.

The engineering properties of various agricultural products need to be understood and are very important to the design of machine structure, process and control. The engineering properties are physical, mechanical, thermal, electrical, optical, aerodynamic and hydrodynamic properties. All these properties are very useful in handling, storage, processing, preservation, quality evaluation distribution and marketing of crops. But in the course of this research, only the physical and mechanical properties were considered. To design equipment used in planting, storage, transportation, harvesting, processing, and oil extraction of agricultural oil seeds, it is necessary to know various physical and mechanical properties

(Bamgboye & Adebayo, 2012). The physical and mechanical properties are important in the sizing, separating, grinding, and oil extraction machines. As the true density, bulk density and porosity are used in the design of storage bins and silos, separation of desirable materials from impurities, cleaning and grading and quality evaluation of the products. The static friction coefficient of the grinding against the various surfaces is also necessary for designing conveying, transportation and storage structures. Moreover, moisture content, volume and density play important roles in numerous technological processes and in evaluating product quality during drying, and also in the design of silo and other storage structures (Olaniyan & Oje, 2002).

African yam bean seeds have a wide range of applications and have great potential. There is little information on the basic physical and mechanical properties of the seeds, which is an identified problem in the development of a new method of handling and processing the seeds. There is no equipment specifically designed and used in handling and processing African yam bean seeds. This is probably due to the lack of relevant data and information on the physical and mechanical properties of the seeds with different moisture contents. Therefore, this study aims to determine the effect of moisture content on some engineering properties of African yam bean seeds locally grown in Taraba State, Nigeria.

MATERIALS AND METHODS

Sample preparation

Fresh samples of the seeds were obtained from a local market in Jalingo, Taraba State, Nigeria. The seeds were manually clean to remove foreign materials and dirt. Hundreds of seeds were randomly selected for various experiments and conditioned to different moisture contents and their physical and mechanical properties were determined.

Moisture content determination

The initial moisture content (7.8% dry basis) of the seeds was determined using the standard hot air oven method at 105 ^{o}C±1 ^{o}C for 24 hours till there were no more changes in the weight. The initial moisture content (Dry basis) was obtained using Equation 1 (Mirzabe et al., 2016).

$$M_c = \frac{W_1 - W_2}{W_2} \quad (1)$$

Where; M_c = Moisture content (%), W_1 = Weight of seed before oven drying (g), W_2 = Weight of seed after oven drying (g).

Variation of moisture content

The samples (7.8% dry basis) were transferred to separate polythene bags and reconditioned to moisture content levels of 8%, 12%, 16%, 20% and 24%. A calculated amount of distilled water was added to each sample and the bags were sealed tightly. The samples were refrigerated for a week to enable the moisture to distribute uniformly throughout the samples. The prepared samples were then taken out of the refrigerator and placed at room temperature for about 2 hours. The samples of the preferred moisture contents were prepared by adding the pre-determined quantity of distilled water by using Equation 2 (Hazbavi, 2013; Audu et al., 2020).

$$Q = \frac{W_i(m_f - m_i)}{100 - m_f} \quad (2)$$

Where; Q = Mass of distilled water to be added (g), W_i = Initial mass of sample (g), m_i = Initial moisture content of the sample in dry basis (%), m_f = Final moisture content of the sample in dry basis (%).

Determination of physical properties

The physical properties of African yam bean seeds determined include; geometrical properties (size and shape), gravimetric properties and frictional properties.

Determination of size

A Mitutoyo absolute digimatic vernier caliper with 0.001 mm accuracy was used to measure the Length (major diameter), Width (intermediate diameter) and Thickness (minor diameter) of the seeds. The average of each measurement was taken as the reading for each of the samples (Dauda et al., 2015; Balami et al., 2016).

Arithmetic mean diameter (Da)

The arithmetic mean diameter of the African yam bean seed was determined from the Length (L), Width (W) and Thickness (T) using the relationship in Equation (3) as reported by Hazbavi (2013).

$$D_a = \frac{L+W+T}{3} \quad (3)$$

Geometric mean diameter (D_g)

The geometric mean diameter of the African yam bean seed was determined from the Length (L), Width (W) and Thickness (T) using the relationship in Equation (4) as reported by Hazbavi (2013).

$$D_g = (LWT)^{\frac{1}{3}} \quad (4)$$

Equivalent diameter (D_e)

The equivalent diameter of the African yam bean seed was determined from the Length (L), Width (W) and Thickness (T) using the relationship in Equation (5) as reported by Mirzabe *et al*. (2016).

$$D_e = \left[\frac{(T+W)^2}{4} L\right]^{\frac{1}{3}} \quad (5)$$

Surface area (S_a)

Surface area is defined as the total area over the outside of the African yam bean seed. The surface area was determined by analogy using Equation 6 (Hazbavi, 2013).

$$S_a = \frac{\pi B L^2}{2L-B} \quad (6)$$

But, $B = (WT)^{0.5}$

Where; S_a = Surface area (mm^2), L = Length (mm), W = Width (mm), T = Thickness (mm).

Specific surface area (S_S)

The specific surface area of African yam bean seed (S_S) in cm^2/cm^3 was calculated using Equation 7 according to Idowu et al. (2012).

$$S_s = \frac{S \times \rho_b}{M} \qquad (7)$$

Where; S_s = Specific surface area (cm^2/cm^3), S = Surface area (cm^2), ρ_b = Bulk density of seeds (g/cm^3), M = Mass of one unit of seed (g).

Frontal area (F_a)

The frontal area and the related diameters are essential for the determination of terminal velocity, Reynold's number and drag coefficient. The frontal area was obtained using Equation 8 given by Idowu et al. (2012).

$$F_a = \frac{\pi}{4}(D_g)^2 \qquad (8)$$

Transverse surface area (A_t)

Transverse surface was calculated from Equation 9.

$$A_t = \frac{\pi WT}{4} \qquad (9)$$

Projected area (A_P)

The flat surface or projected area was determined from Equation 10.

$$A_P = \frac{\pi WL}{4} \qquad (10)$$

Volume of seeds (V)

The volume of seeds was calculated using Equation 11 (Abano & Amoah, 2011; Hazbavi, 2013).

$$V = \frac{\pi B^2 L^2}{6(2L-B)} \qquad (11)$$

But, $B = (WT)^{0.5}$

Determination of shape

Sphericity

This is a method to measure how close the material is to a sphere. The sphericity (Ø) of African yam bean seed was calculated by using the values of the length, width and thickness of the seed from the expression in Equation 12 (Balami et al., 2016).

$$\emptyset = \frac{(LWT)^{\frac{1}{3}}}{L} \qquad (12)$$

***Aspect* ratio**

The aspect ratio (R_a) of African yam bean seed at a natural flat position was calculated by using the following equations according to Werby and Mousa (2016).

$$R_a = \frac{W}{L} \qquad (13)$$

***Flakiness* ratio**

The flakiness ratio (F_r) is the ratio of the thickness to the width of a particle and was calculated by using the following Equation according to Mirzabe et al. (2016).

$$F_r = \frac{T}{W} \qquad (14)$$

***Elongation* ratio**

Elongation ratio (E_r) is the ratio of the effective length to the width of a particle. It was calculated from Equation 15 (Mirzabe et al., 2016).

$$E_r = \frac{L}{W} \qquad (15)$$

Determination of gravimetric properties

Thousand seed mass

Hundreds of seeds weight was measured by counting 100 seeds and then weighed in the digital weighing balance of 0.001 g accuracy. The resulting value was multiplied by 10 to give the mass of 1000 seeds (Hazbavi, 2013).

Bulk density

The bulk density of African yam bean seed was determined by pouring the seed into a container of known weight and volume. The content was weighed with a digital balance with a sensitivity of 0.001 g and the bulk density was calculated using Equation 16 (Hazbavi, 2013).

$$\rho_b = \frac{W_s}{V_b} \qquad (16)$$

Where; ρ_b = Bulk density (g/cm^3), W_s = Weight of the sample (g), V_b = Bulk volume occupied by the sample (cm^3).

True density

The true density was determined according to the method described by Hazbavi (2013). The true volume of the seeds was determined using the liquid (kerosene) displacement method. Kerosene was poured into a measuring cylinder of 1000 cm^3 to one-half of its volume. Pre-weighed African yam bean seeds were filled inside the cylinder and the change in the level of kerosene in the measuring cylinder was recorded. The mass of each seed was obtained by using an electronic balance with a sensitivity of 0.001 g. The true density was calculated by using the relationship in Equation 17 (Hazbavi, 2013).

$$\rho_t = \frac{M_s}{V_t} \qquad (17)$$

Where; ρ_t = True density (g/cm^3), M_s = Mass of seed (g), V_t = True volume (cm^3).

Porosity

The Porosity of the bulk seed was computed from the values of the true density and bulk density of the seeds by using the relationship adopted by Hazbavi (2013).

$$P = \left(1 - \frac{\rho_b}{\rho_t}\right) \times 100 \qquad (18)$$

Where; P = Porosity, ρ_b = Bulk density, ρ_t = Solid density.

Determination of frictional properties

Static coefficient of friction

The static friction coefficient of African yam bean seeds was determined on five different surfaces (plywood, glass, plastics, stainless steel and mild steel) for all the samples. A cylinder of the height of 50 mm and diameter of 50 mm open at both the top and bottom was filled with the seeds after placing on an adjustable tilting surface. The cylinder was raised slightly so as not to touch the surfaces. The structural surface with the cylinder on its top was gradually raised until the cylinder just started to slide down. The angle of the surface made with the horizontal was taken. The friction coefficient (μ) was obtained using Equation (19) by finding the tangent of the angle (Hazbavi, 2013).

$$\mu = \tan \theta \qquad (19)$$

Where; μ = Static coefficient of friction, θ = Angle of inclination (degrees).

Static angle of repose

Flow-ability of African yam bean seeds was measured using the angle of repose that will be useful in material handling equipment. The static or filling angle of repose with the horizontal at which the material will stand when piled was determined using the open-ended cylinder method. A topless and bottomless cylinder of 5 cm diameter and 7 cm height was placed at the centre of a raised circular plate, having a diameter of 35 cm. The cylinder was filled with bean seeds and raised slowly until it formed a seed cone on the circular plate. The height and base (diameter) of the cone were measured. The static angle of repose was determined using Equation 20 (Galedar et al., 2010; Hazbavi, 2013; Aliyu et al., 2017).

$$\theta_s = \tan^{-1}\left(\frac{2H}{D}\right) \qquad (20)$$

Where; θ_s = Static angle of repose of the seed cone (Degrees), H = Height of cone formed (mm), D = Diameter of cone formed (mm).

Dynamic angle of repose

To determine the dynamic or emptying angle of repose, a fibre glass box of 20 × 20 × 20 cm, having a removable front panel was used. The box was filled with the African yam bean seed samples at the moisture content being investigated, and then the front panel quickly slid upwards allowing the samples to flow out and assume a natural heap. The dynamic angle of repose (θ_d) was obtained from measurements of the height of samples at two points (H_1 and H_2) in the sloping African yam bean seeds heap and the horizontal distance between two points (X_1 and X_2) using Equation 21 (Galedar et al., 2010).

$$\theta_d = \tan^{-1}\left[\frac{(H_2 - H_1)}{(X_2 - X_1)}\right] \qquad (21)$$

Determination of mechanical properties

Mechanical properties of African yam bean seeds were determined using the Universal Testing Machine. This machine has three main components, which are a stable forced and moving platform, a driving unit (AC electric motor, electronic variator and reduction unit) and a data acquisition (load cell, PC card and software) system. The machine was equipped

with a load cell of 500 N at a compressive rate of 25 mm/min. The test type conducted was compression test and the parameters tested include: force at peak, deformation at peak, strain at peak, force at yield, deformation at yield, strain at yield, force at break, deformation at break, strain at break, energy at peak, yield and break.

Experimental design and statistical analysis

The experimental design for the statistical analysis follows a One-treatment effect (moisture content) in a Completely Randomized Design (CRD) with ten observations (replications) per experimental unit. All data collected were compared using One-way analysis of variance (ANOVA) at $P \leq 0.05$ and treatment means were separated using the F-LSD at $P \leq 0.05$. All the data were analyzed using the SPSS statistical software.

RESULTS AND DISCUSSION

Effects of moisture content on physical properties

Table 1. Results of the physical properties of African yam bean seeds.

Properties	Moisture content				
	8%(db)	12%(db)	16%(db)	20%(db)	24%(db)
Geometrical Properties					
Size					
Length (mm)	7.603±0.66	8.211±0.42	8.237±0.28	9.152±0.81	9.722±0.35
Width (mm)	5.755±0.56	6.415±0.46	6.712±0.73	8.108±0.76	8.681±0.69
Thickness (mm)	4.705±0.14	5.706±0.26	6.102±0.44	7.380±0.58	7.990±0.27
Arithmetic Mean Diameter (mm)	6.021±0.81	6.777±0.93	7.017±0.76	8.213±0.55	8.798±0.68
Geometric Mean Diameter (mm)	5.905±0.43	6.698±0.38	6.961±0.61	8.181±0.66	8.769±0.27
Equivalent Diameter (mm)	5.925±0.33	6.706±0.42	6.967±0.15	8.187±0.55	8.774±0.21
Surface Area (mm^2)	94.475±6.32	123.552±5.84	135.406±8.55	192.598±6.05	222.476±4.63
Specific Surface Area (cm^2/cm^3)	3.354±0.85	4.185±0.97	4.497±0.64	5.887±0.77	6.580±0.92
Frontal Surface Area (mm^2)	27.383±3.32	35.240±2.88	38.061±4.21	52.571±5.11	60.396±4.33
Transverse Surface Area (mm^2)	21.266±3.18	28.749±3.46	32.167±2.85	46.996±4.63	54.476±6.08
Projected Area (mm^2)	34.365±2.87	41.370±3.56	43.422±5.70	58.280±6.13	66.285±4.24
Volume (mm^3)	81.935±3.21	124.584±5.33	144.427±4.86	248.305±6.15	308.810± 9.26
Shape					
Sphericity	0.777±0.073	0.816±0.053	0.845±0.092	0.894±0.069	0.902±0.085
Aspect Ratio	0.757±0.052	0.781±0.019	0.815±0.073	0.886±0.025	0.893±0.066
Flakiness Ratio	0.818±0.026	0.889±0.041	0.909±0.038	0.910±0.021	0.920±0.054
Elongation Ratio	1.321±0.212	1.280±0.091	1.227±0.104	1.129±0.124	1.120±0.214
Gravimetric Properties					
Thousand Seed Mass, M1000 (g)	236.6±7.81	245.9±9.24	248.7±5.96	266.3±6.88	272.5±5.78
Bulk Density, ρ_b (g/cm^3)	0.840±0.045	0.833±0.057	0.826±0.078	0.814±0.063	0.806±0.074
True Density, ρ_t (g/cm^3)	1.268±0.083	1.260±0.097	1.252±0.068	1.243±0.088	1.238±0.079
Porosity, P (%)	33.75±3.55	33.89±2.72	34.03±4.17	34.51±2.30	34.89±3.80
Frictional Properties					
Angle of Repose (θ)					
Static Angle of Repose (Deg.)	5.597±1.26	5.622±0.85	5.824±1.32	5.878±1.05	5.962±0.97
Dynamic Angle of Repose (Deg.)	22.132±2.16	23.006±1.95	23.775±3.17	24.502±2.31	25.212±4.08
Coefficient of Static Friction (μ) on Various Structural Surfaces					
Glass	0.249±0.043	0.264±0.061	0.277±0.017	0.291±0.041	0.295±0.091
Stainless Steel Sheet	0.268±0.012	0.273±0.027	0.281±0.014	0.294±0.036	0.298±0.037
Mild Steel Sheet	0.298±0.026	0.315±0.052	0.319±0.023	0.328±0.072	0.334±0.054
Wood	0.325±0.033	0.341±0.058	0.356±0.025	0.360±0.035	0.365±0.019
Plastic Sheet	0.342±0.030	0.353±0.039	0.359±0.044	0.363±0.083	0.366±0.038

A summary of the results obtained for the physical properties of African yam bean seed at different moisture content is shown in Table 1. As it can be seen, the average length (L), width (W), thickness (T), arithmetic mean diameter (D_a), geometric mean diameter (D_g) and equivalent diameter (D_e) was found to increase with the increase in moisture content. With increasing moisture content from 8% to 24% (d.b), the length (L), width (W), thickness (T), arithmetic mean diameter (D_a), geometric mean diameter (D_g) and equivalent diameter (D_e) of the seeds increased significantly ($P \leq 0.05$) from 7.603±0.66 to 9.722±0.35 mm, 5.755±0.56 to 8.681±0.69 mm, 4.705±0.14 to 7.990±0.27 mm, 6.021±0.81 to 8.798±0.68 mm, 5.905±0.43 to 8.769±0.27 mm and 5.925±0.33 to 8.774±0.21 mm, respectively. The increase in dimensions could be attributed to the expansion of the seeds as a result of moisture absorption in the intracellular spaces inside the seeds (Sologubik et al., 2013). These dimensions are important in determining the aperture size of machines, particularly for the separation of different materials. The major axis is indicative of the natural rest position of the material and hence in the application of compressive force to induce mechanical fracture. Also, this dimension will be useful in applying shearing force during slicing (Owolarafe & Shotonde, 2004). The average values obtained from this study compares satisfactorily with that reported by Asoiro and Ani (2011).

The surface areas and volume of the seeds were also found to increase with the increase in moisture content. With increasing moisture content from 8% to 24 % (d.b), the surface area (S), specific surface area (S_S), frontal surface area (F_a), transverse surface area (A_t), projected area (A_P) and volume (V) of the African yam bean seeds increased significantly ($P \leq 0.05$) from 94.475±6.32 to 222.476±4.63 mm^2, 3.354±0.85 to 6.580±0.92 mm^2, 27.383±3.32 to 60.396±4.33 mm^2, 21.266±3.18 to 54.476±6.08 mm^2, 34.365±2.87 to 66.285±4.24 mm^2 and 81.935±3.21 to 308.810± 9.26 mm^3, respectively. The increase in surface areas and volume could be attributed to the expansion of the seeds as a result increase in dimensions.

The average values of sphericity, aspect ratio and flakiness ratio increase with increasing seed moisture content while the elongation ratio decreased with increasing seed moisture content. With increasing moisture content from 8% to 24 % (d.b), the sphericity, aspect ratio and flakiness ratio of the African yam bean seeds increased significantly ($P \leq 0.05$) from 0.777±0.073 to 0.902±0.085, 0.757±0.052 to 0.893±0.066 and 0.818±0.026 to 0.920±0.054, respectively. Generally, in the present study, the African yam bean seed is treated as an equivalent sphere. Considering the high aspect ratio (which relates the seeds width to length) and sphericity, it may be deduced that African yam bean seeds would roll on flat surfaces. This tendency to either roll or slide is very important in the design of hoppers, dehulling and thresher equipment for the seed because most of flat seeds slide easier than spherical seeds, which roll on structural surfaces (Sharma et al., 2011). Furthermore, the shape indices indicated that the African yam bean seed may be treated as a sphere for an analytical prediction of its drying behavior.

The mass of 1000 seeds was found to increase from 236.6±7.81 to 272.5±5.78 g as moisture content increased from 8% to 24% (d.b). This parameter is useful in determining the equivalent diameter that can be used in the theoretical estimation of seed volume and in cleaning using aerodynamic forces. The mean values of the bulk density and true density were found to decrease significantly ($P \leq 0.05$) with the increase in seed moisture content. With increasing moisture content from 8% to 24 % (d.b), the bulk density decreased from 0.840±0.045 to 0.806±0.074 g/cm^3 and the true density decreased from 1.268±0.083 to 1.238±0.079 g/cm^3. The decrease in seed densities with the increase in moisture content shows that the increase in mass resulting from the moisture gain of the sample is lower than the accompanying volumetric expansion of the seeds (Sologubik et al., 2013). Based on the true density value, there is a tendency for African yam bean seeds to be partially submerged

in water. These properties may be useful in the separation and transportation of the seed by hydrodynamic means. The porosity of African yam bean seed increased from 33.75±3.55 to 34.89±3.80% with an increase in moisture content from 8% to 24% (d.b). This could be attributed to the expansion and swelling of seeds that might have resulted in more voids space between the seeds and increased the bulk volume. This is also exhibited in the reduction of bulk density with an increase in moisture content.

The static and dynamic angle of repose were found to increase significantly ($P \leq 0.05$) with increasing seed moisture content. With increasing moisture content from 8% to 24 % (d.b), the static angle of repose increased from 5.597±1.26 to 5.962±0.97° and the dynamic angle of repose increased from 22.132±2.16 to 25.212±4.08°. At higher moisture content seeds might tend to stick together due to the plasticity effect (stickiness) over the surface of the seeds resulting in better stability and less flow ability thereby increasing the angle of repose. The angle of repose is of paramount importance in designing hopper openings, side wall slopes of storage bins and bulk transporting of seeds using chutes (Irtwange & Igbeka, 2002). Therefore, the moisture content of seeds should be taken into account while designing such types of equipment and structures.

The static coefficient of friction of the seed was found to increase significantly ($P \leq 0.05$) as moisture level increased for all contact surfaces. The static coefficient of friction increased from 0.249±0.043 to 0.295±0.091, 0.268±0.012 to 0.298±0.037, 0.298±0.026 to 0.334±0.054, 0.325±0.033 to 0.365±0.019 and 0.342±0.030 to 0.366±0.038 for glass, stainless steel, mild steel, wood and plastic sheet, respectively, as the moisture content increases from 8% to 24% (d.b). This is due to the increased adhesion between the seed and the material surfaces at higher moisture values. The design and the dimension of hoppers, bunker silos and other bulk solid storage and handling structures should ensure non-arching (avoid stoppage of the flow of bulk solids) phenomena. The coefficient of mobility represents the freedom of motion of a substance and is inversely related to the coefficient of friction (tangent of angle of internal friction) (Irtwange & Igbeka, 2002). The higher the coefficient of friction the lower the mobility coefficient hence requiring a larger hopper opening, larger hopper side wall slope and a steeper angle of inclination in inclined grain transporting equipment like chutes (Elaskar et al., 2001; Irtwange & Igbeka, 2002) to avoid immature flow (where some depth of granular particles remain stationary) and the arching phenomena to ensure a fully developed sliding flow. The average values obtained from this study compares satisfactorily with that reported by Asoiro and Ani (2011).

Table 2. Results of the mechanical properties of African yam bean seeds.

Properties	Moisture content				
	8% (db)	12% (db)	16% (db)	20% (db)	24% (db)
Force at Peak (N)	176.564±20.137	118.452±76.791	110.560±55.044	107.710±32.430	104.860±9.814
Deformation at Peak (mm)	1.358±0.245	1.056±0.355	0.784±0.132	0.732±0.182	0.679±0.231
Strain at Peak (%)	15.767±5.303	11.805±2.133	11.362±1.917	10.672±2.655	9.982±3.393
Energy at Peak (N.m)	0.072±0.019	0.059±0.026	0.030±0.023	0.028±0.018	0.025±0.012
Force at Yield (N)	168.548±24.049	107.010±59.952	102.878±11.318	93.786±32.391	84.694±53.464
Deformation at Yield (mm)	1.322±0.304	1.004±0.251	0.757±0.173	0.666±0.237	0.574±0.300
Strain at Yield (%)	14.991±3.754	11.499±2.640	10.968±2.512	9.702±3.462	8.435±4.411
Energy at Yield (N.m)	0.069±0.024	0.050±0.013	0.024±0.013	0.022±0.016	0.019±0.018
Force at Break (N)	172.880±19.506	117.692±77.272	109.462±43.140	103.773±34.790	96.986±14.536
Deformation at Break (mm)	1.411±0.259	1.065±0.369	0.780±0.131	0.756±0.172	0.680±0.230
Strain at Break (%)	15.899±5.505	12.273±2.250	11.577±1.627	11.117±2.648	9.994±3.378
Energy at Break (N.m)	0.078±0.021	0.060±0.028	0.036±0.013	0.030±0.015	0.025±0.012

Effects of moisture content on mechanical properties

According to the results (Table 2), the force, deformation, strain and energy for African yam bean seeds decreased with an increase in moisture content. It can also be observed that the force required to cause a given deformation decreased as the moisture content increased. This may be because at higher moisture content, the seed became softer and required less force. With increasing moisture content from 8% to 24 % (d.b), the force at peak, force at yield and force at break were found to decrease significantly ($P \leq 0.05$) from 176.564±20.137 to 104.860±9.814 N, 168.548±24.049 to 84.694±53.464 N and 172.880±19.506 to 96.986±14.536 N, respectively. The small rupture forces at higher moisture content might have resulted from the fact that the seed became more sensitive to rupture at high moisture.

CONCLUSION

The variation in the moisture content increased the linear dimensions, arithmetic mean diameter, geometric mean diameter, equivalent diameter, surface areas, volume, sphericity, aspect ratio, flakiness ratio, porosity, static angle of repose, dynamic angle of repose and static coefficient of friction along the five surfaces, but decreased the bulk density and true density. The variation in the moisture content also decreased the mechanical properties. Statistical analyses ($P \leq 0.05$) revealed that the variation in the moisture content had a significant effect on the physical properties and the mechanical properties. The effects of other factors such as accessions, varieties, cultivars and species, and development of predictive models for engineering properties of African yam bean is recommended for further studies.

Conflict of interest

The authors have no conflict of interest to report.

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Influence of cannonball tree (*Couroupita guianensis* Aubl.) leaf extract and electrolyzed oxidizing water on postharvest quality of tomato

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ABSTRACT

Purpose: Managing postharvest losses to extend shelf life and cut down on waste is of paramount importance nowadays, especially when resources are scarce. Extracts from the leaves of the cannonball tree and electrolyzed oxidizing water were employed to improve postharvest handling procedures. **Research method:** The experiment consisted of cannonball tree leaf extracts (5 ml/L, 10 ml/L, 15 ml/L, 20 ml/L) and two pH levels of electrolyzed oxidizing water (pH 3, pH 5). Tomato treated with distilled water was considered as a control. The experiment was conducted as a Completely Randomized Design under a factorial arrangement with three replications. **Findings:** Cannonball tree leaf extracts (10 ml/L) significantly retained acceptable fruit color, firmness, high level of titratable acidity, flavonoid, carotenoid, anthocyanin, vitamin C, IC_{50} and prolonged shelf life by more than three days over other treatment combinations. Compared to untreated fruit, treated fruit decayed at a slower rate (30.7±0.4%) and lost less weight (35.4±0.7%). Additionally, electrolyzed oxidizing water (pH 5) significantly outperformed alternative postharvest management techniques to lower postharvest losses, IC_{50} (121.6±2.1 mg/Kg) activity, enhancing titratable acidity and vitamin C content, and other physico-chemical attributes and thereby increasing tomato shelf life by more than two days. **Research limitations:** No limitations were encountered. **Originality/Value:** Electrolyzed oxidizing water (pH 5) or cannonball tree leaf extract (10 ml/L) appears to be the most promising sustainable solution for reducing postharvest tomato losses.

INTRODUCTION

In terms of production, yield, and commercial value, tomatoes rank near the top of the list of most essential vegetables (Sinha et al., 2019). The annual production of tomatoes is about 387.65 thousand metric tons from 28.21 thousand hectares of land with an average yield of 50.00 $MTha^{-1}$ in Bangladesh (YAS, 2020). Poor postharvest practices lead to a loss of 15-42% of vegetables between the farm, the wholesaler, the store, and the consumer, leading to an oversupply and lower prices for consumers and fewer profits for farmers (Arah et al., 2015). As a result, enormous quantities of tomatoes are harvested and sold at throwaway prices. Additionally, microbial degradation significantly contributes to the substantial postharvest loss (Odeyemi et al., 2021). The subtropical regions' tomato output has expanded significantly in recent years (YAS, 2020), but due to the dearth of novel postharvest technology in use, postharvest management strategies could not follow a similar trajectory. Thus, sustainable postharvest loss management approaches might be the lingering strategies to reduce postharvest losses by extending the shelf life of tomatoes.

It is reflected that plants are a reliable supply of natural compounds. The Lecythidaceae family includes huge tropical deciduous trees like the cannonball tree (*Couroupita guianensis* Aubl.). The bioactive composites present in various cannonball tree plant components, include leaves, stems, flowers, bark, etc., may be employed for a variety of healing purposes. The plant's medicinal potential is due to the presence of essential oils, glycosides, ketosteroids, isatin, indurubin, and phenolic chemicals (Pandurangan et al., 2018). The chemical constituents of *C. guianensis* leaves are hydroxycinnamic acids, rosmarinic acid, triterpenic ester β-amirin palmitate, kaempferol-3-O-neohesperidoside, 4-hydroxybenzoic acid, 20,40-dihydroxy-60-methoxy-30, 7-hydroxy-5-methoxy-6,8-dimethylflavanone, 50-dimethylchalcone (Martinez et al., 2012). The phenolic and volatile substances from the leaf extracts of *C. guianensis* showed antibacterial and antifungal properties that cure several diseases (Elumalai et al., 2012).

Electrolyzed oxidizing (EO) water is produced by electrolysis of sodium chloride to yield mainly chlorine-based oxidizing products (Dewi et al., 2017; Zhang et al., 2021). It has currently been proposed as the alternative to conventional sanitation and cleaning agent as well as novel antimicrobial agents (Iram et al., 2021). It has been stated to be extremely microbiocidal against bacteria, viruses, fungi, and may signify an alternative to synthetic chemicals and traditional chlorine-based sanitizers. EO water exerts its antimicrobial effects due to its high oxidation-reduction potential (ORP) (Kim et al., 2000; Len et al., 2000). Microbial cell membranes lose electrons when exposed to an oxidizing solution with a high ORP, leading to cell death (Suslow, 2004). Due to its minimal use of the salt solution and lack of additional chemical additives, EO water has less impact on the environment chemically. (Kim et al., 2000). The effect of EO water was evaluated to improve the shelf life and quality as well as to reduce the microbial population of kumquat citrus (*Fortunella* sp.) (Kassim et al., 2016), date (*Phoenix dactylifera*) (Bessi et al., 2014). It has been proved that EO water was used for improving the postharvest quality of several horticultural products like mushrooms (Aday, 2016), and avocados (Hassan & Dann, 2019).

There are several methods that are correctly used to enhance the quality of postharvest fruits and vegetables, but they have drawbacks such as high energy consumption, complicated spraying procedures, and chemically manufactured fungicides. Consumers' worries about the presence of chemically manufactured fungicides in postharvest produce have been well-founded (Wisniewski et al., 2016). Therefore, the application of non-residue, low energy consumption and inexpensive physical preservation methods in postharvest fruits and vegetables has attracted increasing attention, including EO water (Fallanaj et al., 2016). There

are no reports on the efficacy of cannonball tree leaf extract and EO water in lowering tomato postharvest losses, but a few studies have shown that field applications or postharvest treatment of EO water decreases the onset of disease and increases the shelf life of some harvested fresh produces. Therefore, the present study attempted to assess the ameliorative role of *C. guianensis* leaf extract and EO water on the postharvest physico-chemical attributes of tomatoes.

MATERIALS AND METHODS

Location of experiment, design, treatment

An experiment was carried out at the Horticulture Laboratory, Khulna University (22°80´ N, 89°53´ E), Bangladesh, from December 2021 to March 2022. In this study, the Minto Super tomato was selected as the experimental material for the investigation which was collected from the field laboratory of the Agrotechnology Discipline. The experiment was conducted as a factorial arrangement of CRD (completely randomized design) with three replications. The experiment consisted of four cannonball tree leaf extracts (5 ml/L, 10 ml/L, 15 ml/L, 20 ml/L) and two pH levels of electrolyzed oxidizing water (pH 3, pH 5) which were compared with control (distill water).

Preparation of *C. guianensis* leaf extract

Fresh leaf of *C. guianensis* was collected from the nursery of Forestry and Wood Technology Discipline of Khulna University before preparing leaf extract. Ten leaves were removed from the stem and the leaf surface was cleaned properly. A total of ten leaves were poured into the blender (Sahara Pride Blender, BD) to make the juice. No additional water was added to prepare the juice. Fresh leaf juice was filtered using Whatman No 1 filter paper, and the extract was collected to make the final solution. The four different solutions were prepared viz. 5 ml leaf extract per 1 liter of water (1:200), 10 ml leaf extract per 1 liter of water (1:100), 15 ml leaf extract per 1 liter of water (1:67), 20 ml leaf extract per 1 liter of water (1:50), respectively. The freshly harvested tomatoes were dipped for 30 seconds in different solutions that were made from *C. guianensis* leaf extract. After that, the tomatoes were sprayed with a fresh solution of leaf extract at a 2-day interval. The treatment application procedure was modified from that used by Batu and Thompson (1998). Dipping with appropriate disinfection of tomatoes not only decreases the microbial loads of the fruits but also boosts the superior quality of the tomatoes during storage (Workneh et al., 2012). The tomatoes were stored at the Horticultural Laboratory in ambient conditions (Temperature: 24°C, RH: 65-75%) to evaluate the physico-chemical attributes of tomatoes.

Preparation of EO water

Electrolyzed oxidizing water was collected from the Animal Husbandry Laboratory of Agrotechnology Discipline and the pH of water (pH 3 and pH 5) was adjusted by adding diluted HCL or NaOH and the reading was monitored using the digital pH meter (ASONE ORP Desktop Economy pH meter PH700, Japan) to obtain desire pH. The freshly harvested tomatoes were dipped for 30 seconds in EO water having pH 3 and pH 5, respectively. After that, the tomatoes were sprayed with a fresh solution of EO water at a 2-day interval. The treatment application procedure was modified from that used by Ding et al. (2015). Distill water was used for dipping in case of untreated control.

Determination of the shelf life of tomato

By detecting and judging the quality parameters like appearance (color chart), shriveling, disease incidence (scale rating: 0 means no infection, 5 means 50 % fruit area infected), etc. (Sinha et al., 2019), the shelf life of tomato fruit was assessed with respect to storage days.

Assessment of weight loss

The tomato was chosen at random from each treatment to weigh every other day and compare the weight difference from the fresh weight on the first day. The following equation (1) was used to assess weight loss:

$$\text{Weight loss (\%)} = \frac{M_0 - M_1}{M_0} \times 100 \quad (1)$$

M_0 is the initial fresh weight of the tomato, and M_1 is the individual sampling day measured weight (Qin et al., 2015).

Determination of moisture and dry matter content

Fifty grams (50 g) of fresh fruit sample from each treatment was taken and cut into small pieces on an aluminum foil and oven-dried at 70°C until the constant weight was attained. Percent moisture content was calculated according to the following formula (2), and dry matter content (%) was calculated using the following formula (3) (Khatun et al., 2022).

$$\text{Moisture content (\%)} = \frac{\text{Fresh weight of sample (g)} - Dry\ eeight\ oof\ sample\ (g)}{\text{Fresh weight of sample (g)}} \times 100 \quad (2)$$

$$\text{Dry matter content (\%)} = 100 - \text{moisture content (\%)} \quad (3)$$

Assessment of fruit decay percent

The visual quality losses (fungal decay, brushing, softening, rupturing skin) of stored tomato fruits were evaluated by visual inspection and disease scale rating, 0 means no infection, 5 means 50% fruit area infected (Sinha et al., 2019) for every two days. The fruits infected with visible fungal mycelia or 1/3 damaged was discarded from the container, and the decay percentage was determined from the total number of tomatoes.

Evaluation of fruit firmness

A food texture analyzer (Shimadzu EZ-SX, USA) was used to determine the firmness of treated tomatoes (2 mm diameter). Each fruit was penetrated at two different equilateral locations to assess its firmness. The probe speed was 2 mm/s and the penetration depth was 5 mm. The maximum force firmness was recorded in N/cm^2.

Determination of fruit color

The surface color of the tomato was determined using a chromo meter (CR-410, Konica Minolta, USA) by calculating L*, a*, b* values, where L* represents brightness, a* means redness, b* means yellowness, and hue angle ($h°$), chroma (C). The following equation (4) was used to assess the hue angle, and equation (5) was used to evaluate the chroma:

$$h° = \tan^{-1}(b^*/a^*) \quad (4)$$

$$C = \sqrt{a^2 + b^2} \quad (5)$$

Determination of pH, total soluble solids and titratable acidity of tomato fruit pulp

The pH of tomato pulp was determined using a Benchtop pH meter (HI2210, Hanna Instrument, USA, 0.01 pH resolution) using the procedure described by Mazumdar & Majumdar (2001) and Saini et al. (2006). The percentage of total soluble solids (TSS) was assessed from the reading of the digital Brix meter (Digital/Brix/RI-Check Reichert Technologies, USA). Similarly, tomato titratable acidity (TA) was evaluated using the following procedure described by Mazumdar and Majumdar (2001) and Saini et al. (2006).

Determination of flavonoid in tomato fruit pulp

Ten grams (10 g) of tomato from the sample was taken and crushed finely. Then 100 ml of 80 % methanol was added and kept in a water bath for 10 hours at 40° C. The whole solution was filtered through filter paper (Whatman No. 42). After that, the filtrate was transferred to a crucible and then evaporated to dryness over a water bath at room temperature. The final finding was weighed as a flavonoid (Mazumdar & Majumdar 2001; Saini et al., 2006).

Determination of carotenoid in tomato fruit pulp

The carotenoid content was evaluated using the procedure described by Mazumdar & Majumdar (2001) and Saini et al. (2006). The following equation (6) was used to determine the carotenoid:

$$\text{mg carotenoid/ g tissue} = 7.6\,(A.480) - 1.49\,(A.510) \times \frac{V}{1000 \times 10} \quad (6)$$

Here, A= Absorbance of the specific wavelength, V=Final volume of the carotenoid in 80 % acetone, W= Fresh weight of the tissue extracted

Determination of anthocyanin in tomato fruit pulp

Anthocyanin was extracted with ethanolic-hydrochloride. The total procedure was described by Mazumdar and Majumdar (2001) and Saini et al. (2006). The following calculation (7 and 8) was used to assess the anthocyanin content of the tomato pulp.

$$\text{Total absorbance } (/100g\ sample) = \frac{e \times b \times c}{d \times a} \times 100 \quad (7)$$

a = sample weight, b = volume constructed for color determination c = total volume, d = aliquot volume taken for assessment, and e = 535 nm volume

$$\text{Anthocyanin } (mg/100\ g\ FW) = \frac{Total\ absorbance}{98.2} \quad (8)$$

Determination of vitamin C in tomato fruit pulp

In order to determine tomato vitamin C contents, 30 g of tomato pulp was weighed and melded for 3 to 4 minutes with 6 % Meta phosphoric acid. Then 15 g of the mixture was combined with 85 g of 3 % Meta phosphoric acid in a 100 ml volumetric flask. After that, the mixture was filtrated with filter paper (Whatman No. 42) and titrated immediately following the procedure described by Mazumdar & Majumdar (2001) and Saini et al. (2006). Finally, the following equation (9) was used to determine the ascorbic acid content:

$$\text{Ascorbic acid } (mg/100\ g\ FW) = V \times T \times \frac{100}{W} \quad (9)$$

V = In titration volume of dye used, T = standardized dye value, and W = pulp weight.

Determination of free radical scavenging activity of tomato fruit pulp

The free radical scavenging activity of tomato after treatment with cannonball tree leaf extracts and electrolyzed oxidizing (EO) water were analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the researchers (Jadid et al. 2017; Dash et al., 2022). The DPPH solution was prepared in methanol and subsequently added to various concentrations of the various extracts (25, 50, 100, 200

and 400 mg/Kg). Ascorbic acid was used as a positive control (standard). The following equation (10) was used to calculate the inhibition percentage:

$$\text{Inhibition (\%)} = \frac{\text{Blank absorbance} - \text{extract absorbance}}{\text{Blank absorbance}} \times 100 \quad (10)$$

The IC_{50} values were calculated using a linear regression model (y=ax+b) and used to specify the antioxidant activity of different treatments.

Statistical analysis

Postharvest data were subjected to a two-way ANOVA to determine statistical differences among the treatments and treatment combinations identified by *f*-test, while their pairwise mean comparisons were estimated using Tukey's HSD (Honestly Significant Difference) test at $p \leq 0.05$ (OriginLab Corporation, Version 9.6.5, USA). All quality parameters were analyzed using a model that as cannonball tree leaf extract and electrolyzed oxidizing (EO) water as the main effects and their-2-ways interactions. To determine the relations between the variables and treatments, the principal component analysis was performed using the raw data.

RESULTS

Shelf life

The effect of postharvest treatment with *C. guianensis* leaf extract and EO water on the shelf life of tomatoes varied significantly ($p \leq 0.01$) from each other (Table 1). The interaction effect of *C. guianensis* leaf extracts and EO water was not varied significantly for the shelf life of tomatoes. Tomatoes treated with *C. guianensis* leaf extract (10 ml/L) had a maximum shelf life (16.0 $\pm$1.2 days), while untreated fruits (control) had a minimum shelf life (12.9 $\pm$ 0.5 days). The results revealed that tomatoes treated with *C. guianensis* leaf extract (10 ml/L) prolonged their shelf life by more than three days than untreated (control) fruits. Similarly, tomatoes treated with EO water (pH 5) had maximum shelf life (15.3 $\pm$ 1.0 days), while untreated fruits (control) had a minimum shelf life (13.3 $\pm$ 0.7 days). Tomatoes treated with EO water (pH 5) extended their shelf life by more than two days than untreated fruits.

Weight loss

The various *C. guianensis* leaf extracts and EO water effects on the weight loss of tomatoes were highly significant ($p \leq 0.01$) that as demonstrated in Table 1. The interaction effect of *C. guianensis* leaf extracts and EO water was not significant for the weight loss of tomatoes. During the storage period, gradually increased weight loss. Tomatoes treated with *C. guianensis* leaf extracts (10 ml/L) was significantly lower weight loss (35.01%) than that untreated one (control). The fruit weight loss treated with several concentrations of *C. guianensis* leaf shown as control > 20 ml/L > 15 ml/L > 5 ml/L > 10 ml/L, respectively. Alike, *C. guianensis* leaf extracts, EO water (pH 5) was significantly lower in weight loss (29.33 %) than that of untreated control. The fruit weight loss treated with EO water was ranked as control > pH 3 > pH 5.

Moisture and dry matter

The main and interaction effect of *C. guianensis* leaf extract and EO water on moisture and dry matter content were not significant ($p \leq 0.53$) (Table 1).

Fruit decay

A significant ($p \leq 0.01$) variation in fruit decay percentage was detected due to the differences between postharvest treatments with *C. guianensis* leaf extract and EO water (Table 1). The

interaction effect of *C. guianensis* leaf extracts and EO water were not significant for fruit decay of tomato. The maximum (18.9 ± 1.4 %) decay occurred in untreated control and the minimum (13.3 ± 0.9 %) at *C. guianensis* leaf extract (15 ml/L) followed by (10 ml/L) (13.1 ± 0.8 %). In the case of EO water, the maximum (16.7 ± 0.9 %) decay occurred in control and the minimum (13.5 ± 0.5 %) at EO water (pH 5).

Table 1. Effect of *C. guianensis* leaf extract and EO water on shelf life, weight loss, moisture and dry matter, decay of tomato.

Treatments	Shelf life (days)	Weight loss (%)	Moisture (%)	Dry matter (%)	Fruit decay (%)
C. guianensis leaf extract (A)					
Control	12.9±0.5 d	17.5±1.3 a	13.2±0.1	86.8±0.5	18.9±1.4 a
5 ml/L	14.7±0.9 bc	12.0±0.5 c	13.1±0.1	86.5±0.6	15.5±1.2 b
10 ml/L	16.0±1.2 a	11.3±0.4 c	13.6±0.2	86.9±0.5	13.1±0.8 d
15 ml/L	14.9±0.8 b	14.6±0.8 b	13.5±0.2	86.9±0.5	13.3±0.9 d
20 ml/L	13.9±0.7 c	15.5±0.9 b	13.1±0.1	86.4±0.6	14.4±1.0 c
EO water (B)					
Distill water (Control)	13.3±0.7 c	17.2±1.2 a	12.9±0.2	86.2±0.4	16.7±0.9 a
pH3	14.5±0.9 b	13.3±0.8 b	13.1±0.3	86.9±0.5	14.9±0.6 b
pH5	15.3±1.0 a	12.1±0.5 c	13.8±0.2	87.1±0.6	13.5±0.5 c
Significance					
A	**	**	NS	NS	**
B	**	**	NS	NS	**
A×B	NS	NS	NS	NS	NS

Note: Means followed by the same letters within a column do not differ significantly whereas means having dissimilar letters differ significantly as per Tukey's HSD test at $p \leq 0.05$, ± standard error, NS: non-significant, ** significant at $p \leq 0.01$.

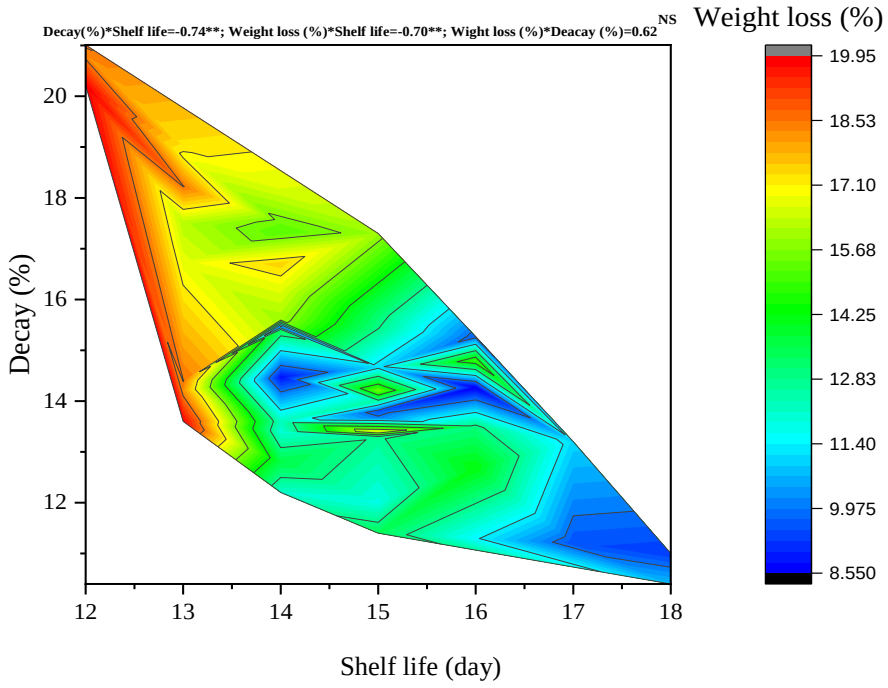


Fig. 1. Three variables contour plot representing the joint interaction among shelf life (day), fruit decay (%) and weight loss (%) of tomato. Note: Numeric value with ** specified correlation between variables significant at $p \leq 0.01$, NS: non-significant.

Relationship among shelf life, fruit decay and weight loss of tomato

A three-variable contour plot highlighted that shelf life was negatively correlated to both the decay (%) and weight loss (%) of tomatoes indicating reduced decay (%) and weight loss (%) helped to prolong the shelf life of tomatoes and vice versa (Fig. 1). The findings showed that fruits with blue color had longer shelf lives because they conserved more water and lost less weight, while tomatoes with red color had shorter shelf lives because more weight loss and deterioration were visible.

Firmness

Firmness is a key quality indicator of tomatoes. The effect of postharvest treatment with *C. guianensis* leaf extract and EO water on the firmness of tomato varied significantly ($p \leq 0.01$) from each other. The interaction effect of *C. guianensis* leaf extracts and EO water was not significant for the shelf life of tomatoes. Tomatoes treated with *C. guianensis* leaf extracts either 10 ml/L or 15 ml/L significantly overweighed the firmness level incurred by both untreated and 5 ml/L. On the contrary, when fruits treated with EO water (pH 5) exhibited a better firmness level than those untreated fruits. Between two EO water treatments, EO water with pH 5 offered a significantly higher firmness to tomato fruits than pH 3.

Tomato fruits color evaluation

Tomato appearance differs by surface color. Discoloration primarily occurs on the surface of the tomato during storage, resulting in spoilage. The brightness (L*) of the tomato was significantly ($p \leq 0.01$) varied due to differences in treatments on different days of storage. At 2-days of storage, the maximum L* value was observed when the fruits were treated with *C. guianensis* leaf extracts (10 ml/L) than that of others. A similar trend was documented for the rest of the storage period. The EO water also significantly ($p \leq 0.01$) influenced the brightness (L*) of tomatoes. The maximum L* value was found when the fruits were treated with EO water (pH 5) compared to the untreated control and the trend was consistent during the storage period.

The evolution of the red fruit color (a*) was more rapid at control as well as fruits treated with *C. guianensis* leaf extracts (5 ml/L) than that of others. On the other hand, the yellowness (b*) of the tomato decreased with the increase of storage time. The minimum b* value decreasing trend was noticed in *C. guianensis* leaf extracts (10 ml/L water) as well as EO water (pH 5). The chroma and hue angle are the most used parameters to indicate the color development of stored tomato fruits. The chroma value and hue angle significantly ($p \leq 0.05$) increased with increase of storage time of the tomato. When the fruits were treated with either *C. guianensis* leaf extracts (10 ml/L) or EO water (pH 5) gave rise to a higher hue angle value (less red) compared to the other treatments.

pH, total soluble solids and titratable acidity of tomato

The main and interaction effect of *C. guianensis* leaf extract and EO water on tomato fruit pH and total soluble solids were not significant after 10 days of storage ($p \leq 0.28$) (Fig. 2). However, a significant ($p \leq 0.05$) variation of TA was observed when fruits treated with *C. guianensis* leaf extracts. The maximum TA was found when fruits were treated with *C. guianensis* leaf extracts (10 ml/L) followed by 15, and 5 ml/L, respectively and the minimum was observed at control followed by *C. guianensis* leaf extracts (20 ml/L). However, when the tomatoes treated with EO water (pH 5) exhibited the highest TA value compared to the untreated fruits.

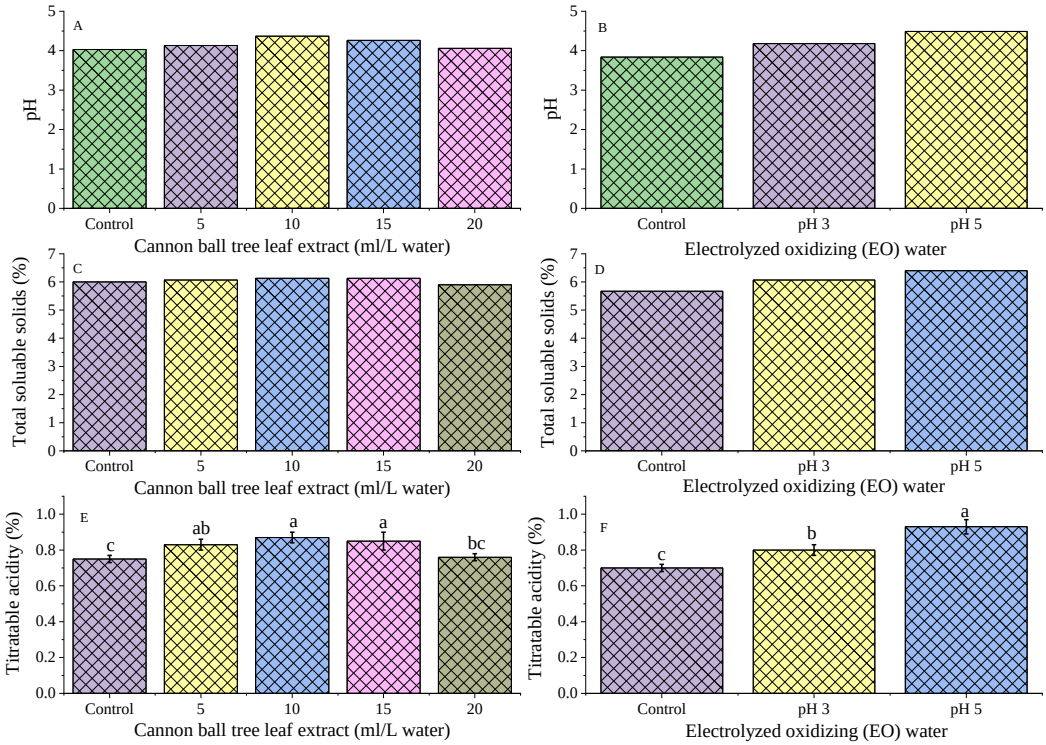


Fig. 2. Effect of *C. guianensis* leaf extracts and EO water on pH, total soluble solids and titratable acidity after 10 days of storage. Note: vertical bar represents standard error, bars that do not contribute to a letter are significantly unique based on Tukey's HSD test at $p \leq 0.05$.

Flavonoid, carotenoid, anthocyanin and vitamin C of tomato

There was significant ($p \leq 0.05$) variation in flavonoid levels among concentrations of *C. guianensis* leaf extracts (Fig. 3). The maximum flavonoid level was observed when the fruits were treated with *C. guianensis* leaf extracts (10 ml/L) followed by 15 ml/L whereas the minimum was found in untreated ones. In the case of EO water, both EO water treatments (pH 3 and pH 5) significantly ($p \leq 0.01$) demonstrated more flavonoid levels than the control. Effects of *C. guianensis* leaf extracts and EO water were significant ($p \leq 0.05$) for the carotenoid content of tomato after 10 days of storage. Tomatoes treated with *C. guianensis* leaf extracts showed better carotenoid content compared to the untreated ones. Also, the EO water (pH 3 and pH 5) treated tomatoes displayed better carotenoid content compared to the control. In the case of anthocyanin, a significant ($p \leq 0.05$) variation of anthocyanin content due to the differences in treatments. The interaction effect of *C. guianensis* leaf extracts and EO water was not significant. The highest anthocyanin content was found when the fruits were treated with *C. guianensis* leaf extracts (10 ml/L), whereas the lowest was observed in control followed by *C. guianensis* leaf extracts (20 ml/L). Alike carotenoid, EO water (pH 3 and pH 5) treated tomatoes displayed better anthocyanins content compared to the control. A significant ($p \leq 0.05$) difference in vitamin C content was found when the fruits were treated with *C. guianensis* leaf extracts and EO water. The highest vitamin C content was observed when the fruits were treated with *C. guianensis* leaf extracts (10 ml/L), whereas the lowest in control was followed by *C. guianensis* leaf extracts (20 ml/L). Similar to flavonoid and carotenoid, EO water (pH 3 and pH 5) treated tomatoes displayed better vitamin C content compared to the control.

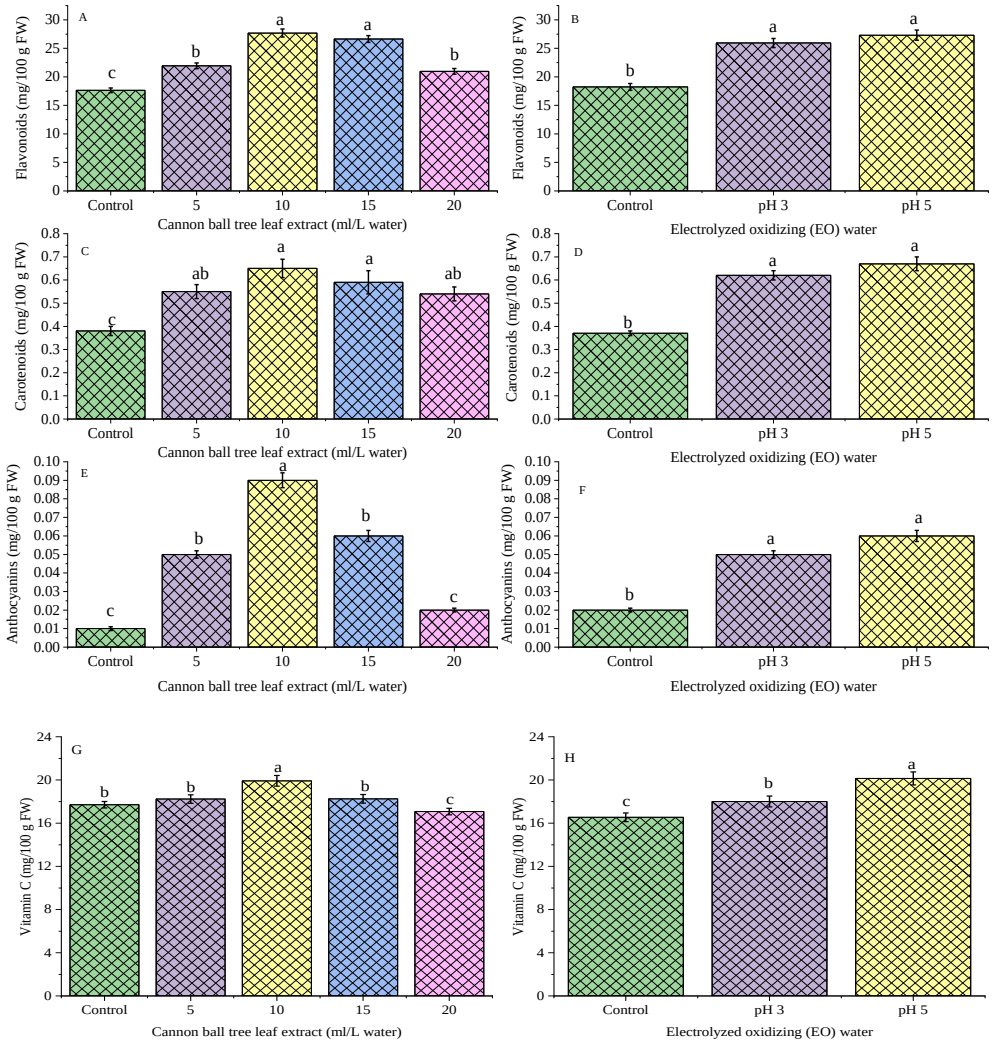


Fig. 3. Effect of *C. guianensis* leaf extracts and EO water on flavonoids, carotenoids, anthocyanins, and vitamin C after 10 days of storage. Note: vertical bar represents standard error, bars that do not contribute to a letter are significantly unique based on Tukey's HSD test at $p \leq 0.05$.

Radical scavenging activity determination

The percentage of inhibition was calculated to assess the antioxidant activity of the tomatoes treated with *C. guianensis* leaf extracts and EO water which could obstruct free radicles. Five varying concentrations (25, 50, 100, 200 and 400 mg/Kg) of different *C. guianensis* leaf extracts, as well as EO water, showed various percentages of inhibition in tomatoes. The scavenging activity of tomatoes treated with *C. guianensis* leaf extracts and EO water was increased with the rise of concentration. Tomatoes were treated with both *C. guianensis* leaf extracts and EO water and showed the best antioxidant activity at 400 mg/Kg concentrations. Among the *C. guianensis* leaf extracts, the 10 ml/L concentration (96.1 $\pm$ 2.5 %) was the highest inhibition followed by 15 ml/L, 20 ml/L, 5 ml/L, control, respectively. At the highest concentration (400 mg/Kg), the scavenging activity of 10 ml/L and 15 ml/L were higher than the ascorbic acid (standard). Similarly, the EO water at pH 5 was the highest inhibition (97.3 $\pm$ 2.2 %) followed by pH 3 and control, respectively. Alike, *C. guianensis* leaf extracts, at the highest concentration (400 mg/Kg), the scavenging activity of EO water at pH 5 was higher than the ascorbic acid (standard).

The IC_{50} value of DPPH radical scavenging activity

The results showed that tomatoes treated with *C. guianensis* leaf extracts (10 ml/L) exhibited the highest antioxidant activity (121.6 ± 2.1 mg/Kg) than the other concentration of leaf extract. Also, the EO water showed better results (Fig. 4). The IC_{50} value was measured to assess the concentration of the extract required to inhibit 50 % of radical.

Principal component analysis

Measured quality parameters were subject to PCA to assess the association among the variables. The principal component-1 (PC-1) explained 65.32 % of the total variation in the measured quality parameters as influenced by treatments (Fig. 5). Analysis of the PCA biplot displaying the loading plot indicated that a*, b*, hue angle, chroma, L*, weight, shape index (SI), vitamin C, carotenoid, anthocyanin, $IC_{50,}$ and shelf life were positively correlated with the treatments whereas weight loss (%), pH, Brix, titratable acidity, flavonoids, moisture (%), dry matter (%), and decay (%) were negatively correlated with the treatments. Among the variables fruit weight, L*, and shelf life are more positively influenced by the treatments whereas weight loss (%) and fruit decay (%) are more negatively impacted by the treatments.

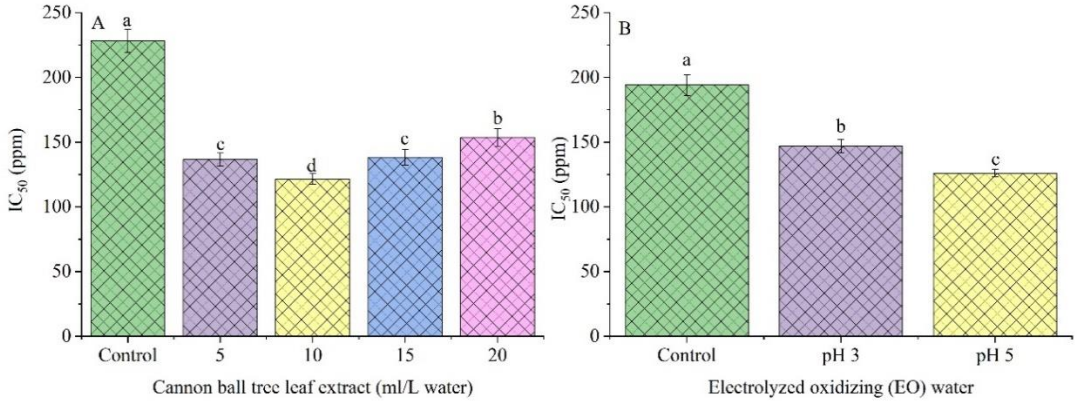
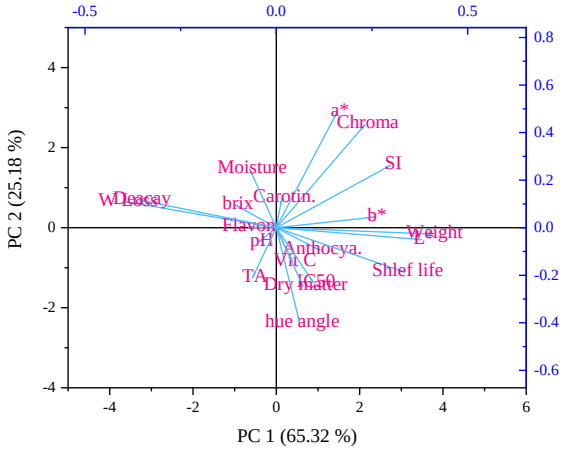


Fig. 4. Effect of tomato treated with cannonball tree leaf extract and electrolyzed (EO) water on IC_{50} value of DPPH radicle scavenging activity. Note: vertical bar represents standard error, bars that do not contribute to a letter are significantly unique based on Tukey's HSD test at $p \leq 0.05$.



Variable	PC1
a*	0.221
b*	0.283
hue angle	0.031
Chroma	0.293
L*	0.391
Weight	0.421
Weight loss (%)	-0.398
Shape Index (SI)	0.350
pH	-0.048
Brix	-0.010
TA	-0.093
Vit. C	0.035
Carotenoid	0.037
Anthocyanins	0.113
Flavonoid	-0.080
IC50	0.081
Moisture (%)	-0.056
Dry matter (%)	-0.056
Decay (%)	-0.351
Shelf life	0.336
Eigenvalue	7.895
Proportion	**0.653**
Cumulative	0.653

Fig. 5. PCA biplot (Principal component-1 PC1 vs Principal component-2 PC2) visualizing the correlations among the quality parameters affected by the cannonball tree leaf extracts and electrolyzed oxidizing (EO) water.

DISCUSSION

The leaf extract of *C. guianensis* extends the freshness of tomatoes. The leaf extract contains numerous phytochemicals that ameliorate several ailments and assist to keep fruits fresh for longer, which suggests that it may decrease the rapid metabolic rates of tomatoes. It was quite effective against both *E. coli* and *B. subtilis* in antibacterial tests (Pandurangan et al., 2018). Tomatoes' longevity can be extended with the help of EO water, which also tastes great. Previous studies have shown that EO water-treated mushrooms aid in delaying senescence (Aday, 2016). The EO water treatment delayed softening of blueberries most likely through the deactivation of cell wall-degrading enzymes (Chen et al., 2017) and regulation of reactive oxygen species (ROS) (Chen et al., 2019). It also helps maintain cellular integrity for a longer period compared with untreated fruit. The cellular integrity of treated tomatoes might help to increase the shelf life. The EO water effectively controlled citrus diseases reported by the researchers (Hussain et al., 2019). During electrolysis of EO water, HOCl is produced that penetrates cell membranes and evolves as hydroxyl radicles, which exert antimicrobial activity through the oxidation of important metabolic compounds (Mahmoud, 2007). EO water is widely used in the food industry to solve storage-related problems and prevents the growth of microbial organisms, which in turn escalates the shelf-life of food (Iram et al., 2021). In addition, water infused with EOs is also used to kill harmful bacteria and viruses on newly harvested crops and vegetables. Freshly cut carrots, bell peppers, spinach, cauliflower, tomatoes, apples, and oranges all have benefited from this method of cleaning (Huang et al., 2008). It was observed that EO water at a pH of 2.5 was extremely good at preventing the spread of *E. coli* in tomatoes (Issa-Zacharia et al., 2010). Due to its high oxidizing potential, EO water has attracted a lot of attention in the postharvest sector as a means to slow the deterioration of fruits and vegetables across a wide pH spectrum (Ippolito et al., 2021). The results revealed that *C. guianensis* leaf extracts and EO water reduced weight loss. It is possibly the postharvest treatment of tomatoes slowing down respiration and other metabolic processes and assists to retain moisture content in the fruits by inhibiting water loss from the surface. Tomatoes treated with either *C. guianensis* leaf extract or EO water reduced postharvest decay. The phytochemical ingredient in *C. guianensis* leaf extract is essential for the treatment of several diseases (Pandurangan et al., 2018). The fungus-caused avocado anthracnose disease was greatly decreased by the EO water (Hassan & Dann, 2019). Additionally, they stated that sodium hypochlorite's direct inhibitory action on the avocado fungal infection was mostly responsible for this. Similar to this study, another one reported that during tomato storage, EO water greatly decreased the occurrence of rot symptoms in tomatoes inoculated with *Fusarium oxysporum*, *Galactomyces geotrichum*, and *Alternaria* sp. (Vasquez-Lopez et al., 2016). The EO water could advance the disease resistance of postharvest citrus by stimulating the resistance of citrus. This theory was supported by the up-regulation of gene expression of a series of defense-related enzymes including chitinase, peroxidase, and phenylalanine ammonia-lyase (PAL) at 12 h after EO water treatment (Fallanaj et al., 2016). The EO water may be an effective treatment to enhance fruit disease resistance for suppressing the disease development of postharvest longans (Tang et al., 2021). Therefore, the effect of EO water on fungal diseases of postharvest fruits might be a double mechanism including the direct inhibition of pathogens and the activation of the host defense system.

Increased firmness in ripe blueberries with a lower weight loss (1%) after 21 days of storage whereas 4%-5% weight loss during storage resulted in softening of blueberries (Miller & McDonald, 1993). The findings suggested that maintaining the firmness of blueberries for an extended storage period could also be achieved by preventing weight loss. In the current

study, tomatoes exhibited an increase in firmness with weight loss (%), which was consistent with previous studies. The results releveled that those tomatoes treated with either *C. guianensis* leaf extracts (10 ml/) or EO water (pH 5) promote maintaining the good color of fruits and also delay over-ripening. It might ameliorate several diseases of tomato treatments and helps to retain the glossiness of fruits (Pandurangan et al., 2018; Vasquez-Lopez et al., 2016). The chemical qualities of preserved tomatoes were enhanced by the use of EO water with the ideal pH and *C. guianensis* leaf extracts at the ideal concentration. This shift in chemical characteristics may be due to *C. guianensis* leaf extracts and EO water, which could speed up the metabolism of postharvest fruits through the up-regulation of a number of metabolic enzymes' gene expression.

The DPPH assay was used to evaluate the free radical scavenging activity of tomatoes treated with *C. guianensis* leaf extracts and EO water. It is a rapid and efficient method to determine the free radical scavenging activity. The DPPH forms a stable diamagnetic molecule after accepting an electron or hydrogen radicle (Jadid et al., 2017; Dash et al., 2022). The color changes from purple to yellow specifies a reduction in absorbance of DPPH radicle. This evidences that antioxidants found in the extract interact with the free radicals (Kedare & Singh, 2011). The lower the IC_{50} value, the higher the antioxidant activity of the extract (Li et al., 2009). Among the three extracts a leaf, flower, and fruit, flower extracts of *C. guianensis* exhibited higher activity than others signifying the existence of more antioxidants in the flowers (Pandurangan et al., 2018). On the contrary, fruit extract showed maximum inhibition (67.85 %) than others resulting in more antioxidant acidity. The fruits of *C. guianensis* contain more polyphenols which are responsible to accelerate antioxidant activity would have made it more effective than other extracts (Raveendra et al., 2016). The increased inhibition mechanism of tomatoes treated with *C. guianensis* (10 ml/L) might be the activation of defense enzymes. The EO water aids to increase shelf life and reducing fruit decay of blueberries by activating antioxidant enzymes and alleviating oxidative damage (Lin et al., 2017; Xu et al., 2016). Higher antioxidant activity moderately denotes the capacity of scavenging ROS and improve oxidative damage in plant tissues, and thereby contributing to the defeat of pathogenic infection and decay in blueberries (Wang et al., 2017; Chanjirakul et al., 2006). The EO water (pH 5) treatment could preserve anthocyanin and phenolic content, enhance ROS scavenging capacity leading to cell membrane integrity, disease resistance capacity, and reduce the incidence of tomato fruit decay. In comparison to the untreated tomatoes, the EO water (pH 5) treated tomato fruits displayed higher antioxidant activity and lower firmness and fruit decay.

CONCLUSION

C. guianensis leaf extract (10 ml/L) and EO water (pH 5) were found to effectively prevent the loss of weight, decay, firmness, and surface color in tomatoes, while also preserving titratable acidity, flavonoids, carotenoids, anthocyanins, vitamin C content, and DPPH radical scavenging, and extending shelf life by more than three days compared to untreated fruits. Therefore, the results indicated that harvested ripe tomatoes treated with either *C. guianensis* leaf extract (10 ml/L) or EO water (pH 5) would reduce postharvest losses of tomatoes and open up a new avenue for postharvest management sectors

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Conflict of interest

The authors have no conflict of interest.

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Effect of edible tragacanth coating on fruit quality of tomato cv. Falkato

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ABSTRACT

Purpose: Considering the high perishability of agricultural products, especially vegetables, it is very important to use edible coating materials that increase their postharvest life and are edible and non-chemical. **Research method**: In this study, the effect of tragacanth gum coatings (0, 5, 7.5, and 10 $g.L^{-1}$) on the edible quality of tomato fruits cv. Falkato was investigated during 35 days of storage (15 °C and 85-95% relative humidity). The fruits were immersed in tragacanth gum solutions for three minutes and carefully weighed and labeled and packed after drying. During storage time, every one week (7 days) fruits were removed and the fruit weight loss, pH, soluble solids concentration (SSC), titratable acidity (TA), fruit firmness, shrinkage, and decay index were measured and compared with the uncoated sample (distilled water treatment). **Findings**: According to the results the tragacanth gum coating significantly reduced the percentage of fruit weight loss and improved the quality of tomato fruits such as firmness, SSC and TA compared to the control sample. So, coated fruits showed better edible quality than uncoated fruits. Then tragacanth gum is recommended for use after harvesting the tomato fruit**. Research limitations**: No limitations were founded. **Originality/Value**: In this research, for the first time, the effect of tragacanth gum coatings on the storage life of tomatoes was investigated.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) fruits have nutritional value such as micronutrients, carotenoids, and natural antioxidants (Oshima et al., 1996; Yahia et al., 2007; Peralta et al., 2008), so it is among the top vegetables in the world. It has allocated for more than 15% of world vegetable production (FAO, 2007). Tomato is a climacteric fruit and has a shorter shelf life after harvest due to some factors such as high respiration rate, then its quality such as flavor, firmness, color, and shelf life changes continuously after harvesting (Zapata et al., 2008; Ju et al. 2000). Tomato fruits are susceptible to frost damage, so they should be stored at temperatures over 11 °C (Cheng and Shewfelt, 1988). Therefore, maintaining the quality of fresh tomatoes is still a major challenge in the post-harvest period.

Edible coatings and films are made from natural polymers and edible films form a thin layer of food and prevent the transfer of moisture, gases, soluble and aromatic substances. These films must have favorable mechanical properties and be chemically stable (Zapata et al., 2008). Edible films and coatings are used as carriers of antimicrobial agents to control microbial contamination of food (Flores et al., 2007). Film or edible coatings reduce the transfer of moisture, oxidation, and respiration of the fruit, thereby preserving their quality and prolonging their shelf life. The coverage of tomato fruits with gum arabic has delayed the process of ripening and maintaining the antioxidant capacity (Ali et al., 2013). Also, the effect of hydroxypropyl coating on tomato shelf life was investigated. The edible coating delayed the tomato color from pink to red during storage, texture, and color changes (Zhuang and Huang, 2003). The rate of respiration and production of ethylene in tomatoes coated with alginate-based edible films was lower than in uncoated samples (Zapata et al., 2008). In one research it was concluded that coatings prevented significant changes in firmness, weight loss, soluble solids concentration, titratable acidity, and the percentage of decay compared to uncoated control tomato fruits (Mahfoudhi et al., 2014). Tragacanth gum also has appropriate coating properties. Its use as an edible coating has been reported on certain fruits such as sweet cherry (Esmaeili et al., 2022) and mango (Ali et al., 2022). Results exhibited that mango fruits coated with 1.5% TG showed substantially lower disease incidence and weight loss (Ali et al., 2022). In a recent study, effect of guar, Persian and tragacanth gums on the surface characteristics of biopolymer-coated tomato and cucumber epicarps was investigated (Mostafavi, 2019). Therefore, based on the investigations that have been done, no research has been done regarding the effect of tragacanth gum on fruit characteristics of tomato. Then, in this study the effect of tragacanth gel coating on storage life and quantitative and qualitative properties of tomato fruit cv. Falkato was investigated.

MATERIALS AND METHODS

Materials

Tomato fruits were provided from a traditional greenhouse located in Dehaghan (Isfahan, Iran) and transferred to cold storage immediately. The 180 identical and healthy fruits were harvested at the mature red stage having no visible stain. Tragacanth gum was supplied from the local market in Isfahan (Iran). They were grinded and sieved for producing powder and this powder was used for the preparation of different concentrations of coating solutions.

Preparation of coating treatments

The powdered tragacanth gum at different concentrations (0, 5, 7.5, and 10 $g.L^{-1}$) was added gently to the distilled water, and then stirring the solution was done to obtain a complete dissolving of the gum (Jahanshahi et al., 2018).

Coating and storage

The fruits were immersed in the treatment solution for three minutes at room temperature. Sterile distilled water was used as the control solution. After that, the fruits were air-dried and packed in boxes after weighing them with digital scales (China Note Book Model) and labeling the weight of the fruit. The fruits were stored in cold storage (15 ±1 °C and 85-95% relative humidity) for 35 days and during storage time the fruits were removed from storage at 7-day intervals and the desired traits were measured.

Physicochemical properties

Weight loss and Firmness

To evaluate weight loss, all fruits were weighed at the beginning of storage (day 0) and all sampling days. Water losses were calculated as a percentage of weight loss using the following formula (1):

$$\text{Weight loss (\%)} = \frac{Wi - Wf}{Wi} \times 100 \qquad (1)$$

W_i: Fruit weight before storage, W_f: Fruit weight at each sampling day

A manual fruit penetrometer (model GY3, China) was used to measure the firmness of the fruit tissue. At first, a fruit was randomly selected from each replicate and the probe was used on the fruit tissue and pressed to determine the amount of tissue firmness.

Titratable acidity (TA), Soluble solids content (SSC), and pH

To measure the TA, 10 ml of fruit juice was used and diluted to 100 ml with distilled water. The diluted extract was titrated with 0.1 N NaOH. The following formula (2) was used to calculate the amount of titratable acidity, and it was expressed as a percentage of citric acid per 100 ml of fruit juice (AOAC, 2002). The SSC was analyzed by a refractometer (Model N1, Atago, Japan) at room temperature (25 °C) and expressed as a degree of Brix. The pH of the fruit juice was measured by the pH meter (AOAC, 2002).

$$\text{\% Acid (wt/vol)} = N \times V1 \times \text{Eq wt} / y \times 10 \qquad (2)$$

V: Volume of consumption for titrant (ml), N: Normality of titrant (0.1 normal), Eq.Wt: Equivalent weight of citric acid (70 mg/mEq) and y: volume of Sample (ml).

Fruit shrinkage and decay evaluation

The shrinkage area of the fruit was calculated and expressed in terms of the percentage of shrinkage. Shrinkage of fruits is not homogeneous, so the direct evaluation method requires several measurements at different parts of a fruit, to obtain representative results (Sahin & Sumnu, 2006). Also, the fruit decay was evaluated visually. The degree of surface decay was measured through the same scale of browning judgment. For this purpose, each time the samples were taken out of the cold storage, the fruits were carefully evaluated and graded for fungal contamination (Cao et al., 2010).

Statistical analysis

The experiment was factorial in a split-plot design with three replications. The main factor was tragacanth gum concentrations and the storage time was submitted as a sub-factor. The data analysis was performed using SAS version 9.3. Data normality was tested by the Kolmogorov-Smirnov test and data normality was confirmed. The mean comparison was analyzed by LSD test.

RESULTS AND DISCUSSION

Weight loss

The highest percentage of weight loss occurred in the control treatment (distilled water). The tragacanth coating caused less weight loss in fruits and the results of this research showed that at 7.5 $g.L^{-1}$ tragacanth gum, less weight loss occurred during storage time (Fig. 1). The tomato juice is 90-95% at harvest time. But after fruit harvesting, a significant amount of fruit juice is lost by evapotranspiration (Meidani, 2003; Mazaheri et al., 2007). Fruits coated with 10 and 15% gum had less weight loss compared to the control, and weight loss gradually increased during storage time (Ali et al., 2010).

The pH of fruit juice

The results showed that 7.5 $g.L^{-1}$ tragacanth coating was better-preserved pH during storage time (Fig. 2). The highest increase in pH occurred in the control treatment (distilled water). Thus, the tragacanth coating caused the pH to increase more slowly during the storage time. The pH of tomato fruit increases during ripening and storage (Flores et al., 2007). The significant effect of tragacanth gel on pH in this study was in agreement with the results of *Aloe vera* gel research on grapefruit (Asghari & Ahadi, 2013).

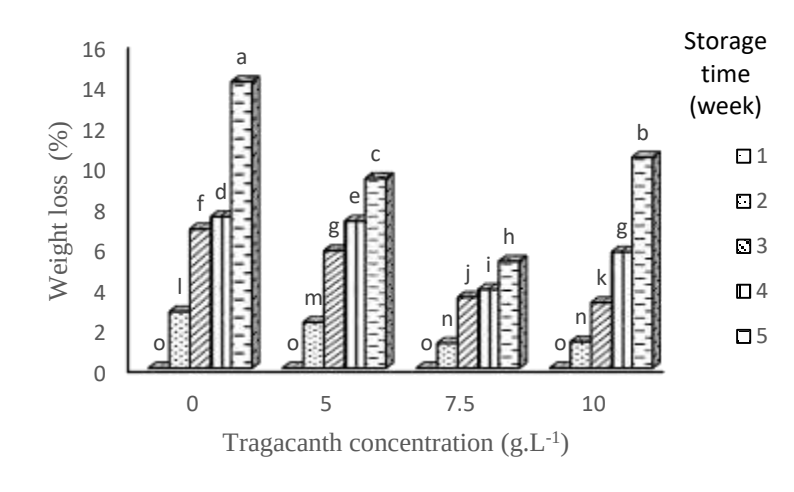

Fig. 1. Effect of tragacanth gum concentration on the weight loss of tomato fruits during storage time.

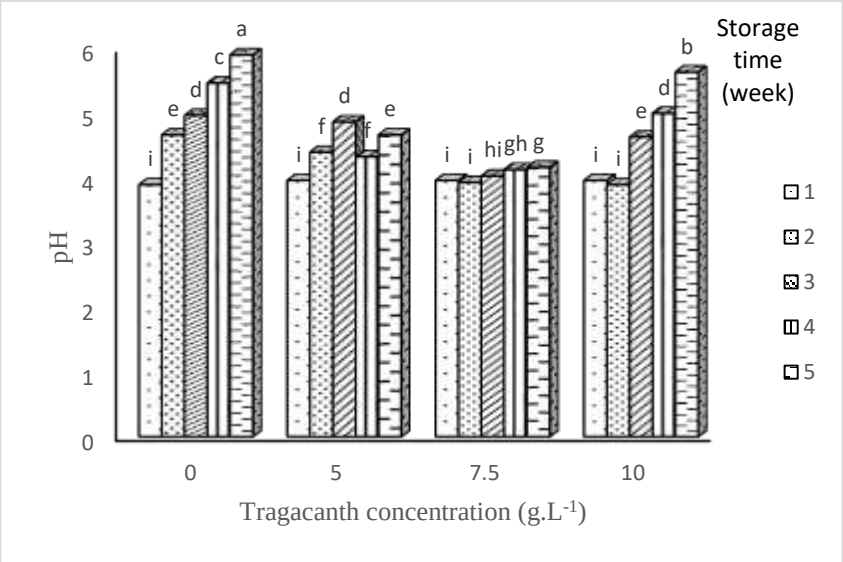

Fig. 2. Effect of tragacanth gum concentration on the pH of tomato fruits during storage time.

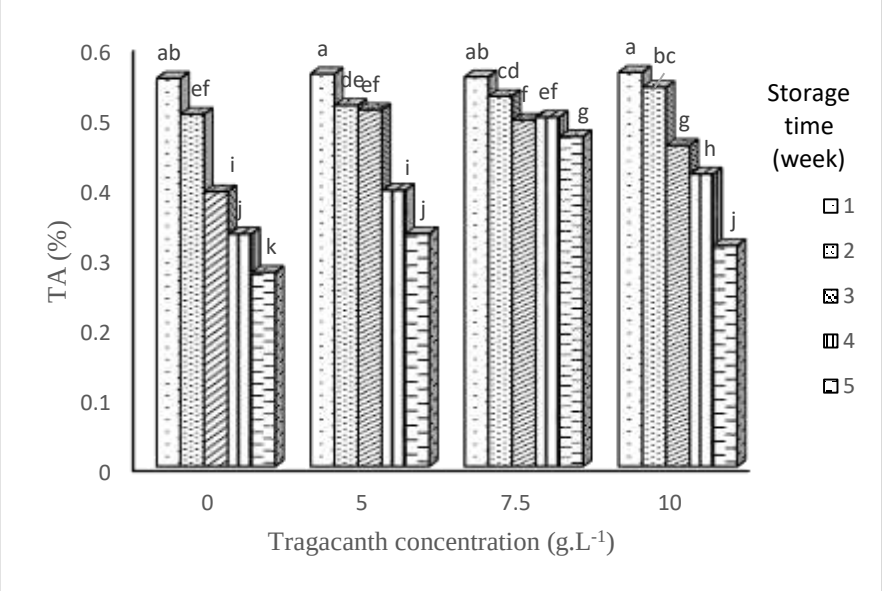

Fig. 3. Effect of tragacanth gum concentration on the TA of tomato fruits during storage time.

TA

The highest decrease in acidity occurred in the control treatment (distilled water). This result also showed that 7.5 g.l^{-1} tragacanth gum had the best effect in this fruit attribute and this level of concentration reduces fruit respiration and decomposition of organic acids by creating a coating on the surface of the fruit (Fig. 3). The TA is associated with the fruit ripening and causes a sour taste in the fruits. As the fruit matures, the organic acids decreases and the fruit harvesting period depends on the soluble solids, and the rate of acid decomposition. The breakdown of organic acids during fruit ripening is dependent on the rate of respiration, as these acids are used in respiratory enzymatic activity. In general, the titratable acidity of tomato fruits decreases during storage time (Mazaheri et al., 2007).

According to our results, the titrated acidity of covered and uncovered fruits decreases during storage time. Garousi (2010) achieved similar results by coating apricot fruit with whey and gellan gum.

SSC

The SSC was constant during 14 days of storage time in both coated and uncoated fruits. In the control treatment (distilled water), the SSC increased after 14 days of storage. However, the SSC increased more slowly in coated fruits. Thus, the tragacanth gel retained the soluble solids at an almost constant level. The SSC remained constant at 7.5 $g.L^{-1}$ tragacanth concentration. However, at 5 and 10 $g.L^{-1}$ of coating treatments, the SSC significantly increased during the last month of storage (Fig. 4).

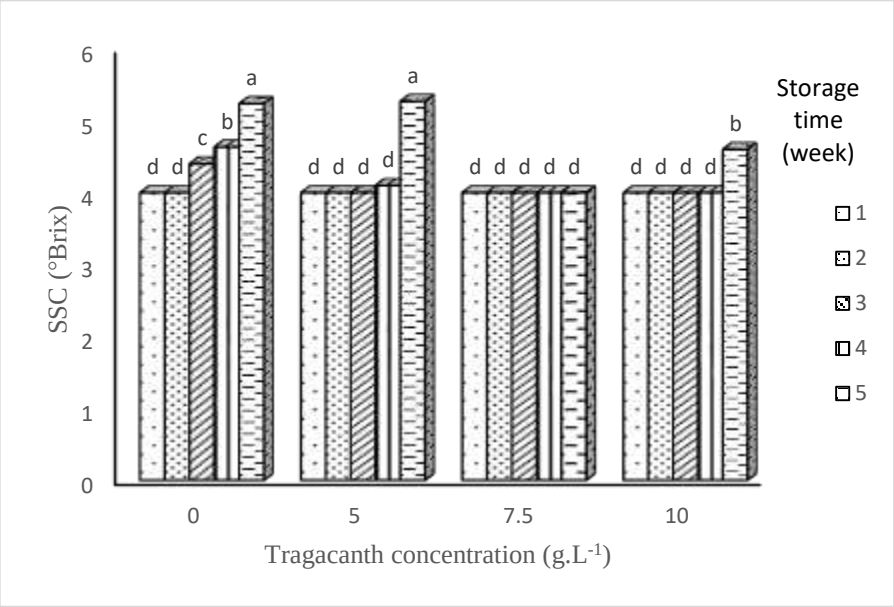

Fig. 4. Effect of tragacanth gum concentration on the SSC of tomato fruits during storage time.

Most soluble solids in fruits contain sugars and a small percentage of amino acids, organic acids, vitamins, and minerals. Fruit ripening and moisture loss in tomato fruits usually increase the SSC in them (Flores et al., 2007; Lee et al., 2003). The increase in SSC during storage time may be related to the weight loss of the fruit, which in turn increases the soluble solids concentration (Tanda-Palmu et al., 2005). However, because the lowest weight loss of the fruits was observed at 7.5 $g.L^{-1}$ tragacanth concentration, so SSC was kept constant. Research on tomato (Chrysargyris et al., 2016) and grape (Asghari & Ahadi, 2013) fruits have also reported that fruits without Aloe vera gel coating had higher SSC after storage time. The SSC was significantly lower in 10% and 15% Aloe-coated fruits after 7 days of storage (Chrysargyris et al., 2016). The SSC in the control treatment was higher compared to coated fruit.

Fruit firmness

According to the results, the reduction in fruit firmness was observed with high speed over time in the control treatment (distilled water). However, in the fruits that were coated, the firmness of tomato fruits was maintained at a higher level. The results showed that at 7.5 $g.L^{-1}$ tragacanth gum, the firmness decreased slower during storage time. Whereas, the firmness of

fruit coated with both concentrations of 5 and 10 $g.L^{-1}$of tragacanth decreased more than 7.5 $g.L^{-1}$ concentration of (Fig. 5).

Our results showed that the tragacanth can delay the softening and ripening process of tomato fruit. Fruit firmness is one of the important qualitative characteristics of many fruits, including tomatoes, which expresses the fruit's surface and internal properties. The tissue firmness of tomato fruits is reduced during the ripening process by the decomposition of insoluble protopectins into pectic acid and soluble pectins (Ali et al., 2010).

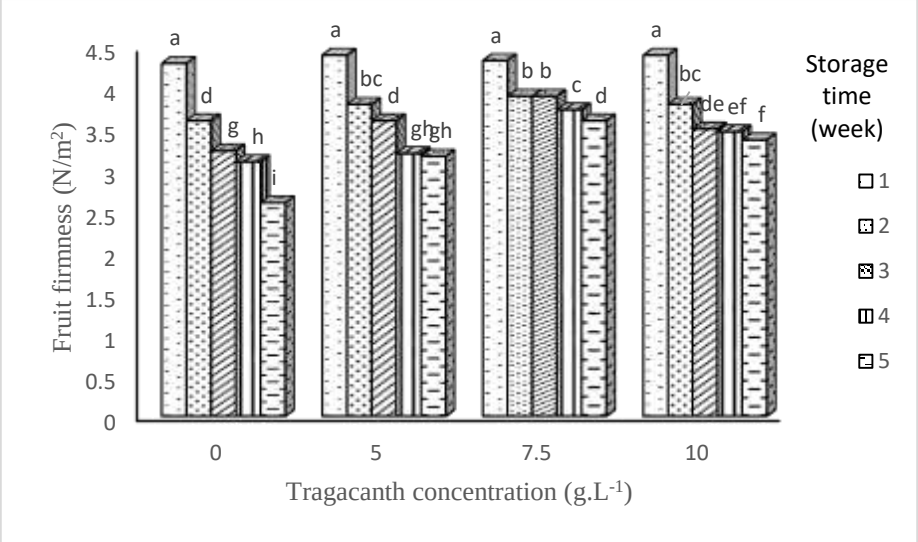

Fig. 5. Effect of tragacanth gum concentration on the fruit firmness of tomato fruits during storage time.

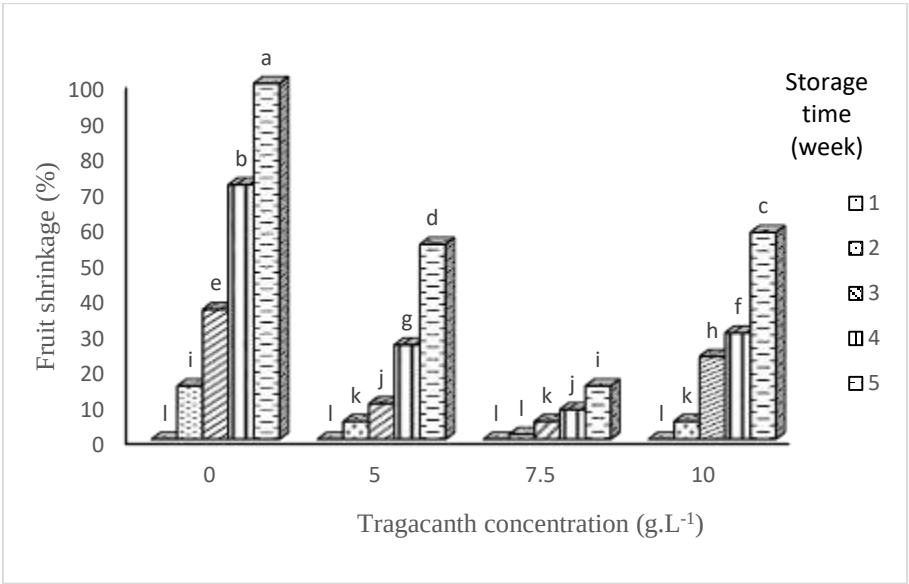

Fig. 6. Effect of tragacanth gum concentration on the fruit shrinkage of tomato fruits during storage time.

The use of edible coatings in many cases controls the softening of fruit tissue during storage time (Zhuang & Huang, 2003). In one research, fruit firmness was reduced during storage time for both coated and uncoated fruits. At the end of storage time, the control fruits showed the lowest firmness. The maximum firmness was maintained with 20% gum arabic until the 12th day (Ali et al., 2010). Martınez-Romero et al. (2005) reported that Aloe vera gel coating reduces weight loss of cherry fruit and thus preserves tissue firmness. Also, Hernandz-Munoz et al. (2006) reported similar results regarding the effect of chitosan and calcium coating on fruit firmness of strawberries, which is in agreement with this result. Aloe vera gel-treated grapes retained their firmness 50% higher than control grapes after 21 days of cold storage (Valverde et al., 2005).

The fruit shrinkage

Based on the results of the correlation between fruit shrinkage and weight loss percentage, these two traits were positively correlated with a 99% probability level (data not shown). This means that with increasing weight loss, shrinkage increases. So it can be concluded that the shrinkage of the fruit surface is directly caused by the loss of water and moisture.

The fruit shrinkage increased more rapidly compared to the coated fruits, so all fruit surface was shrinkage during the last week of storage. However, in the coated fruits, the shrinkage occurred less slowly and at a smaller surface area. The fruits coated with 7.5 $g.L^{-1}$ tragacanth have the least shrinkage area (about 15%) compared to other treatments in the last week of storage (Fig. 6).

Decay evaluation

Until day 14 of the storage, no decay was observed in uncoated or coated fruits. In the control treatment, the decay index of fruit increased faster during storage time, so that in the last week of storage many of the fruits in this treatment were completely crushed. However, in coated fruits, less contamination was observed. The results showed less contamination at 7.5 $g.L^{-1}$. However, at both concentrations of 5 and 10 $g.L^{-1}$, the rate of fruit decay increased during storage time (Fig. 7). Edible coatings can affect the growth of microorganisms due to the control of respiratory gases and fruit atmosphere. Some of them such as *Aloe vera* gel and chitosan have antimicrobial effects. Also, the addition of different antimicrobial agents to these coatings can prevent the growth of fungi and other microorganisms on the fruit surface (Hernandz-Munoz et al., 2006). It has also been reported that carvacrol in tragacanth has a wide range of antimicrobial effects. It inhibits ATPase activity and increases the nonspecific permeability of the bacterial membrane and not only inhibits the bacterial population but also increases the membrane permeability of the bacterium, making them susceptible to other antibacterial substances (Gill et al., 2006).

Similar results were observed by reducing the fruit surface microorganisms by using edible coatings such as carrageenan (0.5 g/100 ml) and whey protein concentrate (Lee et al., 2003). Chrysargyris et al. (2016) reported the same results by using Aloe vera coating on tomato fruits. Also until the fourth day of storage, no significant decay was observed in the coated or control fruits. After that, the coatings significantly reduced decay compared to control and fruits with 10% gum arabic coating, and remained unaffected even after 20 days of storage (Ali et al., 2013).

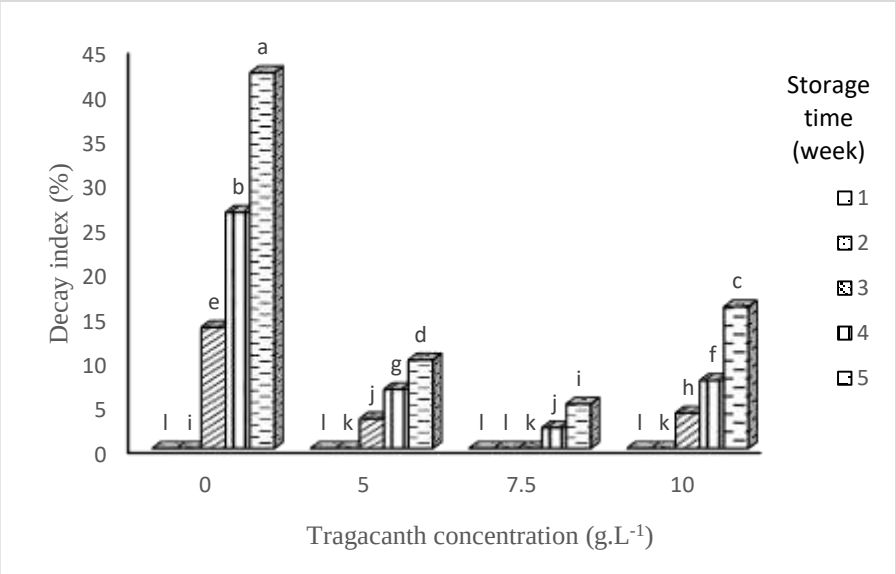

Fig. 7. Effect of tragacanth gum concentration on the decay index of tomato fruits during storage time.

CONCLUSION

According to our results, the fruits coated with tragacanth gum showed less weight loss, and shrinkage compared to the control treatment. The percentage of weight loss of the non-coated sample was about three times higher than coated fruit with 7.5 $g.L^{-1}$ tragacanth gum. Fungal contamination in uncoated fruits was high in the third and fourth weeks of storage so some samples were eliminated. In addition, fruits coated with tragacanth gum were higher fruit firmness. So, the tragacanth coating improved the appearance of the fruit, which can influence the marketability and product sales, and then it could be used for increasing the postharvest life of tomato fruits.

Conflict of interest

The authors declare that they have no conflict of interest.

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The physiological effect of fruit maturity and 1-methylcyclopropene on 'Hass' avocado fruit exocarp colour and chilling injury during ripening

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ABSTRACT

Purpose: This study was undertaken to investigate the influence of harvest maturity and 1-methylcyclopropene (1-MCP) treatment on exocarp colour development and chilling injury of 'Hass' avocado fruit during ripening. **Research method:** 'Hass' avocado fruit harvested at three different maturity stages, early (21% DM), mid- (28% DM) and late (35% DM) were treated with 300 g mol^{-1} of 1-MCP for 16 hours and stored at 5.5 °C for 28 days, subsequently, ripened at 21 °C. The physico-chemicals quality parameters evaluated every 2 days includes exocarp subjective (visual colour), objective (lightness-L^*, chroma-C^* and hue angle-h^o), chlorophyll-*a* and -*b*, total anthocyanin, cyanidin 3-*O*-glucoside and chilling injury. **Findings:** 1-MCP delayed ripening for early and mid-matured fruit and extended ripening by 2-4 days when compared with untreated fruit. In this study, exocarp colour development of 'Hass' avocado fruit was improved by 1-MCP treatment as measured visual and objectively. The accumulation of total anthocyanins and cyanidin 3-*O*-glucosides in 1-MCP-treated fruit was slower than that in untreated fruit, however, 1-MCP treatment was associated with higher concentrations after fruit had reached 'eat-ripe' firmness, irrespective of maturity. The study found that 1-MCP reduced the development of chilling injury symptoms for early harvested and mid-harvested 'Hass' avocado fruit. **Research limitations:** The main limitation of the present study is the lack of evaluation of ethylene production. **Originality/Value:** The study found that different maturity stages of 'Hass' avocado fruit responded differently to 1-MCP treatment. Thus, 1-MCP had a positive effect on early and mid-harvest fruit exocarp colour and CI development during ripening.

INTRODUCTION

Avocado (*Persea americana* Mill.) 'Hass' is a member of the Lauraceae family (Chanderbali et al., 2008) with spherical-shaped and medium size fruit that pleasant, creamy and smooth texture edible covered by the thick dark green exocarp (Hurtado-Fernández et al., 2016). Its unique exocarp colour development from green to purple then black during ripening has made 'Hass' very popular among avocado cultivars (Cox et al., 2004). The exocarp colour is used as a visual quality parameter for assessing postharvest fruit quality and marketability of 'Hass' avocado fruit. The importance of this quality attribute in limiting marketability and consumer satisfaction has been repeatedly studied (Mathaba et al., 2015; Mathaba et al., 2017). Consumers associate purple colour development with ripeness and good quality thus, driving preference and purchase at retail stores. Colour development for 'Hass' avocado fruit can be strongly affected by harvest maturity and postharvest treatments used during storage (Mathe et al., 2018).

In avocado fruit, harvest maturity is important for quality and handling and markedly influences colour development during ripening. Therefore, harvesting 'Hass' avocado fruit at an early maturity stage results in poor colour development during ripening and as such fruit quality becomes insufficient to fulfil traders and consumer preference (Mathaba et al., 2015). The purple and black exocarp colours are mainly determined by anthocyanin pigments identified as cyanidin 3-*O*-glucoside (Cox et al., 2004). Previous studies showed that the concentration of cyanidin 3-*O*-glucoside, which contributes to 'Hass' avocado fruit purple exocarp colouration, was significantly influenced by harvest maturity (Ashton et al., 2006; Cox et al., 2004). Donetti & Terry (2014) found that 'Hass' avocado fruit harvested at early maturity showed reduced exocarp cyanidin 3-*O*-glucoside concentration during ripening. However, fruit harvested at late maturity recorded higher cyanidin 3-*O*-glucoside concentrations accompanied by improved colour change (Cox et al., 2004).

Postharvest treatment such as 1-methylcyclopropene (1-MCP) has been used to extend the storage-life of avocado fruit during cold storage (Jeong et al., 2002). In general, 1-MCP application inhibits ethylene perception, consequently altering the ethylene-dependent process including softening and colour development (Hershkovitz et al., 2005; Jeong et al., 2002; Mubarok et al., 2022). Moreover, the influence of 1-MCP on fruit quality attributes such as softening, and colour development is by delaying their metabolic rate. Several studies showed that 1-MCP treatment for avocado fruit resulted in delayed softening, colour development and incidence of physiological disorders (Woolf et al., 2005). Research findings have revealed that the effectiveness of 1-MCP was ascribed to harvest maturity (Blankenship & Dole, 2003; Satekge & Magwaza, 2022). Moreover, avocado fruit are sensitive to low temperatures, and prolonged storage under 10 °C causes chilling symptoms such as darkening of the surface, pitting, discolouration of the mesocarp, uneven ripening, and poor fruit quality (Setagane et al., 2021). the development of chilling injury symptoms for 'Hass' avocado fruit exacerbates the desynchronization of fruit firmness with exocarp colour change during ripening. During ripening, Mathaba et al. (2015) found that the development of CI symptoms was closely related to poor exocarp colour changes for 'Hass' avocado fruit. According to studies, 1-MCP can increase or decrease chilling injury, and has been widely used to reduce postharvest chilling injury in climacteric fruit crops. However, little has been documented about its relationship with anthocyanin accumulation subsequently leading to exocarp colour development in 'Hass' avocado fruit during ripening. The objective of this study was to investigate the influence of harvest maturity and 1-MCP treatment on exocarp colour development and chilling injury of 'Hass' avocado fruit during ripening.

MATERIALS AND METHODS

Site and plant material

This study was conducted at an avocado commercial orchard at Nico Swart estate (25° 04' 12.7" S 31° 00' 35.8" E), Kiepersol, Mpumalanga, South Africa. In the area, the average yearly temperature is 22.26 °C and the average rainfall is < 667 mm. Fruit were harvested from 11 years old 'Hass' avocado trees at different harvest maturity stage based on dry matter); early maturity (≈ 21% dry matter), mid-harvest (≈ 28% dry matter) and late harvest (≈ 35% dry matter). During these three-harvest maturities, fruit were immediately transported to the Agriculture Research Council-Tropical and Subtropical Crops (ARC-TSC) postharvest laboratory (25° 27' 04.6" S 30° 58' 09.1" E), Nelspruit, Mpumalanga, South Africa for storage and analysis.

Postharvest experimental design and treatment

The experimental design was carried out as 3 × 2 factorial factors A (early, mid- and late harvest maturity) and factor B (1-MCP at 300 g mol^{-1} and control), arranged in a completely randomized design (CRD) and replicated three times. Fruit were sorted, graded and then packed into avocado crates each containing 30 fruit, therefore, divided into six sample groups, each treatment had three replicated at each harvest maturity. Three groups were untreated and served as control [early (3 × 30 fruit), mid-(3 × 30 fruit) and late (3 × 30 fruit)]. The other three sample groups were treated with 1-MCP [early (3 × 30 fruit), mid-(3 × 30 fruit) and late (3 × 30 fruit)] at 300 g mol^{-1} in a closed plastic container for 16 hours. All six sample groups were cold stored at 5.5 °C for 28 days. After removal from cold storage, fruit were ripened at 21 °C. During ripening fruit were sensory evaluated every second day until they reached 'eat ripe' firmness. Fruit quality evaluated includes firmness, colour (subjective and objective colour parameters), and 5 fruit per treatment were sampled, freeze-dried in liquid nitrogen and subsequently, cold-stored at -21 °C for further analysis of total carotenoids and chlorophyll-*a* and-*b*, total anthocyanin and cyanidin 3-*O*-glucoside.

Determination of physicochemical parameters

Fruit firmness

Fruit firmness was determined using non-destructive digital bench top Sinclair IQ™ desktop automated machines (51DFTB, International LTD, Jarrold, Bowthorpa, Nonwich, NR5, 9.D, England). Fruit were measured three times along the equatorial region and values were expressed in Newton (N).

Subjective and objective exocarp colour parameters

Avocado fruit 'Hass' exocarp colour change was determined subjectively using eye colour change (1 – emerald-green, 2 – forest-green, 3 – olive-green, 4 - violet; 5 - purple, and 6 – black) as previously described by Mathaba et al. (2015). The same fruit samples were also used for objective colour assessment using Minolta chromameter (Model: CR-400, Minolta, Sensing Incorporation, Japan) with a white calibration plate (Y = 87.00; x = 0.3146; y = 0.3215) L^* = lightness, a^* = greenness/redness and b^* = yellowness/blueness and thereafter, converted to chroma and hue angle ($h°$) using the necessary equations according to McGuire (1992).

Total carotenoids, chlorophyll-a and chlorophyll-b

Chlorophyll and total carotenoids were determined using a UV-visible spectrophotometer as previously described by Lichtenthaler (1987). Freeze-dried 'Hass' avocado exocarps tissue 0.5 g was extracted with 10 ml of 80% acetone. The extraction tubes were kept on ice for 30

minutes and thereafter, vortexed for 30 seconds and centrifugation at 2500 × g for 5 minutes. The absorbance values of the supernatant were measured at 470, 646 and 663 nm. Calculation of content of chlorophyll-a and -b and carotenoids were as follows.

$$C_a = 12.25\ A_{663} - 2.79\ A_{646} \quad (1)$$

$$C_b = 21.50\ A_{646} - 5.10\ A_{663} \quad (2)$$

$$C_{x+c} = (1000\ A_{470} - 1.82\ Ca - 85.02\ C_b) / 198 \quad (3)$$

Where C_a and C_b represents chlorophyll *a* and *b*, and C_{x+c} total carotenoids

Total anthocyanin and cyanidin 3-O-glucoside

Extraction was done according to Cox et al. (2004), avocado exocarp tissues were milled to powder under liquid nitrogen and 0.5 g was extracted with 5 ml of 10% acetic acid/methanol (v/v) at room temperature. The extract was centrifuged at 3000 × g for 10 min, the supernatant was diluted 1:1 with methanol: water: acetic acid (50:50:10, v/v/v). The pH differential method previously described by Giusti & Wrolstad (2001) was used to determine total anthocyanin content. The diluted 1:1 supernatant was filtered through 0.45 µm nylon filters into clean vials and diluted with 1 µl of potassium chloride buffer ($pH_{1.0}$) and sodium acetate buffer ($pH_{4.5}$), separated in triplicate. The mixtures were allowed to settle in the dark for 10 minutes subsequently, absorbance values of each buffer mixture were measured at 530 and 700 nm in a UV-visible spectrophotometer. The total anthocyanin was calculated using the equation.

$$A = (A_{510} - A_{700})\ pH\ _{1.0} - (A_{510} - A_{700})\ pH\ _{4.5} \quad (4)$$

$$\text{Total anthocyanin (mg/ml)} = (A \times MW \times DF) / (\varepsilon \times L) \quad (5)$$

Where A = Absorbance, ε = Cyd-3-glucoside molar absorbance (26,900), MW = anthocyanin 164 molecular weight (449.2), DF = dilution factor, L = cell path length (1 cm).

Furthermore, using the above-described extraction method cyanidin 3-*O*-glucoside concentration was measured by HPLC as previously described by Cox et al. (2004). The HPLC system was equipped with JASCO units (LG-980-02 ternary gradient controller, AS-950 auto sampler, and a UV-975 UV/Vis detector). The chromatography column was a Phenomenex AQUA 5u C18 125A 5 um PR-18e 4.6 × 150 mm (California, United States of America), maintained at 35 °C. Where mobile phase (A) 1.5% $H_3\ PO_4$ and (B) acetic acid: acetonitrile: $H_3\ PO_4$: water (20: 24: 1.5: 54.5, v/v/v/v) was used. The solvent program started with solvent (B) at 20%, increasing to 70% after 25 minutes then 90% at 30 minutes. After 35 minutes the solvent composition was returned to the initial 20% solvent (B) and ready for the next injection. The sample injection volume was 2 µl and detection was at 530 nm.

Exocarp chilling injury

Exocarp chilling injury was measured according to the International Avocado Quality Manual (White et al., 2009) where a benchmark percentage and severity were derived as level 1= 10%; 2 = 30% and 3 = 50% chilling severity. The chilling injury was recorded only on the second day of fruit ripening as previously described by Mathe et al. (2018).

Statistical analysis

Statistical analyses were carried out using statistical software (GenStat, version 16th, VSN International, UK) and means separated using Duncan multiple range tests (DMRT) at the 5% level of significance. Furthermore, data were subjected to principal component analysis (PCA) using Unscrambler version 9.8 (Camo Process AS, Oslo, Norway). In addition, the relationship between the measured postharvest fruit quality parameters were determined by subjecting data to Pearson correlation test in Statistix software version 10.1.

RESULTS

Fruit firmness

The firmness of avocado 'Hass' fruit declines continuously during ripening, with maturity and 1-MCP treatment contributing significantly ($p < 0.05$) (Fig. 1). This study found that early harvest fruit ripened steadily thus, taking longer to ripen when compared to mid- and late harvest. Moreover, 1-MCP treated fruit ripened slowly for early and mid-harvested when compared with late-harvested fruit (Fig. 1). The ripening patterns of late harvest 1-MCP and untreated fruit were comparable, but 1-MCP treated fruit extended their ripening by 2 days.

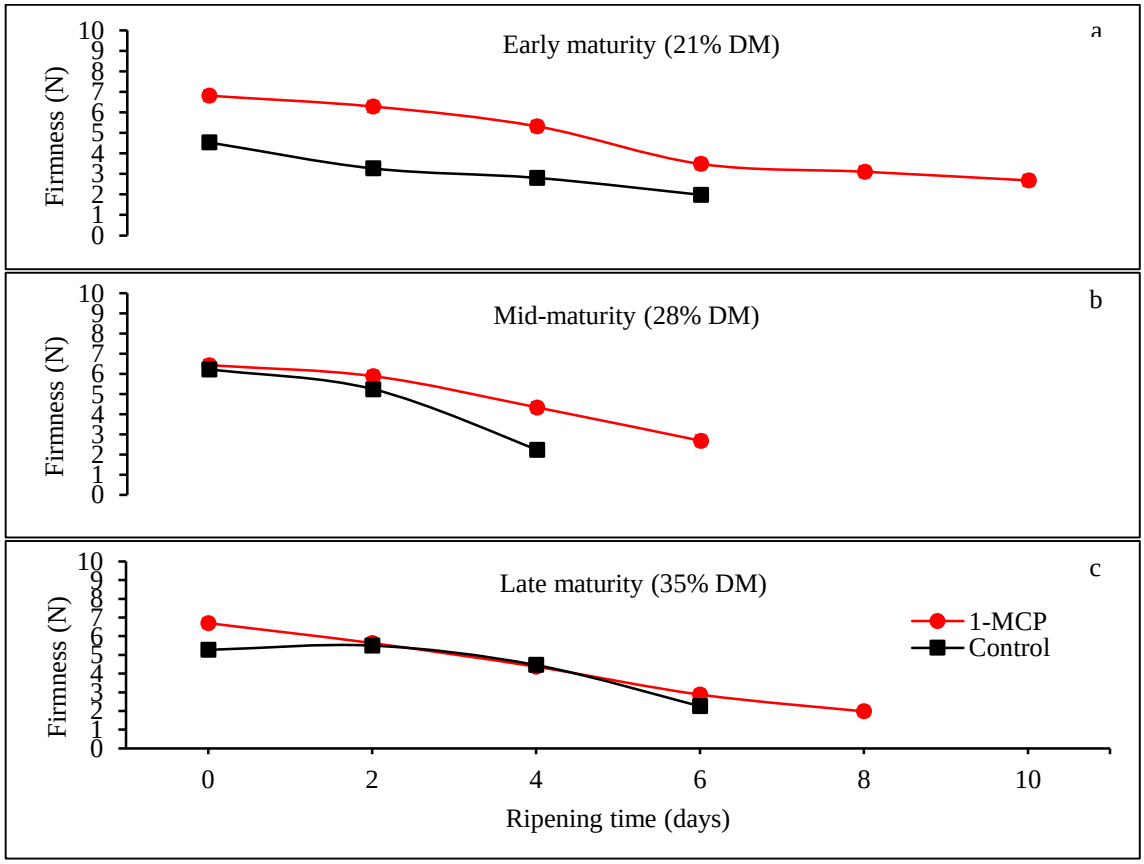

Fig. 1. Changes in fruit firmness of 'Hass' avocado fruit during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 3).

In this study, Pearson correlation was conducted to examine the relationship between firmness and visual colour and objective colour parameters during ripening (Table 1). According to Pearson correlation, firmness and visual colour were significantly correlated with each at early, mid and late harvest (R^2 = -0.953**, -0.894** and -0.928**, respectively) for untreated fruit. For 1-MCP treated fruit, a significant negative correlation was observed at early (R^2 = -0.833**), mid (R^2 = -0.944**) and late-harvest (R^2 = -0.911**). There was a significant positive correlation between objective colour parameters (L^*, C^* and $h°$) and firmness throughout harvest time and treatments (1-MCP and untreated) during ripening. In the early season, 1-MCP fruit were significantly different from untreated fruit, especially in terms of lightness (L^*) and chroma (C^*) (Fig. 3a and Fig. 4a). The exocarp colour of 'Hass' avocado fruit showed no significant differences at late harvest, therefore, the effect of 1-MCP treatment was not significant when compared with the untreated fruit for all objective colour parameters (L^*, C^* and $h°$).

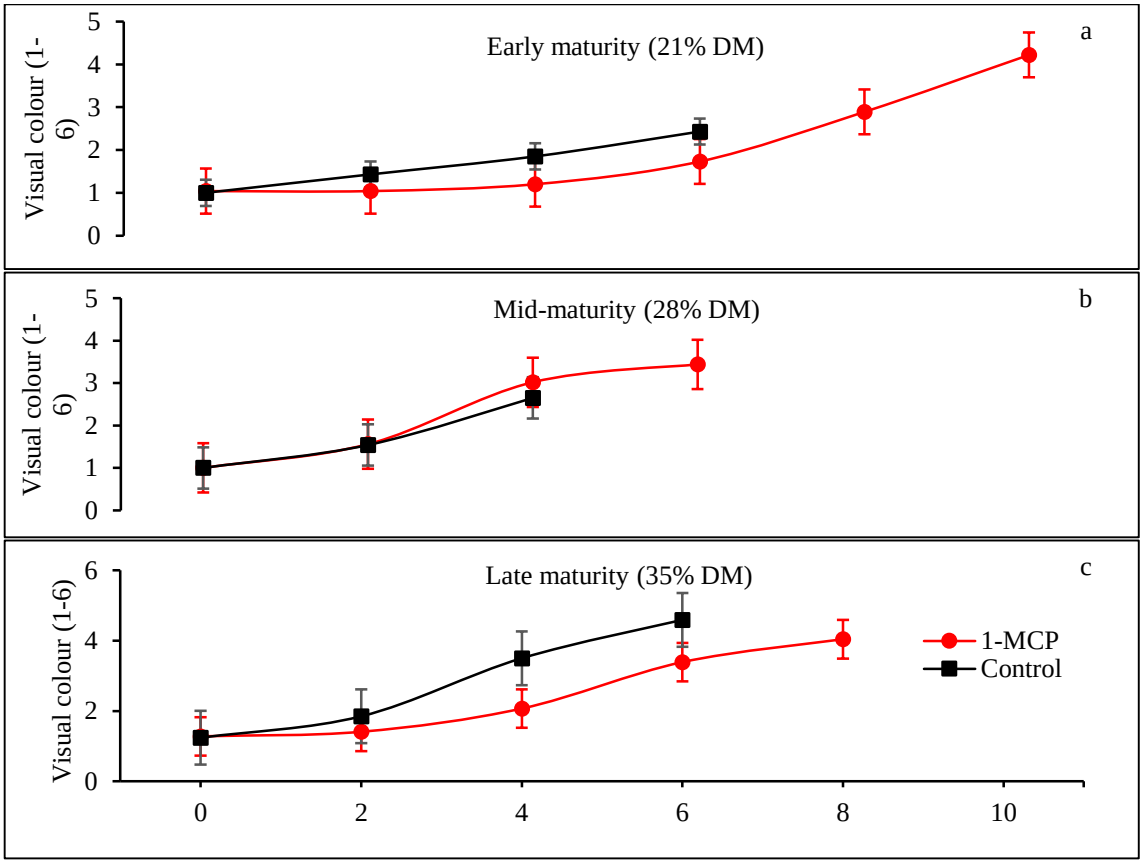

Fig. 2. Change in subjective colour (visual colour rating) of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 3).

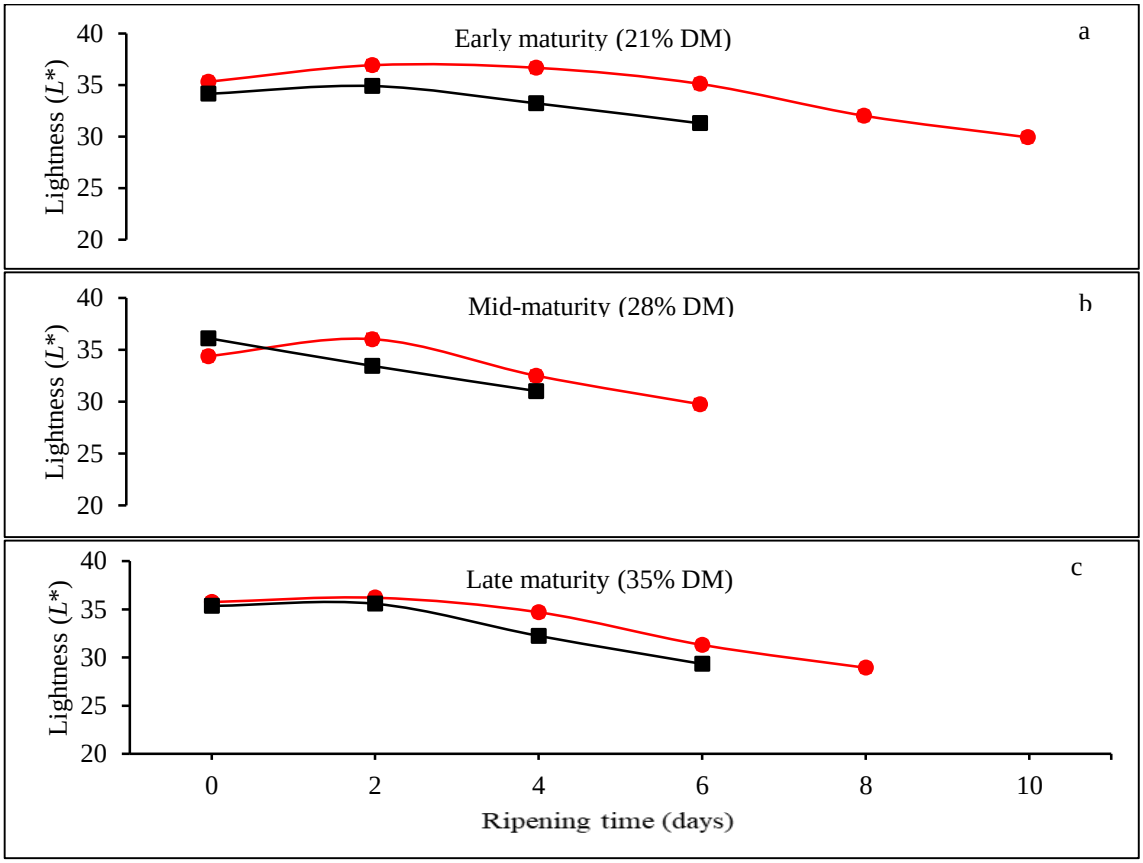

Fig. 3. Change in lightness-*L** of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 3).

Furthermore, colour intensity was evident by the decline in objective (*L**, *C** and *h**) values, irrespective of treatment (Fig. 3, Fig. 4 and Fig. 5). In general, objective (*L**, *C** and *h*°) values decreased with days to ripening in all investigated treatments. A decrease in *h*° values reflected a change in exocarp colour from emerald-green to purple, then black, and a decline in *L** and *C** values reflected a decrease in colour intensity, resulting in a darker colour. In the present study, exocarp *h*° colour negatively correlated with cyanidin 3-*O*-glucoside at early (R^2 = -0.731**), mid (R^2 = -0.796*) and R^2 = -0.839**) for untreated fruit, and early (R^2 = -0.857**), mid (R^2 = -0.727**), late (R^2 = -0.735**) for 1-MCP treated fruit (Table 1). According to these observations, the colour of 'Hass' avocado fruit during ripening was associated with cyanidin 3-*O*-glucoside accumulation, which was increased by late harvest and 1-MCP treatment.

Subjective and objective exocarp colour parameters

In this study, the subjective exocarp colour parameters visual colour (Fig. 2) and objective (*L**, *C** and *h*°) (Fig. 3, Fig. 4, and Fig. 5) changed for all investigated treatments during ripening. It appeared that visual colour significantly ($p < 0.05$) increased due to a combined effect of fruit maturity and 1-MCP treatment on visual colour (Fig. 2). Late harvested fruit recorded higher visual colour rating when compared with early and mid-season, consequently, the fruit showed purplish to purplish black exocarp colour after reaching 'eating ripe' firmness.

Table 1. Pearson correlation coefficient between objective (Minolta chromameter values; *L**, *C**, *h°*) and subjective (visual colour rating) of 'Hass' avocado fruit exocarp colour measurement/firmness and total anthocyanin and cyanidin 3-*O*-glucoside concentrations in response to harvest maturity and 1-MCP treatment during ripening at 21 ± 2 °C.

Correlations	Early maturity		Mid-maturity		Late maturity	
	Control	1-MCP	Control	1-MCP	Control	1-MCP
	R^2		R^2		R^2	
Firmness × Vis colour	-0,953**	-0,833**	-0,894**	-0,944**	-0,928**	-0,911**
Firmness × *L**	0,703**	0,768**	0,917**	0,885**	0,944**	0,899**
Firmness × *C**	0,971**	0,912**	0,846**	0,965**	0,822**	0,959**
Firmness × *h°*	0,918**	0,841**	0,719*	0,798**	0,828**	0,897**
Firmness × Total anthoc	-0,937**	-0,827**	-0,958**	-0,946**	-0,925**	-0,753**
Firmness × Cyan 3-gluc	-0,686**	-0,665**	-0,957**	-0,965**	-0,926**	-0,858**
Vis colour × *L**	-0,788**	-0,959**	-0,890**	-0,849**	-0,956**	-0,935**
Vis colour × *C**	-0,961**	-0,955**	-0,856**	-0,989**	-0,953**	-0,968**
Vis colour × *h°*	-0,958**	-0,989**	-0,920**	-0,945**	-0,964**	-0,943**
Total anthoc × Vis colour	0,944**	0,914**	0,916**	0,824**	0,829**	0,769**
Total anthoc × *L**	-0,813**	-0,814**	-0,858**	-0,838**	-0,885**	-0,804**
Total anthoc × *C**	-0,963**	-0,875**	-0,770**	-0,870**	-0,679*	-0,733**
Total anthoc × *h°*	-0,851**	-0,712**	-0,817**	-0,623*	-0,673*	-0,603*
Cyan-3-gluc × Vis colour	0,793**	0,900**	0,882**	0,898**	0,936**	0,883**
Cyan-3-gluc × *L**	-0,795**	-0,900**	-0,827**	-0,903**	-0,942**	-0,895**
Cyan-3-gluc × *C**	-0,687**	-0,859**	-0,705*	-0,922**	-0,851**	-0,863**
Cyan-3-gluc × *h°*	-0,731***	-0,857**	-0,796*	-0,727**	-0,839**	-0,735**
Total anthoc × Cyan-3-gluc	0,787**	0,834**	0,933***	0,921**	0,894**	0,866**

Vis colour = visual colour, *L** = lightness, *C** = chroma, *h°* = hue angle, Total anthoc = Total anthocyanin, Cyan 3-gluc = Cyanidin 3-*O*-glucoside, *significant different at *p ≤ 0.05, **p ≤ 0.01, and ns = non-significant.

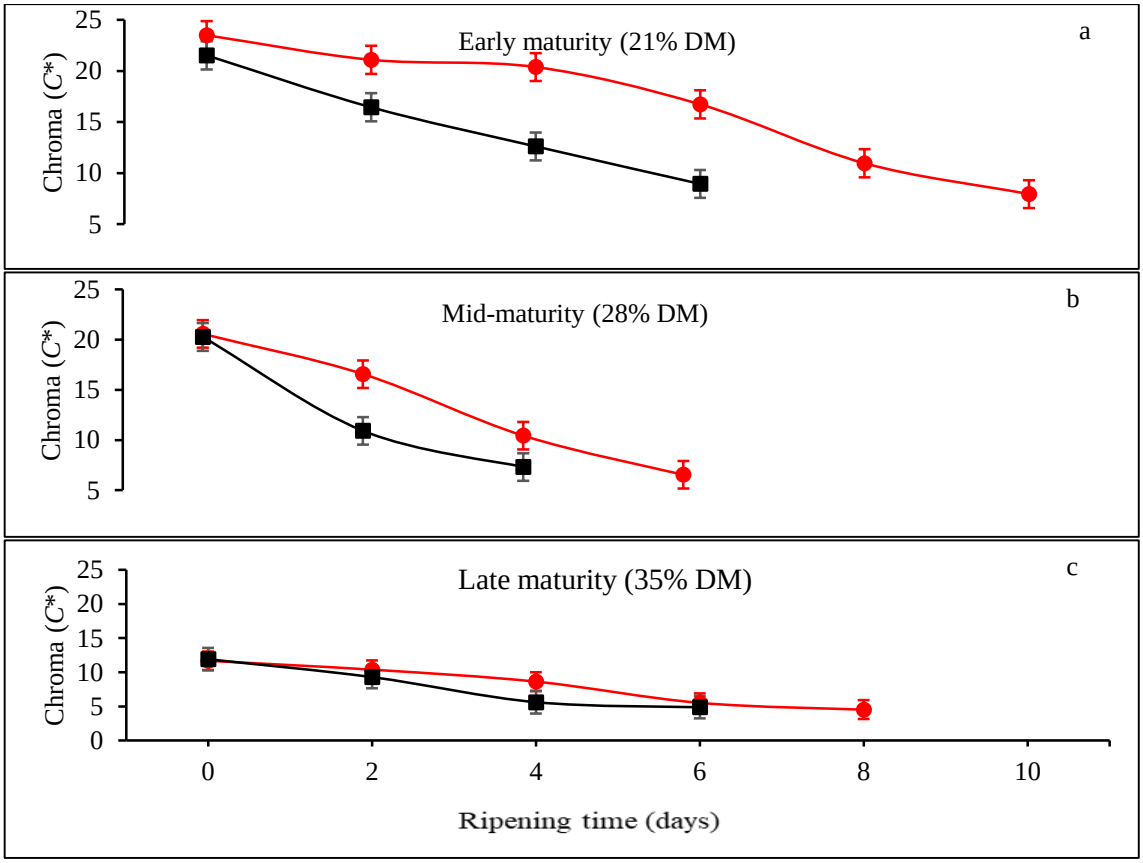

Fig. 4. Change in chroma-*C** of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 3).

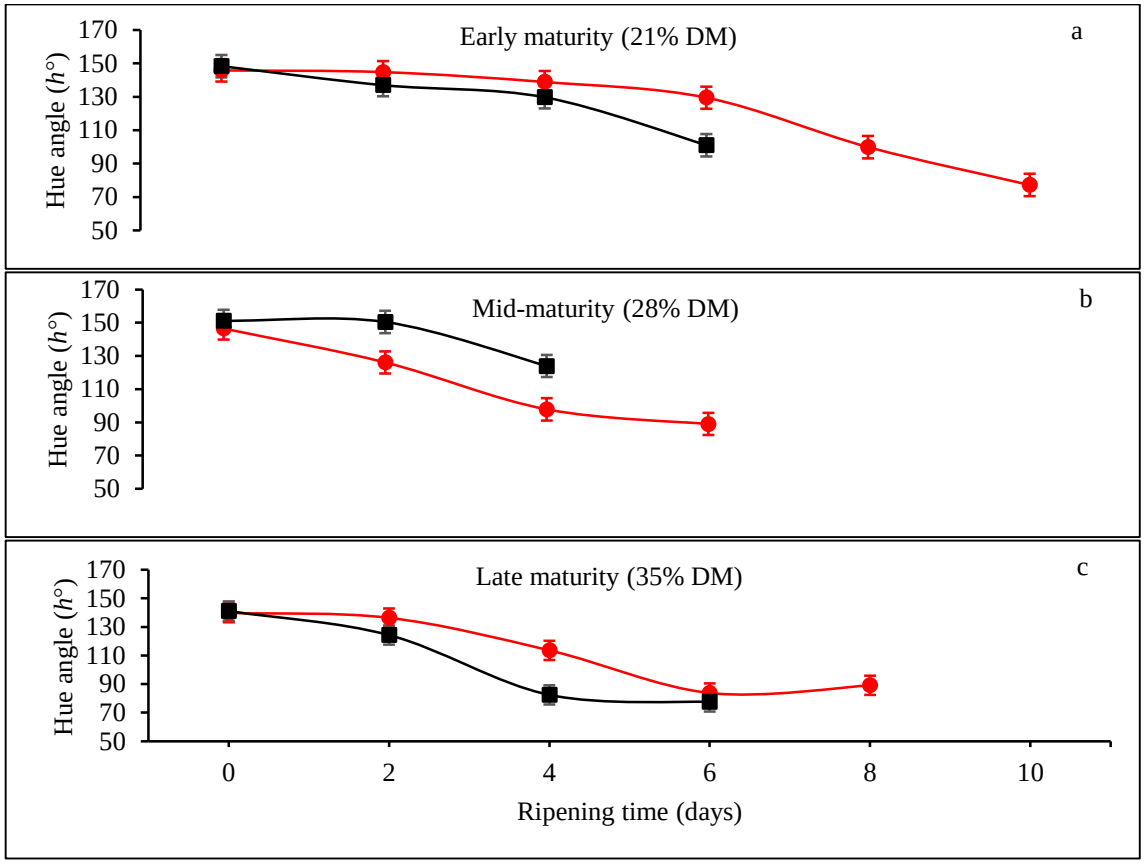

Fig. 5. Change in hue angle-*h*° of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 3).

Total carotenoids (TC), chlorophyll-a and chlorophyll-b

There was a continuous accumulation of total carotenoids, concurrently with a reduction of chlorophyll-*a* and chlorophyll-*b* content in both untreated and 1-MCP-treated fruit during all harvest maturity (Fig. 6, Fig. 7, and Fig. 8). However, during early and mid-harvest, total carotenoids and chlorophyll-*a* and -*b* contents did not decrease significantly during ripening when compared with late-harvest time. In this study, chlorophylls-*a* and -*b* content decreased in both untreated and 1-MCP treated fruit at all fruit maturities, indicating exocarp green colour degradation as the fruit ripens (Fig. 7 and Fig. 8). Furthermore, control fruit harvested late recorded the lowest total carotenoids and chlorophyll-*a* and -*b* content when compared with 1-MCP treated fruit during ripening.

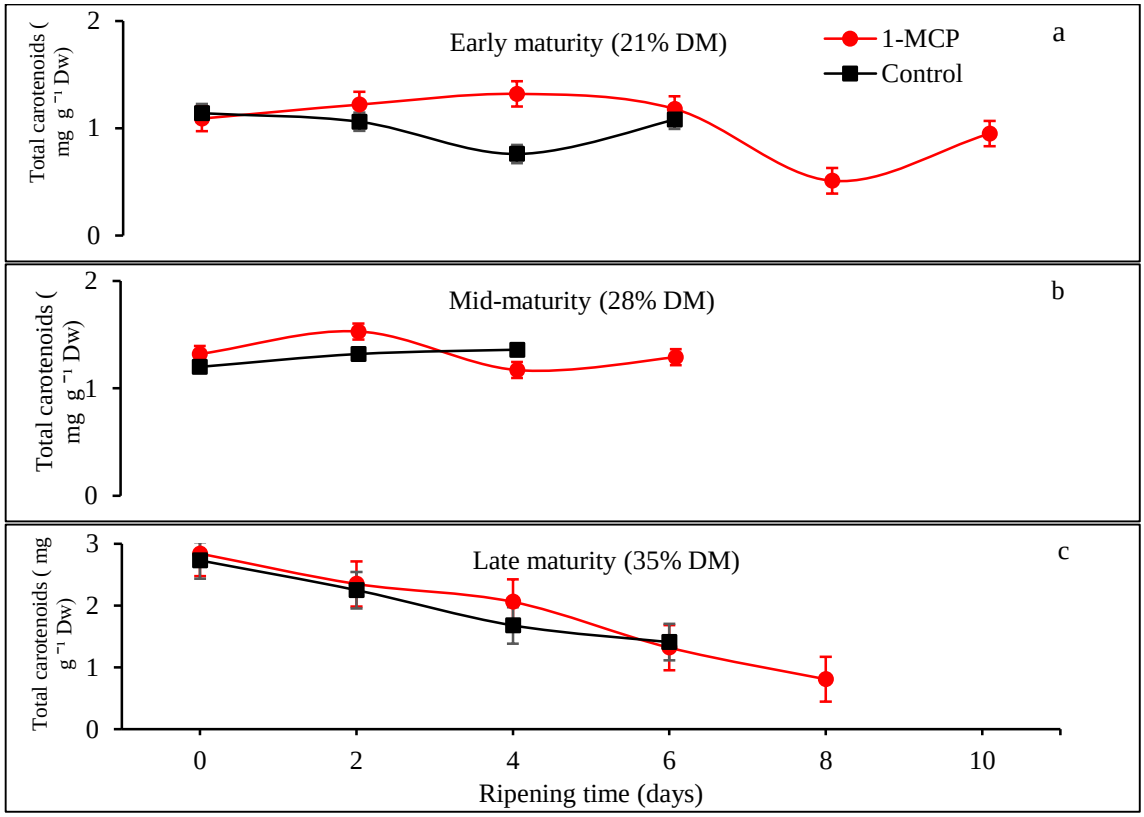

Fig. 6. Change in total carotenoids of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 5).

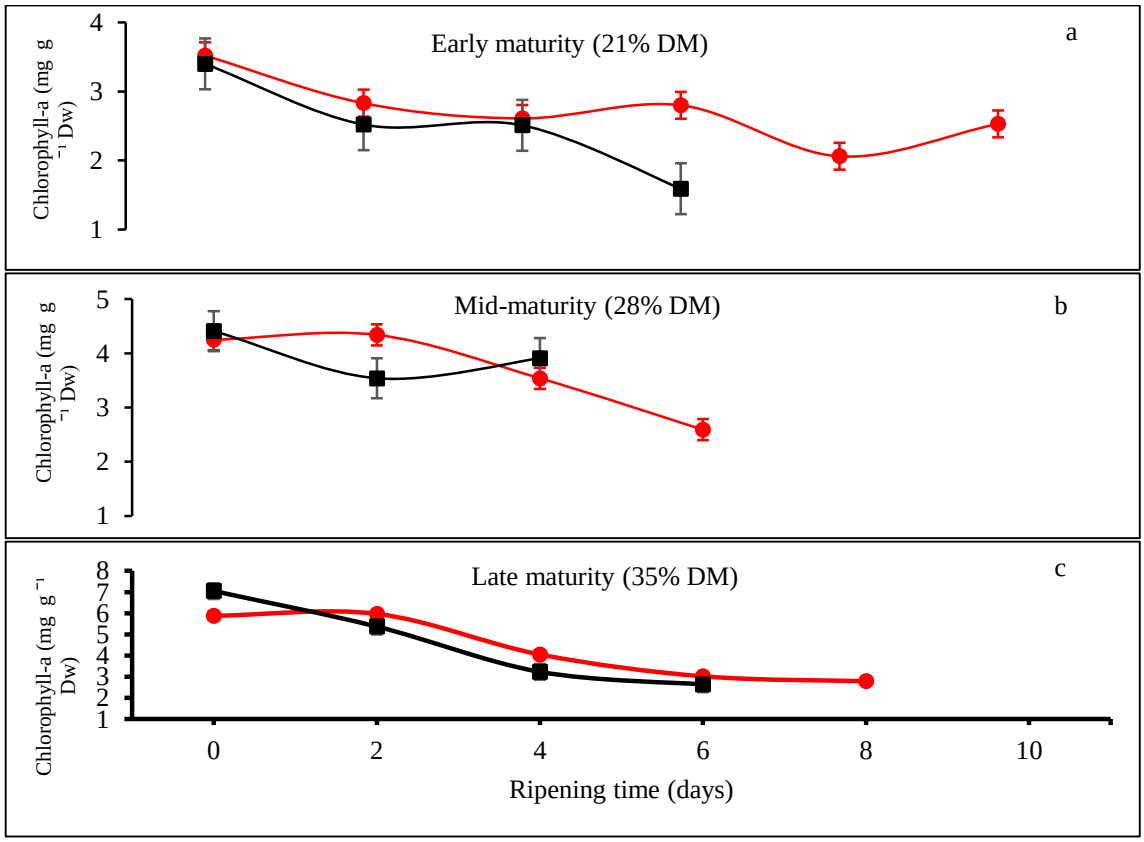

Fig. 7. Change in chlorophyll-a of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 5).

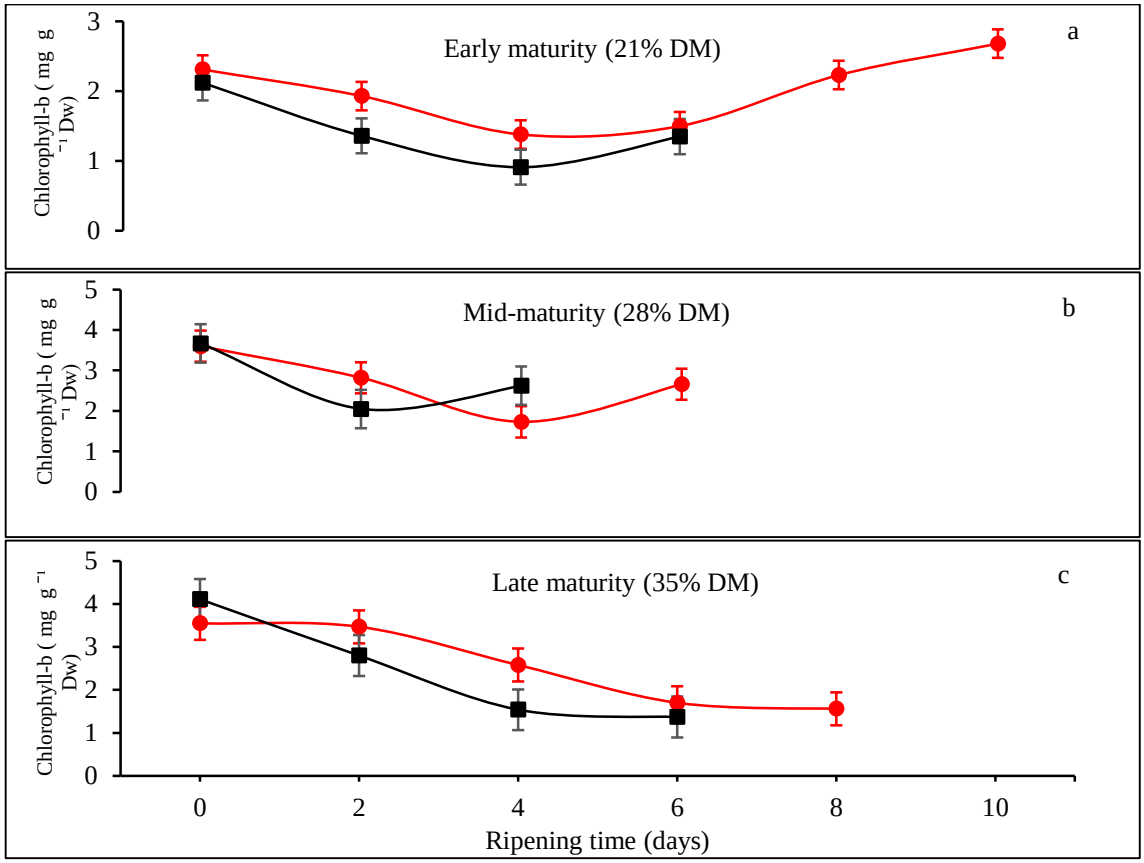

Fig. 8. Change in chlorophyll-b of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 5).

Total anthocyanin (TA) and cyanidin 3-*O*-glucoside (Cy3G)

This study found that late-harvest fruit had higher total anthocyanin and cyanidin 3-*O*-glucoside when compared with early and mid-harvested fruit (Fig. 9 and Fig. 10). In terms of treatment effect, 1-MCP fruit showed higher total anthocyanin and cyanidin 3-*O*-glucoside accumulation on the final day of ripening, irrespective of maturity. According to the Pearson correlation, exocarp total anthocyanin concentration positively correlated with cyanidin 3-*O*-glucoside at early (R^2 = 0.787**), mid (R^2 = 0.933**) and late (R^2 = 0.894**) for untreated fruit, and early (R^2 = 0.834**), mid (R^2 = 0.921**) and late (R^2 = 0.866**) for 1-MCP treated fruit (Table 1).

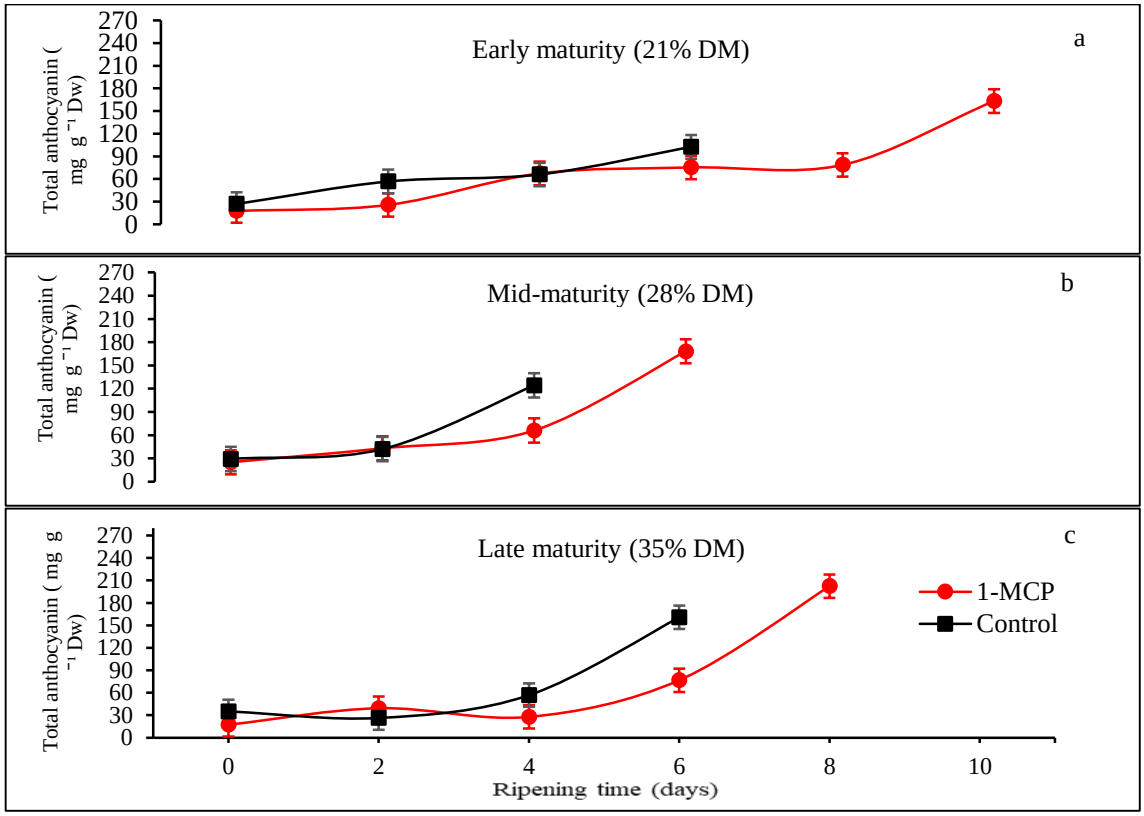

Fig. 9. Change in total anthocyanin of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 5).

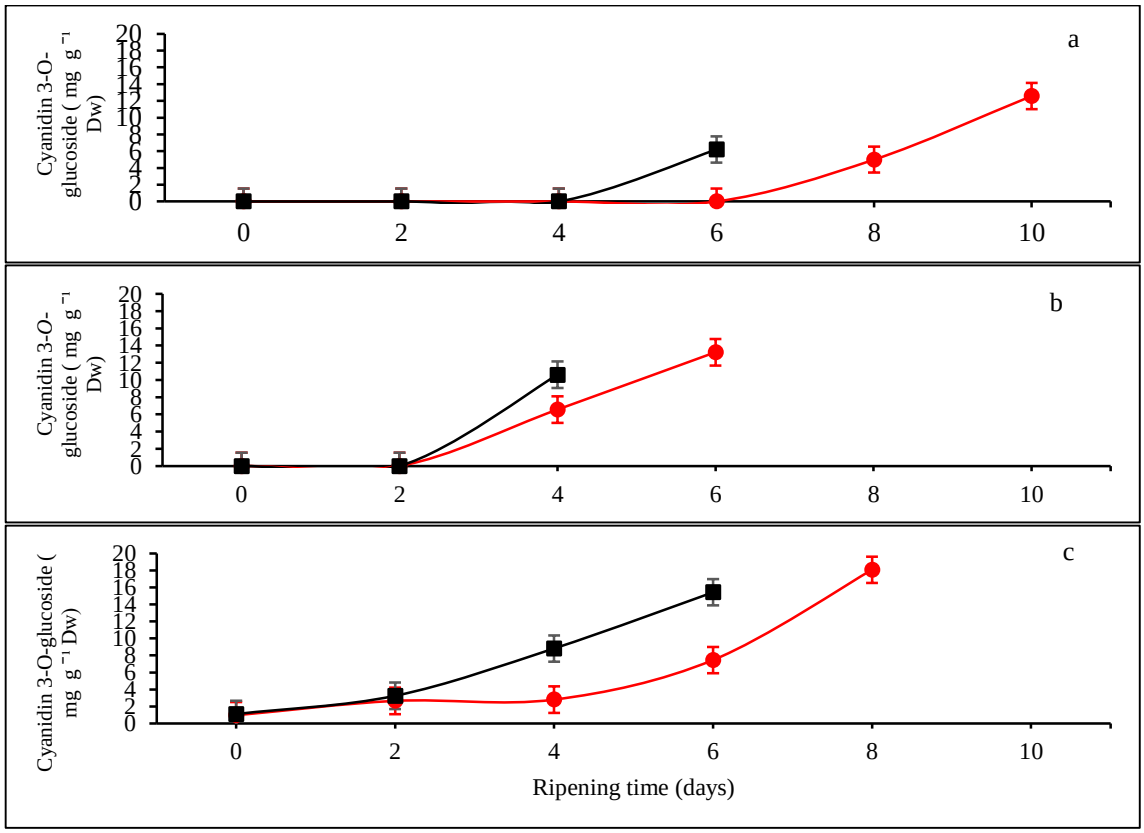

Fig. 10. Change in cyanidin 3-*O*-glucoside of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 5).

Exocarp chilling injury Index (CII)

In this study, chilling injury (CI) incidence decreased with advanced fruit maturity (Fig. 11). The chilling injury index (CII) significantly ($p < 0.05$) decreased during ripening as a result of an interactive effect between fruit maturity and 1-MCP (Fig. 11). Fruit harvested early was highly susceptible to chilling injury, which resulted in a higher proportion of poor coloured fruit than those harvested mid or late. The CI symptoms in untreated fruit were higher when compared with 1-MCP-treated fruit throughout all harvest periods. CI symptoms were significantly suppressed by 1-MCP treatment as reflected in lower CII throughout harvest.

Principal component analysis (PCA)

The interrelationships between the measured parameters concerning the effect of harvest maturity, 1-MCP and ripening time were evaluated using principal component analysis (PCA) (Fig. 12). The PCA showed a total variation of 93%, whereby PC-1 explained 8% (Component 1) while PC-2 explained 85% of the observed variation. It was used to describe the interrelationship of the exocarp colour parameters to fruit ripening in response to maturation, 1-MCP treatment and ripening time (Fig. 12). In this case, the first principal component (PC-1) explained 8% of the total variation and was related to total anthocyanin, cyanidin 3-*O*-glucoside, visual colour, lightness, and firmness. The second principal component (PC-2) explained 85% of the total variation and mainly related to the hue angle.

In addition, fruit ripening characteristics were grouped with the PCA, into seven distinct clusters (Fig. 12). The first cluster included firm, emerald-green fruit that had been treated with 1-MCP at day 0, early harvest 1-MCP treated after day 2, mid-harvest control at day 0 and late harvest control at day 0. The second cluster consisted of firmer and showing forest green colour, which included, early harvest control after day 2, mid-harvest 1-MCP treated at day 2, late 1-MCP treated at day 4 and late harvest control after day 2. The third cluster consisted of fruit which were half-ripe but showed forest green colour; and included early harvest control at day 4 and early season 1-MCP treated at day 6. The fourth cluster consisted of fruit that were half-ripe and showing olive-green colour (early 1-MCP treated day 8). The fifth cluster included those that were half-ripe and showing purple colour (mid-harvest 1-MCP treated after day 4, early 1-MCP treated after day 10 and late 1-MCP treated after day 6). The sixth cluster included avocado fruit that reached eat-ripe and showed purple and black exocarp colour (late 1-MCP treated after day 8, early 1-MCP treated after day 10, late control day 6 and early control day 6). In the seventh cluster, fruit were overripe and showed black exocarp colour (mid-control after day 4 and early control after day 6). According to these clusters, 1-MCP treated fruit showed purple and black exocarp colour upon reaching ripe firmness when compared to untreated fruit, irrespective of fruit maturity.

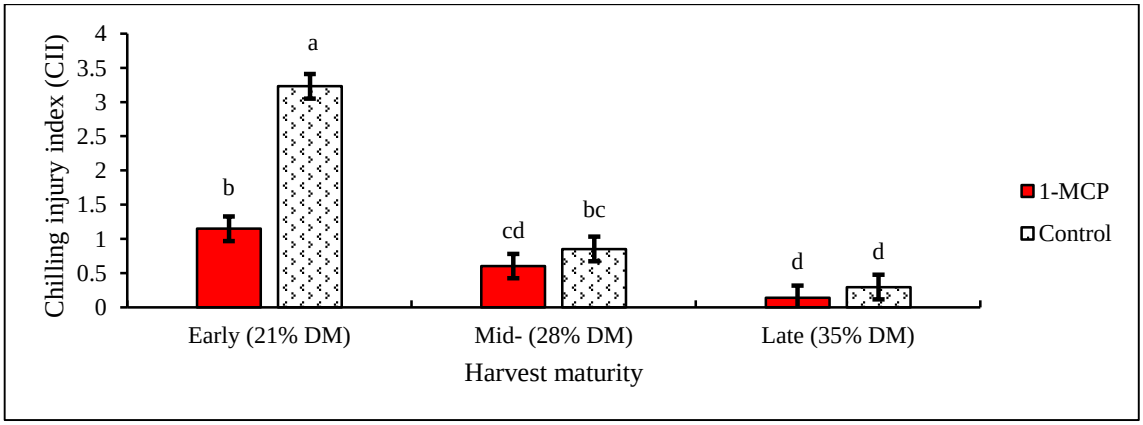

Fig. 11. Chilling injury index (CII) of 'Hass' avocado fruit during ripening at different harvest seasons. Each bar represents means of three replications and vertical bars represent standard error (±SE, *n = 30*).

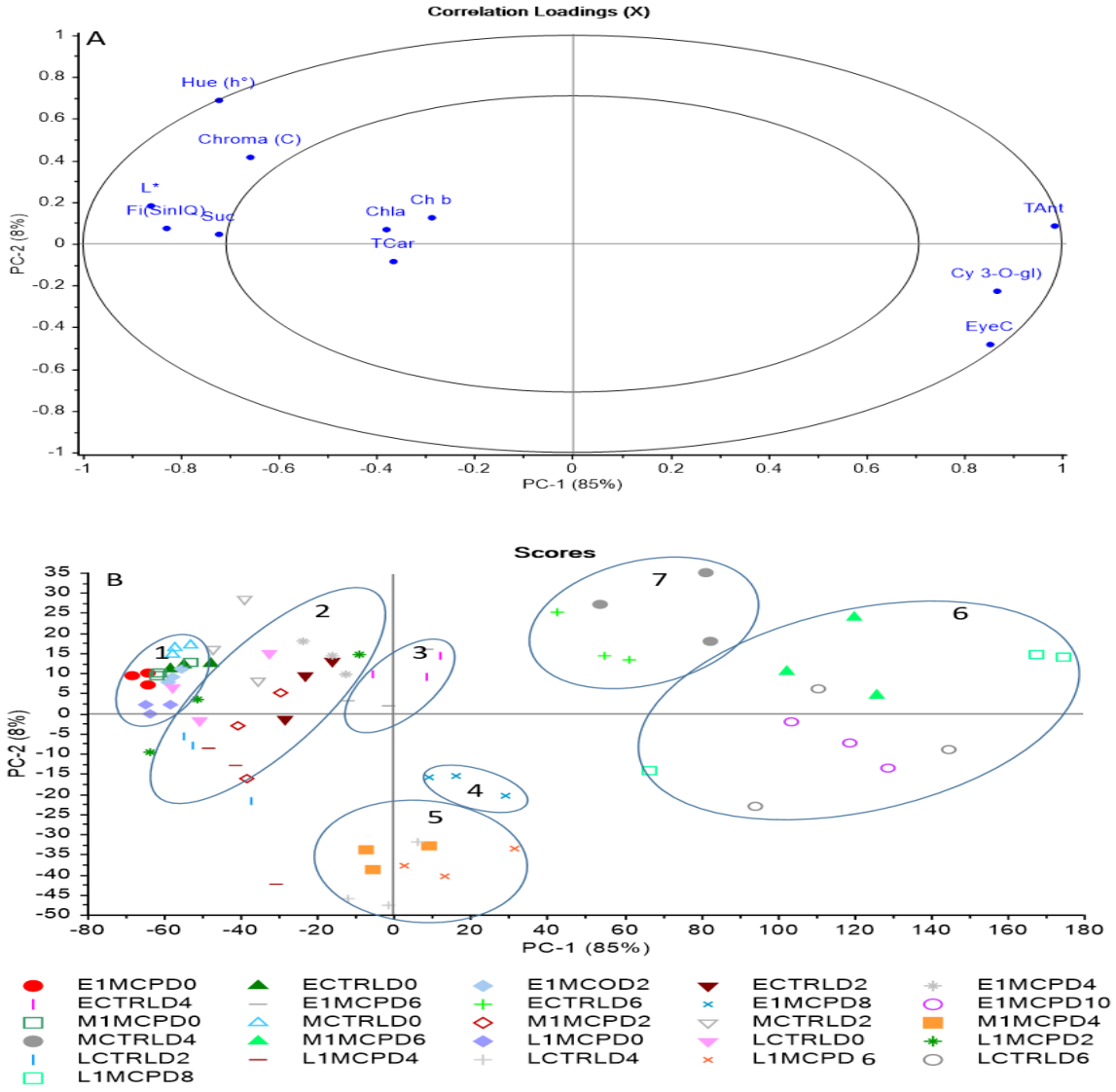

Fig. 12. Principal component analysis (PCA) showing correlation loadings. **A:** Score plot on colour physicochemical properties **B:** Score plot for the groups of 'Hass' avocado fruit exocarp colour in response to maturity and 1-MCP treatment during ripening.

DISCUSSION

The maturity of avocado fruit plays an important role in softening during ripening (Kassim et al., 2013). A study by Zauberman et al. (1977) found that fruit ripening rate was highly dependent on fruit maturity and observed that fruit harvested at early season took longer to soften when compared with fruit harvested at late maturity. The present study also found that early matured fruit ripened slower, therefore, took longer to ripen when compared with mid- and late-matured fruit (Fig. 1). Similarly, Vuthapanich et al. (1995) also reported that fruit harvested late ripened rapidly, especially when grown in warmer regions. The same study further showed that the time to ripening was influenced by moisture content (Vuthapanich et al., 1995). In this study, 'Hass' avocado fruit quality was improved during ripening following 1-MCP application, presumably due to a delay in fruit softening. Thus, 1-MCP treated fruit ripened slowly for early and mid-harvested when compared with late-harvested fruit. These results concur with observations reported by Feng et al. (2000) and Jeong et al. (2002), whereby 1-MCP treatment delayed ethylene-induced fruit softening on avocado fruit during ripening. The results suggest that late harvested fruit has high enzyme activity for ethylene

biosynthesis and high ethylene production, resulting in reduced 1-MCP effectiveness (Satekge & Magwaza, 2022).

In general, fruit softening has been reported to be resultant of cell wall degradation by enzymes such as polygalacturonase (PG), pectate lyase (PL), pectin methylesterase (PME), cellulose and β-galactosidase (Payasi et al., 2009). These enzymes are operative by breaking down the cell wall polysaccharides, which cause the pectin and hemicellulose to solubilize and depolymerize, subsequently, leading to cell wall loosening (Wakabayashi, 2000). Studies further showed that the activities of PG, PME and cellulose enzymes are highly dependent on ethylene production. For instance, softness inhibition in transgenic melon fruit was restored following treatment with exogenous ethylene (Nishiyama et al., 2007). Therefore, the activities of these enzymes can be inhibited and delayed during ripening by 1-MCP treatment. These observations were confirmed in a study reported by Jeong et al. (2002), whereby PG activity was delayed for up to 10 days in avocado fruit treated with 1-MCP. Similarly, Nishiyama et al. (2007) found that treatment with 1-MCP completely inhibited softening in melon fruit.

Avocado 'Hass' exocarp colour development has previously been linked with fruit harvest maturity advancement (Mathaba et al., 2015). This study found that fruit harvested at mid and late maturity developed purple and black colour better than early matured fruit (Fig. 2). These observations were also reported by Donetti and Terry (2014) and Mathaba et al. (2015). Their studies found that mid-harvest fruit developed purple exocarp colour during ripening, while late harvested fruit reached the desired black colour when compared with early harvest fruit (Fig. 2). This effect was ascribed to the higher accumulation of cyanidin 3-*O*-glucoside for mid and late harvest fruit during ripening (Cox et al., 2004). In addition, early maturing fruit had lower total anthocyanin concentration, resulting in reduced accumulation of cyanidin 3-*O*-glucoside for exocarp colour development.

In this study, 'Hass' avocado fruit treated with 1-MCP showed delayed colour development during ripening when compared with untreated, regardless of fruit maturity. Although 1-MCP treatment delayed colour development, it ultimately yielded a better and improved exocarp colour for early and mid-matured fruit. Similar results were reported by Feng et al. (2000) and Jeong et al. (2002), who also found that 1-MCP treatment delayed colour change in 'Hass' avocado fruit during ripening. In general, 1-MCP application improves fruit quality by inhibiting ethylene production and delaying ripening processes such as firmness, chlorophyll degradation, anthocyanin biosynthesis and final colour development (Xu et al., 2021; Zhang et al., 2021).

In this study, the decrease in L^*, C^* and $h°$ and high visual colour rating indicated an improvement in exocarp colour from green to purple then black for 1-MCP treated fruit during ripening (Fig. 3, Fig. 4 and Fig. 5). As previously observed by Cox et al. (2004), whereby, the decrease in objective colour parameters (L^*, C^* and $h°$) indicated chlorophyll degradation, and concomitantly, anthocyanin accrued leading to 'Hass' avocado exocarp colour development during ripening. In addition, PCA 1 (85% variation) showed that total anthocyanin and cyanidin 3-*O*-glucoside were interrelated with colour change (visual rating) during ripening. This was further explained by a negative correlation obtained between colour intensity ($h°$) and cyanidin 3-*O*-glucoside at early (R^2 = -0.857**), mid- (R^2 = -0.727**) and late (R^2 = -0.735**) for 1-MCP treated fruit (Table 1).

According to studies of fruit colour changes, sucrose and glucose are responsible for regulating anthocyanin biosynthesis and cyanidin 3-*O*-glucoside accumulation (Solfanelli et al., 2006; Teng et al., 2005). Moreover, sucrose and glucose can be phosphorylated by sucrose non-fermenting-related kinase enzyme (SnRK1) and hexokinase (HXK1) to produce UDP-glucose in the cytoplasm, respectively (Peng et al., 2016). The resultant UDP-glucose interacts with MYB transcriptional factors to regulate genes encoding enzymes related to

anthocyanin biosynthesis within the flavonoid's pathways (Shin et al., 2013). In this work, the 1-MCP application supposedly suppressed ethylene production, concomitantly, delaying UDP-glucose glycosylation by SnRK1 and HXK1 during early ripening when compared with the untreated fruit (Liu et al., 2017; Shin et al., 2013; Solfanelli et al., 2006).

In 'Hass' avocado fruit, the development of CI symptoms is closely associated with poor exocarp colour change during ripening (Mathaba et al., 2015). In this study, exocarp CI symptoms development decreased with fruit maturity (Fig. 11). Therefore, early matured fruit were highly susceptible to cold chilling injury damage, as they showed higher fruit proportion with poor colour when compared with mid- and late-matured fruit (Fig. 11). In previous studies, it was established that early matured 'Hass' avocado fruit were highly susceptible to CI when compared with late matured fruit (Bower & Magwaza, 2004; Faubion et al., 1992; Mathaba et al., 2015). Furthermore, Mathaba et al. (2015) reported that CI development was closely associated with exocarp colour de-synchronization with softening in early harvested fruit than late during ripening. In addition, control fruit showed higher CI symptoms when compared with 1-MCP treated fruit during ripening. Previous studies on 'Hass' avocado fruit also reported a similar trend (Hershkovitz et al., 2005; Jeong et al., 2002; Pesis et al., 2002).

Additionally, ethylene production is arguably involved in chilling injury development during ripening (Hong & Gross, 2000; Cocetta & Natalin, 2022). The production of reactive oxygen species (ROS) induced by chilling storage stress reduced membrane integrity, increase lipid peroxidation damage and molandialdehyde (MDA) accumulation, subsequently, leading to the development of chilling injury symptoms (Endo et al., 2019). However, 1-MCP treatment alleviates chilling induced mesocarp discolouration and CI symptoms development in avocado fruit (Pesis et al., 2002). Our results in the current study supported these findings, whereby, 1-MCP treatment significantly suppressed CI symptoms as indicated by a lower CI index throughout fruit maturity stages (Fig. 11). Another study supporting these observations showed that 1-MCP treatment prior to cold-storage reduced the CI incidence in 'Hass' avocado fruit (Hershkovitz et al., 2005). Woolf et al. (2005), however, did not find that 1-MCP could reduce external chilling injury at 0 °C. It has been reported that chilling injury is associated with reactive oxygen species (ROS) in the exocarp of the 'Hass' avocado fruit (Pesis et al., 2002).

Tesfay et al. (2011) found that exposure of 'Hass' avocado fruit to oxidative stress such as chilling temperature-induced ROS production, subsequently leading to oxidative damage. In avocado fruit, the phenols merged with antioxidants are the major defence systems responsible for scavenging ROS, thereby, protecting the cells against oxidative stress (Tesfay et al., 2010). Avocado fruit exocarps are rich in phenolic compounds that contribute to their antioxidant properties (Rodríguez-Carpena et al., 2011; Wang et al., 2010). Catechin and epicatechin phenols dominate the antioxidant system (Tesfay et al., 2009). Therefore, the ability of avocado exocarp to tolerate oxidative stress is highly dependent on its ability to produce catechin and epicatechin (Tesfay et al., 2011).

Furthermore, the exposure of avocado fruit to chilling stress results in an up-regulation of phenylpropanoid, which is an important pathway for the synthesis of both the phenols and anthocyanin pigment (Mahattanatawee et al., 2006). In general, phenylalanine ammonia-lyase (PAL) enzyme is a rate-controlling enzyme in phenylpropanoid pathway leading to phenols and anthocyanin synthesis (Villa-Rodríguez et al., 2011). Studies further showed that PAL enzyme activity is highly dependent on ethylene production (Martinez & Whitaker, 1995). Therefore, the potential benefit of postharvest 1-MCP treatment is to delay ethylene production during chilling stress. Physiologically, delayed ethylene synthesis and action down-regulate PAL activity, thereby delaying phenols synthesis and their antioxidant capacity during cold storage. This was supported by Zhang et al. (2013), who found changes in the proportion of antioxidant parameters in 'Booth 7' avocado fruit were delayed subsequent to 1-

MCP treatment due to suppression of ethylene production and fruit softening. Consequently, ethylene production increase the activity of PAL, subsequently, leading to the synthesis of phenols, as a result, scavenging ROS, therefore, reducing the development of exocarp CI symptoms during ripening (Fawbush et al., 2009; Zhang et al., 2013). In addition, sugars and antioxidants protect the chlorophyll systems against chilling damage during cold storage, while 1-MCP reduce respiration rate, inhibit ethylene production, and delay chlorophyll degradation during ripening (Lv et al., 2020).

In general, chlorophyll degradation involves chlorophyll carbolic enzymes such as chlorophyllase and Mg-dechelatase (Cheng et al., 2012). Alternatively, chlorophyll degradation could also be triggered through oxidative stress induced by ROS, particularly peroxidise (POD) (Kariola et al., 2005). Sharma et al. (2012) reported that excess ROS result in lipid peroxidation, protein denaturation and DNA damage. In addition, ROS acts as a signal transduction messenger triggering protective response through the accumulation of carotenoids and polyphenols compounds (Lukaszewicz et al., 2004). Frequently, the action of ROS results in PAL promoting enzymes, consequently, synthesis of antioxidant compounds such as phenols, phenolic acid and anthocyanin pigments (Karuppanapandian et al., 2011). It was reported that ROS signalling strength depends on the balance between oxidant production and removal by antioxidant properties (Theocharis et al., 2012).

During normal chlorophyll degradation, ROS are generated at a low level, which constitutes a balance between production and removal (Sharma et al., 2012). The counterbalance is disrupted by chilling stress when intracellular ROS levels increase, resulting in senescence peroxidation and CI damage (Vellosillo et al., 2010). This study found that early matured fruit exhibited poor colour change due to damage to the chlorophyll system. Fruit that were mid- and late-matured were probably protected from chlorophyll degradation by high exocarp sugar concentration and accumulation of antioxidants, in particular cyanidin 3-*O*-glucoside. In contrast, early matured 'Hass' avocado fruit poor colour development can be attributed to low sugar accumulation at harvest and anthocyanin synthesis during ripening. 1-MCP treatment conserved pre-harvest sucrose, which, assumably, promoted PAL activity, leading to higher phenol antioxidant accumulation and anthocyanin pigment accumulation during ripening than untreated fruit. This resulted in improved colour change in 1-MCP fruit, regardless of its maturity. As a result of 1-MCP treatment, ethylene synthesis was suppressed, reducing ROS formation, and protecting the chlorophyll system (chlorophyll-*a* and -*b*) against damage during chilling stress in early and mid-matured fruit.

The PCA analysis showed that the ripening period of untreated and 1-MCP treated ‘Hass’ avocado fruit was grouped into seven clusters. These clusters represented ripening as influenced by the combined effect of firmness and exocarp colour change *viz*: unripe-emerald-green, unripe-forest green, partially ripe-olive green, ripe-purple, ripe-black and senescence-black. In this study, ripening followed a climacteric pattern; therefore, clusters 1, 2 and 3 can be grouped as pre-climacteric whereas cluster 4, 5 and 6 as climacteric and cluster 7 as senescence stage. In addition, these clusters demonstrated that colour change makes an ideal non-destructive reference for classifying fruit ripeness of ‘Hass’ avocado fruit during ripening. The difference between clusters in this study corroborated a distinctive change in cell wall enzymatic activities, anthocyanin and cyanidin 3-*O*-glucoside synthesis and sucrose degradation rate during ripening. In this study, although 1-MCP treatment delayed these physiological processes, it resulted in improved fruit quality particularly early-season when compared with untreated fruit.

CONCLUSION

The study found that different maturity stages of 'Hass' avocado fruit responded differently to 1-MCP treatment. Results showed that 1-MCP treatment significantly reduced the damage caused by CI during storage for early and mid-harvest maturities. In this study, 1-MCP treatment delayed firmness, colour, and chilling injury development for early and mid-matured fruit, resulting in improved exocarp colour development at the end of ripening. Thus, it is concluded that maturity and 1-MCP had a positive influence on avocado fruit's exocarp colour pigments (total anthocyanin and cyanidin 3-*O*-glucose) synthesis and accumulation during ripening.

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Conflict of interest
The authors declare that there is no conflict of interest.

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Changes in the quantitative and qualitative characteristics of seedless barberry (*Berberis Vulgaris* L.) fruit as influenced by fruit thinning

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ABSTRACT

Purpose: Barberry has been used for many centuries due to its highly nutritious benefits, ornamental value, and medicinal properties. Iran is the largest producer of seedless barberry and this has been growing in various regions with dry climates, poor soil conditions and severe water shortages. Alternative bearing is a frequent problem in seedless barberry production. To avoid it, thinning has been employed as a common cultural practice in orchard management. **Research method:** In this study, three chemical thinners including gibberellic acid (GA_3) at 75, 100 and 150 mg/L, naphthalene acetic acid (NAA) at 10, 20 and 40 mg/L and ethephon at 50, 100 and 200 mg/L, and hand thinning (20%) were applied in a commercial orchard in Birjand, Iran, one week after petal fall. Thinning rate, quantitative and qualitative traits were investigated in comparison to the control in the split plot arrangement in a randomized complete block design during 2015 and 2016. **Findings:** Results showed that NAA at 10 mg/L caused the highest fruit abscission. Vegetative traits such as shoot length, number of leaves per shoot and leaf area showed significant increases under the treatments while shoot diameter had no significant difference with control. Chemical thinning remarkably enhanced the starch and sugar of the shoots, especially in "on" year (2015). The minimum chlorophyll content in "off" year was observed in control and the highest by NAA at 10 ppm. All the treatments increased seedless barberry shrub yield in the "off" year (2016). Biochemical traits such as ascorbic acid, total soluble solids, titratable acidity and anthocyanin improved in most treatments. **Research limitations**: No limitations were encountered**. Originality/Value:** To avoid alternative bearing in seedless barberry shrubs, thinning has been employed as a common cultural practice in orchard management. So, the application of NAA 10 mg/l is recommended for control of alternative bearing and also better fruit quality.

INTRODUCTION

An important aspect of horticulture is the cultivation of plants for food, fiber, biofuel, medicine, and other products used to sustain and enhance human life (Vijayan et al., 2008; Sengul et al., 2011). Seedless barberry (*Berberis vulgaris* L.) is an important underutilized horticultural crop that belongs to the family of Berberidaceae and is native to Iran (Zargari, 1990). Iran is the largest producer of seedless barberry with a production quantity of more than 22322 tons of dried berries in around 19220 ha (Ahmadi et al., 2021). Most of the consumption of seedless barberry is in a form of dried berries. Seedless barberry is usually propagated by two- or three-year-old suckers or stem cutting and It can be grown in any soil especially in poor and alkaline ones with pH up to 8.5 and very drought and salinity tolerant in which most of the other trees cannot produce satisfactory fruits (Alemardan et al. 2013). Apart from its agronomical value, it has been used in landscape design, plant disease control, textile dying (Alemardan et al., 2013) and for its medicinal properties such as anticancer, antimicrobial (Alimirzaee et al., 2009), decreasing addiction and dependency to morphine (Hashemzaei et al., 2012) and rehabilitation, hypoglycemic, anti-diabetic (Shidfar et al., 2012) and reducing LDL and total cholesterol (Ebrahimi-Mamaghani et al., 2009).

One major problem in seedless barberry production is alternate bearing (Kafi et al., 2002). In fruit trees, the alternate bearing mechanism shows that in years with a higher yield, fewer branches with generative buds are produced. Lack of sufficient nutrients to produce flower buds or phytohormones imbalance are the major causes of alternate bearing, Intense frequency in productivity in this crop results in a delay in fruit maturity and the loss of fruit size and crop quality. To control alternate bearing in fruit trees, a technique called thinning is used. In fruit trees, if an extra fruit set happens, the insufficient resources cannot support the demands of fruit lets leading to the production of low-quality fruits and alternate bearing. To avoid these phenomena fruit thinning is an essential orchard cultural practice to improve the partitioning of photosynthetic resources and tree performance (Costa et al., 2019). In this method, excessive flowers or young fruit lets are eliminated from branches (Taghipure et al., 2011). Fruit thinning is usually employed by hand thinning which is accurate but laborious, expensive and slow or by chemical thinning which is easier and more promising but not environmentally friendly (Costa et al., 2019).

In an experiment on the effects of GA_3 and ethephon on seedless barberry, it was revealed that these chemicals can reduce the alternate bearing (Balandary, 1995). Milić et al. (2018) reported that both naphthalene acetic acid (NAA) and benzyl adenine (BA) stimulated vegetative growth and the development of leaf area in northern high-shrub blueberries. NAA treatment at 20 and 40 mg/L two weeks after anthesis significantly increased the thinning of apricot ‘Khiary’ fruit (Taghipure & Rahemi, 2009).

There is little information regarding the alternate bearing of seedless barberry and the feasibility of using hand thinning technique and chemicals thinners application for berry thinning. Therefore, the present study aimed to study the effects of hand thinning, NAA, GA_3 and ethephon for fruit thinning and to investigate their influences on vegetative growth, fruit quality and yield of seedless barberry fruit.

MATERIALS AND METHODS

Experimental site

This study was carried out in an orchard on 12 km of Birjand-Kerman road (latitude 32°56' N., longitude 59°13' E., elevation 1480 m.) with arid and semi-arid climate under the natural conditions in 2015 (“on” year) and 2016 (“off” year), Birjand, Iran. Irrigation in this orchard

was carried out over, every 14 days with a surface irrigation system. The result of the soil analysis is shown in Table 1. Mineral deficiencies are compensated with fertilization during the growing season.

Plant material and treatments

A similar 15-year-old shrub in terms of vegetative and reproductive characteristics was used in this experiment. The following treatments were applied on all branches was performed: hand thinning (20% of fruit lets removed by hand), NAA (10, 20 or 40 mg/L, Merck, Germany), GA_3 (75, 100 or 150 mg/L, Merk, Germany), ethephon (50, 100 or 200 mg/L, Merck, Germany) and untreated control. For each treatment, five shrubs were used and the density in the orchard was 2 × 3m. One week after the petal fall in early spring, the shrubs were sprayed with a backpack sprayer in the afternoon, with the amount of solution of 2.8 L per each treatment. From each shrub, four branches which had similar lengths and diameters were selected and labelled to measure quantitative and qualitative traits. The same shrubs were subjected to the same treatments in both experimental years.

Fruit drop percentage and vegetative growth characteristics

Berries drop percentage (overall fruit set) was obtained by calculating the berries number at four clusters before treatment and the final stage of ripening. At the end of the growing season, four one-year-old branches per shrub which were similar in all shrubs were selected and the length, diameter and number of leaves in each shoot were measured. To measure the leaf area (LA), 20 leaves were taken from the middle part of the shoots from each shrub (80 leaves in total per treatment). The LA was measured using Image J (v.1.51f) software (Easlon & Bloom, 2014).

Shoot sugar and starch and chlorophyll content

Shoot sugar and starch were calculated at the end of the growing season. The concentration of starch was estimated with the procedure described by Hedge and Hofreiter (1962) and the light absorption of the solution was read using a spectrophotometer (UV-Win X- ma 2000) at 630 nm. Dissolved sugar was calculated using the phenol and sulfuric acid method introduced by Kochert (1978) at 485 nm. The content of leaf chlorophyll was estimated with the procedure described by Lichtenthder (1987) using a spectrophotometer (UV-Win X- ma 2000) at 646.6 and 663.6 nm.

Yield per shrub

To calculate the yield of each branch, all berries of each branch were harvested separately. To measure the fresh weight of each shrub, the fruits of each shrub were harvested in November and weighed with a digital 0.001 g scale (model KB120-3N, Kern, the UK).

Table 1. Chemical analysis of the soil of the seedless barberry orchard in Birjand.

Texture soil	pH	Saturation (%)	EC (ds/.m)	Depth (cm)	K (ppm)	P (ppm)	N (%)
Sandy- clay	7.9	25	7.8	0-30	198	15	0.1

Fruit characteristics

To measure fruit fresh and dry weight, each sample consisted of 20 fruits randomly taken out from each shrub, making the sample 100 fruits in total per each treatment. Samples with 100 already harvested fruits were weighed with a digital 0.001g scale (model KB120-3N). Then, the fruits were oven-dried at 80°C for 24 hours to estimate their dry weight. The water displacement measurement method was used to determine the volume of 10 berries. Fruit anthocyanin was calculated using the method introduced by Wrolstad (1976) using a spectrophotometer (UV-Win X- ma 2000) at 510 and 700 nm. Total acidity (%) was determined by titration using the sodium hydroxide method (Ranjana, 1986). Ascorbic acid was measured by titration using2,6-dichlorophenol-indophenol (Kassem et al., 2011). Total soluble solids (TSS) were measured using a handheld refractometer (Model Abbe, Waj, China). Fruit acidity was measured using a digital pH meter (Model 110, Jenway, and the UK).

Statistical analysis

The experiment was a split-plot arrangement where thinning was the main plot and year was a subplot laid out in a randomized complete block design with five replicates. We used the GLM procedure of SAS statistical software for data analysis and Duncan's multiple range tests at 5% level for means comparison.

RESULTS

According to ANOVA results, the interaction effects of thinning and year were significant for all the traits except fruit drop, shoot length and diameter**,** TSS and fruit width while the simple effects of thinning or year were significant in these traits (Table 2 and 3). There wasn't a significant difference in shoot diameter in this experiment.

Fruit drop percentage

The results showed that the interaction effects of thinning and year were not significant for the fruit drop parameters while the simple effect of thinning shows the significant difference with the control. All of the chemical treatments resulted in a significant increase in fruit drop as compared to the control. The highest rate of drop was observed in 10 NAA ppm treatment that exhibited 29.9% fruit the drop and the lowest of it related to control (3.41%) (Fig. 1).

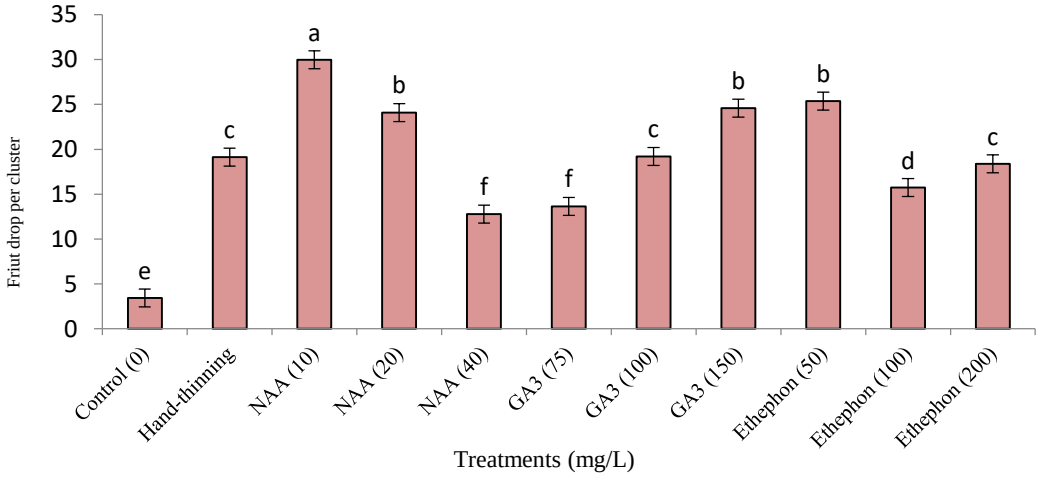

Fig. 1. Effect of different thinning techniques on fruit drops of seedless barberry. Columns with common letters are not significantly different according to Duncan's test.

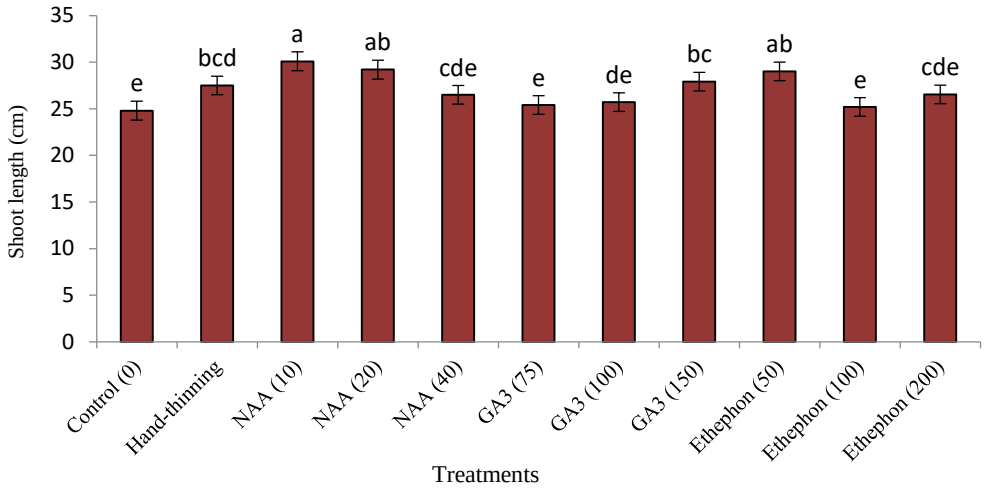

Fig. 2. Effect of different thinning techniques on shoot length in seedless barberry. Columns with common letters are not significantly different according to Duncan's test.

Table 2. Analysis of variance (ANOVA) of the effect of thinning and time on some growth and morphological parameters of barberry.

SOV	df	Shoot length	Shoot diameter	No. of leaves	Leaf area
Replication	4	1.7 ^{ns}	3.66 ^{ns}	0.22 ^{ns}	0.002^{ns}
Thinning (a)	10	712.2**	34.3 ^{ns}	251.7**	17.98**
Whole Plot Error	1	0.361	5.98	0.153	0.15
Time (year) (b)	10	32.5**	2.69 ^{ns}	29.45**	4.15**
a × b	4	1.1 ^{ns}	3.16 ^{ns}	1.61*	0.10**
r × b	40	2.5 ^{ns}	3.91 ^{ns}	0.94*	0.005^{ns}
Subplot error	4	2.25	4.13	0.364	0.31
CV		6.1	20	2.4	1.19

Table 2. (Continued).

SOV	df	Leave chlorophyll	Yield per Shoot	Fruit drop	Shoot Starch	Shoot sugar
Replication	4	0.37**	78081 ^{ns}	0.13 ^{ns}	0.88*	0.08 ^{ns}
Thinning (a)	10	25.30**	18777051**	539.04**	18.41**	5.55**
Whole Plot Error	1	0.21	359083	1.05	0.57	0.28
Time (year) (b)	10	9.89**	3280247952**	216.03**	26.86**	19.90**
a × b	4	**9.15	8395813**	2.4 ^{ns}	2.55**	**12.72
r × b	40	0.04 ^{ns}	167764 ^{ns}	3.38 ^{ns}	0.41 ^{ns}	0.11*
Subplot error	4	0.01	122981	1.91	0.28	0.04
CV		1.28	86.2	7.37	7.71	1.78

Table 3. Analysis of variance (ANOVA) of the effect of thinning and time on some growth and chemical parameters of barberry fruit.

SOV	df	Length	Width	Volume	Fresh W.	Dry W.
Replication	4	0.04**	0.24 ^{ns}	0.08^{ns}	0.05 ^{ns}	0.35*
Thinning (a)	10	0.07**	0.94^{ns}	0.45**	4.22**	0.95**
Whole Plot Error	1	0.10	0.21	0.28	0.99	0.88
Time (year) (b)	10	0.44**	0.45^{ns}	1.33**	9.73**	9.16**
a × b	4	0.21**	0.61^{ns}	0.69**	2**	0.54**
r × b	40	0.006*	0.52 ^{ns}	0.00005^{ns}	0.06^{ns}	0.06^{ns}
Subplot error	4	0.002	0.27	0.12	0.03	0.09
CV		0.64	11.02	7.51	1.17	6.72

*, ** represents significant at probability levels of 0.05 and 0.01 respectively; ns means non-significant.

Table 3. (Continued).

SOV	df	Ascorbic acid	pH	TSS	TA	Anthocyanin
Replication	4	1312^{ns}	0.08**	4.40**	0.0006^{ns}	0.0002 ^{ns}
Thinning (a)	10	611184**	0.02**	25.02**	1.30**	0.02**
Whole Plot Error	1	1401.1	0.02	10.47	0.02	0.0004
Time (year) (b)	10	341051**	0.007*	6.44**	19.40**	0.04**
a × b	4	8100**	0.002*	0.93 ^{ns}	0.22**	**0.001
r × b	40	1086.4 ^{ns}	0.0002 ^{ns}	0.27 ^{ns}	0.02*	0.0004*
Subplot error	4	1334.2	0.001	0.76	0.006	0.0002
CV		1.72	0.77	4.22	2.08	3.54

*, ** represents significant at probability levels of 0.05 and 0.01 respectively; ns means non-significant.

Shoot length and diameter, number of leaves per shoot and leaf area

It is evident in Figure 2 that the maximum shoot length observed in NAA (10 and 20 mg/L) and ethephon 50 mg/L at about 30, 29.2 and 29 cm, respectively. A minimum shoot length was observed in control it showed no significant difference with NAA acid 40 mg/L and ethephon 50 mg/L. NAA (10 mg/L) caused the maximum leaf areas at about 10.1 and 10.5 cm in the "on" and "off" year, respectively a minimum leaf area was observed in control at about 5.1 cm in on year (Fig. 3). Maximum number of leaves per shoot observed in NAA (10 and 20 mg/L) and ethephon 50 mg/L at about 29.5, 28 and 28.1 cm, respectively that they showed no significant difference with GA_3 (150 mg/L) in "on" year. Minimum of number of leaves per shoot was observed in control in "off" year (Fig. 3).

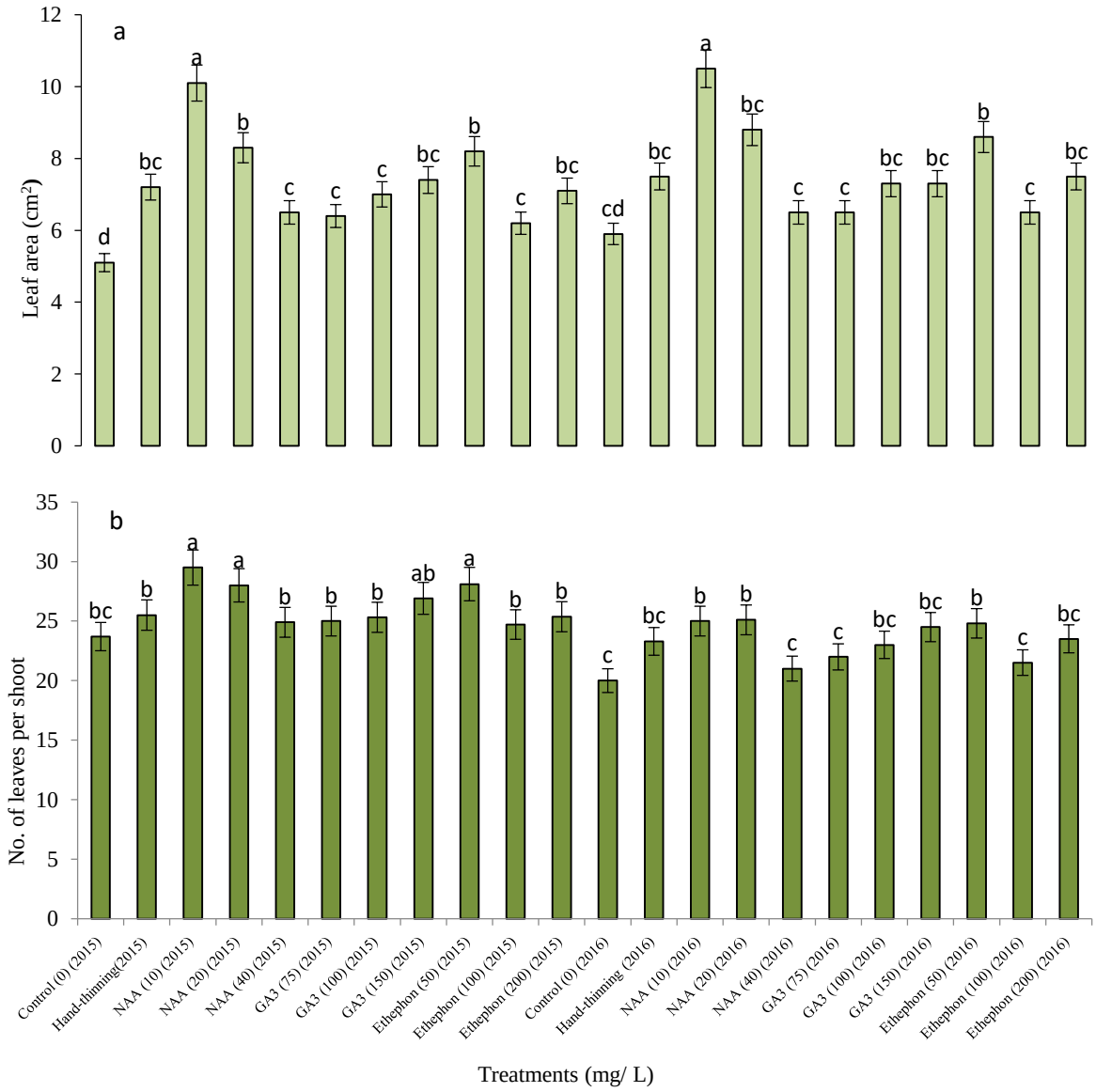

Fig. 3. Effect of different thinning techniques and time on leaf area (a), and No. of leaves per shoot (b) in seedless barberry. Columns with common letters are not significantly different according to Duncan's test.

The starch and sugar of shoots

Figure 4 shows that shoot starch and total sugar content (at the end of the growing season) in the "off" year was significantly more than the "on" year in control and other treatments. Most of the treatments increased starch and total sugar in shoots at the end of the growing season in the "on" year. The lowest percentage of sugar and starch in the shoot was related to control treatment and the maximum percentage of shoot starch was observed in plants treated with NAA (10 and 20 mg/L), ethephon (200 mg/L) and GA3 (150 mg/L), respectively. The highest shoot sugar related to NAA (20 mg/L), ethephon (200 mg/L) and GA_3 (150 mg/L), respectively. Chemical thinning enhanced starch and sugar to a greater extent than hand-thinning.

Chlorophyll content

Figure 5 shows that in the "off" year, leaves chlorophyll content was significantly lower than "on" year in control. It was found that chlorophyll content was decreased with the application

of thinning in the "on" year while it was increased in "off" year. In the "on" year, maximum chlorophyll was related to the control and NAA (10 mg/l). Other thinning treatments except hand thinning, GA_3 (75 mg/l) and ethephon (100 mg/l) resulted in a significant reduction of chlorophyll content in seedless barberry leaves. Minimum chlorophyll was observed in plants treated with GA_3 (150 mg/L) and NAA (20 mg/L) treatments, respectively (Fig. 5). In the "off" year, minimum chlorophyll was related to the control and maximum of it was observed in plants treated with NAA (10 mg/L) (Fig. 5).

Morphological traits of berries and shrub yield

Length, width, volume, fresh and dry weight of berries

Results presented in Figure 7 and Table 4 shows that all of the measured morphological traits except fruit dry weight of berries were significantly higher in the "off" year than "on" year. In the "on" year, all thinning treatments significantly increased the length, width and volume of fruits. The highest fruit width related to NAA (10 mg/L) and other treatments showed no significant difference with the control (Fig. 7). Maximum and minimum fruit length was related to the control in "off" and "on" year, respectively. NAA (10 mg/L), hand thinning and ethephon (50mg/L) caused the highest length of fruit in on year. The highest fruit volume was related to control in off year and NAA (10 mg/L) and ethephon (200 mg/L) in on year and the lowest of it related to control in "on" year. Maximum fresh weight of fruits was observed in ethephon (200 mg/L) in "off" year while the maximum dry weight of fruits related to NAA (40 mg/L) that it showed no significant difference with NAA (10 mg/L), and ethephon (200 mg/L) in "on" and a minimum of them were observed in the control in "on" year (Table 4).

Table 4. Effect of different thinning techniques and time on some physical and biochemical traits of seedless barberry fruit.

Year	Treatments	Length (mm)	Volume of 10 berry (cm^3)	Fresh weight of 100 berry (gr)	Dry weight of 100 berry (gr)
2015	Control (0)	7.57±0.06^{d}	3.48±0.06^{c}	14.04 ±0.07^{c}	3.86±0.03^{d}
	Hand-thinning	8.2±0.06^{bc}	4.44±0.01^{b}	15.88 ±0.03 ^{b}	4.9±0.06b^{bc}
	NAA (10)	8.3±0.02^{b}	5.15±0.03^{a}	17.05 ±0.02 ^{ab}	5.21±0.06 ^{ab}
	NAA (20)	8.03±0.02 ^{c}	4.54±0.01^{b}	15.22±0.06^{bc}	4.66±0.06^{cd}
	NAA (40)	8.04±0.03^{c}	4.74±0.01^{ab}	17.08±0.003 ^{ab}	5.5 ±0.1 ^{a}
	GA_3 (75)	8±0.03^{c}	4.57±0.1^{b}	15.81±0.3^{b}	4.3 ±0.06^{cd}
	GA_3 (100)	8.1±0.003^{c}	4.9±0.2^{ab}	16.46±0.15^{ab}	4.15 ±0.009 ^{cd}
	GA_3 (150)	8.11±0.003^{c}	4.91±0.1 ^{ab}	16.02±0.03^{b}	4.03±0.2^{d}
	Ethphon (50)	8.17±0.01^{bc}	4.9±0.03 ^{ab}	16.03±0.04^{b}	4.78 ±0.01^{bc}
	Ethphon (100)	8.13±0.01^{c}	4.9±0.06 ^{ab}	17.21 ±0.06 ^{ab}	4.94 ±0.01 ^{bc}
	Ethphon (200)	8.14±0.3^{c}	5.12±0.1 ^{a}	17.21±0.07^{ab}	5.21 ±0.06^{ab}
2016	Control (0)	8.5±0.3^{a}	5.18±0.3^{a}	17.19±0.06 ^{ab}	4.33±0.1^{cd}
	Hand-thinning	8.3 ±0.03^{b}	4.97±0.008 ^{ab}	16.88 ±0.03 ^{ab}	4.25±0.2^{cd}
	NAA (10)	8.28±0.02^{b}	5±0.3 ^{ab}	16.96 ±0.1 ^{ab}	4.25±0.1^{cd}
	NAA (20)	8.06±0.06^{c}	4.56±0.03^{b}	15.74 ±0.06^{b}	3.98±0.06^{d}
	NAA (40)	8.02±0.05^{c}	4.8±0.03 ^{ab}	17.04 ±0.05 ^{ab}	4.29±0.1^{cd}
	GA_3 (75)	8.07±0.01^{c}	4.81±0.09 ^{ab}	16.08 ±0.08^{b}	4.05±0.03^{d}
	GA_3 (100)	8.11±0.003^{c}	4.82±0.3 ^{ab}	16.76 ±0.22^{ab}	4.27±0.07^{cd}
	GA_3 (150)	8.14±0.003^{c}	4.97±0.1 ^{ab}	16.5 ±0.15 ^{ab}	4.22±0.02^{cd}
	Ethphon (50)	8.15±0.06^{c}	4.96±0.06 ^{ab}	16.47 ±0.13 ^{ab}	4.15±0.06^{cd}
	Ethphon (100)	8.12±0.03^{c}	4.78±0.06 ^{ab}	17.36 ±0.06 ^{ab}	4.37±0.01^{cd}
	Ethphon (200)	8.22±0.03^{bc}	5.06±0.3 ^{ab}	17.56 ±0.07^{a}	4.35±0.06^{cd}

Different letter(s) in each row indicates significant differences according to Duncan's multiple range tests at $P < 0.01$.

Table 4. (Continued).

Year	Treatments	Ascorbic acid (mg/100g)	pH	TA (%)	Anthocyanin (mg/l)
2015	Control (0)	1537±3.1^{e}	4.25±0.07^{b}	4.16±0.1^{b}	0.340 ±0.0008^{cd}
	Hand-thinning	1908±0.6^{c}	4.3±0.07^{b}	3.35±0.05^{bc}	0. 399±0.003^{c}
	NAA (10)	2438±3.1^{ab}	4.23±0.07^{b}	3. 75±0.04^{b}	0.4±0.01^{c}
	NAA (20)	2226±0.3^{b}	4.24±0.01^{b}	3.66±0.06^{b}	0.481±0.001^{ab}
	NAA (40)	2226±0.6^{b}	4.22±0.06^{b}	3.38±0.003^{bc}	0.417±0.003^{bc}
	GA_3 (75)	1902±0.6^{c}	4.21±0.03^{b}	3.04±0.01^{c}	0.434±0.001^{bc}
	GA_3 (100)	1918±0.4^{c}	4.22±0.07^{b}	3.33±0.04^{bc}	0.409±0.003^{c}
	GA_3 (150)	2020±0.6^{bc}	4.23±0.07^{b}	3.31±0.03^{bc}	0.410±0.003^{c}
	Ethphon (50)	2120±6b^{b}	4.27±0.006^{b}	3.82±0.02^{b}	0.435±0.001^{bc}
	Ethphon (100)	2385±1.5^{ab}	4.26±0.01^{b}	3.6±0.04^{bc}	0.443±0.0004^{b}
	Ethphon (200)	2332±0.5^{ab}	4.35±0.01^{ab}	3.6±0.05^{bc}	0.503±0.001^{ab}
2016	Control (0)	1688±3.5^{de}	4.32±0.04^{b}	4.99±0.1^{a}	0.405±0.0008^{bc}
	Hand-thinning	2093±2.4^{bc}	4.3±0.08^{b}	4. 1±0.05^{b}	0. 415±0.02^{bc}
	NAA (10)	2521±6^{a}	4.24±0.07^{b}	4.69±0.04^{ab}	0.406±0.01^{bc}
	NAA (20)	2346±5^{ab}	4.22±0.02^{b}	4.5±0.06^{ab}	0.502±0.01^{ab}
	NAA (40)	2319±4^{ab}	4.22±0.06^{b}	4.17±0.003^{b}	0.478±0.003^{ab}
	GA_3 (75)	2024±6^{bc}	4.21±0.03^{b}	4.04±0.01^{b}	0.498±0.009^{ab}
	GA_3 (100)	2099±6^{bc}	4.22±0.07^{b}	4.21±0.04^{b}	0.456±0.01^{b}
	GA_3 (150)	2183±8^{b}	4.23±0.07^{b}	4.3±0.03^{ab}	0.41±0.003^{bc}
	Ethphon (50)	2184±1.5^{b}	4.26±0.01^{b}	4.68±0.02^{ab}	0.501±0.0004^{ab}
	Ethphon (100)	2391±1.5^{ab}	4.28±0.003^{b}	4.5±0.04^{ab}	0.510±0.0004^{a}
	Ethphon (200)	2386±2.5^{ab}	4.4±0.03^{a}	4.5±0.05^{ab}	0.520±0.01^{a}

Shrub yield

Hand and chemical thinning were done in "on" year (2015) and caused to decrease in yield in hand thinning treatment but, didn't decrease yield in other treatments and control in the same year. This indicated that hand thing isn't suitable in on year because of decreased yield while chemical thinning is suitable because it caused no difference in yield in comparison to the control. On the other hand, all thinning treatments induced a significant increase in yield of the "off" year. The lowest shrub yield (13980 g) was observed in the hand-thinning treatment in "on" year while the minimum yield was observed in the control (1793g) in "off" year. It is noticeable that the entire treatments improved seedless barberry shrub yield in the "off" year compared to the control this means approaching a balanced bearing every year (Fig. 6).

Biochemical traits of berries

Ascorbic acid (Vitamin C), and pH

Table 4 shows that all thinning treatments significantly improved ascorbic acid content versus control in both years. The highest amount of ascorbic acid was observed in NAA (10 mg/L) in "off" and "on" year, respectively they showed no significant difference with ethephon 100 and 200 mg/L in both years and NAA (20 and 40 mg/L) in "off" year. The lowest amount of ascorbic acid was observed in the control in the "on" and "off" year. Table 4 indicates that the amount of fruit pH in all treatments except ethephon (200mg/l) was not significantly different in comparison to the control.

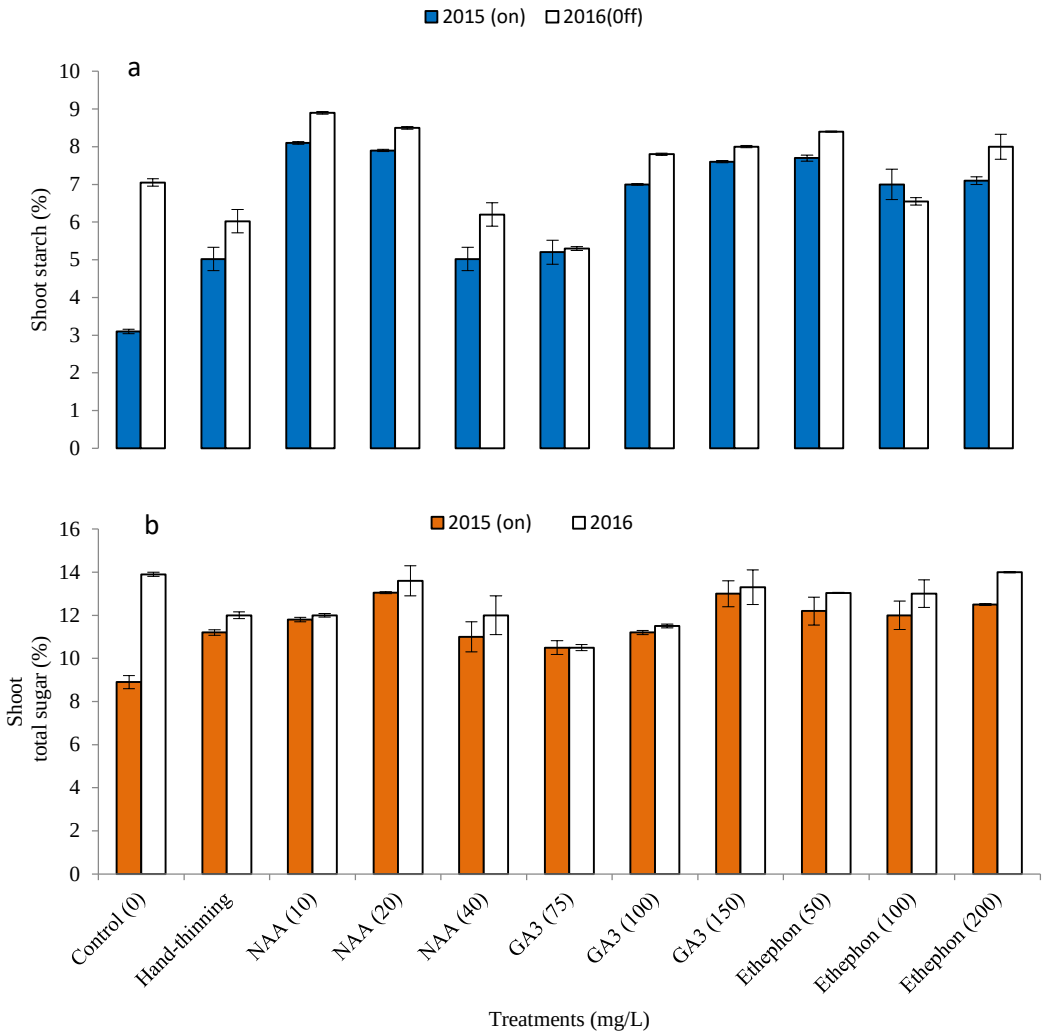

Fig. 4. Effect of different thinning techniques and time on shoot starch (a), and total sugar content (b) in seedless barberry.

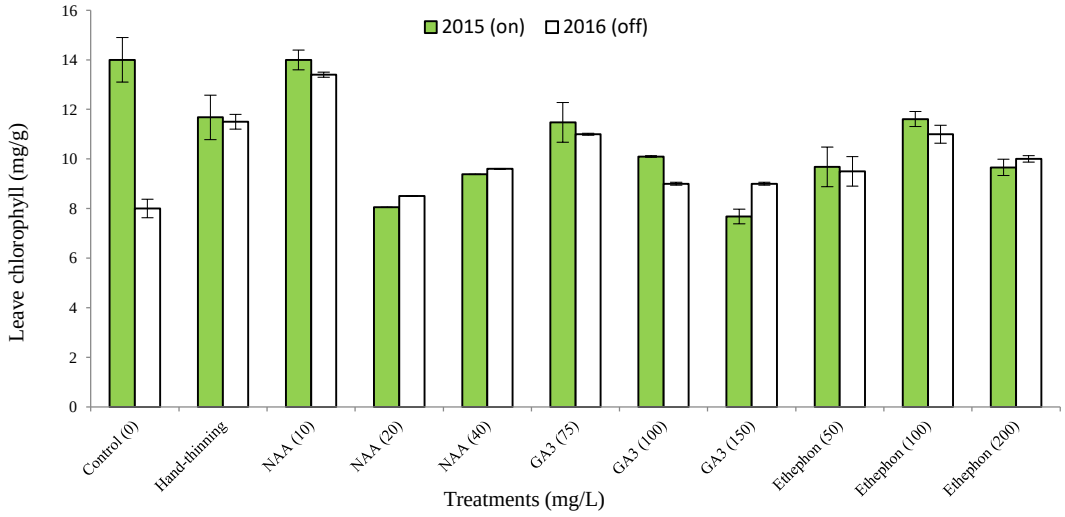

Fig. 5. Effect of different thinning techniques and time on leave chlorophyll content in seedless barberry.

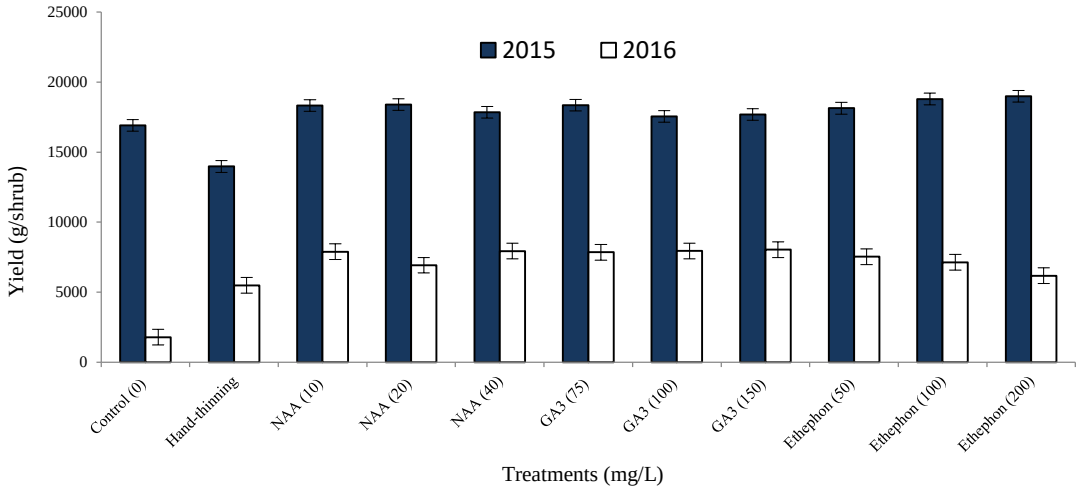

Fig. 6. Effect of different thinning techniques and time on shrub yield in seedless barberry. Columns with common letters are not significantly different according to Duncan's test.

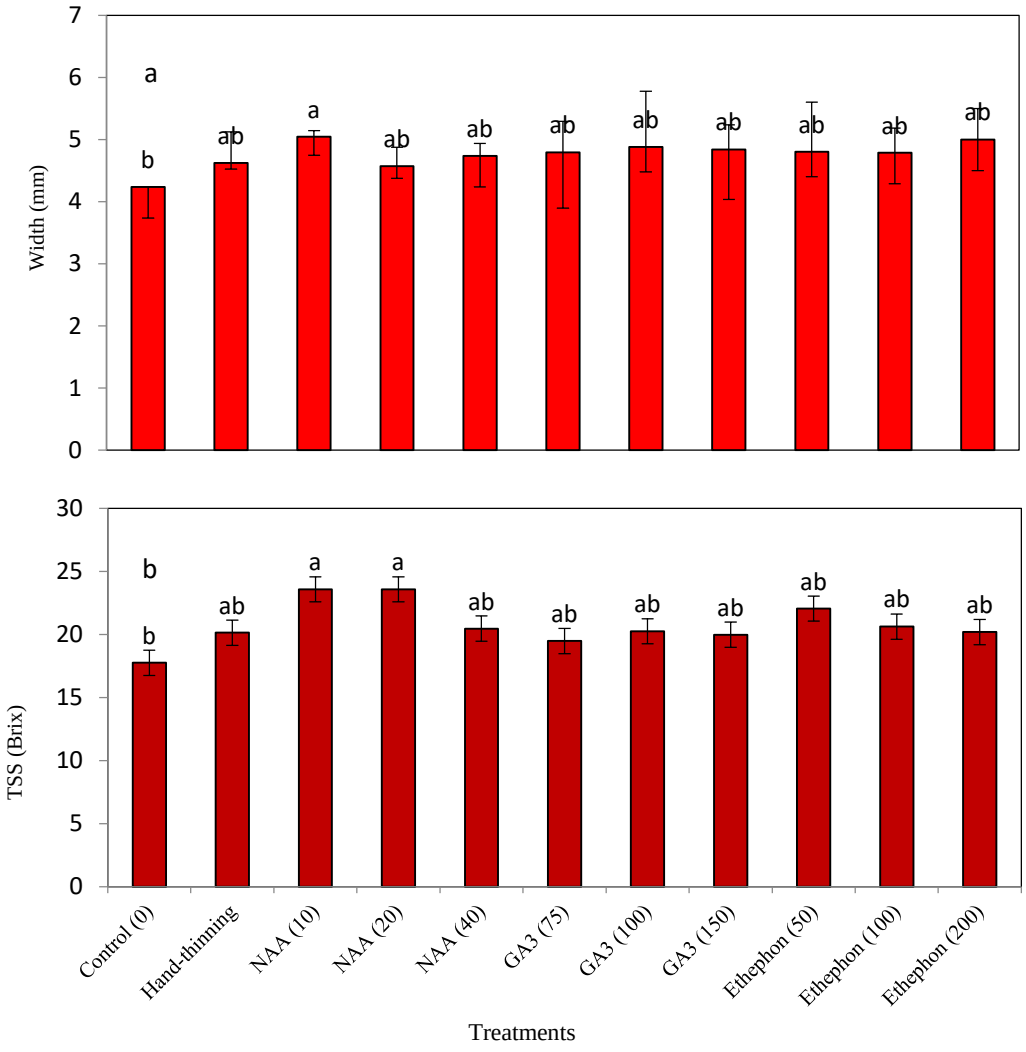

Fig. 7. Effect of different thinning techniques on fruit width (a), and total soluble content (TSS) (b) of seedless barberry. Columns with common letters are not significantly different according to Duncan's test.

Total soluble solids (TSS) and titratable acidity (TA)

According to Figure 7 and Table 4, the treatments resulted in higher total soluble solids and lower total acidity of barberries than the control, respectively. The highest total soluble solid was observed in NAA (10 and 20 mg/L) at about 23.57% Brix that they showed no significant difference with other treatments and the lowest one was related to control at about 17.75% Brix (Fig. 7). Table 4 indicates maximum titratable acidity was related to control at about 4.99 % in the "off" year and the minimum was related to GA_3 (75 mg/L) at about 3.04 % in "on" year.

Fruit anthocyanin

The results in Table 4 showed that the anthocyanin content of berries was considerably higher in the "off" year than "on" year. The lowest amount of anthocyanin content was observed in control and the highest one was observed in ethephon (100 and 200 mg/L) in "off" year it showed no significant difference with ethephon (200 mg/L) and NAA (20 mg/L) in on year and NAA (20 and40 mg/L), GA_3 (75 mg/L) and ethephon (50 mg/L) in the "off" year (Table 4).

DISCUSSION

Application of chemical thinning in this experiment showed that NAA and ethephon were more effective on fruit drop in lower concentrations, while the highest fruit drop was related to the highest rate of GA_3. Taghipure et al. (2011) showed that the application of NAA resulted in fruit thinning of apricot. NAA application disrupts the hormone balance in plants and stimulates ethylene synthesis, so this chemical thinning substance indirectly increases fruit drop (Bertelsen, 2002). It has been shown that seedless barberry yield fluctuates around 40% during five years of study which indicates the biennial bearing phenomena (Rezaei & Balandary, 2011). Seedless barberry morphological traits increased in all thinning treatments and especially in lower concentrations of NAA and ethephon, and higher concentrations of GA_3. The loss of fruit could be one of the important reasons for higher leaf area in barberry.

This experiment showed that chlorophyll content decreased with the application of thinning in the "on" year while it increased in "off" year. In the "on" year chlorophyll content was decreased significantly in most thinning treatments except in hand thinning, GA_3 (75 mg/L), ethephon (100 mg/L) and NAA (10 mg/L). Chlorophyll plays an important role in sunlight absorption and photosynthesis. It has been indicated that chlorophyll content is lower in low-yielding shrubs than in high-yielding shrubs (Choi et al., 1997).

Most of the treatments especially NAA treatment increased starch and total sugar in shoots at the end of the growing season in the "on" year. The loss of fruit after thinning decreased different sinks and consequently, assimilates such as starch and carbohydrate were stored in shoots or remaining fruitlets. On the other hand, leaf area was increased after thinning and assimilated more carbohydrates and stored them in shoots. In shrubs with high carbohydrate storage, more fruits will be produced in the subsequent year and alternate bearing will be decreased while the loss of carbohydrate reserve because of higher yield increases alternate bearing (Spann et al., 2008).

The results of this experiment showed that the orchard management of fruitlet thinning by chemical thinner reagents can increase the yield of seedless barberry, particularly in "off" years. In the "on" year, all the treatments except hand thinning increased the yield. However, hand thinning enhanced the yield even in the "off" year as well. It has been reported that GA_3 could improve fruit set, plant growth, flowering and as a result, increase yield (Williamson &

NeSmith, 2007; Zang et al., 2016). Milić et al. (2018) reported that NAA increased the number of berries per cluster and yield per shrub in blueberries.

Evaluation of different fruit traits showed that chemical thinning was more effective than hand thinning compared to the control and also NAA and ethephon had more positive effects on the improvement of these fruit traits than GA_3. GA_3 at the concentration of 50 mg/L showed the most effective as a thinner chemical on *Prunus salicina* and improved fruit size, colour, TSS and fruit firmness (Erogul & Fatih Sen, 2015). According to Suzuki et al. (1998), fruit weight in blueberries is positively correlated with shoot vigour and the number of leaves that increased morphological fruit traits by chemical thinning. On the other hand, earlier and greater expansion of the LA, which might be the case with NAA treatment, could be the reason for the increased fruit size in barberry. In our study application of NAA and ethephon confirm it. Due to the fruit's abscission after NAA and ethephon application, fewer fruits remained on trees until harvest time, the more photosynthetic resources lead to the larger fruits with higher dry weight. Blueberry hand-thinning and NAA caused a significant increase in fruit fresh and dry weight (Gough et al., 1976). Foliar application of ethephon (100 mg/L) on tangerine (Gallash, 1978) increased fruit size by around 65% which is consistent with our findings.

Evaluation of different biochemical fruit traits showed that ascorbic acid content and total soluble solids were increased under thinning treatments. The role of chemical thinning treatments on increasing the total soluble solids of fruit juice is associated with an increase in the ratio of leaf to fruit after fruit thinning. In this experiment, leaf area was increased after fruitlet abscission and as a result, more assimilates reached the remaining fruits which resulted in higher soluble solids and sweeter berries. Moreover, auxins stimulated the expansion and growth of cells, resulting in leaf area expansion. The increase in total soluble solids and ascorbic acid after hand thinning has been reported in grapes by Singh (1995). Measurement of total acidity showed that thinning treatments decreased total acidity in fruit juice. The application of 20 mg/L NAA on blueberries increased the ratio of total soluble solids to total acidity in its juice (Gough et al., 1976).

Fruit anthocyanin was increased in all treatments which ethephon (200 mg/L) being the most effective one. Anthocyanin is considered one of the indicators for quality assessment in seedless barberry (Rezvani Moghaddam et al., 2013). Ban et al. (2007) revealed that ethephon could promote the fruit ripening process in blueberry. In another study on grapes, it was reported that the colour synthesis decreased when the number of branches was increased (Choi et al., 1997). The increase in anthocyanin pigment after hand or chemical thinning could be related to the increase of soluble solids in fruit. Also, Whiting et al. (2006) found that hand thinning of sweet cherry increased the fruit's red colour and Guidoni et al. (2002) found that hand thinning of bunch of grape increased anthocyanin and flavonoid in berries it is speculated that the interference with other endogenous hormones, the transient rise of ABA, the inhabitation or down-regulation of IAA export from fruitlets after the application of thinning chemicals such as NAA, ethephon or BA initiate the correlatively triggered response and eventually fruitlet abscission (Bangerth, 2000).

CONCLUSION

Alternative bearing is a frequent problem in seedless barberry production. To avoid it, thinning has been employed as a common cultural practice in orchard management. In this study vegetative traits such as shoot length, the number of leaves per shoot and leaf area showed significant increases under the treatments while shoot diameter show no significant difference with control. Chemical thinning remarkably enhanced the starch and sugar of the

shoots, especially in on year. The minimum chlorophyll content in "off" year was observed in control and the highest by NAA at 10 ppm. All the treatments increased seedless barberry shrub yield in the "off" year (2016). Biochemical traits such as ascorbic acid, total soluble sugar, total acidity and anthocyanin improved in most treatments. So, the application of NAA 10 mg/l is recommended for control of alternative bearing and fruit quality. In conclusion, we have revealed that the exogenous application of chemical thinning significantly reduced the alternate bearing in barberry. In both years, 2015 and 2016, the quality of berries was improved markedly by treatments compared to the control. NAA at 10 mg. L^{-1} showed the most promising results in barberry. Chemical thinning especially by NAA which is inexpensive and straightforward to use, offers a suitable alternative to reduce the cost of seedless barberry production and will boost farmers' economic benefits.

Conflict of interest

Authors declare that they have no conflict of interest.

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The effect of white and red LED lights on coloring and antioxidant capacity of Japanese persimmon at postharvest stage

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ABSTRACT

Purpose: One of the most important subtropical fruits in Iran is Japanese persimmon. Persimmon is a climacteric fruit and continues to ripening after the harvest. One of the main quality components in persimmon fruit is its appearance due to the presence of different carotenoid pigments. Persimmon has also high antioxidant capacity. The use of LED lights is one of the most important commercial techniques to increase the quality and postharvest life of the fruits. In the present study, the effect of LED lights on the coloring and antioxidant capacity of persimmon fruit was studied. **Research method:** The Karaj genotype of persimmon was harvested at commercial stage and exposed to white and red LED lights (at intensity of 40-60 candle/W) up to 21 days at 10°C and 80% RH, as the control fruit was stored at dark conditions. **Findings:** The results showed that the samples of red LED light had higher color index and lower hue° and L * values as compared to control or white LED light samples. Therefore, the red light induced the coloring in persimmon fruit, but the white light did not show such an effect. Also, both white and red LED lights have resulted in better preservation and increased antioxidant capacity in persimmon fruit as compared to control. **Research limitations:** No limitations were encountered. **Originality/Value:** based on the results of this experiment, LED lights are effective treatments in maintaining and increasing the quality of Japanese persimmon at the postharvest stage, and have potential to further investigation and commercialization.

INTRODUCTION

Japanese or oriental persimmon (*Diospyros kaki* Thunb) is the most famous species from the Ebenacea family (Guan et al., 2020). In recent years, the cultivation of persimmon has been developed in Iran and it is being produced and consumed considerably, but useful research has not been conducted in this regard (Khademi et al., 2022). Most persimmons in Iran are astringent type, but the astringency removal treatments are not common in Iran and therefore, the persimmon is harvested at over ripe stage in order to decrease the astringent taste; as a consequence, the fruits lose their high quality with low storability and are not popular in the markets (Khademi et al., 2010).

Persimmons have high antioxidant capacity due to carotenoid, phenolic and ascorbic acid compounds and can be effective in avoiding cardiovascular diseases (Ozen et al., 2004). One of the significant qualitative properties of Japanese persimmon is the beautiful color resulting from lots of carotenoid pigments. Among carotenoids, persimmons are rich of β-crytoxanthin, β-carotene, lutein, zeaxanthin and lycopene, that β-carotene and β-crytoxanthin are provitamin A, although 31 specific carotenoids were detected in persimmon cultivars. The carotenoid content is strongly affected by cultivar, ripening stage, and processing method. In persimmon, the color varies in different cultivars from yellow, and orange to deep red, which is due to the differences between the compositions and contents of carotenoids in the different persimmon cultivars; however, researchers demonstrated that β-cryptoxanthin was the most abundant carotenoids in most persimmon cultivars (Plaza et al., 2012; Zhou et al., 2011).

Plants react to a wide range of lights ranges from ultraviolet to infrared. Sunlight is the main source of light in terms of photosynthesis and physiological processes in plants (Taiz & Zeiger, 2010). However, LED lights are a modern technology, which are of lots of useful applications in food industry and agriculture. LEDs are the members of the family diodes. When in diodes, the electricity passes, the energy is changed in to the light. LEDs formerly could produce red, blue and green lights, which has led to the limitations in use, but recently, LED with white light has been produced, which can produce the white light with a halo of blue color. Now, LEDs have more advantages as compared to the other light resources involving; production of specific wavelengths, production of monochrome light, production of less heat, high durability or high useful life, low production expenses, low consumption of energy, production of cold light, small size, safety, regulation of light intensity and quality (Singh et al., 2015; Yan et al., 2020). Nowadays, LED lights have been converted to an accessible, attractive and economic technology in the field of postharvest of horticultural products, so that the application of LED light is one of important strategies for the increased postharvest life of fruits and vegetables as well as the improved coloring (Barta et al., 1992; Cho et al., 2008).

In the studies conducted on the citrus (Ma et al., 2012), tomato (Nájera et al., 2018; Liu et al., 2009), cherry tomatoes (Ngcobo et al., 2021) and bell peppers (Martínez-Zamora et al., 2021), it has been shown that the LED light treatment has led to the increased carotenoids and fruits color. In fact, LED lights play a role in the evolution of fruits color after the harvest because they affect the metabolism of pathways involved in pigments biosynthesis. The properties of light spectrum influence the synthesized pigments, which play a determining role in the quality of products (Nájera et al., 2018).

On the other hand, LED lights are effective treatments in inhibiting senescence and maintaining the product quality during storage or shelf life by the improved antioxidant properties (Maroga et al., 2019; Song et al., 2020). Exposure to LED lights induced different antioxidant systems in the plant cells such as phenylpropanoid or carotenogenesis pathways via the related enzymes activations. The positive effect of LED lights on increasing the

functional bioactive compounds has been shown in bell peppers (Martínez-Zamora et al., 2021) and cherry tomatoes (Ngcobo et al., 2021), and in fact, light quality, light intensity, and irradiation duration affect the effect of light treatment (Song et al., 2020). Since the senescence is an oxidative stress, the improved antioxidant compounds can delay the senescence (Maroga et al., 2019).

LED lights technology may affect the harvested fruits and vegetables considerably. However, to determine the effect of LED light mechanism on the postharvest quality of fresh products, more research is required. So far, no reports have been published in the field of LED lights impact on the coloring and qualitative properties of persimmon; in the current study, the impact of red and white LED lights has been investigated on the color properties and antioxidant activities in the Japanese persimmon at postharvest stage.

MATERIALS AND METHODS

The persimmon fruit with common name of "Karaj" genotype at firm and orange color stage was harvested from an orchard near to Karaj city and immediately transported to the postharvest laboratory of Department of Horticulture, Shahed University (Khademi et al., 2012). The intact and defect-free fruits were selected and divided into three groups, each group containing 60 fruits, the first group was exposed to the darkness as control, the second group was exposed to the red LED light treatment (660nm, 40-50 candle/W), and the third group was exposed to white LED light treatment (449nm, 50-60 candle/W). For light treatments the apparatuses were made from a steel frame in the dimensions of 1 m length × 0.5 m width × 0.5 height, and LED lamps for each treatment were mounted on each frame, and distance between the fruits and lamps was set at 20 cm. The surroundings of the apparatuses were covered with black polyethylene to prevent lights from the outside (Arslan et al., 2021). The fruits were continuously subjected to lights treatments at 10°C and RH above than 80% for up to 21 days, inside the cold chamber. At 0 (harvest time), 7, 14 and 21 days of the storage, 15 fruits from each treatment were taken out and evaluated.

The firmness (penetration test) of the fruits was evaluated using a hand penetrometer (model VBR80, Italy) with 8-mm plunger at 3 equatorial points after removing peel and the result was expressed as N. The color parameter of *L**, *a** and *b** was determined at various points of each fruit using a colorimeter (model TES-300, Taiwan) and hue angle and color index was calculated by the following equations (1 and 2) (Khademi et al., 2013).

$$\text{Hue}^\circ = \tan^{-1}(b^*/a^*) \quad (1)$$

$$\text{Color Index} = (1000a/Lb) \quad (2)$$

For the determination of antioxidant capacity, 0.2 gr of frozen sample was homogenized in 3 mL of 80% methanol and the homogenate was centrifuged at 8000 g for 10 min at 4°C. To 250 µL of resultant supernatant, 250 µL of 1mM DPPH (1, 1-diphenyl-2-picryl hydrazyl) was added and after 30 min incubation at room temperature in dark conditions, the absorbance (ABS) of the resulting solution was spectrophotometrically measured at 515 nm and the percentage of reduction in DPPH was calculated based on the following equation (3) (Naser et al., 2018):

$$\text{DPPH}(\%) = (1-(\text{ABS}_{\text{sample}}/\text{ABS}_{\text{control}})) \times 100 \quad (3)$$

A randomized completely design with three replicates per treatment was used in this experiment. To determine the effects of light treatments and storage time on each dependent

variable, a two-way analysis of variance was carried out using SAS software (version 9.3). Mean values of the treatments were compared by Least Significant Difference test (*LSD*, *P*=0.05).

RESULTS

The ANOVA results related to LED lights treatments and storage time (ST) factors and their interaction on Karaj persimmon have been shown in Table 1.

Fruit firmness

Suitable tissue firmness is one of the main quality components in persimmon fruit, therefore, maintaining firmness and preventing softening of the fruit are the most important factors in the postharvest of persimmon (Salvador et al., 2008). In this fruit, softening takes place under the control of ethylene, in which the up-regulated enzymes involved in degradation of cell wall material (Khademi et al., 2014; Nakano et al., 2001).

The result of this study showed that the firmness value of the samples decreased significantly over the time of the experiment. At 7-day of the experiment, the firmness value of persimmons treated with red and white LED lights was significantly higher than that of control samples. However, at 14- and 21-day of the experiment, there was no significant difference between the samples in terms of firmness value (Fig. 1). In similar results on the tomato, it has been shown that the treatment with red and white lights was more effective than blue or green lights in preserving the fruit firmness as compared to control at postharvest stage (Arslan et al., 2021). In other study, it was shown that tomatoes exposed to higher red/far red light ratio had increased firmness value more than tomatoes exposed to lower red/far red light ratio (Nájera et al., 2018). However, in this study, LED lights were only effective in preservation of fruit firmness in the first week of the study; since persimmon is very sensitive to softening during the postharvest conditions; no positive effect of LED lights was observed in the continuation of the experiment (Khademi et al., 2014).

Table 1. Statistical analysis of parameters studied: LED lights treatments and storage time (ST) and their interaction for 'Karaj' persimmon.

Treatment	Firmness	L*	Color index	Hue angle	Antioxidant capacity
LED lights	ns	**	**	**	*
ST	**	**	**	**	*
LED × ST	*	**	**	*	ns

*, ** and ns represent significance at the 0.05 and 0.01 levels and non-significance respectively.

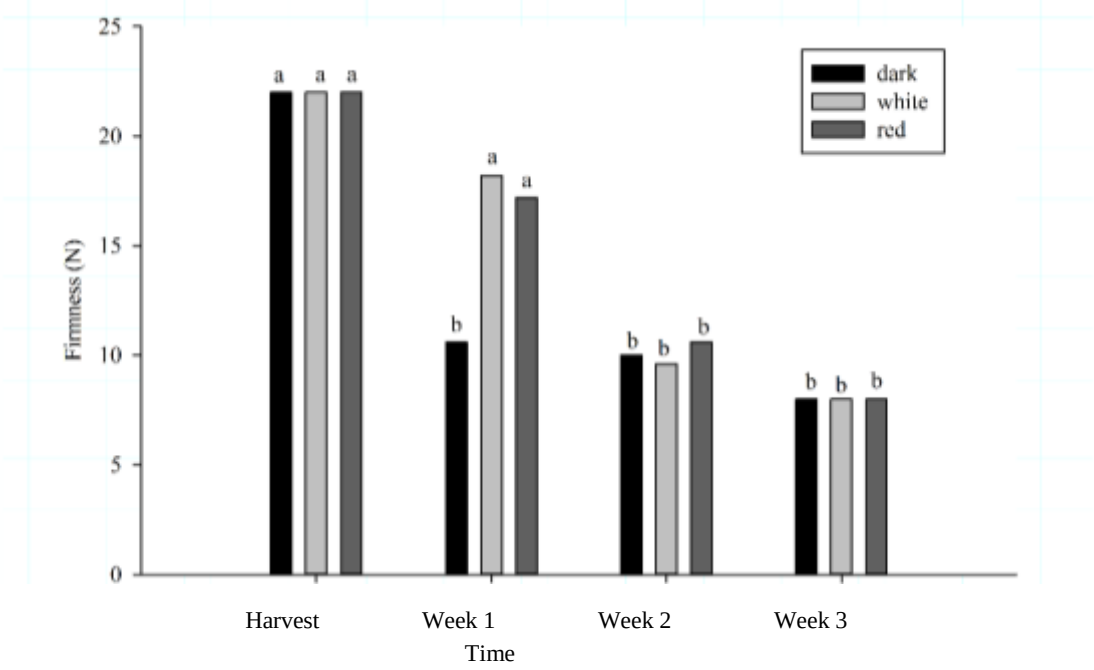

Fig. 1. Firmness value of 'Karaj' persimmon exposed to red and white LED lights for 21 days at 10 °C. Means with the same letter are not significantly different at 5% level of the *LSD* test.

Color properties

Persimmon fruit is a rich source of carotenoids, and a lot of changes occur in the amount of carotenoids during the growth and development in this fruit. The highest amount of carotenoid accumulation occurs at full ripening stage in persimmon fruit, which is specified by red-orange color in Karaj persimmon (Naser et al., 2018).

The results showed that the L* value of all samples decreased over the time of the experiment. At 7-day of the experiment, no significant difference was observed between the red light and control samples in L* value, but the white light samples had a higher L* value than control at this time. A similar trend was observed at 14-day of the experiment, however, at the end of the experiment, the samples treated with red LED light had significantly lower L* value than other samples, while no significant difference was observed between the white light and control samples in terms of L* value. In general terms, red light decreased the L* value of persimmon compared to the control in this study, but white light did not show such an effect (Fig. 2).

The color index of all samples significantly increased over the time of the experiment. Based on the results, no significant difference was observed between the samples in terms of color index at 7th day of the experiment. At 14th day of study time, no significant difference was observed between the red light and control samples in terms of color index, but at this time, the lowest color index was detected in white light samples. At 21st day of study time, red light samples had higher color index than others, while no significant difference was observed between the white light and control samples in terms of color index (Fig. 3). In general, red light increased the color index of persimmon fruit in this study, but white light did not show such an effect.

The Hue angle of all samples decreased over the time of the experiment. The results also showed that at 7- and 14- day of the experiment, there was no significant difference between the control and white light or red light samples in terms of Hue angle. But, at the end of the experiment, the Hue angle in the red light samples was significantly lower than that of white light or control samples, while no significant difference was observed between the white light

and control samples in terms of the Hue angle (Fig. 4). Therefore, in line with the L* and color index results, the Hue angle result showed that red light increased the coloring of persimmon fruit as compared to control at this experiment, but white light did not show such an effect.

In persimmon fruit, coloring and carotenoid accumulation are usually associated with a decrease in the L* and Hue angle values and increase in the color index value (Khademi et al., 2013). In a similar study, Zhou et al. (2011) in comparisons between 46 different persimmon cultivars showed that lower values of L* and hue angle in astringent cultivars reflected the deeper color in them as compared to non-astringent cultivars, which was caused by higher abundance of carotenoids in astringent types.

In this experiment, red LED light increased the coloring of persimmon fruit, but white LED light did not show a similar effect. Consistent with this results, several studies on tomatoes (Liu et al., 2009; Nájera et al., 2018), cherry tomatoes (Ngcobo et al., 2021), mandarins (Ma et al., 2012) and bell peppers (Martínez-Zamora et al., 2021) have demonstrated that irradiation with red LED light increased color formation by inducing the accumulation of carotenoids. Furthermore, Arslan et al. (2021) found that coloring of tomatoes harvested at breaking stage was not affected by white LED light treatment.

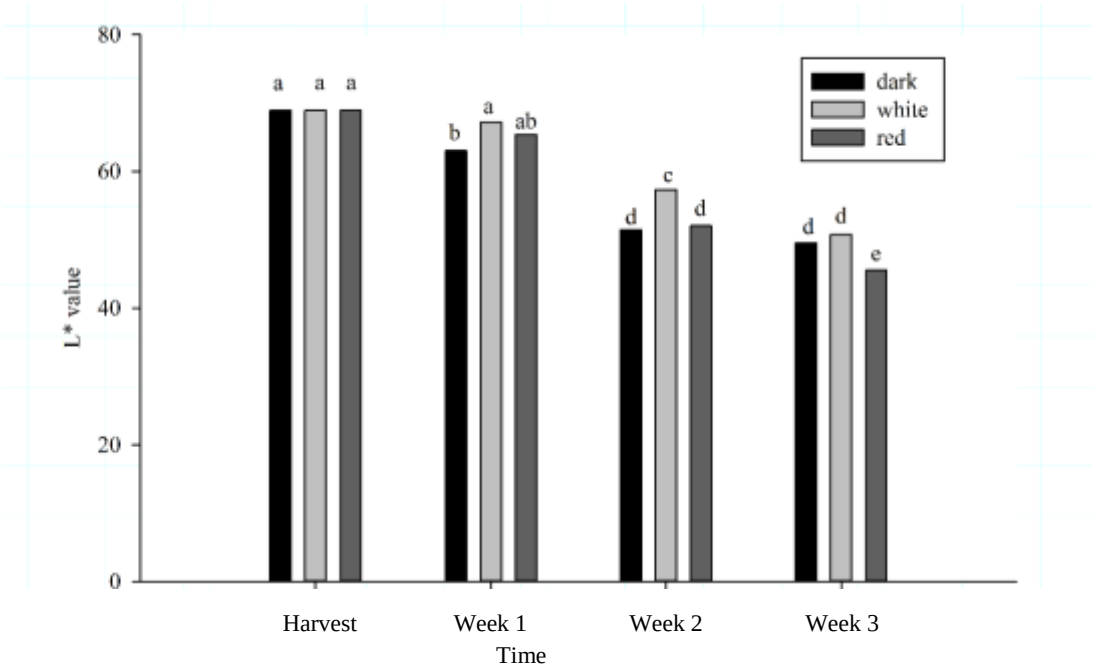

Fig. 2. L* value of 'Karaj' persimmon exposed to red and white LED lights for 21 days at 10 °C. Means with the same letter are not significantly different at 5% level of the *LSD* test.

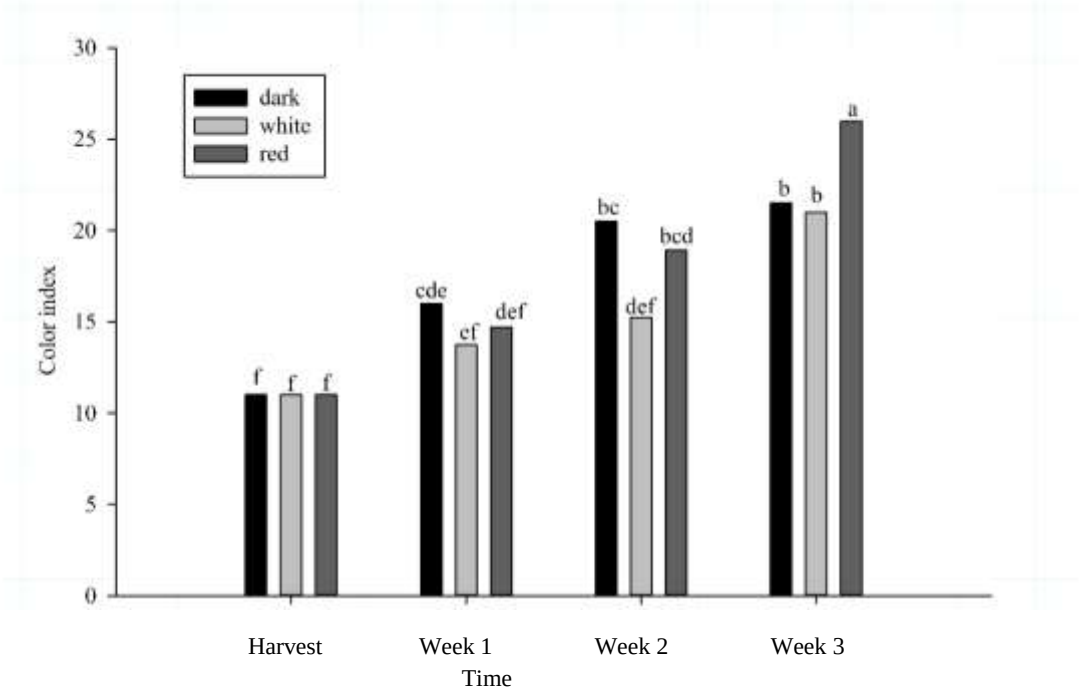

Fig. 3. Color index of 'Karaj' persimmon exposed to red and white LED lights for 21 days at 10 °C. Means with the same letter are not significantly different at 5% level of the *LSD* test.

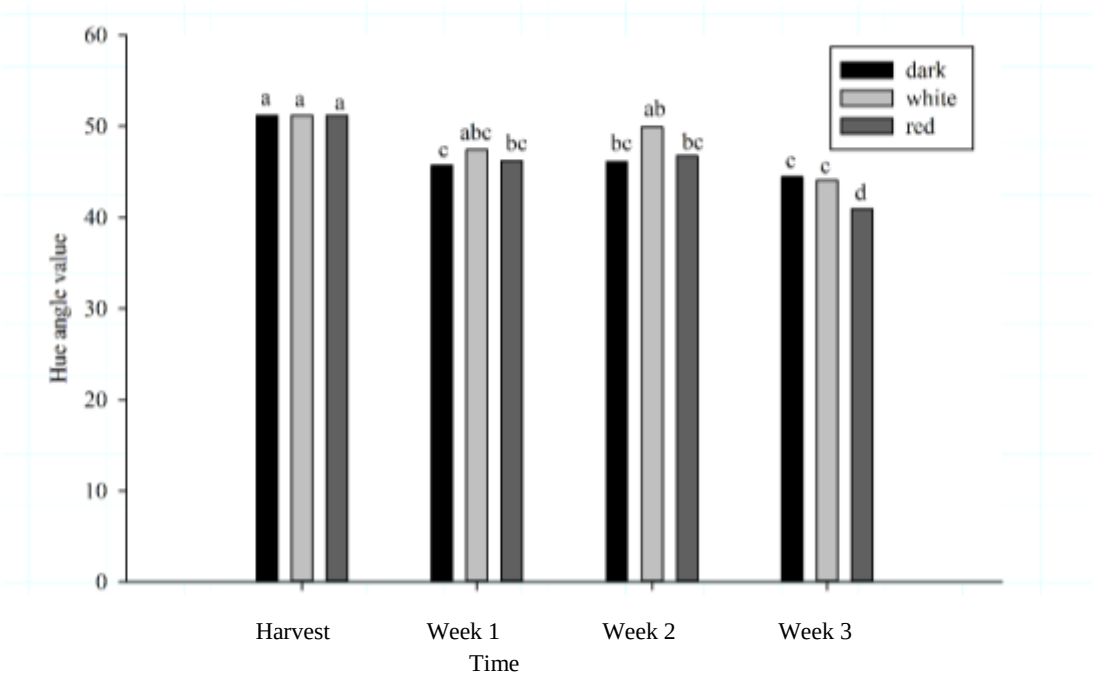

Fig. 4. Hue angle value of 'Karaj' persimmon exposed to red and white LED lights for 21 days at 10 °C. Means with the same letter are not significantly different at 5% level of the *LSD* test.

Liu et al. (2009) in a research on the harvested tomatoes exposed to different lights up to 21 days found that red light treatment had greater effect than sun light on carotenoids contents and coloring in tomatoes. Stimulating effect of red light on the carotenogenesis could be

explained by the light-dependent synthesis of certain genes or enzymes involved in the formation of carotenoids such as phytoene synthase. Phytoene synthase is the first enzyme in carotenoids production pathway and increased its expression leading to the enhanced carotenoids production (Martínez-Zamora et al., 2021; Ngcobo et al., 2021).

Antioxidant capacity

The antioxidant capacity of all samples decreased over the time of the experiment (Fig. 5-A). The results also showed that; the antioxidant capacity of LED lights treated samples was higher than control samples, however, there was no significant difference between the red and white lights samples in terms of antioxidant capacity in this experiment (Fig. 5-B).

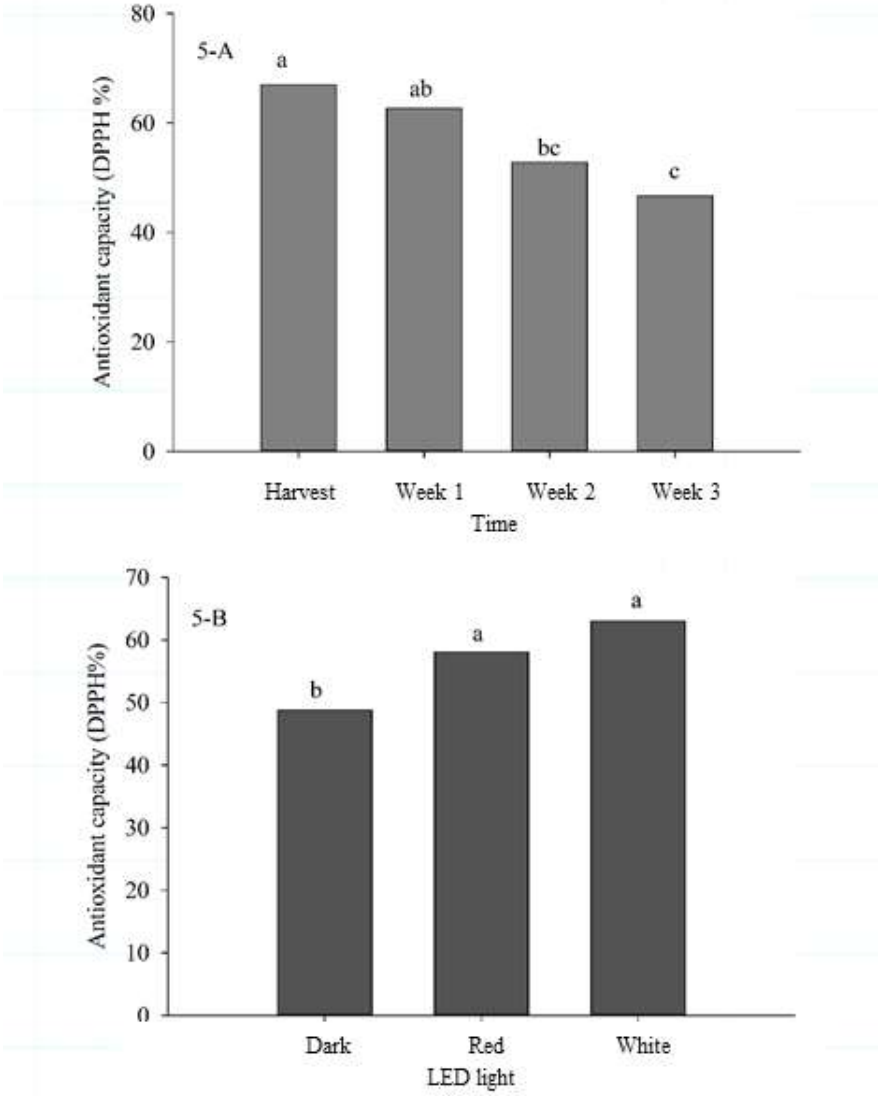

Fig. 5. Effects of studying times (5-A) and LED lights (5-B) on the antioxidant capacity of 'Karaj' persimmon exposed to red and white LED lights for 21 days at 10 °C. Means with the same letter are not significantly different at 5% level of the *LSD* test.

An important part of fruits nutritional value is related to the antioxidant compounds which refer to a group minimizing the oxidative damage to the alive creatures through the reactions with free radicals and reactive oxygen species, which are considerably dangerous to the living cells (Vicente et al., 2005). Persimmon has higher antioxidant capacity than such fruits as apples, blueberries, grapes, tomatoes. Even though, persimmon is regarded as a rich source of dietary fiber, minerals, vitamin A, vitamin C, carotenoids and phenolic components, it is well known that antioxidant capacity of persimmon is significantly correlated with phenolic components. (Li et al., 2011). The antioxidant capacity in persimmon fruit is usually reduced under the storage condition, as observed in control samples (Khademi et al., 2014), but according to the result presented here, this reduction has been alleviated by red and white LED lights. The significant positive effect of LED lights on preserving antioxidant capacity agrees with the results from the investigations on fresh-cut sweet peppers (Maroga et al., 2019), intact bell peppers (Martínez-Zamora et al., 2021), habanero peppers (Pérez-Ambrocio et al., 2018), pak chois (Song et al., 2020), tomatoes (Baenas et al., 2021) and bananas (Huang et al., 2018). Light is an essential factor in biosynthesis of functional compounds, and the quantity and quality of applied light can affect the bioaccumulation of antioxidant compounds (Martínez-Zamora et al., 2021). It has been reported that exposure to LED lights induced the activity of phenylalanine ammonia lyase (PAL), PAL enzymes converts phenylalanine to trans-cinnamic acid in the top of phenylpropanoid pathway and this pathway is responsible for the production of different types of phenolic components (Huang et al., 2018; Maroga et al., 2019) which have been directly related to the antioxidant capacity in persimmon fruit (Li et al., 2011); therefore, increased biosynthesis of phenolic components under red and white LED lights postponed antioxidants depilation in persimmon fruit under the postharvest conditions (Baenas et al., 2021).

CONCLUSION

The research results indicated that the red LED light has had positive impact on the preservation of firmness and antioxidant capacity in persimmon fruits, in addition to the increased color of the fruits. The white LED light has had no significant effect on the color of fruits, but it was effective in preserving the firmness and antioxidant capacity in persimmon. Therefore, LED lights can be considered as the effective treatments for the postharvest of Japanese persimmon fruit, and they require further investigations in order to be commercialized.

Conflict of interest

The authors declare that there is no conflict of interest.

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Effect of postharvest microwave heat treatment of pomegranate on carob moth, *Ectomyelois ceratoniae*, control and quality parameters

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ABSTRACT

Purpose: In some orchards in the harvest season, the pomegranates are suspected to carob moth infestation. Visible infested fruits are removed but there is a possibility of hidden infestation. The effect of microwave heating on this hidden infestation was investigated in this study. **Research Method:** The mature pomegranate fruits were artificially infested with the eggs or larvae (1st, 2nd and 3rd) of the carob moth inside the crown. Microwave radiation was focused on the crown zone of infested pomegranate with a novel setup and its effect on pest mortality was studied. The experiments were carried out at three microwave powers (540, 720 and 900 W) and three heating times (4, 6 and 8 minutes). In order to study the effect of microwave heating treatments on pomegranate, quality parameters including appearance, weight loss, total soluble solids (TSS), pH, titratable acidity (TA) and taste index (TSS/TA) of the samples were investigated after 60 days' storage. **Findings:** The egg and larval mortality rose with microwave power and heating time. The mortality was reduced with increasing the age of pest. There were no significant differences among values of quality factors except for titratable acidity. Because of 100% mortality with no significant differences on quality parameters, 6 min treatment time with 720 microwave power was selected as the optimum treatment. **Research limitations:** Energy consumption in microwave heating limits the application of this method in practice. **Originality/Value:** Microwave local heating of the pomegranate crown is an effective novel method to remove carob moth in hidden infestation.

INTRODUCTION

The scientific name of pomegranate, *Punica granatum*, is derived from the name *Pomum* (apple) *granatus* (grainy) or seeded apple. Pomegranate is native to Persia (Iran) and surrounding areas where, it spread to the rest of the world (Teixeira da Silva et al., 2013; Fadavi et al., 2006). Iran is one of the most important pomegranate producers and exporters in the world (Khajehei et al., 2015; Khodabakhshian et al., 2016).

The carob moth, *Ectomyelois ceratoniae*, is a worldwide polyphagous destructive pest witch attacks pomegranate, pistachio, date, fig and some other fruits (Khodabakhshian et al., 2016; Sobhani et al., 2015). This herbivorous insect is the major field and storage pest of the pomegranate causing 30-80% yield loss (Hosseini et al., 2017). The infestation begins in the orchard. The adult female lays its eggs in the crown (calyx) of the pomegranate. After egg's hatching, the first-instars larvae (1^{st}, 2^{nd} and 3^{rd}) feed inside the crown and then burrow into the fruit and feed on internal parts (Hashemi Fesharaki et al., 2011). The pest infestation and saprophytic fungi contamination result from larvae penetration to the fruit cause the internal hidden decay of pomegranate. The degree of fruit decay grows until reaching the rind where symptoms are readily visible. In the harvest season, visibly rotted fruits are removed but the hidden infested ones not detectable. After harvesting (during handling, shipping, storage and marketing) the hidden decayed fruits grow and the symptoms become visible. Non-destructive tests (NDT) have been used to detect hidden rotted pomegranate fruits (Jamshidi et al., 2019; Khodabakhshian et al., 2016).

Postharvest heat treatments of fruits (hot water dips, vapor heat and hot air) are being widely used for quality maintenance of stored fruit and disease/pests control (Fallik & Ilic', 2019; Ferguson et al., 2000; Hansen et al., 2011; Lurie, 1998; Tang et al., 2007). For the pomegranate fruits in cold storage (90 days at 2 or 5 °C), one day intermittent warming at 20 °C, every 6 days, or curing at 33 °C for 3 days before storage, reduced decay and chilling injury symptoms (Artés, et al., 2000). Juice characteristics (soluble solids content (SSC), titratable acidity (TA) and pH) and visual appearance show the advantage of the pomegranates heat treating rather than conventional cold storage (Artés et al., 2000). Heat treating of pomegranates by hot water dip at 45 °C for 4 min, and then stored at 2 °C for 90 days significantly reduced chilling injury symptoms (Mirdehghan et al., 2007). In Iran, there are some local publications (in Persian) that confirm the effect of pre-storage thermal heating of pomegranate on quality characteristics improvement after storage. They find that the pre-storage hot water treatment of pomegranate at 45-50 °C for 1-5 minutes has a significant effect on decay control during storage.

Heat treatment has been used for carob moth control on harvested dates. Hot air (55, 60 and 65 °C for 20-40 minutes) and hot water (50, 55 and 60 °C for 0-30 minutes) had a significant effect on pest mortality (Ben-Amor et al., 2016a; Ben-Amor et al., 2016b; Zouba et al., 2013).

Microwave heating is a useful technique for postharvest thermal treatments of fruits and vegetables (Marsaioli Jr et al., 2009). Accordingly, it is an effective method for pest control (García-Mosqueda et al., 2019; Ling et al., 2015; Nelson & Trabelsi, 2016). Microwave treatment for postharvest control of the date infested by carob moth showed significant mortality of pests (Zouba et al., 2009). In the microwave, due to volumetric heating, thermal gradient on the product is lower rather than conventional heating (Datta, 2001; Feng et al., 2012; Mirzabeigi Kesbi et al., 2018).

Integrated pest management (IPM) is the careful consideration of all available pest control techniques for the economic control of pests. For carob moth, applying postharvest techniques in combination with the field approach will be useful. In this study, postharvest

heat treatment was applied to control hidden carob moth infestation on pomegranate fruit. In hidden infestation, there are eggs and/or first-instars of pest inside the crown (calyx). Therefore, local heating around the crown zone was applied by microwave radiation focus with a novel setup. By the crown zone local heating, there is low temperature increasing on the edible part of the pomegranate (arils) that causes lower unwanted changes in fruit quality.

MATERIALS AND METHODS

Samples Preparation

Mature pomegranate fruits, Galubarik variety were harvested on 15 October 2018 in a commercial orchard in Varamin, Tehran Province, Iran (Latitude 35°N and Longitude 51° E) and immediately transported to the laboratory. Fruits with defects (crack, bruise, and decay) were removed. The fruits with sound appearance were kept 10 days at room temperature in order to detect the hidden naturally infestation by carob moth in the orchard. Naturally infested samples were discarded and the sound pomegranates were selected for tests. Fruits were artificially infested with two eggs or larvae (first-instars, 1^{st}, 2^{nd} and 3^{rd}) of carob moth inside the crown (calyx). To simulate hidden infestation, eggs and first-instars larvae of carob moth were used. The larvae were put into the crown after four hours of starvation. Larvae infested samples were kept at room temperature for 24 hours prior to heat treatments. This allows the larvae to embed in the fruit crown. The crown opening was closed with a fabric net piece to prevent the larvae from leaving the fruit (Fig. 1).

Experimental design

A novel setup based on a domestic 2450 MHz microwave oven (Fig. 2) was used to focus microwave radiation on the pomegranate crown zone (Mirzabeigi Kesbi et al., 2018). Focusing the radiation makes local heating of the crown and the carob moth mortality without unwanted heating on other parts of the fruit.

The power of the oven was set to values of 180, 360, 540, 720 and 900W in the control panel. The maximum output power supply (nominally 900W) was measured by two liters water load method (IEC, IMPI, (Buffler, 1993)). The mean of three measurements was 581 W. The microwave was generated by the magnetron and was fed into the chamber with an aperture in the sidewall. For preventing random reflection in the chamber, 1.2 liters' water load was used. The water load container (made from Teflon, microwave transparent) was placed in the chamber, adjacent to the sidewall. There is a cylindrical path (30 mm diameter) in the water container in front of the aperture. The propagating waves from the aperture were guided into the cylindrical path. In the oven chamber, infested pomegranate samples were placed on the sample holder and the crown was guided into the cylindrical path (Fig. 2). The waves parallel to the path axes radiated on the sample crown zone, whereas the unparalleled (random) waves entered into the water load and damped (for further information, refer to Mirzabeigi Kesbi et al., 2018). This causes local heating of the pomegranates crown and carob moth mortality.

Preheating of the chamber was carried out prior to the tests. During preheating, the water container with full capacity (1.2 liters) was placed in its location and the microwave oven was operated with 900 W power level for two minutes (without sample). After each test, the water container was taken out from the chamber and the water load replaced with room temperature water.

Experimental Procedure

Carob Moth Mortality

The carob moth infested samples (24 hours after infestation) were treated by the microwave setup. In each test, one infested pomegranate was placed in the setup (Fig. 2) and the experiments were carried out at three microwave powers of 540, 720 and 900 W, three heating times of 4, 6 and 8 minutes and three pest's age treatments of egg, 1st-2nd and 3rd instar (due to difficulty of 1st and 2nd larva instars separation, both of them were placed in one treatment). Treated samples were kept in the room temperature for 48 hours. After 48 hours, the eggs and larvae were separated by cutting the crown of fruits. In larval infested samples, live and dead pest stage were manually counted. The live larvae were fed and after four weeks, the larvae with incomplete life cycles were considered as the perished pests. Total dead and incomplete life cycle larvae were included as final mortality. In eggs infested samples, unhatched eggs were considered as final mortality and used to calculate the percentage of mortality. The microwave heat treatments were analyzed using a completely randomized design in factorial layout (3×3×3) with 10 replications. Analysis of variance (ANOVA) procedure was performed to determine the significant effects of the experimental factors on carob moth mortality. The analysis was carried out in SAS statistical software (SAS Institute Inc., NC, USA). Mean values were compared using Duncan's multiple range test to find significant differences among treatments and interactions between factors (p<0.05). Infested samples by three pest's age with no heating treatment were considered as control treatments. The mortality of control treatments was compared with treated samples (final mortality) using the least significant difference (LSD) test (p<0.05).

At the end of the heating process, the surface temperature on the crown zone of samples was measured with a non-contact infrared thermometer (ST350, Thermometer superstore, UK).

Quality Parameters

In order to study the effect of microwave heating treatments on pomegranate quality, the sound pomegranate fruits were treated in the separate microwave heating tests with the same condition of carob moth mortality investigations (at 540, 720 and 900 W microwave powers and 4, 6 and 8 minutes heating times).

The appearance of fruits was observed 48 hours after heat treatment. Damaged samples, because of overheating, were removed and others were stored at 5±0.1°C and 85±3% relative humidity. After 60 days' storage, quality factors (weight loss, total soluble solids, pH and titratable acidity) of samples were measured. The results were compared with control (without heat treatment before storage) and fresh samples using a completely randomized design with a factorial arrangement of variables.

All sample weights were measured before (w_1) and after (w_2) storage using a digital balance (KERN, KB, Germany). Weight loss was calculated by Eq (1).

$$Weight\ loss\ (\%) = \frac{w_1(g) - w_2(g)}{w_1(g)} \times 100 \quad (1)$$

After storage, the fruits were cut, peeled and arils were separated. The arils were manually pressed and the extracted juice was filtered. Sugar content or total soluble solids (TSS) of samples' juice were determined by a handheld refractometer (A. Krüss Optronic, Germany). 10 ml of sample juices were diluted with 90 ml distilled water and the pH was measured using a pH meter (Sartorius, PB-11, Germany). The diluted juices were titrated with 0.1 N NaOH (to pH 8.2) and the titratable acidity (TA) were expressed as the percent of citric acid (Elyatem & Kader, 1984; Fadavi et al., 2005; Sadler & Murphy, 2010). The ratio of

TSS/TA (taste or maturity index) as a numerical value that represents the taste condition of pomegranates was calculated for samples. Low and high values of taste index indicate sour and sweet taste respectively (Fadavi et al., 2005; Martinez et al., 2006).

RESULTS AND DISCUSSION

Carob Moth Mortality

Table 1 displays the mortality of carob moth in the microwave heat treated pomegranates. It shows the mortality of the larvae after 48 hours and the final mortality of three ages of pest against the control treatments. As mentioned, in pomegranate samples the pest was embedded inside the crown, but penetration ability of microwave radiation causes considerable mortality of carob moth.

According to Table 1, for larvae, the final mortalities are higher than the values after 48 hours. It means that 48 hours after treatments, there are some live larvae that cannot complete their life cycle. It demonstrates that microwave heating makes a persistent effect in addition to immediate mortality. Larva with incomplete life cycle is the result of persistent effect while larva death due to overheating is the result of immediate effect. In some cases, overheating makes the larva's body disintegrating (Fig. 3).

According to the LSD test (Table 1), the final mortality of all heat treated samples is significantly higher than control treatments. It shows the satisfactory effect of microwave heating on carob moth mortality in pomegranate samples.

Analysis of variance (ANOVA) showed significant differences in carob moth mortality among simple factors (pest's age, treatment time and microwave power) and no significant differences among interactions. Non-significant interactions mean that the change in each factor has a similar effect on others.

Table 1. Carob moth mortality of heat treated pomegranates.

	Mortality after 48 hours (%)		Final mortality (%)		
Treatments	1st-2nd instars	3rd instar	Egg	1st-2nd instars	3rd instar
Control	20	20	15	35	30
540 W-4 min	50	30	95	85	60
540 W-6 min	85	65	95	90	85
540 W-8 min	80	90	100	95	95
720 W-4 min	80	45	95	90	80
720 W-6 min	100	70	95	100	100
720 W-8 min	100	100	100	100	100
900 W-4 min	40	50	100	100	100
900 W-6 min	95	95	100	100	100
900 W-8 min	100	100	100	100	100
LSD*	---	---	11	19	23

†Least significant difference test for comparing the values of mortality with control treatments ($p<0.05$).

Table 2. Effect of pest's age, treatment time and microwave power on carob moth final mortality after microwave heat treatments.

	Age			Treatment time (min)			Microwave power (W)		
	Egg	1st-2nd instars	3rd instar	4	6	8	540	720	900
Mortality (%)	97.8^{a}	95.6^{ab}	91.1^{b}	89.4^{a}	96.1^{b}	98.9^{b}	88.9^{a}	95.6^{b}	100^{b}

†Mean values were compared using Duncan's multiple range test ($p<0.05$). Similar letters are not significantly different.

The mean values of simple factors display in Table 2. As shown, the mortality of pest decreases with age growth. It demonstrates the higher susceptibility of pest's egg and lower age larvae to the microwave heating. These results are in accordance with the findings of Zouba et al. (2009). Since the microwave radiation creates more heating in the larvae body due to the aqueous tissue, it seems that the microwave treatment causes higher mortality in larvae rather than eggs but the susceptibility of pest in lower age is the dominant factor. As expected, the mortality has risen by treatment time and microwave power increase. The higher level of power and time cause a higher temperature in samples and increase carob moth mortality. Increasing temperature in a higher level of microwave power and time, confirmed by the measured values (Table 3). In some other studies, increasing the mortality at the higher temperature were noted in hot water, hot air and microwave heat treatment of carob moth infested samples (Ben-Amor et al., 2016a; Ben-Amor et al., 2016b; Zouba et al., 2013; Zouba et al., 2009).

According to the data in Table 2, increasing microwave power from 540 to 720 W caused significant and 720 to 900 W caused non-significant differences in mortality. Also in treatment time, increasing from 4 to 6 min made significant effects and 6 to 8 min made non-significant differences in mortality. Hence, applying 720 W microwave power with 6 min treatment time caused considerable mortality with energy saving.

Quality Parameters

In pomegranate quality tests, 48 hours after heat treatments the appearance of samples was investigated. The observations showed severe heat damage of samples in four treatments (from 9 treatments). There were severe damages in all 8 min (540, 720 and 900 W) treatments and 900 W-6 min. In all damaged samples the temperature has reached above 47 °C after heat treatment (Table 3). It demonstrates that the critical temperature of pomegranate samples is between 42 to 47 °C while higher values caused over heating damage.

Table 4 shows the quality parameters of stored pomegranates (after removing the damaged samples). As shown, there is no significant difference in weight loss, TSS and pH values. Except for 900 W-4 min, TA values of treated samples and control treatment are very close to each other and placed in one group. In stored pomegranates, no considerable differences among treated and control samples demonstrated that heat treatment has no significant effect on pomegranate quality (unless for the removed treatments). In all stored samples, pH increased and consequently, TA decreased rather than fresh pomegranates while the significant difference has occurred only in 900 W-4 min. In treated pomegranates with 900 W, a high level of microwave power caused the chemical reactions to speed up and makes a great change at TA level. pH rise and TA reduction during conventional cold storage of pomegranate have been reported by other researchers (Artés et al., 2000; Ehteshami et al., 2021; Fawole & Opara, 2013). With approximately a constant amount of TSS among all treatments, TA values were used to determine the variations of taste index (TSS/TA). Except for 900 W-4 min, all TSS/TA values were minimal together and represented sour-sweet taste. In 900 W-4 min, lower values of TA caused a higher taste index and appeared to have a sweet taste. Depending on customer demand, both sour and sweet tastes are marketable.

Table 3. The surface temperature on the crown zone of pomegranates at the end of the heating process.

Treatments	540 W 4 min	540 W 6 min	540 W 8 min	720 W 4 min	720 W 6 min	720 W 8 min	900 W 4 min	900 W 6 min	900 W 8 min
Temperature (°C)*	33.7	42.5	47.7	36.3	40.9	47.6	40.2	49.4	57

†Average of 30 replications (10 replications for each pest's age).

Table 4. Quality factors of fresh, control and microwave heat treated samples after storage.

	Weight loss (%)	TSS (%)	pH	TA (%)	TSS/TA
Fresh sample	---	15.3	3.18	1.72^{a}	8.90^{a}
Control	11.56	15.1	3.57	1.54^{ab}	9.81^{a}
540 W-4 min	11.08	15.5	3.39	1.42^{b}	10.92^{a}
540 W-6 min	10.80	15.5	3.48	1.40^{b}	11.07^{a}
720 W-4 min	11.83	13.9	3.35	1.32^{b}	10.53^{a}
720 W-6 min	10.69	15.5	3.29	1.34^{b}	11.57^{a}
900 W-4 min	11.06	14.0	3.47	0.55^{c}	25.45^{b}

†Mean values were compared using Duncan's multiple range test ($p<0.05$). Similar letters are not significantly different.

Fig. 1. Carob moth infested pomegranate sample.

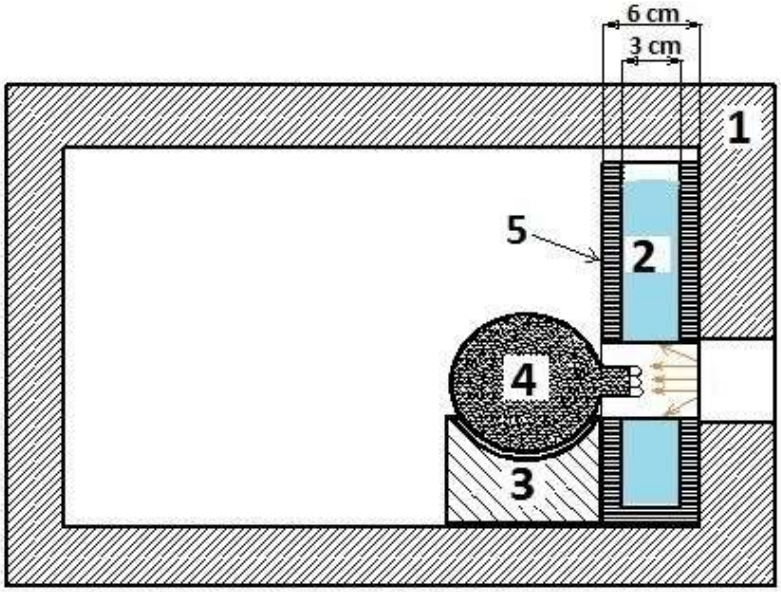

Fig. 2. Cross-sectional view of the microwave heating setup: (1) oven chamber, (2) water load, (3) sample holder, (4) sample, (5) water container.

Fig. 3. Immediate effect of microwave heating on carob moth larva body (3rd instar, 900 W, 4 min).

CONCLUSION

In this study, the significant effect of microwave heat treatment on carob moth mortality in artificially infested pomegranate fruits was studied. The mortality of 720 W-6 min treated sample is about 100 % while quality parameters have no significant differences in comparison with storage control treatment. Higher level of microwave power and treatment time creates higher temperatures and more carob moth mortality but may cause over heating damages.

Conflict of interest

The authors have no conflict of interest to report.

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