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Effect of Sulfur Pesticide on the Quality of Fresh Pistachios in Cold Storage

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ABSTRACT

The efficiency of sulfur in controlling pistachio pests, especially psylla, has been documented in the available literature, but it is essential for pesticides not to affect the quality of the product. In this study, movento (0.5 L/1000 L), confidor (0.4 L/1000 L) and refinery micronized (R) and mineral (M) sulfur (30 and 60 kg/1000 L in water) were sprayed twice on pistachio trees of the cultivar 'Ahmadaghaei' (in mid-May and 50 days thereafter). Pistachios were harvested in late September and were stored at 4 °C for 25 and 50 days to evaluate the effects of pesticides on the quality of hulls and kernels. The taste, aroma, appearance, respiration rate, firmness of hull and kernel, *a** (red-green), *b** (blue-yellow) and chroma of hull and kernel declined over time, while *L** (lightness) and the hue angle of the hull and kernel increased. Ethylene production remained stable over time. Chemical pesticides stimulated hull firmness. Refined micronized sulfur at both concentrations caused higher *L** and hue angle, but lower *a**, *b** and chroma in the hull and kernel (i.e. lighter and yellower in color). All sulfur treatments reduced the firmness of the hull and kernel, while also increasing the respiration rate. R30 preserved the taste and aroma after 50 days. R30 and M30 showed higher ethylene production. The R30 treatment had some positive effects on preserving the taste and aroma of pistachios overtime. Thus, it may be recommended as a reliable treatment, although the ability of sulfur in pest control could be offset by the side effects of sulfur on product quality.

Abbreviations:

R30: Refined micronized sulfur (30 kg/1000 L); R60: Refinery micronized sulfur (60 kg/1000 L); M30: Mineral sulfur (30 kg/1000 L); M60: Mineral sulfur (60 kg/1000 L)

Introduction

Pistachio trees produce valuable commodities that are mainly traded as dry nuts in-shell kernels. The sheer process of drying, however, leads to various degrees of loss in nutrients and antioxidant components of pistachio, so that the demand for fresh pistachios has increased due to its health benefits (Sheikhi et al., 2019). Fresh pistachios can be stored in cold storage for a short time because many factors influence its shelf life. Different kinds of microorganisms can infect the fresh pistachios and cause postharvest spoilage and, thus, disinfection is a crucial step after harvesting to prevent postharvest loss by microbial decay (Sheikhi et al., 2020).

Sulfur as a pesticide with a non-systemic contact mode has been extensively applied in pistachio orchards in recent years, especially against psylla. Common pistachio psylla (*Agonoscena pistaciae*) is an important pest of pistachio trees, which causes a lot of damage to pistachio orchards every year. Pest resistance to chemical pesticides has become a

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problem in controlling psylla. Using different forms of sulfur is a strategy which has been welcomed by farmers (Mehrnejad, 2002). Sulfur is a byproduct of coal, natural gas, petroleum refinement and mining processes (Afrousheh and Hasheminasab, 2018). The advantages of sulfur are its low cost, efficiency, low risk of resistance development, and compatibility with different production systems (Kwasniewski et al., 2014). Sulfur spraying against pistachio psylla is developing in many pistachio-rich regions (Binam, 2017). However, there are different opinions about this compound due to some damages to the leaves and fruits. The application of this compound without conducting preliminary research can have detrimental effects on fruit and yield. Field studies also confirm some negative effects of sulfur on the leaves and fruits of some pistachio cultivars. Therefore, the type, concentration and time of exposure should be determined for each crop to avoid harmful effects. For example, research shows that sulfur may stimulate fruit water loss during storage (Rivera et al., 2013), or that the application of sulfur (100-150 µL L^{-1} h) on blueberry fruits through SO_2 pads inside the packaging caused a reduction in fruit firmness (Rodrigues and Zoffoli, 2016). Sulfur can elevate the respiration rate of some plant (Agrawal and Deepak, 2003), although this is not desirable in cold storage. Besides pistachio psylla, sulfur has been used for managing brown rot in peaches (Holb and Schnabel, 2008), mildew on roses (Hosseininia et al., 2008), powdery mildew of apples (Holb and Kunz, 2016), and peanut leaf spot (Culbreath et al., 2019).

Even though pistachio kernels are protected by the shell and hull, they receive signals from the surrounding tissues and are influenced by the physiological status of the hull and of the whole plant. Thus, in this study, we aimed to evaluate the effects of refined micronized and mineral sulfurs on the quality of fresh pistachio hulls and kernels. The evaluations were made at harvest and during cold storage. Two chemical pesticides, movento and confidor, were compared in their ability to act against psylla.

Materials and Methods

Plant material and treatments

This study began in mid-May 2019 in the orchards of Anar city, Iran. Pistachio trees of the cultivar 'Ahmadaghaei' were sprayed with movento (0.5 L/1000 L), confidor (0.4 L/1000 L) and two types of sulfur, including refined micronized sulfur (30 and 60 kg/1000 L in water) and mineral sulfur (30 and 60 kg/1000 L in water). The concentrations were selected based on suggestions made by orchardists in the area. Trees of the control group were not sprayed. The spraying was repeated after 50 days in July. The spraying was performed at a scale of 3000 L per hectare, with each hectare having almost 200 trees (15 L per tree). Each treatment was performed on three rows of trees. Six neighboring trees were considered as one replicate. Sampling was done from four sides of each tree. Spraying was done in the morning and ended before the weather warmed by noon. The ripening time of pistachios was determined with physical indices such as the color change of hulls, ease of peeling and separation of the fruits from the clusters. In late September and early October, the fruits were sampled and transferred to the laboratory. A portion of the fruits was used for obtaining the data of harvest time and the rest were kept in disposable tableware with a closed lid. They were stored at 4 °C and, after 25 and 50 days, the fruits were analyzed again.

Firmness

Firmness was measured using a Digital Force Tester (Lutron fg5020, Taiwan) that was fitted with a sharp 11 mm probe. Two different measurements were carried out on two opposite sides of the central zone of kernels or hulls. The values were expressed as kilogram-force per centimeter square (kgf/cm^2).

Water activity

One g of kernels or hulls were used as replicates. Water activity was directly measured by a water activity meter (Novasina LabMaster model, Switzerland) and expressed as a_w.

Color indicators

Color values of hulls and kernels (with testa) were evaluated using a color meter (Minolta Chroma Meter Model CR-400, Minolta, Japan). The lightness ($L*$), red-green ($a*$) and blue-yellow ($b*$) were measured as color values. The chroma value and hue angle were calculated by the following equations (Equations 2 and 3).

$$\text{Equation2: Chroma} = \sqrt{(a*)^2 + (b*)^2}$$

$$\text{Equation 3: hue angle} = \tan^{-1}\left(\frac{b*}{a*}\right)$$

Sensory evaluation

A panel test was performed by ten semi-trained panelists. The panelists examined the taste, aroma, appearance and general acceptance of fruit. Excellent was shown with the number 'five', very good with 'four', good with 'three', moderate with 'two', poor with 'one', and extremely poor with '0' (Moradinezhad et al., 2019).

Ethylene production and respiration rate

Fifteen grams of fresh pistachios were sampled from each treatment group and were placed in airtight respiration jars with a volume of 1.6 L at 20 °C. They were allowed to equilibrate for 2 h. One mL of the head-space air was drawn by plastic syringes and injected into a gas chromatograph (Agilent 7890B, USA) equipped with a packed column and a thermal conductivity detector (TCD) for quantification of CO_2 or C_2H_4. Injector, detector and oven temperatures were set at 100, 120 and 80°C, respectively. Nitrogen was used as a carrier gas at a flow rate of 73.7 mL min^{-1}. The respiration rate and ethylene production were expressed as ng kg^{-1} s^{-1} (Gheysarbigi et al., 2020). Ethylene production and respiration rates were only measured at harvest time and after 25 days.

Statistical analysis

A factorial experiment was carried out based on a completely randomized design with four replicates. Sources of variation were storage time (0, 25 and 50 days), treatments (control, movento, confidor, R30, R60, M30, and M60) and their interactions. Mean values were calculated and reported as the mean value ± standard error. Data were analyzed by SAS 9.1 (SAS Institute Inc 2013) statistical software package, and Duncan's multiple range test was used for comparing the mean values at $P \leq 0.01$ probability. The graphs were plotted in MS-Excel software.

Results

Firmness

The interaction of storage time and treatments had a significant effect ($P \leq 0.01$) on hull firmness (Table 1). The quality of this trait decreased through time. Movento and all sulfur treatments caused a reduction in hull firmness, compared to the control at harvest time, but not during storage (Fig. 1 A). The simple effects of treatments and storage time were significant on kernel firmness ($P \leq 0.01$) (Table 1). On day 50, the decrease in kernel firmness had become significantly noticeable by the effect of sulfur treatments (Fig. 1 B, C).

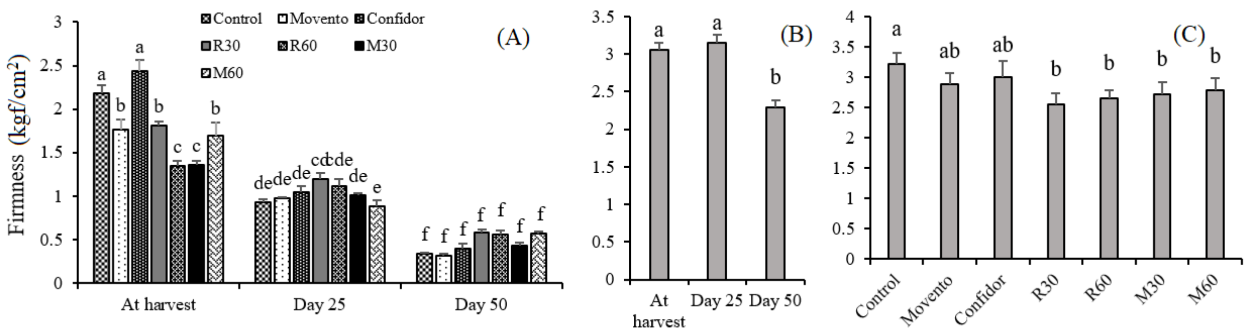

Fig. 1. Firmness of (A) hull and (B, C) kernel of pistachio cultivar 'Ahmadaghaei' treated preharvest with movento (0.5 L/1000 L), confidor (0.4 L/1000 L), refinery micronized sulfur at two concentrations (30 and 60 kg/1000 L) (R30 and R60) and mineral sulfur at two concentrations (30 and 60 kg/1000 L) (M30 and M60) during storage

Table 1. Analysis of variance showing mean squares for treatments and storage time for measured traits of fresh pistachios

Sources of variation	**Degree of freedom**	**Traits Mean squares**									
		Hull firmness (KgF)	**Hull water activity (a_w)**	**Hull *L****	**Hull *a****	**Hull *b****	**Hull chroma**	**Hull hue**	**Kernel firmness (KgF)**	**Kernel water activity (a_w)**	**Kernel *L****
Treatments (T)	6	0.14**	0.00003^{ns}	314.52**	70.86**	11.22**	64.48**	0.02^{ns}	0.46**	0.00004^{ns}	125.34**
Storage (S)	2	9.54**	0.001**	1902.01**	1721.76**	137.41**	1284.74**	0.95**	4.64**	0.0008**	1068.30**
T×S	12	0.20**	0.00003^{ns}	176.71**	223.02**	10.58**	132.46**	0.16**	0.23^{ns}	0.00003^{ns}	267.45**
Error	42	0.01	0.00003	9.80	4.05	1.88	1.18	0.009	0.18	0.00004	14.88
Coefficient of variation (%)		11.10	0.63	6.0	12.08	7.41	4.23	0.05	15.01	0.66	7.82

Sources of variation	Degree of freedom	Traits Mean squares								
		Kernel *a**	Kernel *b**	Kernel chroma	Kernel hue	Taste	Aroma	Appearance	Ethylene (ng kg^{-1} s^{-1})	Respiration (ng kg^{-1} s^{-1})
Treatments (T)	6	199.22**	33.37**	178.80**	0.10**	1.13**	0.46**	2.11**	0.25**	23.98**
Storage (S)	2	2090.33**	407.07**	1781.08**	0.87**	13.20**	5.86**	3.40**	0.04^{ns}	144.18**
T×S	12	198.31**	9.59**	106.32**	0.16**	0.29**	0.23**	1.24**	0.17**	108.21**
Error	42	1.71	3.26	2.0	0.007	0.09	0.05	0.06	0.02	1.80
Coefficient of variation (%)		10.27	9.62	5.95	0.04	8.52	6.08	6.57	16.19	7.45

** and ns mean significant at probability level of 0.01 and non-significant, respectively

Water activity

The simple effects of storage time were significant (P≤0.01) on the water activity of hulls and kernels (Table 1). This trait decreased in value up to the 50th day, compared to the control (Fig. 2 A, B).

Color indicators

The interaction of storage time and treatments was significant (P≤0.01) on the color values of the hull (Table 1). At harvest time, refined micronized sulfur-treated samples (R30 and R60) had higher *L** values but lower *a** and *b** values in their hulls, compared to other samples (Fig. 3 A, B, C and Fig 4). These two treatments had higher hue values (basic color) and lower chroma values (color intensity) (Fig. 3 D, E). Totally, the *L** value of hulls showed an increase until the 25th day, followed by a slight decrease in some samples. The *a** and *b** values revealed a decreasing trend during storage. The hue value of most samples increased until the 50th day. The chroma value, however, decreased over time.

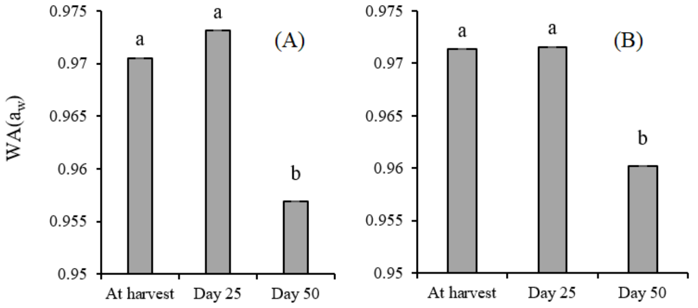

Fig. 2. Water activity of (A) hull and (B) kernel of pistachio cultivar 'Ahmadaghaei' during storage

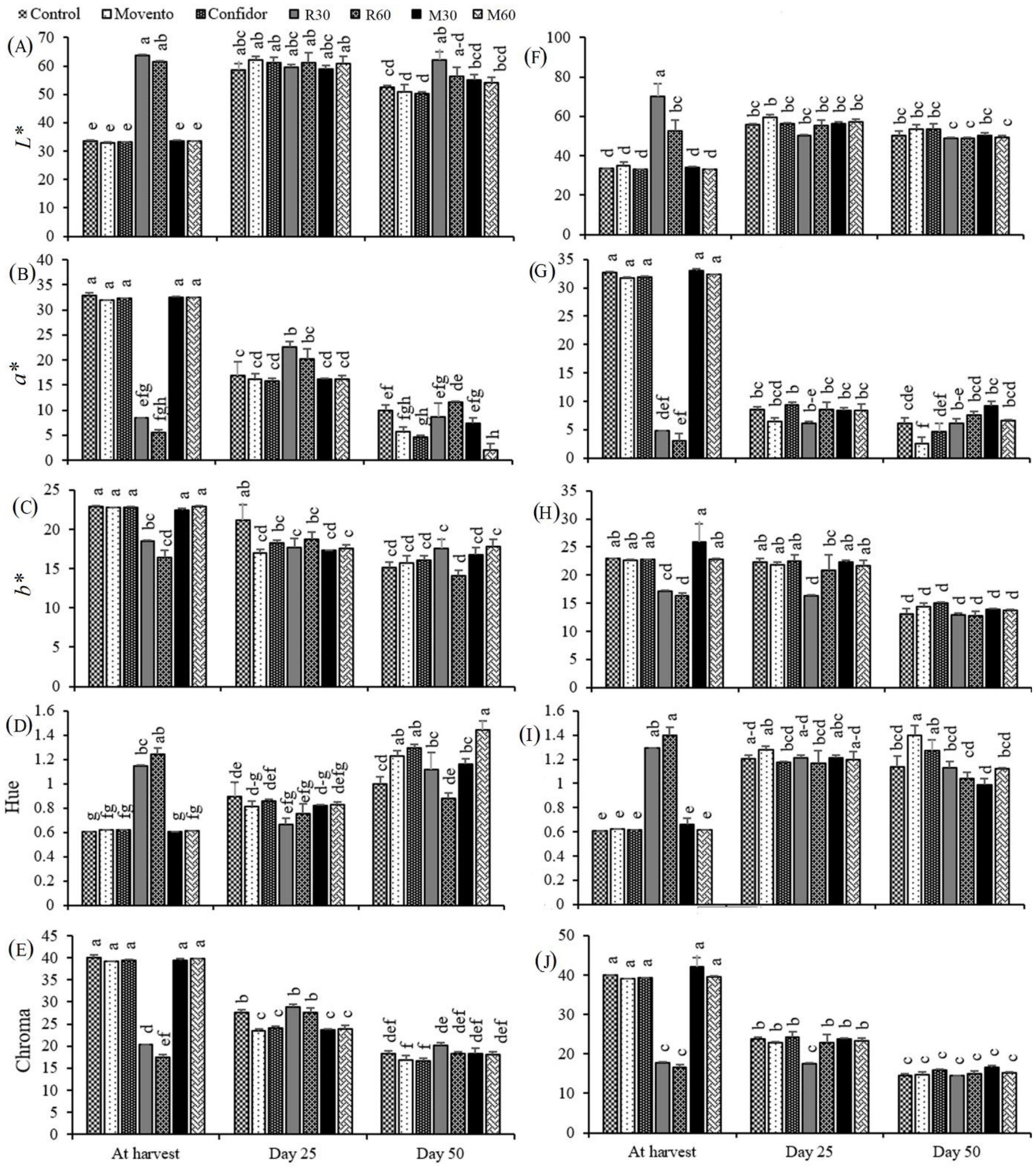

Fig. 3. Color indicators of (A-E) hulls and (F-J) kernels of pistachio cultivar 'Ahmadaghaei' treated preharvest with movento (0.5 L/1000 L), confidor (0.4 L/1000 L), refinery micronized sulfur at two concentrations (30 and 60 kg/1000 L) (R30 and R60) and mineral sulfur at two concentrations (30 and 60 kg/1000 L) (M30 and M60) during storage.

The interaction of storage time and treatments were significant (P≤0.01) on the color indicators of the testa of the kernel (Table 1). R30- and R60-treated samples had higher *L** and hue values but lower *a**, *b** and chroma values of testa at harvest time (Fig. 3 F, G, H, I, J). *L** and hue values increased, but *a**, *b** and chroma values decreased over time.

Fig. 4. A, B and C show the control pistachios at harvest time, on day 25 and on day 50, respectively; D, E and F show fresh pistachios treated with R30 (refinery micronized sulfur with 30 kg/1000 L concentration) at harvest time, on day 25 and on day 50, respectively

Sensory evaluation

The interaction of storage time and treatments was significant (P≤0.01) on taste, aroma and appearance of pistachios (Table 1). All three parameters displayed a decreasing trend during storage, but in some cases this decrease was not statistically significant. The R30 treatment could maintain the taste and aroma of pistachios, even after 50 days (Fig. 5 A, B). The panelists did not record any off-flavor as a result of the treatments in this case. The pistachios that were treated by sulfur (all sulfur treatments) had a higher degree of appearance at harvest time (Fig. 5 C).

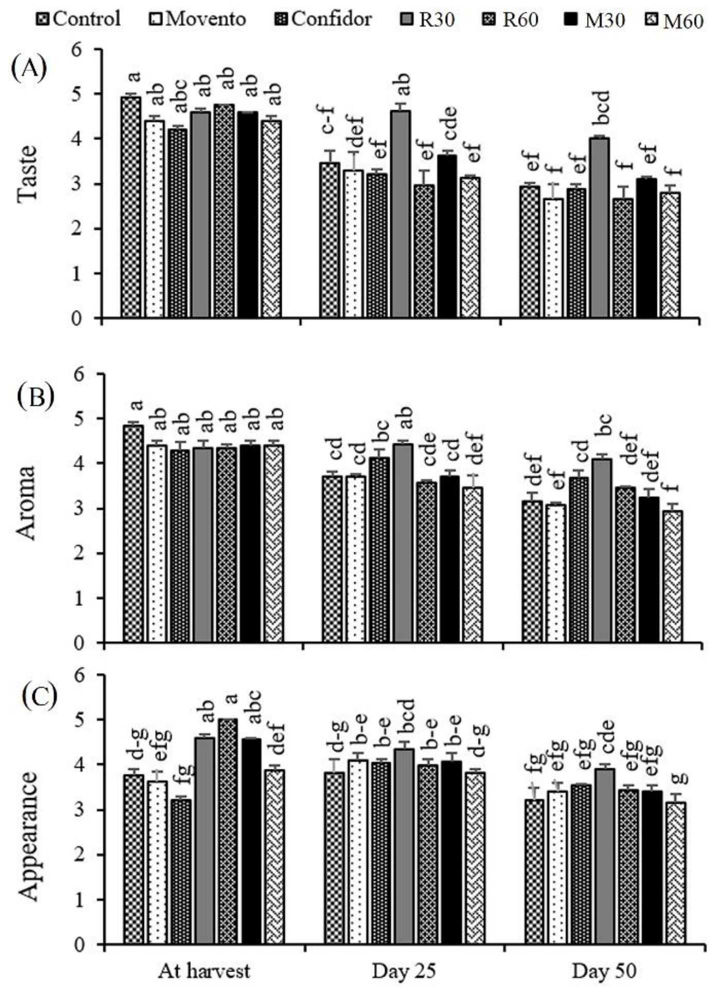

Fig. 5. Sensory indicators of pistachio cultivar 'Ahmadaghaei' treated preharvest with movento (0.5 L/1000 L), confidor (0.4 L/1000 L), refinery micronized sulfur at two concentrations (30 and 60 kg/1000 L) (R30 and R60) and mineral sulfur at two concentrations (30 and 60 kg/1000 L) (M30 and M60) during storage.

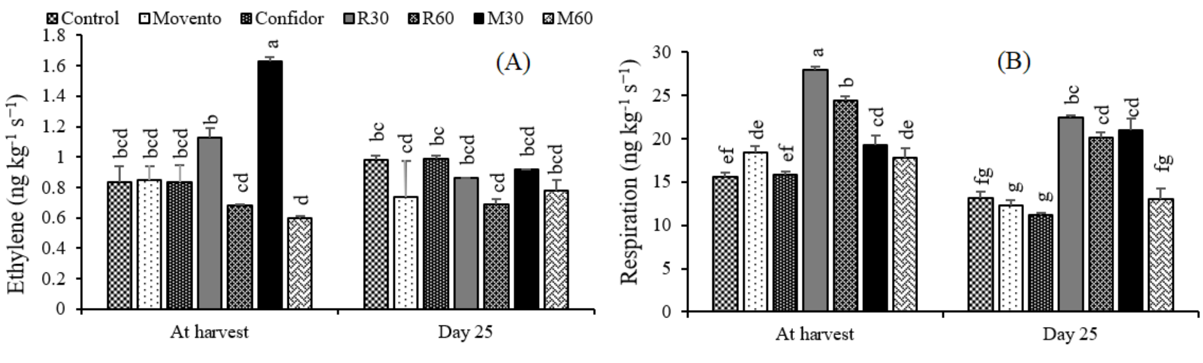

Fig. 6. Ethylene production (A) and respiration rate (B) of pistachio cultivar 'Ahmadaghaei' treated preharvest with movento (0.5 L/1000 L), confidor (0.4 L/1000 L), refinery micronized sulfur at two concentrations (30 and 60 kg/1000 L) (R30 and R60) and mineral sulfur at two concentrations (30 and 60 kg/1000 L) (M30 and M60) during storage.

Discussion

Firmness is a major indicator of fruit quality. Normally, when fruit water content decreases over time, a reduction in firmness is expectable, because shriveling occurs and the cells suffer some degrees of plasmolysis. Paniagua et al. (2013) revealed a negative correlation between water loss and fruit firmness. The lower firmness of sulfur-treated samples at harvest time indicates the lower water content compared to the control. Research shows that sulfur may have adverse effects on fruit water loss (Rivera et al., 2013). In comparison, application of SO_2 pads inside the packaging, that released 100-150 µL L^{-1} h sulfur, resulted in lower firmness of blueberries than the control (Rodrigues and Zoffoli, 2016).

The growth of pathogens in foods is highly affected by water activity (Romero et al., 2007). The water activity of both hulls and kernels was above 0.9 aw which is high and suitable for the growth of most pathogens. Our treatments did not influence the water activity because it is mostly dependent on temperature (Slade et al., 1991). All the samples had relatively the same temperature at harvest time and also at cold storage. Although the water content of a food does not necessarily determine the water activity, the high water loss on the 50th day resulted in a reduction in water activity.

At harvest time, refined micronized sulfur-treated samples (R30 and R60) had higher *L** values but lower *a** and *b** values of hulls than others, which means that R30 and R60-treated hulls were lighter and yellower than others (Fig 4). In coordination, these two treatments had higher hue (basic color) and lower chroma (color intensity) values. The higher hue shows the yellower color and less red pigments, while the lower chroma value reveals the less intense color of these two treatments. Similarly, SO_2 treatment caused a greener color and less red pigments in Arabidopsis leaves (Li and Yi, 2020). Totally, *L** value of hull showed an increasing trend in the 25th day, followed by a slight decrease in some samples. This increasing trend can be due to the gradual synthesis of cuticles on hull epidermal cells and also the gradual removal of sulfur particles. Likewise, fresh cut apples treated with 50% sodium sulfite were lighter than the control after 5 days of cold storage (Ortzi et al., 2018). The *a** and *b** values revealed a decreasing trend during storage. Similarly, the hue value of most samples increased on the 50th day which shows the gradual loss of red pigments. The chroma value revealed a reduction through time that again shows the loss of pigments and loss of color intensity. The results of testa color were very similar to the results of hull color. Accordingly, R30- and R60-treated samples had higher *L** and hue values but lower *a**, *b** and chroma values of testa at harvest time which means that they were lighter and yellower with less red pigments. *L** and hue values showed increasing values, but *a**, *b** and chroma values showed decreasing trends over time. We believe that sulfur particles do not penetrate the kernel if the hull is intact. But the kernels are certainly influenced by the physiological status of hulls and get signals from them through metabolic pathways, a fact that was also reported in the case of walnuts (Pakrah et al., 2021). This can be the reason why the kernels changed their color in a similar way that the hulls did.

As expected, all sensory parameters displayed a decreasing trend during storage. The R30 treatment maintained the taste and aroma of pistachios even after 50 days. Sulfur, in certain concentrations and application times, was applied as a preservative in food technology because it keeps the quality of food constants, meeting market requirements (Rossello et al., 1994). For example, in the wine industry, small doses of SO_2 are needed for extending the sensory quality (Wyk et al., 2018). Herein, the panelists did not record any off-flavor for the treatments. Comparably, no off-flavor was developed after SO_2 fumigation in blueberries (Cantin et al., 2012). The pistachios that were treated by sulfur (all sulfur treatments) had a higher degree of appearance at harvest time, which may be related to the antimicrobial effect of sulfur, keeping the fruit surface safe from disease damages. The lighter color of sulfur-treated hulls was another reason for the higher degree of appearance in these treatments.

Sulfur treatments at lower concentration (R30 and M30) showed higher ethylene production than others. Tomato plants that were fumigated by SO_2 for 1 h revealed an increase in ethylene production and when the treatment prolonged for 4 h, ethylene production was also increased. Ethylene might play a role in SO_2-induced plant injuries in the short term of SO_2 fumigation (Gong Young et al., 1995). Alfalfa plants fumigated by 0.7 µL/L SO_2 for 8 h increased ethylene production 10 times greater than control plants (Peiser and Yang, 1979). Ethylene production by bean plants, after treatment with elemental sulfur, increased before full bloom and before fruit set, and flowering occurred earlier because a slow oxidation of elemental sulfur in air produced SO_2 which enhanced ethylene synthesis in the leaves (Recalde-Manrique and Diaz-Miguel, 1981).

All sulfur treatments had higher respiration rates than the control, which might be the result of high-energy consumption for protection mechanisms and activation under sulfur stress. The respiration rate declined through time as a result of lowered metabolism under cold temperatures. The respiration rate of wheat plants increased because of SO_2 fumigation for 8 h each day, with a concentration of 0.06 ppm. Previous researchers believed that the detoxification reactions against SO_2 are ATP-mediated, and ATP is provided by respiration (Agrawal and Deepak, 2003). In the present study, a probable toxification of the hulls resulted in an increase in respiration.

Conclusion

Practical evidence shows that sulfur application on pistachios is an effective practice to control pests such as psylla, causing huge yield and economic loss each year. At the same time, it is crucial to optimize the type and concentration of sulfur, because the physiological status of hull and the whole plant may influence the kernels and the yield. Firmness, color, taste and aroma are indicators of marketability, and they declined over time in the case of this research. Some sulfur treatments, especially the refined micronized type, provided lighter but yellower color and could preserve the taste and aroma of pistachios over time. At the same time, they reduced the firmness of the nuts. The pistachios that were treated by sulfur had a better appearance at harvest time which can be related to the antimicrobial effect of sulfur. Ultimately, the refined micronized sulfur at 30 kg/1000 L concentration had some positive effects on saving the taste and aroma of pistachios over time. However, orchardists should take into account that besides the benefits of controlling pests, sulfur may have adverse effects on product quality.

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Conflicts of interest

All authors agree on the content of the paper and have no conflict of interest to disclose.

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