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Full Research Paper

Effects of some salts on the shelf life of Shahrood Sorkh-e-Fakhri table grapes stored in cold storage

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Abstract

In this study, some preservative solutions were used as an alternative to sulphur fumigation. For this purpose, sodium metabisulfite, calcium chloride, sodium acetate, and sodium carbonate solutions at concentrations of 0.5, 1 and 2 percent were applied. Grapes (Sorkh-e-Fakhri var.) were dipped in different concentrations of each solution for 2 minutes. After dipping, the surface water of grapes was dried by natural air blowing and then, put in conventional baskets and stored at 0.5-1°C at 85-95% RH for 6 months. The percentage of moldiness, soluble solids, reducing sugar, acidity, and pH of the samples were determined after 2, 4, and 6 months of storage. The results of this study showed that the effect of type of preservative used and the storage time, on the percentage of moldiness and pH and acidity of all samples were significant at 1% level. Sodium metabisulfite had the highest inhibitory effect on mold growth. Calcium chloride had the greatest effect on moisture retention. In addition, the highest pH was observed in samples immersed in sodium carbonate solution. In the case of interactions, the effect of type of preservative and storage time on moldiness, moisture content, brix, sugar content, and acidity, and also the type of preservative and its concentration on moldiness and acidity of samples were significant. According to the obtained results, it was shown that the grapes could be stored for 6 months by dipping them in 0.5% sodium metabisulfite for 2 minutes before cold storage.

Keywords: Cold Storage, Preservatives, Shelf Life, SO_2 Replacement, Table Grapes

Introduction

Molding in fruits is due to the growth of microorganisms on their surface as well as physiological damage. Some of these damages can be fully controlled and delayed by proper storage conditions. Among the different methods of storage, the use of cold stores makes the least changes compared to other methods (Jay, 2005; Fatemi, 2007). Various variables, including non-compliance with technical and hygienic issues, can cause adverse changes in the quality and health of stored foods. In Iran, large quantities of fresh grapes are stored in cold stores in late summer and marketed in late fall and winter (Ghodsvali, 2000). Lack of accurate information about the storage conditions of fresh grapes in cold stores can reduce the quality of the product during storage. Fumigation with Sulfur dioxide is often used to disinfect grapes. It inhibits the growth of decay fungi such as *Botrytis*, *Cladosporium* and *Alternaria* (Zomorodi, 2005). For this purpose, the grapes are disinfected in the cold store with sulfur gas several times. Sulfur dioxide gas combines with high moisture content in the cold store and produces sulfuric acid, which is a very strong acid and causes the evaporator tubes to corrode in the cold store. Also, due to high sulfur dioxide gas consumption in most grape cold stores, residual sulfur dioxide levels exceed the permitted level, causing respiratory problems and sensitization in consumers, while also having a nasty odor (Sharayei *et al*., 2004).

Shahrood Fakhri Red Grape has 20% soluble solids, pH of 3.9 and acidity of 0.8 (Sherafatian, 1999). Storage conditions of this grape cultivar in Shahroud are at temperatures usually 0.5 to 1 °C and their relative humidity is 90-94%. Disinfection of the cold store rooms also begins with sulfur before the grape arrives and continues until the end of May of the following year. So that once a week from late September to early January of the same year and from early January to the end of March every two weeks

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and from April to late May every 20 days disinfection is carried out by burning solid sulfur in cold rooms. The amount of sulfur used in the cold rooms is 8.5 kg of solid sulfur for about 400 tons of grapes (Ghodsvali, 2000). Different methods can be used to reduce the adverse effects of sulfur dioxide. These include the use of preservatives, the use of edible coatings, as well as the proper packaging and use of systems that gradually release sulfur gas (Sharayei *et al*., 2004). Chemicals investigated so far include sodium bicarbonate, sodium carbonate, potassium carbonate, calcium chloride, sodium formate, sodium phosphate, ammonium chloride, sodium acetate, potassium chloride, ammonium sulfate, sodium sulfate, ammonium bicarbonate, sodium silicate, sodium metabisulfite and potassium metabisulfite and so on (Nigro *et al*, 2006). Edible coatings include chitosan (Meng et al, 2008), sodium alginate (Miguel *el al*, 2009) and methyl cellulose (Yeol *et al*, 2002). Various salts have been used to control pre-harvest or post-harvest molds in the cold storage. It has been shown that boron in the form of potassium tetraborate prevents growing of the spores and spreading of the *botrytis cinerea* mycelium in grapes kept in the cold store (Qin *et al*, 2010). Youssef and Roberto (2014) used salts such as potassium sulfate, potassium sorbate, potassium bicarbonate, calcium sulfate, and calcium chloride before and after harvesting to control gray mold in grapes and showed that potassium bicarbonate and sorbate potassium were the most effective in controlling gray mold growth. Carvalho et al. (2008) investigated the effect of calcium chloride concentration and storage time on the amount of reducing sugars in grapes. They showed that the 2% concentration of calcium chloride was able to maintain the quality of grapes in the cold store. Doulatibaneh and Zomorodi (2004) studied the effects of calcium chloride spraying on the quality and storage traits (including maintaining firmness, reducing waste and loss of water and shedding berries during storage) of two grape varieties of Rish Baba and Ghezel Uzum. Results showed that spraying 20 days before harvest increased calcium content of the berries. During storage, the amount of brix, pH and percentage of weight loss increased but the acidity decreased. Fungal contamination was the lowest in 1 and 1.5% of calcium chloride. Nigro et al. (2006) examined the effect of 19 different salts on grape mold growth and showed that calcium chloride, potassium carbonate, sodium bicarbonate and sodium carbonate had the most effect.

The use of grapegard sheets containing 10% sodium metabisulfite solution improved the quantitative and qualitative characteristics of grapes (Zomorodi *et al.,* 2005). Ethanol has also been used to prevent mold growth on grapes and it has been shown that the effect of ethanol on mold prevention is even better than that of sulfur (Lurie *et al.,* 2006). Guzev et al. (2008) investigated the effects of sulfur dioxide and ethanol on Aspergillus fungi in grape storage. They showed that the fungus survived in the grapes stored at 20°C for 7 days, but storing at 0°C for one month using SO_2 gas packs reduced the number of fungi completely. Immersion of grape clusters in ethanol solution before cold storage had no effect on *Aspergillus niger* reduction. Spraying 16% ethanol solution containing 1% calcium chloride before harvesting on fresh grapes reduced the development of gray mold. This can reduce the damage caused by decaying clusters from 15% in the control samples to 5% in the samples treated by the above method. In this way, after 6 weeks of storage in the cold store, mold-induced lesions of gray mold were also reduced by 50% compared to untreated control samples (Chervin *et al.,* 2009). Adding 0.5% and 1% potassium sorbate to 10% and 20% ethanol reduced grape mold growth by 10% or less. After 30 days of storage at 1°C, the combination of 20% ethanol with 0.5 or 1% potassium sorbate had the same effect as the effect of SO_2 releasing sheets on reducing mold in grapes (Karabulut *et al*., 2005). By immersing the grape in 30% ethanol solution for 10 seconds at 24 °C, germination of the spores of *Botrytis cinerea* was completely prevented. Soaking the moldy grapes for 3 minutes in 10% ethanol solution at 30, 40, or 50°C reduced the moldiness to 20.7, 6.7 and 1.7

berries/kg after 30 days of storage at 1°C. The treatments used had no significant effect on the appearance of the berries, their cracking, meat browning, taste, weight loss and color of the berries (Karabulut *et al*., 2004).

Gabler et al. (2010) used a combination of chemical and biological fumigation to control post-harvest gray mold decay of grapes. After fumigating the grapes with ozone or SO_2 during the pre-cooling phase, they continuously treated the fruits during storage with the *Muscador albus* fungi, which produces volatiles. The natural appearance of gray mold in grapes kept at 0.5°C for 1 month was 31%, while ozone fumigation reduced it to 9.7% and biofumigation with *Muscador albus* reduced it to 4.4%. The combination of these two methods reduced the rate of gray mold appearance to 3.4%. However, the effect of using this method was less than using SO_2 gas. The use of this gas reduced the appearance of mold to 1.1%.

Pre-harvest chitosan spraying and post-harvest coating with chitosan had a good effect on controlling mold growth on grapes at 0°C and 2°C and significantly prevented weight loss at 20°C (Meng *et al*., 2008). Edible coatings such as methyl cellulose with antimicrobial agents, n-capric acid, isopropyl ester and sodium nitrate were also sprayed to increase the storage life of grapes (Yeol *et al.*, 2002).

In Shahroud/Iran, SO_2 fumigation is conventionally used in all cold stores for storing grapes, which is uncontrolled and usually more than the permitted level. Our innovation is to use GRAS salts to replace fumigation with SO_2. Thus, the aim of this study was to investigate the effect of some of these safe salts for humans that do not have sulfur problems and harmful effects on the shelf life of Shahrood Sorkh-e-Fakhri grapes and to introduce the best method (salt type and concentration) for elimination of sulfur dioxide gas application. Instead of unhealthy grapes with a nasty and harmful odor of sulfur, the grapes are offered to consumers with good quality and health in the fall and winter seasons.

Materials and Methods

According to the study performed by Ghodsvali (2000), if the grapes are harvested at full maturity, their shelf life will be longer. This time for Sorkh-e-Fakhri grapes in the Shahroud area is in late October. Therefore, in this study, first, the grapes of Sorkh-e-Fakhri cultivar were harvested at full maturity at the end of October and transferred to a cool, shady environment to reduce their initial temperature. During this time, the moisture content, average weight of the berries, soluble solids or brix, reducing sugar percentage, acidity and pH of the samples were measured (Table 1) and then 10 to 15 clusters were placed in each plastic basket.

Table 1. Grape characteristics after harvesting

Reducing sugar (%)	Moisture content (%)	Acidity (%)	pH	Brix (%)	Mean weight of berries (g)
15	78.18	0.48	4.29	20.4	5.41

Experimental treatments consisted of four permitted salts (sodium carbonate, calcium chloride, sodium acetate and sodium metabisulfite) in three concentrations (0.5, 1 and 2%) and total in 12 treatments. Each treatment was applied in three replications. The grapes were immersed in each solution for 2 minutes. The samples were then air-dried in the garden at about 30°C for one hour. One sample was immersed in distilled water as a control. The samples were then stored in a refrigerator at 0.5-1°C and 90-95% relative humidity for 6 months. Each month various tests including mold percentage, average weight of berries, soluble solids content, sugar content, acidity and pH of samples were measured and recorded. After leaving the fridge, the samples were kept in the laboratory for one day and then their mold level was assessed. The results were analyzed using a completely randomized factorial design with three replications and the means were compared by Duncan's test.

To measure mold percentage, the number of decayed and moldy berries in each basket was counted and the percentage of moldiness was determined by dividing the number of molded berries by the total number of studied berries (Sherafatian, 1999).

To measure soluble solids, after extraction of grape extract for experiments, two drops of extract were poured onto a manual refractometer (Atago, Tokyo, Japan) and the percent of soluble solids (Brix) was read from the apparatus.

Sugar content was measured by Fehling method and according to Iranian National Standard for Grape juice No. 2685 (ISIRI, 2007). 25 grams of the sample was transferred to a 100 ml graduated balloon containing 2 mL of saturated lead acetate and a small amount of activated carbon. It was made to volume with distilled water and then filtered. 5 mL of Fehling's "A" and 5 mL of Fehling's "B" was added to 250 ml flask. After adding a few drops of methylene blue and about 20 mL of distilled water, the solution was heated to boiling. The filtered solution was then poured into the burette and gently added to the boiling solution to fade blue to give a brick red color. Regarding the volume of solute consumed, the percentage of reducing sugars was calculated from equation 1:

$$n = \frac{F \times 100 \times 100}{V \times 25} \quad (1)$$

Where, n, is the reducing sugar (percent), F is the Fehling factor and V, is the consumed volume of the Fehling solution for titration.

To measure the acidity, 10 g (m) of grape juice was diluted with 50 mL of distilled water and titrated in the presence of phenolphthalein indicator with 0.1N sodium hydroxide until a pale pink appearance (V). Then the acidity percent was calculated based on tartaric acid from the equation 2 (Doulatibaneh and Zomorodi, 2004).

$$Acidity = \frac{V \times 0.075 \times 100}{m} \quad (2)$$

The Metrohm pH meter was used to measure the pH of the juice extracted from the grapes.

To measure the moisture, about 5 grams of each sample was weighed and completely shredded on a plate, then dried at 70°C for 6 hours to reach constant weight. The plate was weighed again and moisture content was obtained by weight difference from equation 3:

$$Moisture\ Content = \frac{X_1 - X_2}{M} \times 100 \quad (3)$$

Where X_2 is the sample weight and the plate after drying, X_1 is the sample weight and the plate before drying and M is the sample weight.

Results and Discussion

According to the analysis of variance (Table 2), it was observed that the effect of type of salt used and storage time on mold percentage, pH and acidity of all samples were significant at 1% level.

Table 2- Analysis of variance of the effect of different treatments on the studied characteristics

Source Deviation	Mean Squares						
	Acidity	Reducing sugar	Brix	pH	Moisture content	Sample weight	Moldiness
type of salt(A)	0.015**	0.929	0.973	0.028**	0.599*	0.37	45.90**
Salt concentration (B)	0.006	0.639	7.746	0.014	0.171	0.001	0.28
A× B	0.009**	0.189	2.99	0.006	0.284	0.234	0.827**
storage time (C)	0.024**	16.453**	9.156	0.077**	0.049	0.969	43.21**
A× C	0.007*	1.269*	8.5*	0.006	0.405*	0.448	2.923**
B× C	0.006	0.434	3.39	0.002	0.237	0.127	0.432
A× B ×C	0.005*	0.227	3.8	0.008	0.36*	0.189	0.193
Error	0.003	0.519	4.04	0.005	0.185	0.329	0.247

** Significant at 1% level, * significant at 5% level

Interaction effects of salt type and storage time on moldiness, moisture content, brix, sugar content and acidity, as well as salt type and concentration on moldiness and acidity of samples were also significant. The significant effect of storage time on the sugar content of the samples is due to the change in the moisture content of the samples during storage, thereby increasing the sugar concentration. As can be seen from Table 2, the effect of the concentrations used in this study on any of the examined traits was not significant. of course, if higher concentrations were used, this effect could be significant, but at concentrations higher than 2%, the salts used would precipitate on the berries and make their appearance undesirable.

As seen in Figure 1, sodium metabisulfite had the highest inhibitory effect on mold growth and had the lowest molding on samples treated with this salt due to the presence of sulfur in it. The storage time had a significant effect on the molding rate as well, so that the molding after six months was significantly higher than the four months storage (Fig. 1).

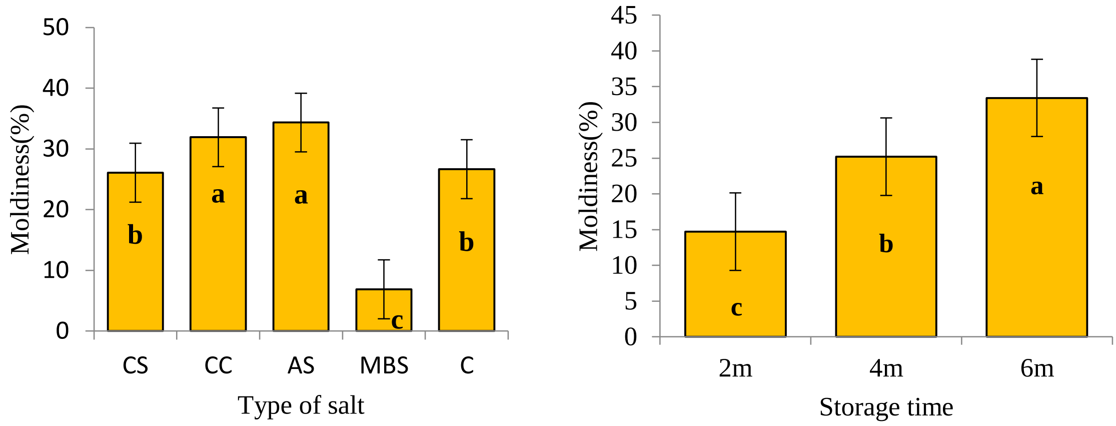

Fig 1. The effect of salt type (left) and storage time (right) on the moldiness of the grape samples; CS=sodium carbonate; CC= calcium chloride; AS= sodium acetate; MBS=sodium metabisulfite; C=control; m=month

The interaction between the type of salt and its concentration as shown in Fig. 2 was the lowest for the different concentrations of sodium metabisulfite and the highest for the different concentrations of sodium acetate and 2% calcium chloride. It can be seen from Fig. 2 that the inhibitory effect of sodium metabisulfite is even greater after six months than the effect of other substances

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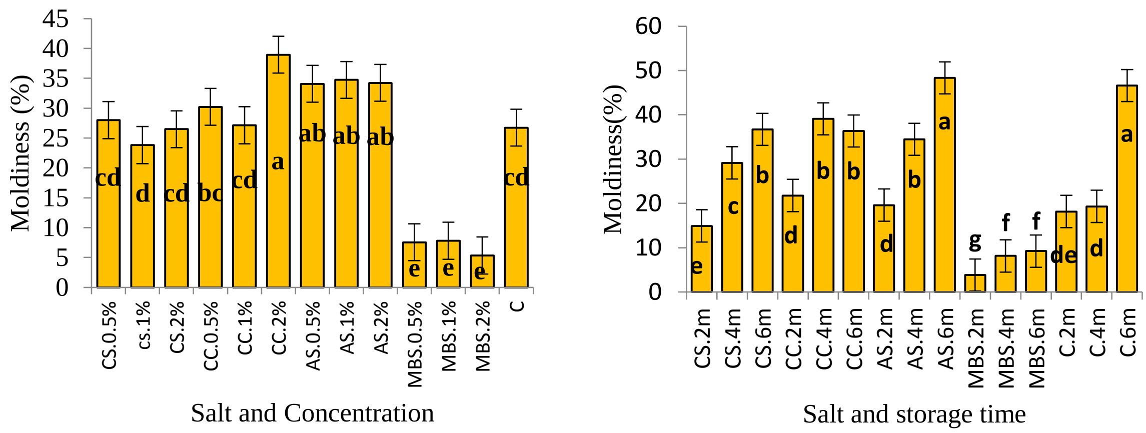

Fig 2. The mutual effect of the salt type and concentration (left) and salt type and storage time (right) on the moldiness of the grape samples; CS=sodium carbonate; CC= calcium chloride; AS= sodium acetate; MBS=sodium metabisulfite; C=control, m=month

Regarding the effect of salt on moisture content of the samples, it was observed that all the materials used had better preservation of the moisture content of the samples compared to the control samples, which calcium chloride had the greatest effect in this regard. The interaction between salt type and storage time also showed that calcium chloride retained more moisture than other materials (Figure 3).

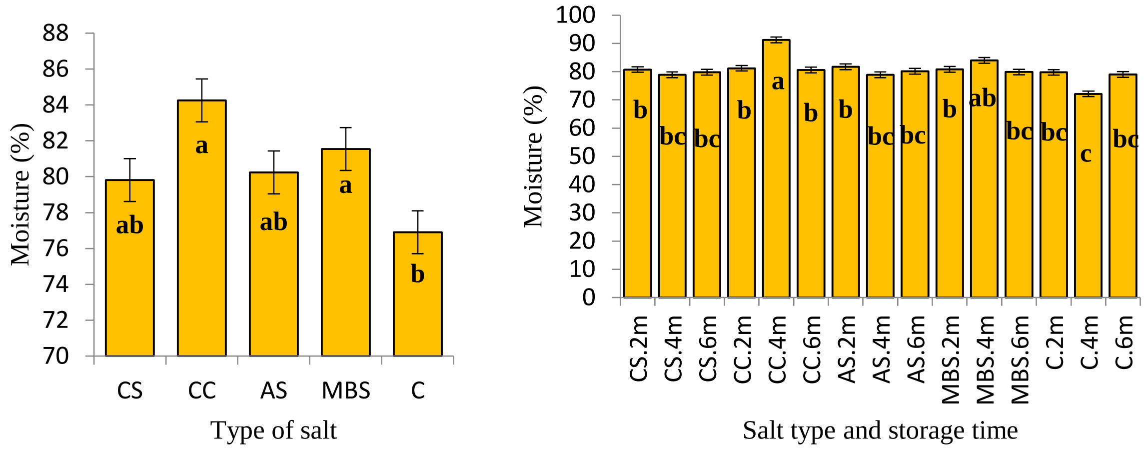

Fig 3. The effect of the salt type (left) and the mutual effect of salt type and storage time (right) on the moisture content of the grape samples; CS=sodium carbonate; CC= calcium chloride; AS= sodium acetate; MBS=sodium metabisulfite; C=control; m=month

According to Fig. 4, the highest pH was for samples immersed in sodium carbonate solution, because sodium carbonate was more alkaline than the other salts used, and even the pH of the samples treated was higher than that of the control sample. There was no significant difference between the other treatments.

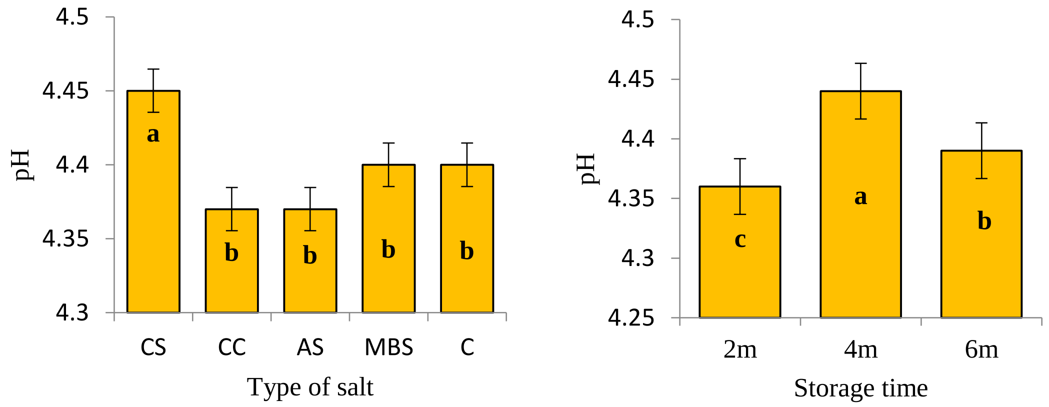

Fig 4. The effect of salt type (left) and storage time (right) on the pH of the grape samples; CS=sodium carbonate; CC= calcium chloride; AS= sodium acetate; MBS=sodium metabisulfite; C=control, m=month

The aim of the current study was to select effective salts to increase the shelf life of Shahrood Sorkh-e-Fakhri grapes. The different salts used had different effects on the mold content of the samples and other shelf-life characteristics. All salts used were effective at different concentrations. Sodium acetate did not have any effect on the reduction of moldiness and even increased moldiness in comparison with the control. This effect is probably due to the fact that these salts provide more nutrients to the mold or provide better environmental conditions for growth (Nigro *et al.*, 2006). Other salts at all concentrations reduced the amount of moldiness that was most affected by sodium metabisulfite and then sodium carbonate. Nigro et al. (2006) showed that the use of salts such as sodium acetate and sodium phosphate increased the growth of *Botrytis cinerea* on small grape clusters. In the case of calcium carbonate, they also showed that using this compound in vitro and in the culture medium did not prevent the growth of *Botrytis cinerea*, but if used for fresh grapes, it inhibits mold growth. This is due to specific biochemical interactions between salt and grape tissue. Sharayei et al. (2004) also mentioned the inhibitory effect of metabisulfite in their research. Chervin et al. (2009) also prevented the development of gray mold rot on grapes during 6 weeks of storage by using ethanol solution containing calcium chloride. Nigro et al. (2006) also showed that the inhibitory effect of calcium chloride was less than other salts such as sodium carbonate and sodium bicarbonate. They obtained the minimum inhibitory concentrations of salts against *Botrytis cinerea* as greater than 2% for sodium acetate and calcium chloride, 0.25% for sodium carbonate and 0.5% for sodium sulfate.

In the current study, it was found that the salts used had no effect on the pH of the samples except sodium carbonate. Sodium carbonate increases the pH due to the alkaline conditions it creates, which may be a reason for the lower salt content to prevent molding. Increasing the storage time from 2 months to 4 months increased the pH value initially, probably because of the increased salt concentration led to the decrease in moisture content. However, by increasing the storage time from 4 months to 6 months the pH value decreased due to the increased contamination of grapes with molds and the effect of molds on the decomposition of alkaline salts. Nigro et al. (2006) showed that pH alone cannot explain the inhibitory effect of salts. Ca^{2+} ions can easily penetrate the fruit epidermis (Schonher, 2000). The mechanism by which calcium increases plant tissue resistance to mold has not been established yet. Most of the calcium that penetrates into the plant tissue accumulates in the middle layer of the cell wall and thereby forms ionic bridges between and inside the pectic polysaccharides that harden the cell wall. The formation of calcium junctions between pectic polysaccharides makes the cell wall more resistant to the hydrolytic enzymes produced by molds (Tobias *et al*., 1993). Various researchers have shown that carbonates and bicarbonates are effective in preventing spore growth, elongation of mold hyphae, and producing pectin-degrading enzymes in many molds (Hervieux *et al.*, 2002; Mills *et al*., 2004; Smilanick *et al.*, 2005). In the current study, it was observed that the storage time was significant on the examined traits, including the percent of grape moldiness. Sharayei et al. (2004) also showed in their research on Kolahdari and Kaj Angor grape cultivars that the effect of the storage time of the grapes treated with sodium metabisulfite was significant on all quantitative and qualitative traits except grape acidity. They also showed that different concentrations of sodium metabisulfite were effective in decaying grapes and recommended 10% sodium metabisulfite concentration for storage of grapes in cold stores. In the current study, it was found that the highest moisture content was in the samples treated with sodium metabisulfite and calcium chloride. Calcium chloride inhibits moisture excretion by affecting fruit texture and hardening it (Schonher, 2000) and sulfur dioxide gas emitted by sodium metabisulfite prevents respiratory and transpiration reactions and prevents loss of product moisture (Sharayei *et al*., 2004). Pretel et al (2006) also showed that the rate of weight loss and moisture loss in grapes treated with gradual release of SO_2 was lower than that control.

Conclusion

According to the results of the experiments and the fact that the most important factor in this study was the number of moldy grapes during storage, to store the grapes in the cold store, it is recommended to immerse them in a 0.5% sodium metabisulfite solution for about 2 minutes before transferring them into the cold store rooms. This allows the grapes to be easily stored for up to 6 months. To replace sulfur dioxide, other researches recommending to consider the possibility of using ozone generators in grape cold stores. In addition, it is recommended to consider the use of other harmless chemicals and in different concentrations or combinations of two or more, to investigate the possibility of spraying grape clusters with harmless antifungal chemicals such as calcium chloride, potassium carbonate, potassium bicarbonate, etc. before harvesting and its effect on increasing the shelf life of grapes.

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اثر برخی از نمک‌ها در افزایش زمان نگهداری انگور سرخ فخری شاهرود در سردخانه

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چکیده

به‌منظور افزایش زمان ماندگاری انگور سرخ فخری و جلوگیری از کپک‌زدگی آن در سردخانه، خوشه‌های انگور در محلول‌های نگهدارنده غوطه‌ور شدند. برای این منظور محلول‌های متابی‌سولفیت سدیم، کلرید کلسیم، استات سدیم و کربنات سدیم با غلظت‌های مختلف ۰/۵، ۱ و ۲ درصد تهیه شد و انگورها پس از قرار گرفتن در سبدهای مناسب، در شرایط دمای حدود ۰/۵ تا ۱ درجه‌ی سانتی‌گراد و رطوبت نسبی ۸۵ تا ۹۵ درصد در سردخانه به مدت ۶ ماه نگهداری شدند. هر دو ماه یک‌بار درصد کپک‌زدگی، مواد جامد محلول، درصد قند، اسیدیته و pH نمونه‌ها بررسی شد. نتایج این پژوهش نشان داد که اثر نوع ماده استفاده شده و زمان نگهداری بر درصد پوسیدگی و مقدار pH و اسیدیته‌ی تمام نمونه‌ها در سطح ۱٪ معنی‌دار می‌باشد. متابی‌سولفیت سدیم بیشترین اثر بازدارندگی را بر کپک‌ها داشت. همچنین، از میان نمک‌های مورد استفاده کلرید کلسیم بیشترین اثر را در حفظ رطوبت نشان داد. بیشترین مقدار pH نیز در نمونه‌هایی به‌دست آمد که در محلول کربنات سدیم غوطه‌ور شده بودند. در مورد اثرات متقابل نیز اثر نوع ماده و زمان نگهداری بر میزان پوسیدگی، مقداررطوبت، بریکس، درصد قند و اسیدیته و همچنین نوع ماده و غلظت آن بر میزان پوسیدگی و اسیدیته‌ی نمونه‌ها معنی‌دار می‌باشد. با توجه به نتایج و داده‌های حاصل از تجزیه و تحلیل آماری شرایط مناسب برای نگهداری انگور در سردخانه، غوطه‌وری آن‌ها به مدت حدود ۲ دقیقه در محلول ۰/۵ درصد متابی‌سولفیت سدیم قبل از سردخانه‌گذاری می‌باشد. با این کار می‌توان انگور را به راحتی تا ۶ ماه نگهداری کرد.

واژه‌های کلیدی: انگور سرخ فخری، جایگزین گوگرد، سردخانه، نگهدارنده‌ها، زمان ماندگاری

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