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RESEARCH PAPER

Vacuum Versus Open Air Storage for Pepper (*Capsicum annuum* L.) Seed Longevity with Low Temperature and Seed Moisture Content Over 48 Months

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Abstract

This study was carried out to test the effect of vacuum and open-air storage on seed germination, oil content, and sugar contents of four pepper cultivars. Seeds were stored at 13°C with 35% relative humidity over 48 months in vacuum packets or in perforated cheese cloth in a storage room. Seed samples were examined at 12, 24, 36 and 48 months. Seed germination, oil content and sugar contents were determined. Seed germination declined gradually as storage time extended. Vacuum storage had significantly higher ($P<0.05$) germination than oxygen storage after 48 months of storage for all cultivars. Differences between the two storage methods were not significant for the other samples, except Yaglik in which vacuum storage had higher values from 24 months onwards during storage. Total oil content declined in all cultivars but the decline was faster in seeds stored in the open air. A similar trend was also observed for sugar contents. Seeds stored in the presence of oxygen lost sugar content faster than vacuum-stored seeds. Results indicated that storage with vacuum conditions (no oxygen) extended the longevity of pepper seeds.

1. Introduction

The principal post-harvest environmental factors influencing seed deterioration are temperature, seed moisture content and oxygen pressure (Roberts and Abdalla, 1968; Roberts, 1972; Krishnan et al., 2004). The relationship between seed longevity temperature and seed moisture were well documented and quantified over a wide range of species (Ellis and Roberts, 1980; Walters et al., 2005; Ventura et al., 2008; Kochanek et al., 2010; Sano et al., 2017; Kim, 2018). However, research about the oxygen effect on seed longevity is relatively limited. There was a tendency in seed storage to ignore the role of oxygen and its effect on longevity is considered to be modest in air-dry storage. However, more recent reports indicated that oxygen reduced seed longevity in various crop seeds (Ellis and Hong, 2007; Barzali et al., 2005; Schwember and Bradford, 2011; Gonzales-Benito et al., 2011; Groot et al., 2015). Ellis and Hong (2007) concluded that oxygen is relatively more deleterious to timothy and sesame seeds at lower rather than higher moisture contents. Groot et al. (2015) also confirmed that longevity of lettuce and celery seeds was extended by anoxia in dry storage. Both studies suggested vacuum storage (anoxic) to extend longevity.

Seed storage conditions for commercial purposes by the seed company use air relative humidity of about 35-50% and temperature of about 13-17°C. In these conditions, seeds of each species equilibrate at different seed moistures; for example, pepper seeds equilibrate to about 7.1-7.3% seed moisture. Open seed storage is common in many seed technology practices in which seeds equilibrate to ambient relative humidity and oxygen is freely available at atmospheric concentrations. This is the case when large amounts of seeds are stored before packaging in air tight packets for sale. For seed germplasm conservation, Groot et al. (2015) suggested that seeds should be stored under dry, cool and low oxygen concentration conditions after harvesting. The objective of this investigation was to determine whether or not seed germination differed between vacuum-sealed containers (hermetic storage/vacuum storage) and open-air seed storage at 35% relative humidity and temperature of 13°C over 48 months.

2. Materials and Methods

2.1. Seed material and storage conditions

Seeds of the pepper (*Capsicum annuum* L.) cultivars Surmeli, K. Dolma, Yaglik and Corbaci were selected for investigation, representing comparatively different fruit shapes and growing habits (Table 1). Seeds were initially equilibrated in a storage room with 35% relative humidity at 13°C over a week. Seeds were weighed daily until no more seed weight changes occurred and seed moisture was determined (wet basis) 103±2°C (ISTA, 2016) and changed between 7.1-7.3% seed moisture. Then, seeds were packed hermetically (vacuumed) or placed in perforated cheese cloth. Eight samples (four vacuum, four open-air) were prepared for each cultivar. Each packet contained 6 g of seeds, except the final sample which only had 2 g of seeds. Seed weight for each sample was measured before storage. Then, 32 samples (4 cultivars × 2 treatments × 4 sampling times) were placed in storage. Seed moisture contents were determined gravimetrically for each sample when taken from storage for germination.

2.2. Germination tests

Seeds were withdrawn from storage at 12, 24, 36 and 48 months. They were then tested for ability to germinate on top of two layers of filter paper (Whatman 42) moistened with 5 ml distilled water in 90 mm Petri dishes at 25°C for 14 days (ISTA, 2016). Radicle emergence was counted after 4, 7 and 14 days during the test. Seedlings were evaluated according to the criterion of normal germination (ISTA, 2016).

2.3. Reduced sugar determination

After adding 5 mL of 15% potassium ferrocyanide and 5 mL of 30% zinc sulphate solution to 2 g powdered pepper seed samples, the mixture was completed to 100 mL with distilled water. The mixture was filtered on filter paper. Then 0.5 mL of the filtrate was taken and 1.5 mL of distilled water and 6 mL of dinitrophenol solution were added. After these processes, the samples were kept in a water bath at 100°C for 6 minutes and then cooled in running water for 3 minutes. The absorbance values of the samples were measured at 600 nm wavelength in a Hitachi brand spectrophotometer. As control in the method, 2 mL of distilled water and 6 ml of dinitrophenol solution were used (Ross, 1959).

2.4. Total oil determination

Dried 2 g pepper seed samples were placed in the extraction cartridge after grinding. The mouth of the cartridge was closed with cotton, preventing the samples from falling out of the cartridge. Cartridges and flasks were placed in the Soxhlet device and extracted in the heating unit (55°C) for 6 hours continuously. After the solvent was completely removed, the balloons were weighed to calculate the percentage of total oil (Cemeroglu, 2010).

2.5. Statistical analysis

The tests were established in accordance with the experimental randomized design. Means for vacuum and open-storage samples in each sampling period were compared using the t test at 0.05% significance. JMP 8.0 statistical package program was used for the analyses.

3. Results and Discussion

Initial normal seed germination of all four cultivars was above 91%. Equilibrated seed moisture content in the storage room resulted in seed moisture content between 7.1 and 7.6%. Mean germination time and seed weight were very similar between cultivars (Table 1). Seed germination percentages gradually declined with storage time in both storage methods. The difference between open-storage and vacuum seeds were not significant ($P>0.05$) until 36 months of storage for K.

Table 1. Changes in germination (%), equilibrated seed moisture content (%), fruit type, mean germination time (MGT, day) and one thousand seed weight (g) of four pepper cultivars.

Cultivar	Germination (%)		Equilibrated seed moisture content(%)	Fruit type	MGT (day)	1000 seed weight (g)
	Total	Normal				
Surmeli	93	91	7.1	Long-green	5.28	6.41
K.Dolma	92	90	7,2	Bell-shaped	6.05	6.56
Yaglik	95	94	7.3	Capia-red	5.70	6.73
Corbaci	95	93	7.3	Long-green	5.15	6.78

Dolma, Surmeli and Corbaci, but was significant ($P<0.05$) in the final sampling (48 months). Differences in normal germination percentages between open-storage and vacuum storage were significant ($P<0.05$) at 24, 36 and 48 months of sampling for the Yaglik cultivar (Figure 1). Final seed germination at the last sampling were 75, 79, 82 and 87 for vacuum storage in the K. Dolma, Surmeli, Yaglik and Corbaci cultivars, respectively, while seeds stored in openly had 67, 69, 68 and 84% germination for these cultivars, respectively. Corbaci cultivar appeared to have the highest germination at the end of the sampling among the four cultivars. Differences between open storage and vacuum storage were greatest at 14% for Yaglik cultivar in the final sampling. This difference was 8, 10 and 3% in K. Dolma, Surmeli and Corbaci cultivars, respectively.

Radicle emergence percentages had differences between vacuum and open-storage methods that were not distinctive, but the difference was more pronounced at the final sampling (Table 2). Corbaci had the highest radicle emergence percentages. The other three cultivars were inferior to this.

Oil contents of pepper cultivars changed between 13.6 and 19.5%, with the lowest for Surmeli and the highest for Yaglik. Total oil content declined gradually with storage time and was lower for open storage than vacuum ones. This was the case for all four cultivars and samples until 36 months. Seeds stored in the open-air lost more than half of their oil content by 36 months of storage. This change was from 13.6 to 6.1%, 17.2 to 8.0%, 19.5 to 8.1% and 17.7 to 6.8% for Surmeli, K. Dolma, Yaglik and Corbaci, respectively. When seeds were

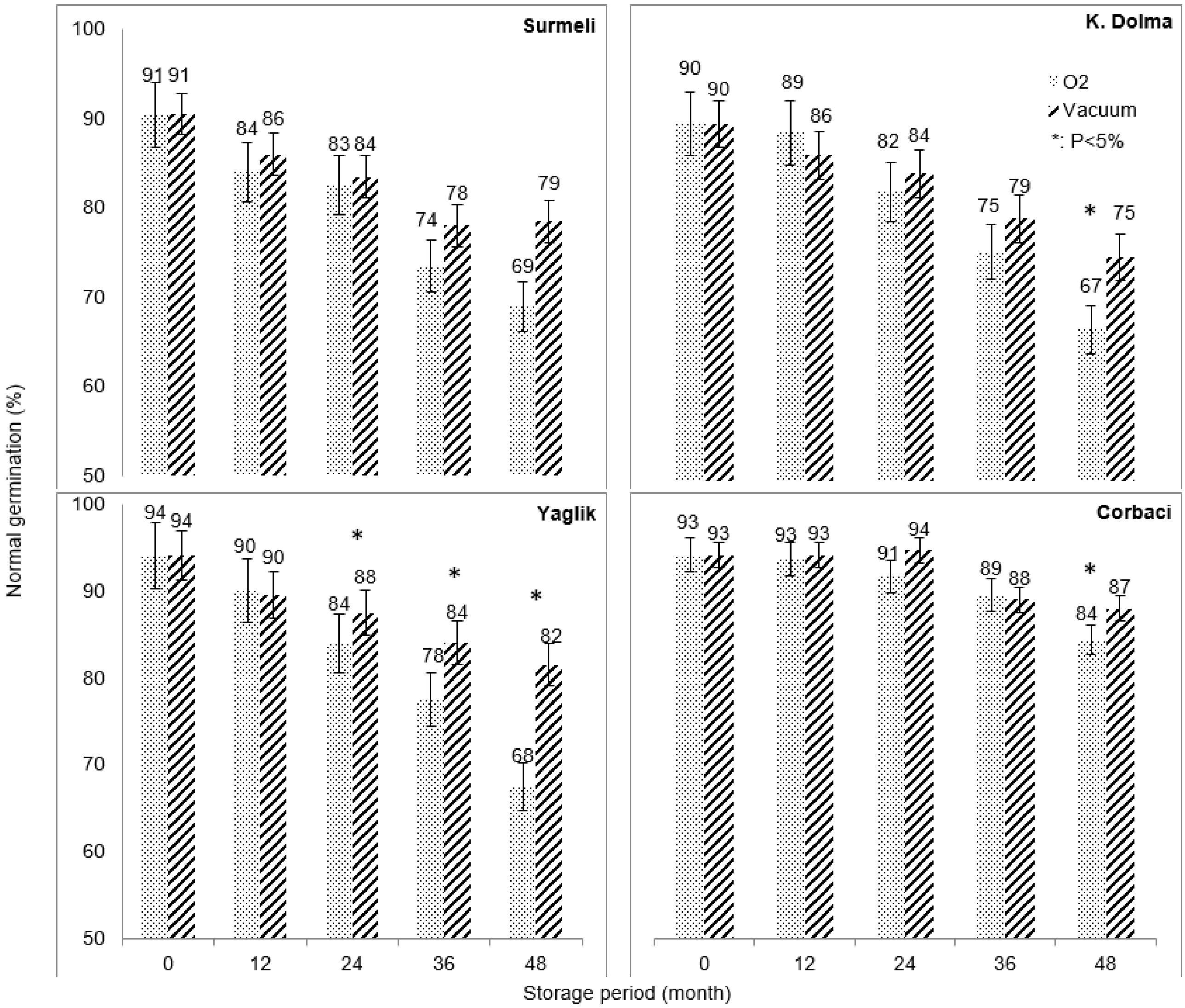

Figure 1. Changes in seed germination of pepper cultivars stored in vacuum and open storage (O_2) conditions during 48 months.

(Bars indicate standard error of mean. Asterisk indicates significant difference at 5% level in each sampling and cultivar).

Table 2. Radicle germination percentages of four cultivars on the fourth day of the germination test.

Cultivar	Storage type	Storage months				
		0	12	24	36	48
Surmeli	Vacuum	62±8.1	63±5.0	57±6.2	58±5.1	58±3.7
	Open-air	62±8.1	53±3.0	53±12.1	58±9.7	41±3.0
K. Dolma	Vacuum	18±0.9	13±1.7	20±2.8	21±5.3	20±3.8
	Open-air	18±0.9	20±3.2	13±4.1	21±7.3	14±4.9
Yaglik	Vacuum	55±6.1	70±3.2	59±.4.0	53±13.9	61±3.3
	Open-air	55±6.1	60±9.2	51±7.3	45±7.8	27±3.3
Corbaci	Vacuum	80±5.4	67±6.2	77±10.7	80±6.5	89±3.0
	Open-air	80±5.4	72±7.5	80±6.2	87±5.0	86±3.0

Table 3. Changes in total seed oil content ±se (%) of four pepper cultivars during 36 months of vacuum or open-storage conditions.

Cultivar	Storage type	Storage months			
		0	12	24	36
Surmeli	Vacuum	13.6±1.3	11.6±2.4	9.8±1.6	8.4±3.4
	Open-air	13.6±1.3	9.5±3.1	7.2±1.7	6.1±2.1
K. Dolma	Vacuum	17.2±2.1	14.4±3.8	12.1±2.6	9.9±1.8
	Open-air	17.2±2.1	12.6±2.4	10.5±1.9	8.0±1.0
Yaglik	Vacuum	19.5±2.0	15.9±2.4	13.1±2.8	10.7±1.7
	Open-air	19.5±2.0	13.2±2.0	9.8±3.2	8.1±2.4
Corbaci	Vacuum	17.7±1.5	13.5±1.8	11.9±1.9	9.5±1.7
	Open-air	17.7±1.5	11.9±3.0	9.1±1.5	6.8±2.1

Table 4. Changes in total sugar content±se (%) of four pepper cultivars during 36 months of vacuum or open-storage conditions

Cultivar	Storage type	Storage months			
		0	12	24	36
Surmeli	Vacuum	7.2±0.8	6.1±0.9	4.3±0.6	3.4±1.0
	Open-air	7.2±0.8	6.4±0.6	2.9±0.3	1.6±0.4
K. Dolma	Vacuum	6.8±1.4	5.1±1.0	3.8±0.8	2.5±0.7
	Open-air	6.8±1.4	5.2±0.4	2.9±.0.4	1.9±0.2
Yaglik	Vacuum	4.6±1.0	3.1±0.8	2.8±0.7	1.4±0.3
	Open-air	4.6±1.0	4.8±1.0	1.7±0.3	0.9±.0.1
Corbaci	Vacuum	5.7±0.8	5.4±1.1	2.9±0.9	2.7±0.9
	Open-air	5.7±0.8	3.0±0.6	1.6±0.2	0.8±0.1

vacuum stored the final oil percentages were 8.4, 9.9, 10.7 and 9.5%, respectively (Table 3).

Sugar content of the cultivars changed between 4.6 and 7.2%, with the lowest for Yağlik and the highest for Sürmeli. Total sugar content reduced with the storage time and reductions were faster in open storage than vacuum storage. By the final sampling, sugar content was 3.4, 2.5, 1.4 and 2.7% for Surmeli, K. Dolma, Yaglik and Corbaci, respectively. When seeds were stored in the open air, these values were 1.6, 1.9, 0.9 and 0.8%, respectively (Table 4).

Results of the present work indicated that vacuum storage was beneficial to pepper seed longevity. Openly stored seeds lose germination earlier than those with vacuum storage. The effect was more prominent in extended samplings, particularly at 48 months of storage. The difference between the two storage methods was significant at the final sampling (48 months) for three cultivars but was significant at 24, 36 and 48 months for the Yaglik cultivar. Our results about the advantages of vacuum storage (oxygen is not available) were reported earlier for various crop seeds (Barzali et al., 2005; Ellis and Hong, 2007; Demir et al., 2009; Schwember and Bradford, 2011; Gonzales-Benito et al., 2011; Soh et al., 2014; Groot et al., 2015; Han et al., 2021) compared to open-air storage ones. The effect of vacuum storage was more prominent at low seed moisture contents than higher one (Ellis and Hong, 2007). Low and high seed moisture contents were not compared in this work. We aimed to obtain results for seed companies. Seeds were equilibrated at 35% relative humidity in both vacuum and open-air conditions where seed moisture ranged between 7.1 and 7.3% (Table 1). The equilibration of pepper seed moisture at this relative humidity was in agreement with Shivhare and Singh (2000). Seed storage temperature was 13°C. In these conditions, medium term storage i.e. 18-36 months is applied at commercial seed production scales. This storage environment is suitable for keeping seed quality for seeds to be sold in the following season. Our results showed that there was an insignificant difference between vacuum and open-storage conditions until 48 months in three out of four cultivars. However, vacuum storage was favourable compared to open-air in the final samples (Figure 1). This shows that even storage conditions which have favourable presence of oxygen may induce aging during prolonged storage periods.

Differences among the species and cultivars were reported in earlier studies (Ozcoban and Demir, 2002; Basay et al., 2006; Nagel et al., 2009; Panayotov and Aladjadjiyan, 2014; Demir et al., 2020; Yildirim et al., 2020). In our work, Yaglik was the most sensitive to longevity among all cultivars. There may be various reasons for this. One may be the higher oil content of the seeds. Yaglik had the highest oil content which may trigger ageing through lipid peroxidation (Copeland and McDonalds, 1995). Corbaci was the more resilient cultivar. The response of cultivars was clearly seen since the initial seed quality, i.e. normal germination and means germination time of the cultivars, were very similar. Thus, we may assume that pre-storage factors were not influential since all had the same quality at the beginning. Variations in response to oxygen among the cultivars may also relate to the seed coat structures, such as the hard and impermeable coat and presence of diffusion barriers. Cuticles are considered to be the main barrier to oxygen diffusion and permeability increases markedly at temperatures above 35°C (Riederer, 2006). The effect of any differences among the cultivar differences in cuticle composition and permeability in low temperature storage in our study may be more influential.

Various metabolic and structural changes occur during ageing. Lipid peroxidation is a crucial element related in longevity control (Xu et al., 2015). During seed storage, respiration processes utilize the energy kept in the seed, so that a seed which has experienced extended storage usually fails to germinate due to insufficient supply of essential soluble sugars (Eastmond, 2006; Zhou et al., 2019). One of the most commonly observed aberrations reported from aged seeds is disruption of lipid bodies. Owing to the low density of water activity, enzyme activity is not or hardly possible in seeds dried under 40% of relative humidity (Labuza, 1970). As a result, oxygen utilization by aerobic respiration is absent or very low as well. For this reason, oxygen consumption by dry seeds is more likely associated with the creation of superoxide or other ROS molecules and later oxidation of macromolecules, for instance, lipids, phospholipids and DNA. This was noted in various species (Harman and Granett, 1972; Smith, 1980; Salisbury and Roos, 1985). According to Li et al. (2005), the basic mechanism for aging of pepper seeds is related with elevated peroxidation of lipid membranes. When the time of seed storage extends, high amount of peroxidation and oxidation of the lipids in the seed cause a diminished concentration of unsaturated fatty acids and soluble sugars that are created from triacylglycerol (TAG) (Bhattacharya et al., 2015; Zhou et al., 2019). Accordingly in our study, pepper oil content decreased during storage but this was faster in open-air storage than vacuum storage. A similar trend was also seen for sugar content during ageing. Metabolic destruction of sugars was also observed during ageing. The presence of oxygen accelerates the decomposition of both oil and sugar content in pepper seeds during storage. Decreases in oil and sugar content occurred as seed germination was reduced by the extended storage. This cannot be due to the activity of hydrolysing enzymes since seed moisture is low. However, respiration, lipid peroxidation and Maillard reactions are likely to have a combined effect of decomposition of stored materials (Colville and Pritchard, 2019).

4. Conclusion

This study indicated that seed germination after open-storage was inferior to vacuum storage in pepper seeds at low 7.1-7.3% seed moisture and 13°C. The decline in germination was associated with reduced amounts of seed oil and total sugar content. While openly stored seeds have lower oil and sugar content than vacuum ones. Vacuum storage is a preferable practice to achieve maximum germination even in storage with low seed moisture and temperature conditions.

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