

Original Research Article

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Enzyme Activity Influenced by Modified Atmospheric Packaging on Onion Seeds (*Allium cepa* L.) cv. Arka Kalyan

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ABSTRACT

The experiment was carried out to understand the storability of onion seeds by subjecting to different modified atmospheric storage conditions with different combination of gases like carbon dioxide, oxygen and nitrogen at different concentrations. The experiment was consisted of seed storage of onion (*Arka Kalyan*) under modified atmospheric storage conditions. There were 15 treatments and the experiment was carried out in Completely Randomized Block Design in four replications. The treatments were T₁- 80 % CO₂ : 05 % O₂ : 15 % N₂, T₂- 70 % CO₂ : 05 % O₂ : 25 % N₂, T₃- 60 % CO₂ : 05 % O₂ : 35 % N₂, T₄- 50 % CO₂ : 05 % O₂ : 45 % N₂, T₅- 40 % CO₂ : 05 % O₂ : 55 % N₂, T₆- 80 % CO₂ : 10 % O₂ : 10 % N₂, T₇- 70 % CO₂ : 10 % O₂ : 20 % N₂, T₈-60 % CO₂ : 10 % O₂ : 30 % N₂, T₉-50 % CO₂ : 10 % O₂ : 40 % N₂, T₁₀-40 % CO₂ : 10 % O₂ : 50 % N₂, T₁₁-50 % CO₂ : 00 % O₂ : 50 % N₂, T₁₂-Atmospheric air, T₁₃-Vacuum, T₁₄-Storage in cloth bag, T₁₅-Cold storage and observations on various enzyme activity were recorded. With an advancement of storage period, various enzyme activities differed significantly due to modified atmospheric storage conditions in all the 12 months of storage irrespective of the modified atmospheric conditions. The seeds stored in Cold storage (T₁₅) recorded maximum dehydrogenase activity of (0.280), amylase enzyme activity of (1.28), catalase enzyme activity of (0.246), superoxide dismutase enzyme activity of (0.900) after twelve months of storage period followed by T₁: 80 % CO₂ : 05 % O₂ : 15 % N₂ and seeds stored in vacuum (T₁₃). The lowest dehydrogenase activity of (0.190), was noticed in seed stored in cloth bag (T₁₄) followed by the second the lowest dehydrogenase activity recorded (0.220), α-amylase enzyme activity of (1.80), catalase enzyme activity of (0.156), superoxide dismutase enzyme activity recorded (0.730) in see ds stored in polythene bag with air (T₁₂) after twelve months of storage.

Keywords

Cold storage,
Enzyme, Onion,
Seed vigor, Vacuum

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Introduction

Onion (*Allium cepa* L.) is a member of family Amaryllidaceae. It is one of the major bulb crops of the world and important commercial

vegetable grown all over the world and occupies a premier position amongst the vegetables due to its high preference in food, remunerative price and regular demand in the market. India ranks first in total area under

onion cultivation (12.25 lakh hectares) with second largest producer (209.91 lakh million tonnes) in the world next to China, but the productivity of onion in India is very low *i.e.*, 17.13 tonnes per ha as compared to China and other countries like, Egypt, Netherland and Iran *etc.*,. Hence, there is a need to enhance the productivity and production. Most of the onion produced in India comes from Maharashtra (28.62 %) state followed by Karnataka (15.94 %), Madhya Pradesh, Bihar, Gujarat Andhra Pradesh and Uttar Pradesh. Karnataka alone occupies around 195.29 thousand ha area with 2,767.98 thousand million tonnes of production and the average productivity is 16.13 tonnes per ha which is again very low compared to national productivity average.

Onion is the only vegetable in which India figures predominantly in the world for production and export. The average productivity of onion is quite low (12.82 t/ha) in India. Among the vegetables, onion seeds are classified as very poor storer, because of low availability of quality seed for planting is a major problem faced by the farmers. After the seeds are harvested, controlling seed deterioration becomes more difficult because, the seeds are much more sensitive to conditions and environments that cause loss of quality.

Modified atmosphere storage of seeds is a suitable alternative to the use of chemical fumigants and contact insecticides that are known to leave carcinogenic residues in the treated products (Bailey and Banks, 1980). Disinfestations of stored seeds using modified atmospheric storage (MA) involves the alteration of the natural storage gases such as carbon dioxide (CO₂), oxygen (O₂) and nitrogen (N₂), to render the atmosphere in the stores lethal to pests. The MA includes neither alteration of the storage atmosphere by addition of toxic gases such as phosphate methyl bromide or regulation or alteration of

the atmospheric water content. The MA may be achieved in several ways: by adding gaseous or solid CO₂, by adding a gas of low O₂ content (*e.g.*, pure N₂ or output from a hydrocarbon burner) or by allowing metabolic processes within an airtight storage to remove O₂, usually with associated release of CO₂. Such atmospheres are referred to as 'high-CO₂', 'low-O₂' and 'hermetic storage' atmospheres, respectively. They are collectively known as 'modified atmospheres' (Banks and Fields, 1995). The effectiveness of modified atmosphere for controlling various stored product pests depends on the temperature and moisture content of the seeds, species and life storage of pests, gaseous composition and uniformity of gas distribution and exposure time of the MA treatment (Lukasiewicz *et al.*, 1999). If the seeds are not sold in time, then it has to be carried to the next season which definitely affects the seed viability.

Materials and Methods

The laboratory experiments were conducted in the laboratory of Seed unit, University of Agricultural Sciences, Dharwad during 2016-2017 and the packaging of the onion seeds were carried out in the Department of Processing and Food Engineering, College of Agricultural Engineering, UAS, Raichur using the Modified Atmosphere Packaging (MAP) Unit and for cold storage seeds of particular treatment was kept in cold storage unit, department of Environmental Science, University of Agricultural Sciences, Dharwad, Karnataka

Experimental details

The storage experiment consisted of totally 15 treatment combinations and details of the treatments are furnished below, out of them 12 treatments were comprised of seed storage under gaseous combinations of carbon dioxide, oxygen and nitrogen and 1 treatment

in cold storage. Rest of two treatments were used to compare gaseous combinations with seeds stored cloth bag and atmospheric air in polythene bag.

Method of modified atmosphere packaging

Polythene bags of 700 gauge measuring 20 cm (length) and 14 cm (breadth) were used for packing purpose. In these bags, 200 grams of onion seeds were packed along with the gases like carbon dioxide, nitrogen and oxygen in different concentrations according to the treatments. Firstly, the valves of the gas cylinders were opened and they were released at a pressure of 7 kg per cm² and the different combinations of carbon dioxide, nitrogen and oxygen were mixed in the mixing chamber. According to the treatments given, the gas flow rate was controlled in the buffer tank which was directly connected to the packaging unit. 200 grams of onion seeds were packed using the packaging unit by evacuating the air and then flushed with the gases of required combinations and automatically sealed.

Composition of the gas *i.e.*, O₂ and CO₂ gas concentrations inside the package was checked by Check mate gas analyser with the help of septum, which prevents leakage of (head space) gas from polyethylene bag while taking readings of change in gas concentration.

Procedure to use MAP instrument

The cylinders containing Carbon dioxide (CO₂), Oxygen (O₂) and Nitrogen (N₂) gas as in Plate 1 were checked for pressure and the pressure of the gases was adjusted by following the steps detailed below.

The top dial in the mixing chamber was adjusted to the required CO₂ gas concentration and the value of X (mentioned below the upper dial) was

noted then adjusted the bottom dial by calculating the value of N₂/X (Plate 10), where N₂ is nitrogen concentration and X is the value or number below the upper dial.

The desired gas concentrations were checked by using check mate gas analyzer (Plate 11). Through the gas sampling port the gases were allowed to pass through needle and the obtained gas concentration from the gas mixing chamber was checked and recorded.

If the required gas concentration was not achieved then dialer was fine tuned to get the exact gas concentration. The sampling port was closed and the gas collected in the buffer tank was evacuated.

Buffer tank (Plate 12) needed to be evacuated to achieve the required gas concentration. The gas was supplied through tube to the modified atmosphere packaging unit for packing of seeds.

In Packaging Unit (Plate 13) the heat level of sealing was adjusted to 2.0 to 2.5 to achieve proper sealing. The packaging material (polyethylene, 700 gauges) was kept in the packaging unit in which the vacuum was created by evacuating the air present in the packaging material and then filled the required gas concentration from buffer tank and sealed (Plate 14 and 15).

Results and Discussion

The results of dehydrogenase activity, α -Amylase enzyme activity, Catalase enzyme activity and Superoxide dismutase (SOD) enzyme activity as influenced by modified atmospheric storage conditions and its effect during storage are presented in Table 1, 2, 3 and 4.

With an advancement of storage period, the mean enzyme activity declined rapidly,

irrespective of modified atmospheric storage conditions. Different enzyme activity differed significantly due to modified atmospheric storage conditions in all the 12 months of storage irrespective of the modified atmospheric conditions. The seeds stored in Cold storage (T_{15}) recorded maximum dehydrogenase activity of (0.280), amylase enzyme activity of (1.28), catalase enzyme activity of (0.246), superoxide dismutase enzyme activity of (0.900) after twelve months of storage period followed by T_1 : 80 % CO_2 : 05 % O_2 : 15 % N_2 and seeds stored in vacuum (T_{13}). The lowest dehydrogenase activity of (0.190), was noticed in seed stored in cloth bag (T_{14}) followed by the second the lowest dehydrogenase activity recorded (0.220), α -amylase enzyme activity of (1.80), catalase enzyme activity of (0.156), superoxide dismutase enzyme activity recorded (0.730) in seeds stored in polythene bag with air (T_{12}) after twelve months of storage.

The present study indicated that seed qualitative characters and biochemical parameters like amylase activity, dehydrogenase activity, catalase, and superoxide dismutase (SOD) varied significantly due to modified atmospheric storage condition under ambient, cold storage and vacuum storage as individual treatments in all the months of storage period. As the storage period advanced, all the seed quality attributes were found to be decreased. Conversely, moisture content, EC values and seed infection increased significantly irrespective of modified atmospheric storage conditions. The seeds stored in ambient condition (T_{14}) recorded lower OD value with respect to dehydrogenase and amylase activity (0.190 and 1.80 u.mol/min/g) compared to cold storage (T_{15}) (0.280 and 1.28 u.mol/min/g) which was followed by T_1 - (80 % CO_2 : 05 % O_2 : 15 % N_2) (0.277 and 1.28 u.mol/min/g) and T_{13} - vacuum (0.272 and 1.27 u.mol/min/g). Higher values in the seeds

stored in cold storage might be due to highest activity of these enzymes that indicate pivotal role played by these enzymes in breaking down the macromolecules of seed to provide soluble precursor for the synthesis of protein and carbohydrates accumulation (Rauf, 1980). Further higher germination and vigour with increase ageing might be due to higher enzyme activity.

In general, ageing is manifested by the decrease of metabolic activity and an increase of catabolic processes (Gorecki *et al.*, 1996). In particular, an oxidative stress might be reduced in lower oxygen storage atmospheres (Justice and Bass, 1978; Wilson McDonald, 1986; Benson, 1990). It should be noted that seed deterioration during storage could result in marked changes in the content and activity of enzymes capable for degrading the stored reserves (Priestley, 1986; Smith and Berjak, 1995; Walters, 1998). In the present investigation, it was observed that the dehydrogenase activity in the seeds was maximum and hence, better maintenance of seed quality in modified atmospheric storage condition has been observed compared to control. Another reason for seed ageing may be the accumulation of deleterious effect on membranes due to oxidative damages to fatty acids and proteins denaturation as a result of Millard reactions (Narayana Murthy and Sun, 2000). The advantage of higher seed reserve utilization efficiency in seeds stored in vacuum, provide energy for a faster growing rate of the seedlings. In the present study also, maximum speed of germination (17.30 and 16.91) was noticed in the treatment T_1 (80 % CO_2 + 5 % O_2 + 15 % N_2) and T_{14} *i.e.*, seeds stored in vacuum package respectively. The similar results were also reported by Rathi *et al.*, (2000), Bera *et al.*, (2004) and Bera *et al.*, (2008).

SOD and CAT activities in onion seeds were observed to decrease with ageing in the present study. These results support the

hypothesis of Bailly *et al.*, (1996) that a decrease in antioxidant enzymes is linked to an increased lipid peroxidation and accelerated ageing. Subsequently, Bailly *et al.*, (2000, 2002) proposed a positive relationship between antioxidant enzyme capacity and the vigour of the seed. Consequently, our results support the hypothesis of inactivation of free radical scavenging enzymes (*i.e.*, SOD and CAT) during ageing and showed a direct relationship with the germination efficiency of ageing onion seeds. Moreover, our results provide convincing evidence that inactivation

of these enzymes strongly increases below the 60% viability level in onion seeds. During storage, free radicals may be formed in the presence of even traces of oxygen. Initially, unsaturated fatty acid moieties get decomposed to form fatty acid hydroperoxide followed by hemolytic breakdown into alkoxy radicals (Hopin *et al.*, 1996). In the absence of active enzymes scavenging free radicals, degradation products of thermo-labile lipid peroxidation accumulate in the ageing seeds, finally resulting in complete loss of seed viability (Rao *et al.*, 2006).

Details of the treatments are:

Treatments	Seed storage in different concentrations of gas combinations
T ₁	80 % CO ₂ : 05 % O ₂ : 15 % N ₂
T ₂	70 % CO ₂ : 05 % O ₂ : 25 % N ₂
T ₃	60 % CO ₂ : 05 % O ₂ : 35 % N ₂
T ₄	50 % CO ₂ : 05 % O ₂ : 45 % N ₂
T ₅	40 % CO ₂ : 05 % O ₂ : 55 % N ₂
T ₆	80 % CO ₂ : 10 % O ₂ : 10 % N ₂
T ₇	70 % CO ₂ : 10 % O ₂ : 20 % N ₂
T ₈	60 % CO ₂ : 10 % O ₂ : 30 % N ₂
T ₉	50 % CO ₂ : 10 % O ₂ : 40 % N ₂
T ₁₀	40 % CO ₂ : 10 % O ₂ : 50 % N ₂
T ₁₁	50 % CO ₂ : 00 % O ₂ : 50 % N ₂
T ₁₂	Atmospheric air
T ₁₃	Vacuum
T ₁₄	Storage in cloth bag control
T ₁₅	Cold storage

Table.1 Effect of modified atmospheric storage conditions on dehydrogenase activity (OD Value) of onion seeds (cv. Arka Kalyan) during storage

Treatment CO ₂ : O ₂ : N ₂	Months after storage		
	2	6	12
T ₁ : 80% CO ₂ : 05 % O ₂ : 15 % N ₂	0.496	0.387	0.277
T ₂ : 70% CO ₂ : 05 % O ₂ : 25 % N ₂	0.495	0.373	0.263
T ₃ : 60% CO ₂ : 05%O ₂ : 35 % N ₂	0.494	0.363	0.253
T ₄ : 50% CO ₂ : 05 % O ₂ : 45 % N ₂	0.493	0.359	0.249
T ₅ : 40% CO ₂ : 05 % O ₂ : 55 % N ₂	0.489	0.356	0.246
T ₆ : 80% CO ₂ : 10 % O ₂ : 10 % N ₂	0.491	0.358	0.248
T ₇ : 70% CO ₂ : 10 % O ₂ : 20 % N ₂	0.487	0.351	0.241
T ₈ : 60% CO ₂ : 10 % O ₂ : 30 % N ₂	0.520	0.342	0.232
T ₉ : 50% CO ₂ : 10 % O ₂ : 40 % N ₂	0.483	0.338	0.228
T ₁₀ : 40% CO ₂ : 10 % O ₂ : 50 % N ₂	0.479	0.337	0.227
T ₁₁ : 50% CO ₂ : 00 % O ₂ : 50 % N ₂	0.494	0.369	0.259
T ₁₂ : Atmospheric air	0.475	0.330	0.220
T ₁₃ : Vacuum	0.496	0.382	0.272
T ₁₄ : Storage in cloth bag (control)	0.471	0.300	0.190
T ₁₅ : Cold storage	0.497	0.390	0.280
Mean	0.490	0.353	0.243
S.Em. ±	0.007	0.011	0.011
C.D. @ 1%	NS	0.031	0.031

(Initial dehydrogenase activity: 0.50)

Table.2 Effect of modified atmospheric storage conditions on α -amylase enzyme activity of onion seeds (cv. Arka Kalyan) during storage

Treatment CO ₂ : O ₂ : N ₂	Months after storage		
	2	6	12
T ₁ : 80% CO ₂ : 05 % O ₂ : 15 % N ₂	2.50	1.89	1.28
T ₂ : 70% CO ₂ : 05 % O ₂ : 25 % N ₂	2.50	1.87	1.26
T ₃ : 60% CO ₂ : 05%O ₂ : 35 % N ₂	2.49	1.86	1.25
T ₄ : 50% CO ₂ : 05 % O ₂ : 45 % N ₂	2.49	1.86	1.25
T ₅ : 40% CO ₂ : 05 % O ₂ : 55 % N ₂	2.49	1.86	1.25
T ₆ : 80% CO ₂ : 10 % O ₂ : 10 % N ₂	2.49	1.86	1.25
T ₇ : 70% CO ₂ : 10 % O ₂ : 20 % N ₂	2.49	1.85	1.24
T ₈ : 60% CO ₂ : 10 % O ₂ : 30 % N ₂	2.52	1.84	1.23
T ₉ : 50% CO ₂ : 10 % O ₂ : 40 % N ₂	2.48	1.84	1.23
T ₁₀ : 40% CO ₂ : 10 % O ₂ : 50 % N ₂	2.48	1.84	1.23
T ₁₁ : 50% CO ₂ : 00 % O ₂ : 50 % N ₂	2.49	1.87	1.26
T ₁₂ : Atmospheric air	2.48	1.83	1.22
T ₁₃ : Vacuum	2.50	1.88	1.27
T ₁₄ : Storage in cloth bag (control)	2.47	1.80	1.80
T ₁₅ : Cold storage	2.50	1.89	1.28
Mean	2.49	1.85	1.29
S.Em. ±	0.01	0.01	0.01
C.D. @ 1%	NS	0.03	0.03

(Initial α -amylase enzyme activity: 2.56 (u.mol/min/g))

Table.3 Effect of modified atmospheric storage conditions on catalase enzyme activity of onion seeds (cv. Arka Kalyan) during storage

Treatment CO ₂ : O ₂ : N ₂	Months after storage		
	2	6	12
T ₁ : 80% CO ₂ : 05 % O ₂ : 15 % N ₂	1.67	1.26	0.243
T ₂ : 70% CO ₂ : 05 % O ₂ : 25 % N ₂	1.67	1.26	0.229
T ₃ : 60% CO ₂ : 05%O ₂ : 35 % N ₂	1.66	1.25	0.219
T ₄ : 50% CO ₂ : 05 % O ₂ : 45 % N ₂	1.66	1.25	0.215
T ₅ : 40% CO ₂ : 05 % O ₂ : 55 % N ₂	1.66	1.25	0.212
T ₆ : 80% CO ₂ : 10 % O ₂ : 10 % N ₂	1.66	1.25	0.214
T ₇ : 70% CO ₂ : 10 % O ₂ : 20 % N ₂	1.66	1.24	0.207
T ₈ : 60% CO ₂ : 10 % O ₂ : 30 % N ₂	1.69	1.22	0.198
T ₉ : 50% CO ₂ : 10 % O ₂ : 40 % N ₂	1.65	1.23	0.194
T ₁₀ : 40% CO ₂ : 10 % O ₂ : 50 % N ₂	1.65	1.23	0.193
T ₁₁ : 50% CO ₂ : 00 % O ₂ : 50 % N ₂	1.66	1.25	0.225
T ₁₂ : Atmospheric air	1.65	1.24	0.186
T ₁₃ : Vacuum	1.67	1.26	0.238
T ₁₄ : Storage in cloth bag (control)	1.64	1.80	0.156
T ₁₅ : Cold storage	1.67	1.28	0.246
Mean	1.66	1.28	0.209
S.Em. ±	0.01	0.01	0.011
C.D. @ 1%	0.02	0.03	0.031

(Initial catalase enzyme activity: 1.70 (mmol/ min/g))

Table.4 Effect of modified atmospheric storage conditions on superoxide dismutase activity (SOD) of onion seeds (cv. Arka Kalyan) during storage

Treatment CO ₂ : O ₂ : N ₂	Months after storage		
	2	6	12
T ₁ : 80% CO ₂ : 05 % O ₂ : 15 % N ₂	1.89	1.16	0.897
T ₂ : 70% CO ₂ : 05 % O ₂ : 25 % N ₂	1.87	1.14	0.883
T ₃ : 60% CO ₂ : 05%O ₂ : 35 % N ₂	1.83	1.13	0.873
T ₄ : 50% CO ₂ : 05 % O ₂ : 45 % N ₂	1.86	1.13	0.869
T ₅ : 40% CO ₂ : 05 % O ₂ : 55 % N ₂	1.84	1.13	0.866
T ₆ : 80% CO ₂ : 10 % O ₂ : 10 % N ₂	1.82	1.13	0.868
T ₇ : 70% CO ₂ : 10 % O ₂ : 20 % N ₂	1.85	1.12	0.861
T ₈ : 60% CO ₂ : 10 % O ₂ : 30 % N ₂	1.84	1.11	0.852
T ₉ : 50% CO ₂ : 10 % O ₂ : 40 % N ₂	1.84	1.11	0.848
T ₁₀ : 40% CO ₂ : 10 % O ₂ : 50 % N ₂	1.84	1.11	0.847
T ₁₁ : 50% CO ₂ : 00 % O ₂ : 50 % N ₂	1.91	1.14	0.879
T ₁₂ : Atmospheric air	1.83	0.98	0.730
T ₁₃ : Vacuum	1.88	1.15	0.892
T ₁₄ : Storage in cloth bag (control)	1.80	0.95	0.700
T ₁₅ : Cold storage	1.89	1.16	0.900
Mean	1.85	1.11	0.848
S.Em. ±	0.02	0.01	0.011
C.D. @ 1%	0.04	0.03	0.031

(Initial superoxide dismutase activity: 1.98)



Plate 10: Mixing chamber



Plate 11: Check mate gas analyser



Plate 12: Buffer tank



Plate 13: Packaging unit



Plate 14: Modified atmosphere packaging instrument



Plate 15: Cold storage



Plate 17: Influence of modified atmospheric packaging on α -Amylase activity after 12 months of onion seed storage by DNSA method



Plate 18: α -Amylase activity estimation using Spectrophotometer at 510 nm



Plate 19: Influence of seed deterioration on dehydrogenase enzyme activity after 12 months of onion seed storage



Plate 20: The intensity of red colour was measured using UV-VIS spectrophotometer at 470 nm

Summary and conclusions are as follows:

The modified atmospheric storage conditions showed significant effect on vigor and viability of onion seeds. The seeds which stored in cold storage (T₁₅) showed better vigor throughout the storage period followed by seeds stored with gaseous combination of 80 % CO₂ : 05 % O₂ : 15 % N₂ (T₁) and under vacuum conditions (T₁₃) under ambient storage conditions. Among the storage conditions tested, the seeds preserved under cold storage recorded higher field emergence and seedling vigor index with less qualitative loss in comparisons to those seeds stored under ambient condition *i.e.* in cloth bag throughout the storage period of 12 months.

The study clearly indicated the importance of onion seed storage under cold storage and higher CO₂ and lowest O₂ for maintenance of seed quality during storage. In case of absence of this facility even seed storage under vacuum is quite satisfactory. The deleterious effect of seeds stored in impervious container and higher oxygen concentration was also revealed during the study.

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