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Effect of Packaging and Storage on Onion (*Allium cepa L.*) Seed Biochemistry

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A lab experiment was conducted from April 2021-Sep 2022 at NSP, Seed unit, UAS, Dharwad. The seed biochemical parameters such as α-amylase activity and catalase activity were evaluated at bimonthly intervals, and the experimental design followed was a factorial completely randomized design (FCRD) with 3 replications and 2 factors, namely storage conditions such as ambient and

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cold storage and storage containers (cloth bag, high-density polythene bag (HDPE), polythene bag (700 gauge), aluminum laminated bag, vacuum-packed bag), the results revealed that the treatment with vacuum-packed bag stored in cold storage gave good results at the end of 18 months of storage period *i.e.,* α-amylase activity (2.00 µ.mol/min/mg) and catalase activity (1.35 mmol/min/g) respectively compared to other treatments stored in ambient and cold storage conditions with different packaging materials.

Keywords: Vacuum-packed bag; ambient storage; cold storage; α-amylase activity; catalase activity.

1. INTRODUCTION

"Onion (*Allium cepa* L.) is one of the most significant vegetables grown from bulbous plants and a member of the Amaryllidaceae family. It holds a significant place in the world due to its widespread cultivation and high demand for its consumption. The seed possesses the highest Vigor at the time of physiological maturity and gradually decreases as the storage period increases" [1]. "Mostly biochemical deterioration during seed aging was conducted, where high temperature and humidity conditions are normally affected" [2-5]. "Many hypotheses have been proposed regarding causes of seed aging such as lipid peroxidation mediated by free radicals, inactivation of enzymes or decrease in proteins, disintegration of cell membranes, and genetic damage" [6-10]. "One of the most crucial hypotheses suggested for the causes of seed aging is the degradation and inactivation of enzymes caused by alterations in their macromolecular structures" [11-14]. Goel et al*.* 2003 Lehner et al*.* [15] Demirkaya and sivritepe [16].

"Majorly the initial quality of seeds, moisture level, relative humidity (RH %), and storage conditions have considerable influence on seed storage. Seeds undergo hydrolysis of sugars during storage seed deterioration" [17]. "However, if the seeds are stored in controlled conditions, it is suitable for maintenance of the seed quality for a longer duration. Different packaging materials available in the market show differences in permeability like permeable, semi-permeable, and impermeable characteristics, each with a different degree of protection concerning variations in air humidity and hygroscopicity" [18].

"Seed vigor and viability may decrease because of deterioration processes as this process is unavoidable and irreversible. It causes an increase in free radicals content and the lengthening of their chain, changes to the structure of proteins, the depletion of food reserves, an increase in fat acidity, changes in the activity of enzymes, damage to the membrane, and finally an increase in the rate of seed respiration. The ability of seeds to germinate decreases as catabolic changes occur with age" [19]. "Seeds use various physiological and biochemical strategies, including regenerating cell membrane lipid compounds, to prevent cell damage caused by decay during storage" [20]. "Usually, as seed moisture levels and storage space temperatures rise, the degree of degradation increases. Seeds are harmed by moist seeds and higher storage temperatures". Ellis et al. [21].

2. MATERIALS AND METHODS

At the NSP, Seed unit, UAS, Dharwad, a lab experiment was conducted from April 2021 to September 2022. The seed biochemical parameters such as α-amylase activity and catalase activity were assessed, and the experiment design used was Factorial Completely Randomised Design (FCRD) with 3 replications and 2 factors, namely storage conditions such as ambient and cold storage and storage containers such as cloth bags, high-density polythene bags (HDPE), polythene bags, aluminum laminated bags and vacuum-packed bags. The seeds were stored for 18 months. Arka Kalyan variety was used for the study. The seed is purchased from the University of Horticultural Sciences, Bagalkot. Every two months, readings were taken.

2.1 Estimation of α-Amylase Activity

Procedure:

- In each test tube, 0.25 ml of starch solution, 0.5 ml of buffer, and 0.25 ml of the enzyme were added. The same amounts of buffer, starch, and enzyme were added to all test tubes for each seed sample. To maintain a blank solution, 0.5 ml of buffer and 0.5 ml of starch were added to a separate test tube. All test tubes, including the blank, were

incubated at room temperature for 15 minutes. After incubation, 0.5 ml of DNSA solution was added to each test tube, and they were placed in boiling water for 5 minutes to stop the reaction. The test tubes were then allowed to cool before the volume was made up to 10 ml with distilled water. The concentration was determined by recording the absorbance at 560 nm, and enzyme activity was calculated using the given formula. The results were expressed in µg of maltose released per minute per 1 ml of enzyme source. Sadasivam and Manickam, [22].

• α-amylase activity = Maltose concentration released by enzyme source (in ug) / Incubation period (15 minutes) × 4

2.2 Catalase activity

Catalase activity was determined spectrophotometrically with the method proposed by Beers and Sizer in 1952.

Procedure

Ten onion seedlings three days old were homogenized in pestle and mortar by adding potassium phosphate buffer (100 mM, pH 7.8) amended with EDTA solution (3 mM). The homogenate was centrifuged at 12,000 rpm for 10 minutes. This was done twice. This supernatant was taken for catalase assay.

The reaction mixture in the test cuvette containing 2.98 ml of 0.051 % substrate (hydrogen peroxide) in 50 mM, pH 7.0 phosphate buffer was read against a control cuvette containing 2.98 ml H_2O_2 free phosphate buffer, 50 mM, pH 7 (substrate blank was used because the enzyme extract observed strongly at 240 nm). This procedure was standardized using pure catalase enzyme from Bovine liver (procured from Sigma Aldrich India) according to the method of Beers and Sizer [23].

Note: The blank was prepared freshly for each assay.

The enzyme activity was calculated using the molar extinction coefficient of H_2O_2 (33.6 M/cm) and expressed as mmol/min/g.

The activity of catalase was calculated by using the following formula,

U/ml/gFW = AΔ x 1,000 x reaction volume (ml) / 33.43 x Time (min) x Volume of enzyme (ml)

Where,

AΔ = Change in the absorbance 240 nm in time t.
33.43 = Molar absorption or extinction coefficient of hydrogen peroxide at 240nm

3. RESULTS AND DISCUSSION

There was a significant effect of storage containers and duration under ambient and cold temperatures on α amylase activity (µ. mol/min/mg) and catalase activity (mmol/min/g) in the enzyme activity of onion var. Arka Kalyan (Table 1 and 2).

3.1 α-Amylase

The results of α- amylase as influenced by storage conditions, packaging materials, and their interactions during the storage period are given in Table 1. As the storage period progressed, the reduction in α- amylase activity from 2.55 in 2nd month to 1.66 µ. mol/min/mg at the end of the 18th month of the storage period was found irrespective of storage conditions and packaging materials. α-amylase activity was influenced by the factors like storage condition in which it is stored and the packaging material in which it is packed. There was a decrease in α-amylase activity from the 2nd month to the 18th month influenced by different factors.

3.2 Storage Conditions (S)

Regardless of initial storage conditions and their packaging material higher mean α-amylase activity was noticed in cold storage compared to ambient storage throughout the storage period (18 months). The mean α-amylase activity decreased from 2.54 to 0.68 u.mol/min/mg and from 2.55 to 1.62 µ. mol/min/mg in ambient storage and cold storage respectively.

3.3 Packaging Material (P)

Among all the packaging materials, lower mean α-amylase activity was noticed in cloth bags followed by HDPE bags, polythene bags, aluminum laminated pouches, and vacuum-packed bags. Mean α-amylase activity decreased from 2.50 to 0.88 µ. mol/min/mg in a cloth bag and from 2.59 to 1.39 µ. mol/min/mg in vacuum-packed bags through the storage period.

Table 1. Influence of packaging material and storage conditions on α-amylase (µ. mol/min/mg) during storage in onion seeds

Treatments	Storage (Months)								
	2	4	6	8	10	12	14	16	18
Storage conditions (S)									
S_1: Ambient	2.54	2.28	2.07	1.90	1.59	1.46	1.22	0.88	0.68
S_2: Cold	2.55	2.47	2.36	2.27	2.18	2.05	1.94	1.78	1.62
S. Em (±)	0.0005	0.007	0.003	0.004	0.005	0.004	0.007	0.008	0.01
C. D. (1%)	0.002	0.031	0.013	0.018	0.022	0.019	0.033	0.033	0.04
Packaging materials (P)									
P_1: Cloth bag	2.50	2.29	2.12	1.96	1.73	1.62	1.41	1.07	0.88
P_2: High-density polythene bag	2.52	2.33	2.16	2.02	1.81	1.68	1.51	1.25	1.03
P_3: Polythene bags (700 gauge)	2.54	2.39	2.23	2.11	1.90	1.78	1.61	1.36	1.17
P_4: Aluminum laminated pouch	2.56	2.41	2.27	2.16	1.97	1.83	1.66	1.46	1.30
P_5: Vacuum packed bags	2.59	2.44	2.29	2.19	2.00	1.86	1.70	1.51	1.39
S. Em (±)	0.0008	0.011	0.005	0.006	0.008	0.007	0.012	0.012	0.01
C. D. (1%)	0.003	0.04	0.021	0.028	0.034	0.030	0.052	0.052	0.06
Interaction (S x P)									
S_1P_1	2.50	2.23	2.02	1.85	1.52	1.38	1.13	0.69	0.49
S_1P_2	2.52	2.26	2.05	1.89	1.58	1.45	1.21	0.88	0.68
S_1P_3	2.54	2.28	2.07	1.91	1.60	1.47	1.23	0.93	0.73
S_1P_4	2.56	2.30	2.09	1.93	1.62	1.49	1.25	0.95	0.75
S_1P_5	2.59	2.33	2.12	1.96	1.65	1.53	1.29	0.99	0.79
S_2P_1	2.51	2.36	2.22	2.08	1.95	1.87	1.70	1.45	1.27
S_2P_2	2.53	2.41	2.29	2.17	2.05	1.92	1.82	1.63	1.40
S_2P_3	2.55	2.52	2.40	2.32	2.22	2.10	2.00	1.80	1.62
S_2P_4	2.57	2.53	2.45	2.40	2.33	2.18	2.08	1.98	1.85
S_2P_5	2.60	2.55	2.47	2.43	2.35	2.20	2.12	2.05	2.00
Mean	2.55	2.38	2.22	2.09	1.89	1.76	1.58	1.34	1.16
S. Em (±)	0.001	0.016	0.007	0.009	0.011	0.010	0.017	0.017	0.02
C. D. (1%)	0.004	0.070	0.029	0.040	0.049	0.043	0.074	0.074	0.09
C.V (%)	0.08	1.23	0.56	0.81	1.09	1.03	1.95	2.33	3.41

Storage conditions (S): S1: Ambient storage, S2: Cold storage, Packaging materials (P): P1: Cloth bag, P2: High density polythene bag, P3: Polythene bags (700 gauge), P4: Aluminum laminated pouch, P5: Vacuum packed bags, (Initial=2.60 u.mol/min/mg)

3.4 Interaction (S x P)

The interaction effect of storage conditions and packaging material on mean α-amylase was found to be significant throughout the storage period. Among all the treatment, combinations S_2P_5 reported significantly the highest α-amylase activity of 2.00 u.mol/min/mg at the end of the storage period. S_1P_1 recorded significantly the lowest α-amylase activity of 0.49 u.mol/min/mg at the end of the 18-month storage period. There was an 81 percent decrease in amylase activity in S_1P_1 followed by S_1P_2 (73.85%) decrease and a 23.08 percent decrease was seen in S_2P_5. α-amylase activity content decreased as the storage progressed. The highest decrease was seen in treatment S_1P_1 (81.15 %) then followed by S_1P_2 (73.85 %) and the lowest decrease was seen in S_2P_5 (23.08 %). (Fig. 1 and 2).

3.5 Catalase Activity

The results of catalase activity, as influenced by storage conditions, packaging materials, and their interactions during the storage period, are given in Table 2. As the storage period progressed, reduction in catalase activity from 1.69 at the 2nd to 1.02 mmol/min/g at the end of the 18th month of the storage period irrespective of storage conditions and packaging materials.

Catalase activity was influenced by the factors like storage condition in which it is stored and the packaging material in which it is packed. There was a decrease in catalase activity from the 2nd month to the 18th month influenced by different factors.

3.6 Storage Conditions (S)

Regardless of initial storage conditions and their packaging material higher mean catalase activity was noticed in cold storage compared to ambient storage throughout the storage period (18 months). The decrease in mean catalase activity was from 1.68 to 0.79 mmol/min/g and from 1.69 to 1.25 mmol/min/g in ambient storage and cold storage respectively (Fig. 3 and 4).

3.7 Packaging Material (P)

Among all the packaging materials, lower mean catalase activity was noticed in cloth bags followed by HDPE bags, polythene bags, aluminum laminated pouches and vacuum-packed bags. Mean catalase activity decreased from 1.67 to 0.67 mmol/min/g in cloth bags and from 1.71 to 1.30 mmol/min/g in vacuum-packed bags through the storage period.

Table 2. Influence of packaging material and storage conditions on catalase activity (mmol/min/g) during storage in onion seeds

Treatments	Storage (Months)								
	2	4	6	8	10	12	14	16	18
Storage conditions (S)									
S_1: Ambient	1.68	1.65	1.50	1.35	1.27	1.17	1.05	0.94	0.79
S_2: Cold	1.69	1.67	1.64	1.62	1.58	1.52	1.47	1.38	1.25
S. Em (±)	0.002	0.001	0.002	0.001	0.004	0.001	0.001	0.001	0.009
C. D. (1%)	0.010	0.004	0.011	0.006	0.017	0.005	0.008	0.008	0.039
Packaging materials (p)									
P_1: Cloth bag	1.67	1.62	1.51	1.39	1.32	1.21	1.09	0.88	0.67
P_2: High-density polythene bag	1.67	1.65	1.52	1.42	1.37	1.23	1.19	1.12	0.95
P_3: Polythene bags (700 gauge)	1.68	1.66	1.59	1.48	1.41	1.36	1.24	1.16	1.05
P_4: Aluminum laminated pouch	1.69	1.68	1.56	1.48	1.44	1.37	1.28	1.20	1.13
P_5: Vacuum packed bags	1.71	1.70	1.68	1.65	1.62	1.57	1.52	1.43	1.30
S. Em (±)	0.004	0.001	0.004	0.002	0.006	0.002	0.003	0.003	0.01
C. D. (1%)	0.016	0.007	0.018	0.009	0.027	0.009	0.012	0.12	0.06
Interaction (S x P)									
S_1P_1	1.67	1.60	1.42	1.22	1.10	0.95	0.76	0.45	0.20
S_1P_2	1.67	1.65	1.45	1.25	1.16	0.98	0.95	0.91	0.70
S_1P_3	1.69	1.66	1.53	1.35	1.25	1.20	1.00	0.95	0.85
S_1P_4	1.69	1.68	1.45	1.33	1.28	1.20	1.05	0.98	0.95
S_1P_5	1.71	1.70	1.67	1.65	1.61	1.56	1.52	1.42	1.25
S_2P_1	1.68	1.65	1.61	1.58	1.54	1.48	1.42	1.32	1.15
S_2P_2	1.68	1.66	1.61	1.60	1.58	1.49	1.44	1.34	1.20
S_2P_3	1.69	1.67	1.65	1.63	1.58	1.53	1.48	1.38	1.25
S_2P_4	1.69	1.68	1.67	1.64	1.60	1.55	1.51	1.43	1.32
S_2P_5	1.71	1.70	1.69	1.66	1.63	1.48	1.53	1.32	1.35
Mean	1.69	1.66	1.57	1.49	1.43	1.35	1.27	1.16	1.02
S. Em (±)	0.005	0.002	0.006	0.003	0.009	0.003	0.004	0.004	0.021
C. D. (1%)	0.023	0.015	0.025	0.013	0.039	0.013	0.018	0.018	0.087
C.V (%)	0.58	0.26	0.68	0.38	1.14	0.40	0.60	0.65	3.57

Storage conditions (S): S1: Ambient storage, S2: Cold storage, Packaging materials (P): P1: Cloth bag, P2: High density polythene bag, P3: Polythene bags (700 gauge), P4: Aluminum laminated pouch, P5: Vacuum packed bags, (Initial=1.72 mmol/min/g)

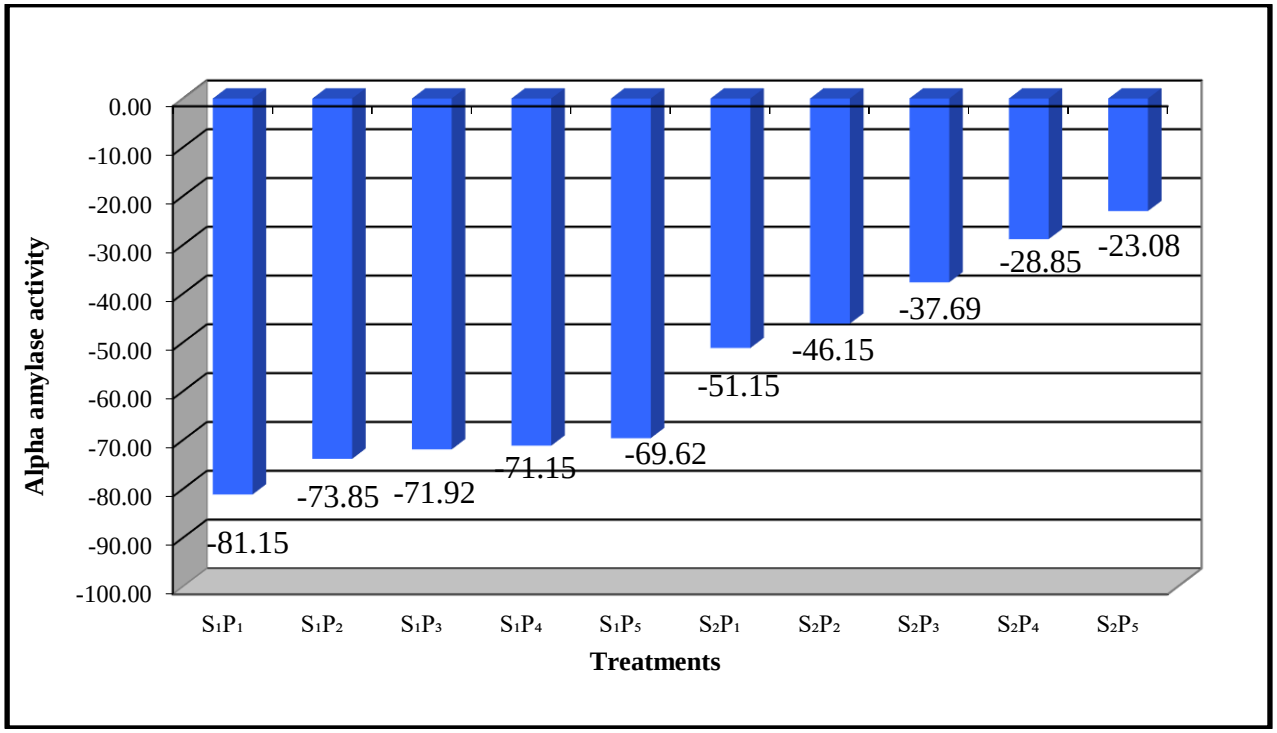

Fig. 1. Per cent reduction in the Alpha amylase activity of treatment interactions after 18 months of storage

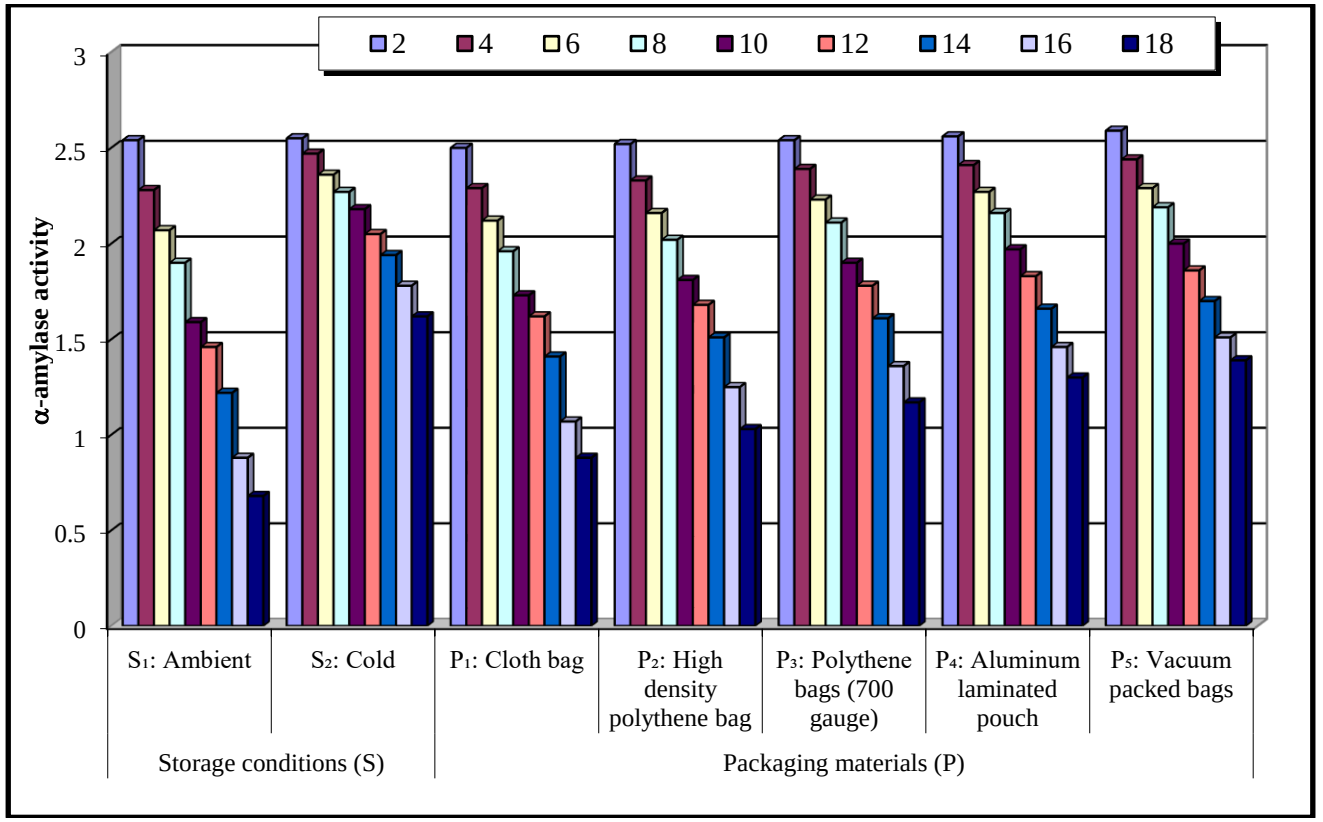

Fig. 2. Effect of packaging and storage conditions on a-amylase activity during storage in onion seeds

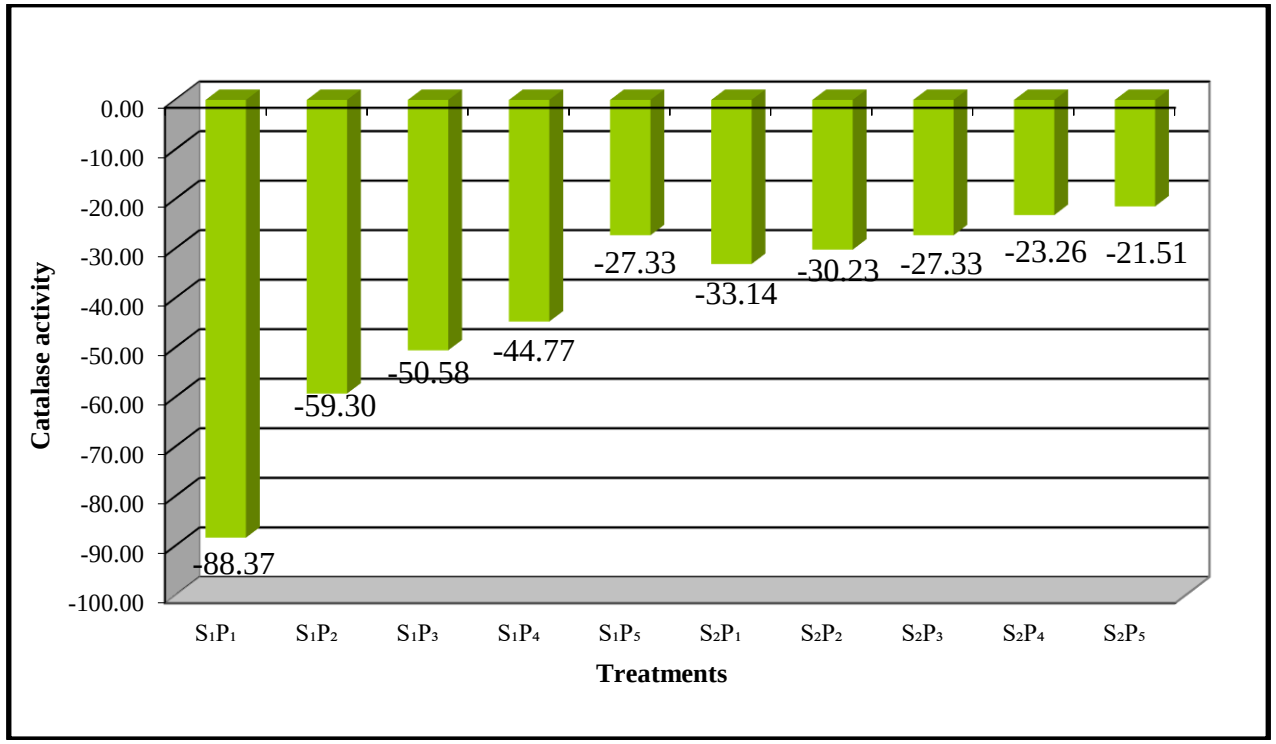

Fig. 3. Per cent reduction in the catalase activity of treatment interactions after 18 months of storage

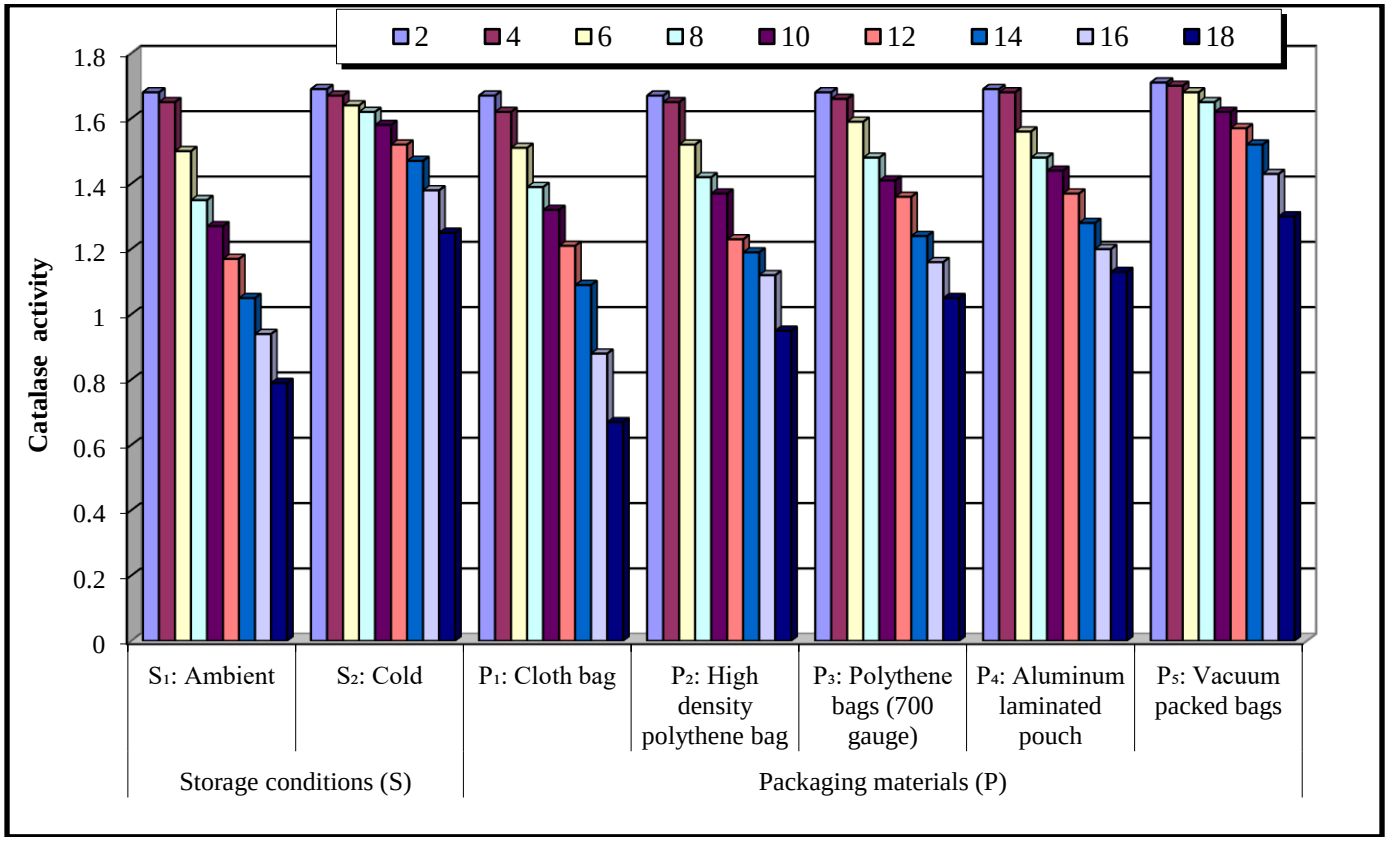

Fig. 4. Effect of packaging and storage conditions on catalase activity during storage in onion seeds

3.8 Interaction (S x P)

The interaction effect of storage conditions and packaging material on mean catalase activity was found to be significant throughout the storage period. Among all the treatment, combinations S_2P_5 significantly reported the highest catalase activity of 1.35 mmol/min/g at the end of the storage period. S_1P_1 recorded significantly lowest catalase activity of 0.20 mmol/min/g at the end of the 18-month storage period. There was an 88.37 percent decrease in catalase activity in S_1P_1 followed by S_1P_2 (59.30%) decrease and a 21.51 percent decrease was seen in S_2P_5. Catalase activity content decreased as the storage progressed. The highest decrease was seen in treatment S_1P_1 (88.37 %) then followed by S_1P_2 (59.30 %) and the lowest decrease was seen in S_2P_5 (21.51 %).

As the storage progressed the deterioration occurred due to the high respiration rate increased due to high moisture content. As the storage period advances the vigor of the seed declines due to the catabolic activity going on in the seed and thus the seed though viable fails to emerge. Decline in seed vigor depends on storage conditions that is temperature, relative humidity, and seed moisture contents. High temperature, relative humidity, and moisture in the storage environment appear to be principle factors involved in the deterioration of seed quality.

The seeds stored in the cloth bag (P1) noticed the lowest α-amylase activity at cold and ambient temperatures respectively. The same container recorded lower mean amylase activity and catalase activity (0.88 µ.mol/min/mg) (0.67 mmol/min/g), respectively. This result is in agreement with Mollah et al*.* [24] and Rao et al*.* [25] in onion seeds as well as Barua et al*.* [26] in chilli seeds. Seeds stored in cloth bags and stored in ambient storage conditions showed less α-amylase activity and catalase activity after 18 months of storage period. Peroxidation of unsaturated fatty acids is one of the main reasons for loss of storability, which occurs due to decreased levels of antioxidants, reduced activity of free radical and peroxide scavenging enzymes, and increased lipid peroxidation. These results are in agreement with the studies of Chiu et al*.* [27] and Hsu and Sung [28] in watermelon and Bailly et al*.* [29] in sunflower seeds.

4. CONCLUSION

It was concluded that storage containers and duration were found to be compatible with packing in vapour-proof containers (plastic bag 700 gauge) and preserved in cold storage for maintaining good quality parameters and the lowest enzymatic activity during storage. There was a gradual reduction in seed quality parameters during the storage in both cold and ambient storage environments, but the reduction process was relatively slower in cold storage environments compared to ambient storage mainly due to lower respiration rate and metabolic activity governed by lower temperature, during the storage period. Significantly lower enzymatic activity was shown in seeds stored in cloth bags after the 18 months of storage duration. Enzyme activity decreased along with the increase in storage duration and decreased seed viability level. Significantly highest enzyme activity was observed in seeds stored in vacuum-packed bags stored in cold storage conditions till the end of the 18 months of storage period.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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