

## Original Research Article

### Effect of Modified Atmospheric Storage on seed longevity of soybean [*Glycine max* (L.) Merr.]

#### ABSTRACT

Seed quality is crucial for boosting seed production and productivity. However, soybean seeds are inherently short lived, quickly loses viability even under optimal storage conditions. Therefore, maintaining their quality during storage is of prime importance. Keeping these in view a laboratory experiment was conducted to study the effect of modified atmospheric storage on seed quality of soybean (cv. DSb 34). The freshly harvested seeds with initial moisture content of 7.6% were stored in different modified atmospheric gaseous combinations for nine months from November 2023- August 2024. Results indicated that the seeds stored with gaseous combination of 90 % CO<sub>2</sub>: 4 % O<sub>2</sub>: 6 % N<sub>2</sub> in 700 gauge polyethylene bag maintained better seed quality in terms of germination (86.77%), shoot length (14.49 cm), root length (12.58 cm), dry weight(0.995g/ 10 seedlings), seedling vigour index I (2349) and II (86.34) upto the end of nine months storage period by recording higher total dehydrogenase (0.438 OD value),  $\alpha$ -amylase (10.72  $\mu\text{mol}$  of maltose released  $\text{min}^{-1} \text{ml}^{-1}$  of enzyme) and catalase activity (1.364  $\mu\text{mol}$  H<sub>2</sub>O<sub>2</sub> decomposed  $\text{min}^{-1}\text{g}^{-1}$  protein) compared to all other gaseous combinations and control. Hence, this technique can be used to maintain seed longevity with little deterioration,ensuring the quality of seeds even after extended storage period.

Key words: soybean, seed longevity, Seed quality, Quality seeds

#### INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is a legume and oil seed crop native to East Asia, belongs to family *leguminaceae*. It is becoming an economically and industrially important crop as it possesses many therapeutic and nutraceutical compounds. Therefore, soybean is referred to as 'Gold from the soil' [1]. These seeds are powerhouse of nutrients consisting of 40-45% protein, 20-22% oil and 20 to 26% carbohydrates [2]. India ranks fifth among major soybean growing countries in the world with 13.08 m ha of cultivated area producing 14.98 m t at a productivity of 1298 kg/ha [3].

Quality seeds are fundamental input in agriculture. Insufficient access to high quality soybean seeds poses a significant obstacle for expanding soybean cultivation. Soybean seed is considered as poor storer, loses viability quickly when stored under ambient condition as compared to other *kharif* oil seed /pulse crops due to its inherent thin seed coat and high fatty acids. In addition to these intrinsic factors, the composition of the surrounding atmospheric air during ambient storage also significantly effects seed longevity. Oxygen levels in the air stimulate metabolic activity in seeds, as well as in insects and microorganisms, accelerating deleterious oxidative processes leading to an accumulation of reactive oxygen species (ROS), ultimately reducing seed viability and physiological quality. Low O<sub>2</sub> and high CO<sub>2</sub> during storage environment reduced metabolic activity favoring quality maintenance of soybean seed [4]. Hence, Modified atmospheric storage (MAS) or storage of seeds with elevated levels of carbon dioxide emerges as a pivotal solution in this regard.

Modified atmospheric storage is one of the seed and food preservation method that maintains the natural quality of seeds and food products besides extending the storage life of seed by reducing the respiration rate [5]. MAS is, modifying the concentration of the storage gases like carbon dioxide, oxygen and nitrogen, which creates an environment different from an atmospheric air condition. Modified atmosphere of elevated carbon dioxide and depleted oxygen is an effective method against insect pests and **microorganisms** attack and seed quality deterioration during storage. MAS slows down the respiration rates of seeds and reduces the insect and micro-organisms activity during seed storage [6]. **MAS with addition of CO<sub>2</sub> inside the hermetic packages favors the maintenance of the physiological quality of soybean seeds during storage [7].**

Keeping in view the above facts the present investigation was undertaken to understand the effect modified atmospheric storage on seed longevity of soybean [*Glycine max* (L.) Merrill]”

## **MATERIALS AND METHODS**

The laboratory experiment was conducted in the Seed testing laboratory, Seed Unit and Post Graduate laboratory, Department of Seed Science and Technology, College of Agriculture, Dharwad during November 2023- August 2024. For the conduct of experiment, freshly harvested 500 g of soybean seeds (*cv.* DSb 34) with 7.6 % moisture content were packed in 700 gauge polythene bag with different concentrations of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> using MAC VAC DUO machine according to treatments in three replications. The packed seeds stored for nine months from November 2023 to August 2024 in Department of Seed Science and Technology, College of Agriculture, Dharwad. The treatments include different concentrations of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> as mentioned under.

**List 1: Concentrations of CO<sub>2</sub>, O<sub>2</sub>&N<sub>2</sub> used for Modified Atmospheric Storage**

Sl. No.	Concentration of gases (%)		
	CO <sub>2</sub>	O <sub>2</sub>	N <sub>2</sub>
T <sub>1</sub>	95	2	3
T <sub>2</sub>	90	4	6
T <sub>3</sub>	85	6	9
T <sub>4</sub>	80	8	12
T <sub>5</sub>	75	10	15
T <sub>6</sub>	70	12	18
T <sub>7</sub>	65	14	21
T <sub>8</sub>	60	16	24
T <sub>9</sub>	55	18	27
T <sub>10</sub>	50	20	30
T <sub>11</sub>	100	0	0
T <sub>12</sub>	0	0	100
T <sub>13</sub>	Atmospheric air (Polythene bag)		
T <sub>14</sub>	Vacuum packaging		
T <sub>15</sub>	Control (HDPE bag)		

### **Method of Modified Atmospheric Packaging**

For the packaging MAP VAC DUO machine of Elixir Technology was used (Plate1a.). The detailed procedure of modified atmospheric packaging is given below

- Initially the gas cylinders of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> were opened, releasing gas at a pressure of 7 kg per cm<sup>2</sup>. The released gases are filtered by the dust and mist filters (Plate1b.).

- The required combinations of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> were mixed in the digital 3 G gas mixing chamber (Plate 1c.).
- 500 grams of soybean seeds were weighed and filled in 700 gauge polythene bag. The filled polythene bag kept in sealing chamber and closed.
- Later, the gas inlet **evacuates** the air from the polyethylene bag and flushes in the required concentration of gases, which are mixed in the mixing chamber
- Subsequently, sealing chamber automatically seals the packet (Plate 1d.).



a. MAP VAC DUO machine



b. CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> gas cylinders



**c. Gas mixing chamber**

**d. Sealing chamber**

**Plate 1. Method of Modified Atmospheric Packaging using MAP VAC DUO machine**

**Effect of modified atmospheric storage on seed quality parameters of soybean during storage**

Seeds stored under different concentrations of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> gases along with T<sub>13</sub>, T<sub>14</sub> and T<sub>15</sub> were used for analysis of following seed quality parameters bimonthly for nine months of storage.

The standard germination test was conducted by adopting between paper method according to ISTA rules for seed testing [8]. The final germination count was recorded on Eighth day and percent germination calculated using following formula:

$$\text{Seed germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds kept for germination}} \times 100$$

From the germination test, Ten normal seedlings were randomly selected from each treatment in Four replications of on Eighth day of germination test and shoot length, root length and seedling dry weight (80 °C for 24 hours) were measured.

The seedling vigour index- I and II was calculated using the formula stated by Abdul-Baki and Anderson [9].

$$\text{Seedling Vigour Index I} = \text{Germination (\%)} \times [\text{Mean Shoot Length (cm)} + \text{Mean Root Length (cm)}]$$

$$\text{Seedling Vigour Index II} = \text{Germination (\%)} \times \text{Mean seedling dry weight (g/ 10 seedlings)}$$

**Effect of modified atmospheric storage on enzymatic activities of soybean during storage**

Seeds stored under different concentrations of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> gases were used for analysis and the following biochemical parameters were analysed for Nine months of storage during initial, mid and final months of storage.

Dehydrogenase activity was determined by using tetrazolium staining method. The OD value obtained was recorded as the dehydrogenase enzyme activity [10]. **α-Amylase activity was analyzed according to Sadasivam and Manickam [11]. Catalase activity was estimated by the decrease in absorbance of H<sub>2</sub>O<sub>2</sub> at 240 nm and expressed as μmol H<sub>2</sub>O<sub>2</sub> decomposed/min/g [12].**

## RESULTS

### **Effect of modified atmospheric storage on seed quality parameters of soybean during storage**

The results of the present experiment revealed that, all the seed quality parameters decreased with advancement of storage period, irrespective of modified atmospheric storage conditions, though the decline varied across the treatments. Seed quality parameters differed significantly in all the months of storage due to modified atmospheric storage and untreated control.

The mean initial seed germination (93.25 %) declined to 74.08 per cent at the end of the storage period. Among the different modified atmospheric storage conditions, maximum seed germination of 86.77 % was recorded in gaseous combination 90 % CO<sub>2</sub>: 4 % O<sub>2</sub>: 6 % N<sub>2</sub> (T<sub>2</sub>) which was on par with T<sub>1</sub>: 95 % CO<sub>2</sub>: 2 % O<sub>2</sub>: 3 % N<sub>2</sub> (85.62 %) and T<sub>11</sub>: 100 % CO<sub>2</sub>: 0 % O<sub>2</sub>: 0 % N<sub>2</sub> (85.10 %) followed by T<sub>12</sub>: 0 % CO<sub>2</sub>: 0 % O<sub>2</sub>: 100 % N<sub>2</sub> (83.18 %). Seeds stored in HDPE bag with atmospheric air (T<sub>15</sub>: Control) recorded significantly lower seed germination percentage of 50.35 % at the end of nine months storage (Table 1).

The maximum shoot length (14.49 cm) and root length (12.58 cm) were recorded in MAS condition of 90 % CO<sub>2</sub>: 4 % O<sub>2</sub>: 6 % N<sub>2</sub> (T<sub>2</sub>) which was on par with T<sub>1</sub>: 95 % CO<sub>2</sub>: 2 % O<sub>2</sub>: 3 % N<sub>2</sub> (14.32 cm and 12.47 cm respectively) and T<sub>11</sub>: 100 % CO<sub>2</sub>: 0 % O<sub>2</sub>: 0 % N<sub>2</sub> (14.25 cm and 12.44 cm respectively) followed by T<sub>12</sub>: 0 % CO<sub>2</sub>: 0 % O<sub>2</sub>: 100 % N<sub>2</sub> (13.45 cm and 11.75 cm respectively). Seeds stored in HDPE bag with atmospheric air (T<sub>15</sub>: Control) recorded significantly lower shoot length (9.61 cm) and root length (8.03 cm) at the end of storage period (Table 2 & 3). Seedling dry weight was also followed the similar trend (Table 4).

At the end of nine months storage, maximum seedling vigour index I (2349) was observed in gaseous combination of 90 % CO<sub>2</sub>: 4 % O<sub>2</sub>: 6 % N<sub>2</sub> (T<sub>2</sub>) followed by T<sub>1</sub>: 95 % CO<sub>2</sub>: 2 % O<sub>2</sub>: 3 % N<sub>2</sub> (2294) whereas, significantly lower vigour index (744) was recorded in seeds stored in HDPE bag with atmospheric air (T<sub>15</sub>: Control). The similar trend was also followed by seedling vigour index II (Fig 1).

**Table 1. Effect of Modified Atmospheric Storage (MAS) on seed germination (%) of soybean (cv. DSb 34) during storage**

Sl. No.	Concentration of gases (%)			Months of storage (Nov 2023- Aug 2024)					
	CO <sub>2</sub>	O <sub>2</sub>	N <sub>2</sub>	0 (Nov)	2 (Jan)	4 (Mar)	6 (May)	8 (Jul)	9 (Aug)
T <sub>1</sub>	95	2	3	93.25	92.49	91.25	89.81	87.79	85.62
T <sub>2</sub>	90	4	6	93.25	92.64	91.45	90.21	88.53	86.77
T <sub>3</sub>	85	6	9	93.25	91.17	87.68	85.07	82.96	78.63
T <sub>4</sub>	80	8	12	93.25	90.85	87.49	84.59	80.15	76.54
T <sub>5</sub>	75	10	15	93.25	90.33	86.79	84.01	79.63	74.72
T <sub>6</sub>	70	12	18	93.25	90.17	86.56	83.58	79.05	73.63
T <sub>7</sub>	65	14	21	93.25	90.05	84.65	80.44	76.86	71.85
T <sub>8</sub>	60	16	24	93.25	89.86	83.58	77.95	73.15	68.52
T <sub>9</sub>	55	18	27	93.25	89.58	82.45	76.28	71.75	67.50
T <sub>10</sub>	50	20	30	93.25	89.49	81.35	75.54	70.02	66.06
T <sub>11</sub>	100	0	0	93.25	92.38	91.15	89.63	87.58	85.10
T <sub>12</sub>	0	0	100	93.25	92.06	90.28	88.17	86.49	83.18
T <sub>13</sub>	Atmospheric air (Polythene bag)			93.25	88.55	80.90	74.06	68.84	60.73
T <sub>14</sub>	Vacuum packaging			93.25	91.84	90.01	87.84	85.89	82.06
T <sub>15</sub>	Control (HDPE bag)			93.25	88.13	75.85	69.02	61.85	50.35
<b>Mean</b>				<b>93.25</b>	<b>90.64</b>	<b>86.10</b>	<b>82.41</b>	<b>78.70</b>	<b>74.08</b>
<b>S.Em ±</b>					<b>0.086</b>	<b>0.098</b>	<b>0.207</b>	<b>0.238</b>	<b>0.347</b>
<b>CD (0.01)</b>					<b>0.275</b>	<b>0.314</b>	<b>0.662</b>	<b>0.967</b>	<b>1.405</b>

HDPE: High Density Polyethylene bag

**Effect of modified atmospheric storage on enzymatic activities of soybean during storage**

The enzymes activities *viz.*, dehydrogenase,  $\alpha$  amylase and catalase showed a positive correlation with seed quality during storage. Initially enzymes activities were maximum of 0.648 OD value, 11.28  $\mu\text{mol}$  of maltose released  $\text{min}^{-1} \text{ml}^{-1}$  of enzyme and 1.428  $\mu\text{mol}$   $\text{H}_2\text{O}_2$  decomposed  $\text{min}^{-1}\text{g}^{-1}$  protein, while it was reduced to 0.189 OD value, 9.66  $\mu\text{mol}$  of maltose released  $\text{min}^{-1} \text{ml}^{-1}$  of enzyme and 1.212  $\mu\text{mol}$   $\text{H}_2\text{O}_2$  decomposed  $\text{min}^{-1}\text{g}^{-1}$  protein as the storage period progressed, respectively. At the end nine months of storage, seeds stored in 90 %  $\text{CO}_2$ : 4 %  $\text{O}_2$ : 6 %  $\text{N}_2$  ( $\text{T}_2$ ) recorded maximum enzyme activities which was on par with  $\text{T}_1$ : 95 %  $\text{CO}_2$ : 2 %  $\text{O}_2$ : 3 %  $\text{N}_2$ , where as it was minimum in the seeds stored in HDPE bag with atmospheric air ( $\text{T}_{15}$ : Control) (Table 5- Table 7).

**Table 2. Effect of Modified Atmospheric Storage (MAS) on shoot length (cm) of soybean (*cv.* DSb 34) during storage**

Sl. No.	Concentration of gases (%)			Months of storage (Nov 2023- Aug 2024)					
	$\text{CO}_2$	$\text{O}_2$	$\text{N}_2$	0 (Nov)	2 (Jan)	4 (Mar)	6 (May)	8 (Jul)	9 (Aug)
$\text{T}_1$	95	2	3	16.08	15.90	15.65	15.32	14.92	14.32
$\text{T}_2$	90	4	6	16.08	15.95	15.72	15.41	15.08	14.49
$\text{T}_3$	85	6	9	16.08	15.68	15.15	14.48	13.72	13.06
$\text{T}_4$	80	8	12	16.08	15.65	15.01	14.25	13.56	12.85
$\text{T}_5$	75	10	15	16.08	15.64	14.87	14.13	13.32	12.51
$\text{T}_6$	70	12	18	16.08	15.61	14.78	13.95	13.04	12.13
$\text{T}_7$	65	14	21	16.08	15.58	14.64	13.81	12.86	11.92
$\text{T}_8$	60	16	24	16.08	15.55	14.47	13.60	12.63	11.60
$\text{T}_9$	55	18	27	16.08	15.53	14.24	13.35	12.36	11.43
$\text{T}_{10}$	50	20	30	16.08	15.50	14.02	13.01	12.12	11.18
$\text{T}_{11}$	100	0	0	16.08	15.87	15.59	15.24	14.85	14.25
$\text{T}_{12}$	0	0	100	16.08	15.75	15.35	14.83	14.18	13.45
$\text{T}_{13}$	Atmospheric air (Polythene bag)			16.08	15.45	13.87	12.55	11.64	10.58

<b>T<sub>14</sub></b>	Vacuum packaging	16.08	15.71	15.28	14.66	13.96	13.32
<b>T<sub>15</sub></b>	Control (HDPE bag)	16.08	15.38	13.74	12.11	10.78	9.61
<b>Mean</b>		<b>16.08</b>	<b>15.65</b>	<b>14.83</b>	<b>14.05</b>	<b>13.27</b>	<b>12.45</b>
<b>S. Em ±</b>			<b>0.021</b>	<b>0.033</b>	<b>0.047</b>	<b>0.062</b>	<b>0.065</b>
<b>CD (0.01)</b>			<b>0.081</b>	<b>0.130</b>	<b>0.181</b>	<b>0.238</b>	<b>0.252</b>

HDPE: High Density Polyethylene bag

**Table 3. Effect of Modified Atmospheric Storage (MAS) on root length (cm) of soybean (cv. DSb 34) during storage**

<b>Sl. No.</b>	<b>Concentration of gases (%)</b>			<b>Months of storage (Nov 2023- Aug 2024)</b>					
	<b>CO<sub>2</sub></b>	<b>O<sub>2</sub></b>	<b>N<sub>2</sub></b>	<b>0 (Nov)</b>	<b>2 (Jan)</b>	<b>4 (Mar)</b>	<b>6 (May)</b>	<b>8 (Jul)</b>	<b>9 (Aug)</b>
<b>T<sub>1</sub></b>	95	2	3	14.76	14.47	14.29	13.70	13.14	12.47
<b>T<sub>2</sub></b>	90	4	6	14.76	14.50	14.33	13.75	13.21	12.58
<b>T<sub>3</sub></b>	85	6	9	14.76	14.02	13.39	12.91	12.30	11.43
<b>T<sub>4</sub></b>	80	8	12	14.76	13.98	13.20	12.73	12.17	11.26
<b>T<sub>5</sub></b>	75	10	15	14.76	13.95	13.15	12.64	12.02	11.09
<b>T<sub>6</sub></b>	70	12	18	14.76	13.87	13.03	12.56	11.89	10.98
<b>T<sub>7</sub></b>	65	14	21	14.76	13.85	12.95	12.41	11.77	10.75
<b>T<sub>8</sub></b>	60	16	24	14.76	13.78	12.82	12.32	11.62	10.57
<b>T<sub>9</sub></b>	55	18	27	14.76	13.75	12.64	12.16	11.55	10.41
<b>T<sub>10</sub></b>	50	20	30	14.76	13.61	12.32	11.95	11.38	10.21
<b>T<sub>11</sub></b>	100	0	0	14.76	14.45	14.27	13.68	13.11	12.44
<b>T<sub>12</sub></b>	0	0	100	14.76	14.36	13.75	13.40	12.61	11.75
<b>T<sub>13</sub></b>	Atmospheric air(Polythene bag)			14.76	13.53	12.17	11.35	10.84	9.91
<b>T<sub>14</sub></b>	Vacuum packaging			14.76	14.32	13.58	13.18	12.49	11.61
<b>T<sub>15</sub></b>	Control (HDPE bag)			14.76	13.46	11.88	11.02	10.28	8.03

<b>Mean</b>	<b>14.76</b>	<b>13.99</b>	<b>13.18</b>	<b>12.65</b>	<b>12.03</b>	<b>11.03</b>
<b>S. Em ±</b>		<b>0.011</b>	<b>0.016</b>	<b>0.021</b>	<b>0.029</b>	<b>0.040</b>
<b>CD (0.01)</b>		<b>0.042</b>	<b>0.061</b>	<b>0.080</b>	<b>0.110</b>	<b>0.152</b>

HDPE: High Density Polyethylene bag

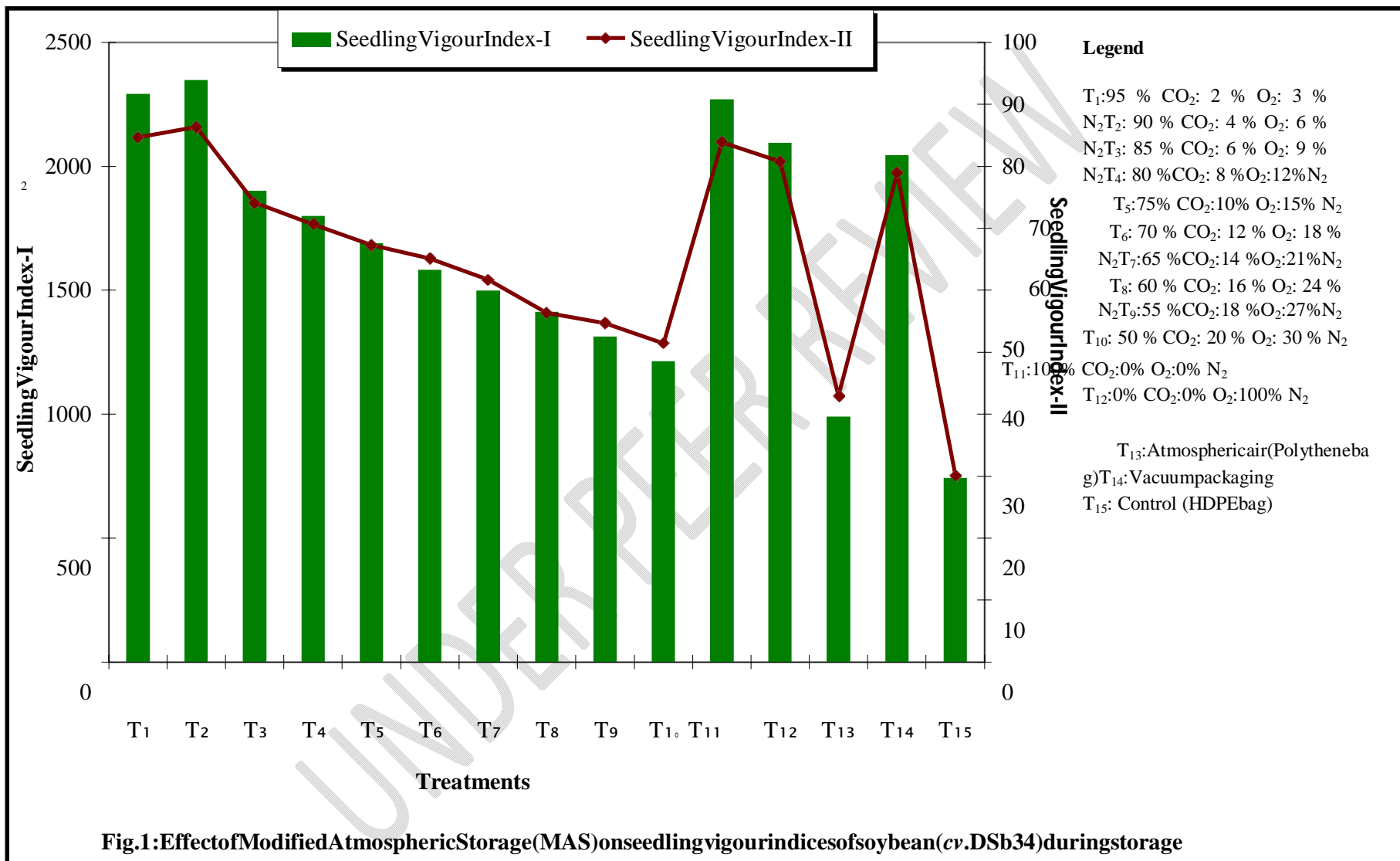
**Table 4. Effect of Modified Atmospheric Storage (MAS) on seedling dry weight (g/ 10 seedlings) of soybean (*cv.* DSb 34) during storage**

Sl. No.	Concentration of gases (%)			Months of storage (Nov 2023- Aug 2024)					
	CO <sub>2</sub>	O <sub>2</sub>	N <sub>2</sub>	0 (Nov)	2 (Jan)	4 (Mar)	6 (May)	8 (Jul)	9 (Aug)
T <sub>1</sub>	95	2	3	1.093	1.083	1.075	1.056	1.033	0.989
T <sub>2</sub>	90	4	6	1.093	1.085	1.076	1.058	1.037	0.995
T <sub>3</sub>	85	6	9	1.093	1.058	1.049	1.024	0.989	0.943
T <sub>4</sub>	80	8	12	1.093	1.055	1.045	1.019	0.972	0.923
T <sub>5</sub>	75	10	15	1.093	1.052	1.041	1.014	0.954	0.901
T <sub>6</sub>	70	12	18	1.093	1.049	1.039	1.011	0.948	0.885
T <sub>7</sub>	65	14	21	1.093	1.046	1.035	0.995	0.916	0.859
T <sub>8</sub>	60	16	24	1.093	1.042	1.032	0.991	0.881	0.823
T <sub>9</sub>	55	18	27	1.093	1.039	1.027	0.986	0.874	0.811
T <sub>10</sub>	50	20	30	1.093	1.035	1.020	0.979	0.852	0.779
T <sub>11</sub>	100	0	0	1.093	1.082	1.073	1.054	1.029	0.986
T <sub>12</sub>	0	0	100	1.093	1.072	1.060	1.048	1.015	0.971
T <sub>13</sub>	Atmospheric air (Polythene bag)			1.093	1.031	1.015	0.971	0.822	0.708
T <sub>14</sub>	Vacuum packaging			1.093	1.070	1.058	1.045	1.008	0.962

<b>T<sub>15</sub></b>	Control (HDPE bag)	1.093	1.030	1.011	0.965	0.793	0.577
<b>Mean</b>		<b>1.093</b>	<b>1.055</b>	<b>1.044</b>	<b>1.014</b>	<b>0.942</b>	<b>0.874</b>
<b>S.Em ±</b>			<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.002</b>	<b>0.003</b>
<b>CD (0.01)</b>			<b>0.003</b>	<b>0.003</b>	<b>0.004</b>	<b>0.008</b>	<b>0.011</b>

HDPE: High Density Polyethylene bag

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**Table 5. Effect of Modified Atmospheric Storage (MAS) on total dehydrogenase activity (OD Value) of soybean (*cv.* DSb 34) during storage**

Sl. No.	Concentration of gases (%)			Months of storage (Nov 2023- Aug 2024)		
	CO <sub>2</sub>	O <sub>2</sub>	N <sub>2</sub>	0 (Nov)	5 (Apr)	9 (Aug)
T <sub>1</sub>	95	2	3	0.648	0.590	0.430
T <sub>2</sub>	90	4	6	0.648	0.593	0.438
T <sub>3</sub>	85	6	9	0.648	0.558	0.387
T <sub>4</sub>	80	8	12	0.648	0.549	0.384
T <sub>5</sub>	75	10	15	0.648	0.535	0.371
T <sub>6</sub>	70	12	18	0.648	0.514	0.357
T <sub>7</sub>	65	14	21	0.648	0.496	0.341
T <sub>8</sub>	60	16	24	0.648	0.477	0.329
T <sub>9</sub>	55	18	27	0.648	0.467	0.294
T <sub>10</sub>	50	20	30	0.648	0.455	0.273
T <sub>11</sub>	100	0	0	0.648	0.588	0.425
T <sub>12</sub>	0	0	100	0.648	0.575	0.412
T <sub>13</sub>	Atmospheric air(Polythene bag)			0.648	0.431	0.248
T <sub>14</sub>	Vacuum packaging			0.648	0.570	0.409
T <sub>15</sub>	Control (HDPE bag)			0.648	0.418	0.189
<b>Mean</b>				<b>0.648</b>	<b>0.521</b>	<b>0.352</b>
<b>S.Em ±</b>					<b>0.003</b>	<b>0.003</b>
<b>CD (0.01)</b>					<b>0.011</b>	<b>0.011</b>

HDPE: High Density Polyethylene bag

**Table 6. Effect of Modified Atmospheric Storage (MAS) on  $\alpha$ - amylase activity ( $\mu\text{mol}$  of maltose released  $\text{min}^{-1} \text{ml}^{-1}$  of enzyme) of soybean (*cv.* DSb 34) during storage**

Sl. No.	Concentration of gases (%)			Months of storage (Nov 2023- Aug 2024)		
	CO <sub>2</sub>	O <sub>2</sub>	N <sub>2</sub>	0 (Nov)	5 (Apr)	9 (Jul)
T <sub>1</sub>	95	2	3	11.28	11.01	10.68
T <sub>2</sub>	90	4	6	11.28	11.04	10.72
T <sub>3</sub>	85	6	9	11.28	10.80	10.22
T <sub>4</sub>	80	8	12	11.28	10.73	9.87
T <sub>5</sub>	75	10	15	11.28	10.62	9.68
T <sub>6</sub>	70	12	18	11.28	10.56	9.59
T <sub>7</sub>	65	14	21	11.28	10.49	9.44
T <sub>8</sub>	60	16	24	11.28	10.41	9.21
T <sub>9</sub>	55	18	27	11.28	10.37	9.09
T <sub>10</sub>	50	20	30	11.28	10.28	8.86
T <sub>11</sub>	100	0	0	11.28	10.99	10.64
T <sub>12</sub>	0	0	100	11.28	10.91	10.53
T <sub>13</sub>	Atmospheric air (Polythene bag)			11.28	9.94	8.28
T <sub>14</sub>	Vacuum packaging			11.28	10.87	10.46
T <sub>15</sub>	Control (HDPE bag)			11.28	9.74	7.62
<b>Mean</b>				<b>11.28</b>	<b>10.58</b>	<b>9.66</b>
<b>S.Em <math>\pm</math></b>					<b>0.002</b>	<b>0.003</b>
<b>CD (0.01)</b>					<b>0.006</b>	<b>0.009</b>

HDPE: High Density Polyethylene bag

**Table 7. Effect of Modified Atmospheric Storage (MAS) on catalase activity ( $\mu\text{mol H}_2\text{O}_2$  decomposed  $\text{min}^{-1} \text{g}^{-1}$  of protein) of soybean (*cv.* DSb 34) during storage**

Sl. No.	Concentration of gases (%)			Months of storage (Nov 2023- Aug 2024)		
	CO <sub>2</sub>	O <sub>2</sub>	N <sub>2</sub>	0 (Nov)	5 (Apr)	9 (Aug)
T <sub>1</sub>	95	2	3	1.428	1.415	1.358
T <sub>2</sub>	90	4	6	1.428	1.419	1.364
T <sub>3</sub>	85	6	9	1.428	1.380	1.301
T <sub>4</sub>	80	8	12	1.428	1.373	1.268
T <sub>5</sub>	75	10	15	1.428	1.365	1.245
T <sub>6</sub>	70	12	18	1.428	1.358	1.228
T <sub>7</sub>	65	14	21	1.428	1.352	1.209
T <sub>8</sub>	60	16	24	1.428	1.340	1.187
T <sub>9</sub>	55	18	27	1.428	1.335	1.168
T <sub>10</sub>	50	20	30	1.428	1.318	1.141
T <sub>11</sub>	100	0	0	1.428	1.413	1.351
T <sub>12</sub>	0	0	100	1.428	1.404	1.334
T <sub>13</sub>	Atmospheric air (Polythene bag)			1.428	1.236	0.895
T <sub>14</sub>	Vacuum packaging			1.428	1.399	1.321
T <sub>15</sub>	Control (HDPE bag)			1.428	1.204	0.811
<b>Mean</b>				<b>1.428</b>	<b>1.354</b>	<b>1.212</b>
<b>S.Em <math>\pm</math></b>					<b>0.002</b>	<b>0.004</b>
<b>CD (0.01)</b>					<b>0.006</b>	<b>0.015</b>

HDPE: High Density Polyethylene bag

## DISCUSSION

In modified atmospheric storage condition, the seeds stored in lower levels of oxygen and higher carbon dioxide concentrations shown higher retention of seed viability during storage period. The differences in storability of seeds is due to the variations in the gas concentrations, where the treatment T<sub>2</sub>(90 % CO<sub>2</sub> + 4 % O<sub>2</sub> + 6 % N<sub>2</sub>) with higher CO<sub>2</sub> and lower oxygen concentration has recorded maximum germination during storage. Higher the levels of carbon dioxide present inside the package, better the maintenance of seed germination, since metabolism during storage is reduced due to decrease in oxygen concentration, thereby maintaining seed viability for a longer period of time [13]. The germination capacity has retained, when seeds were stored in an oxygen-depleted atmosphere which was associated with the inhibition of oxygen-dependent enzymatic activities, allowing greater mobilization of seed reserves for seedling formation has been observed in soybean [14]. Similar findings also reported by Capilheria *et al.*, [15], Raghupathiet *al.*, [16] and Bhattarai *et al.*, [17].

In the present investigation, storage of soybean in high CO<sub>2</sub> concentration coupled with lower O<sub>2</sub> concentrations has been beneficial in maintaining the seedling growth, dry weight and vigour during storage. Seeds stored in elevated carbon dioxide environment within moisture proof containers exhibited high seedling vigour even after 3-5 years of ambient storage. The modified atmosphere storage with carbon dioxide proved superior to air- sealed storage, effectively preserving seed vigour over time [18]. Copeland [19] emphasized the detrimental effects of seed deterioration, which include membrane degradation, the buildup of toxic metabolites, reduced enzymatic activity, lipid auto-oxidation, and the failure of repair mechanisms. These changes can lead to genetic degradation, lower yields, and ultimately, the loss of germination or death. In case of soybean storage, seeds exposed to atmospheric air are vulnerable to auto-oxidation and lipid peroxidation because of high oxygen levels in the air. This process generates free radicals and hydrogen peroxide, which damage cell structures and result in poor seed viability and vigour. The declining seedling vigour could be attributed to oxidative stress, which triggers the generation of reactive oxygen species (ROS). These ROS lead to lipid peroxidation, producing reactive aldehydes like malondialdehyde (MDA) and 4-hydroxynonenal (HNE) as end products. These compounds disrupt cellular metabolism, reducing ATP production, which is essential for the growth and development of the seedling [20]. Similar findings were also reported by Anuja *et al.*, [21] and Divya *et al.*, [22].

Seeds are naturally inbuilt with the various kinds of enzymatic and non enzymatic antioxidants, which play a crucial role in prolonging seed longevity. In general, ageing is characterized by decrease of metabolic activity and an increase in catabolic processes. Seed deterioration during storage can lead to significant changes in the content and activity of enzymes responsible for utilizing stored reserves [23]. The better maintenance of vigour and viability in the seeds stored in modified atmospheric storage confirms a direct correlation between seed germination and enzyme activity. Similar results were also reported by Bera *et al.*, [24], Manjunatha *et al.*, [25] and Punithavathiet *al.*, [26].

## CONCLUSION

The results of modified atmospheric storage effect on seed longevity of soybean confirmed that, all the seed quality attributes and enzyme activities were higher under the modified atmospheric storage conditions of 90% CO<sub>2</sub>, 4% O<sub>2</sub>, and 6% N<sub>2</sub> throughout the nine-months storage period. This demonstrates that, this gaseous combination has a markedly positive impact on preserving the quality and vigour of soybean seeds compared to control condition. In conclusion, storing soybean seeds under modified atmospheric conditions characterized by high CO<sub>2</sub> and low O<sub>2</sub> level proves to be the most effective method for maintaining seed longevity by minimizing the seed deterioration.

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## REFERENCES

1. Ramlal A, Mehta S, Nautiyal A, Baweja P, Sharma D, Lal S K, Vijayan R, Raju D, Subramaniam S, Rajendran A. Androgenesis in soybean [*Glycine max* (L.) Merrill]: a critical revisit. *In Vitro Cellular and Developmental Biology-Plant*.2024; 60(1): 1-15.
2. Rahman MM, Hossain MM, Anwar MP, Juraimi AS. Plant density influence on yield and nutritional quality of soybean seed. *Asian Journal of Plant Science*. 2011; 10(2): 125–132.
3. Anonymous. Area, production and productivity of soybean. 2023. [www.faostat.com](http://www.faostat.com).
4. Alencar ERD, Faroni LR, Peternelli LA, da Silva MT, Costa AR. Influence of soybean storage conditions on crude oil quality. *Brazilian Journal of Agricultural and Environmental Engineering*. 2010; 14: 303-308.
5. Moltos LM, Morretti CL, Ferrira DM. Modified atmosphere packaging for perishable plant products. *Polypropylene*. 2002; 95: 21-22.
6. Jayas DS, Jeyamkondan S. Modified atmosphere storage of grains meats fruits and vegetables. *Biosystems Engineering*.2002; 82: 235–251.
7. Capilheira AF, Cavalcante JA, Gadotti GI, Bezerra BR, Hornke NF, Villela FA. Storage of soybean seeds: Packaging and modified atmosphere technology. *Brazilian Journal of Agricultural and Environmental Engineering*. 2019; 23: 876-882.
8. ISTA. *International Rules For seed testing Switzerland: International Seed Testing Association*; 2013.
9. Abdul-Baki AA, Anderson JD. Vigour determination in soybean by multiple criteria. *Crop Science*. 1973; 13(6): 630-633.
10. Kittock DL, Law AG. Relationship of seedling vigour to respiration and tetrazolium chloride reduction by germination wheat seeds. *Agronomy Journal*. 1968; 60: 286-288.
11. Sadasivam S and Manickam A. *Biochemical methods for agricultural sciences*, New Age International (P) Ltd., New Delhi. 1996; 1-97.
12. Aebi H. Catalase in vitro. *Methods in Enzymology*. 1984; 105: 121-126.

13. Mussi MM. Germination and vigour of sunflower seeds (*Helianthus annuus* L.) subjected to different concentrations of CO<sub>2</sub>, exposure periods, and packaging. Curitiba.2005; 66.
14. Ludwig V, Berghetti MRP, Rossato FP, Wendt LM, Schultz EE, Both V, Brackmann A. Impact of controlled atmosphere storage on physiological quality of soybean seed. Journal of Stored Products Research. 2021; 90: 101749.
15. Capilheira AF, Cavalcante JA, Gadotti GI, Bezerra BR, Hornke NF, Villela FA. Storage of soybean seeds: Packaging and modified atmosphere technology. Brazilian Journal of Agricultural and Environmental Engineering. 2019; 23: 876-882.
16. Raghupathi B, Padmasri A, Narayanamma VL, Ramya V. Effect of modified atmosphere with elevated levels of CO<sub>2</sub> on seed quality parameters of green gram during storage. The Pharma Innovation Journal. 2021; 10(11): 53-59.
17. Bhattarai B, Walker CK, Wallace AJ, Nuttall JG, Hepworth G, Panozzo JF, Partington DL, Fitzgerald GJ. Modified storage atmosphere prevents the degradation of key grain quality traits in lentil. Agronomy. 2023; 13(8): 2160.
18. Doijode SD. Low cost seed storage techniques for certain hardy indigenous pod vegetables. International Conference on Indigenous Vegetables and Legumes. Prospectus for Fighting Poverty, Hunger and Malnutrition. 2006; 752: 589-592.
19. Copeland LL. Seed germination In: Principles of Seed Science and Technology. Surjeet Publications, Delhi. 1985; 55-212.
20. Islavath. Effect of packaging materials and storage conditions on seed longevity in onion (*Allium cepa* L.). Ph.D Thesis, University of Agricultural Sciences, Dharwad, Karnataka, India.2022.
21. Anuja G, Sinha SN, Atwal SS. Modified atmosphere technology in seed health management: laboratory and field assay of carbon dioxide against storage fungi in paddy. Plant Pathology Journal. 2014; 13(3): 193-199.
22. Divya P, Durga KK, Sunil N, Rajasri M, Keshavulu K, Udayababu P. Modified atmosphere storage technique for the management of pulse beetle *Callosobruchus chinensis* in horse gram. Legume Research-An International Journal. 2016; 39(3): 474-478.

23. Vasudevan SN, Shakuntala NM, Teli S, Goud S, Gowda B. Studies on effect of modified atmospheric storage condition on storability of groundnut (*Arachis hypogaea* L.) seed kernels. *International Journal of Research Studies in Biosciences*. 2014; 2(2): 25-36.
24. Bera A, Sinha SN, Singhal NC, Pal RK, Srivastava C. Studies on carbon dioxide as wheat seed protectant against storage insects and its effect on seed quality stored under ambient conditions. *Seed Science and Technology*. 2004; 32:159-169.
25. Manjunatha B, Vasudevan SN, Umesha U, Sravani C. Studies on influence of modified atmospheric storage conditions on biochemical parameters in pigeonpea seeds. *Journal of Applied and Natural Science*. 2016; 8(3): 1249-1252.
26. Punithavathi R, Parameswari K, Umarani R, Eevera T, Anand T, Gomathi V. Exploring the storage potential of groundnut Var. TMV (Gn) 13 under modified atmospheric storage conditions. *International Journal of Environment and Climate Change*. 2023; 13(10): 3982-3989.