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Modified Storage Atmosphere Prevents the Degradation of Key Grain Quality Traits in Lentil

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Abstract: Lentil seed coat colour influences market value, whilst germination is associated with crop establishment and hydration capacity with optimal processing outcomes. Storing lentil grain assists growers in managing price fluctuations; however, exposure to oxygen at higher temperatures during extended storage degrades seed coat colour, germination, and hydration capacity. Depleting oxygen prevents such degradation in other crops; however, studies in lentil are limited. This study examined the effects of oxygen-depleted modified atmospheres and temperatures on seed coat colour, germination, and hydration capacity in two red lentil cultivars, PBA Hallmark and PBA Jumbo2, stored for 360 days. Small volumes of lentil grain were placed in aluminium laminated bags filled with nitrogen (N₂), carbon dioxide (CO₂), or air and stored at either 15 or 35 °C. At 35 °C in an air atmosphere, the lentil's seed coat significantly ($p = 0.05$) darkened after 30 days of storage, whereas germination and hydration capacities decreased after 60 days regardless of cultivar. In contrast, N₂ and CO₂ atmospheres maintained initial seed coat colour, germination, and hydration capacities in both cultivars throughout the study period regardless of temperature. Storing lentil grain in an oxygen-depleted modified atmosphere may assist to maximise returns to grower and maintain key quality traits.

Keywords: post-harvest; oxygen-depleted storage; seed colour; germination; hydration



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1. Introduction

On-farm storage is an important practice for lentil growers to safeguard against price volatility. However, extended periods of storage in an oxygen-rich environment can result in the degradation of the visual and physical traits that determine the market value of grains such as chickpeas [1]. The degradation in the quality traits of grains stored in an oxygen-rich environment is linked with the breakdown of bio compounds such as phenolic compounds, change in metabolic activities and grain respiration in green lentils [2,3]. Modified atmospheric storage refers to limiting the oxygen (O₂) concentration within the storage facility by introducing nitrogen (N₂) and/or carbon dioxide (CO₂) in a controlled manner [4]. These storage systems effectively assist in minimising oxidative processes over time and limit oxygen-dependent biochemical reactions in grain stored at suboptimal conditions, thereby preventing degradation in quality traits as observed in faba beans [5], carioca beans [6] and cowpeas [7]. Nitrogen or carbon dioxide gases are lethal to insects in groundnuts [8], wheat [9] and a range of food grains [10]; therefore, growers adopt N₂ or CO₂-modified atmospheric storage methods to control insect infestation. However, studies

investigating the impact of modified atmospheric storage conditions on key quality traits of lentils are currently limited.

Seed coat colour is an important visual trait that significantly influences the market value of pulse grains [11], as brighter seed coats are preferred by consumers [12]. The seed coat colour of pulse grains has been observed to deteriorate over time during extended storage at high temperatures, as observed in pinto beans [13]. Seed coat darkening is reported to be linked with oxidative biochemical changes, including oxidation of phenolic compounds in green lentils [2], tannins in *Phaseolus* beans [14] and proanthocyanidins in faba beans [15]. These biochemical changes accelerate from as early as 3 weeks after storage at or above 30 °C [13]. Storing grains under N₂-modified atmospheric storage conditions has been identified as reducing seed coat darkening by preventing oxidation of phenolic compounds in faba beans [5] and wheat [9].

Germination capacity in pulses is a significant determinant of quality for growers and consumers [16]. Achieving over 90% germination capacity in pulse grains improves crop establishment [17,18] and reduces seeding rates and input costs [19,20]. In addition, higher germination capacity in pulse grains enhances their nutritional value, as observed in chickpeas [21], mung beans [22] and green peas [23]. Prolonged storage at higher temperatures and in oxygen-rich environments have been identified as increasing biochemical changes such as increases in enzyme activity and protein breakdown, which was reported to link with seed aging and loss of viability, as observed in wheat [24] and maize [25]. In contrast, germination capacity is maintained by depleting oxygen in storage systems using a combination of Nitrogen- (N₂) and Carbon dioxide- (CO₂) modified atmospheres, as reported in groundnut [8]. Additionally, storing grain under reduced oxygen conditions was reported to reduce the rate of loss in germination capacity by reducing enzymatic activity and seed respiration in soybeans [26].

The hydration capacity is an important physical quality trait linked to grain hardness and results in suboptimal milling and cooking outcomes. Lower hydration capacity was linked to increased grain hardness in adzuki beans [27], resulting in an unacceptable milling yield in pigeon peas [28], and reduced cooking time in adzuki beans [29]. The accelerated oxidative breakdown of phenolic compounds in faba beans [15] and starch hydrolysis in cowpeas [7] at higher temperatures have been reported to be associated with cotyledon hardening, which, in turn, is postulated to lead to lower hydration capacity. Whereas storing grains under oxygen-depleted atmospheres has been shown to successfully maintain hydration capacity by preventing lignification in carioca beans [30].

There is limited knowledge of the relationship between oxygen-depleted modified atmospheric conditions and lentil quality when storing grain for extended periods. In semi-arid regions, such as Southern Australia, temperatures often exceed 30 °C during the lentil harvesting season [31], which can result in higher storage temperature conditions. Consequently, there is an increased risk of accelerating oxidative deterioration of quality traits in stored red lentil grains. Quality traits may be maintained in storage environments with limited or no oxygen. In addition, the available research primarily focused on studying the impact of modified storage conditions in a single cultivar or grain type. There is a research gap that needs to be addressed to understand if storage conditions impact different lentil cultivars. This study investigated the impact of modified atmospheres, storage temperature and storage time on the seed coat colour, hydration, and germination capacity of Australian red lentil cultivars over a period of 360 days. This research tested the hypothesis that oxygen-depleted atmospheric storage environments will prevent darkening of the seed coat and loss of germination and hydration capacity in red lentils regardless of storage temperature.

2. Materials and Methods

2.1. Sample Preparation

A single source of grain for two commercial red lentil cultivars (*Lens culinaris* var. PBA Hallmark and *Lens culinaris* var. PBA Jumbo2) classified as 'Grade 1' was sourced at 10%

grain-moisture content from a commercial trader at Horsham, Victoria, Australia, after the December 2019 growing season. These cultivars were selected as they are dominant commercial cultivars grown in southern Australia and carry significant importance to both growers and traders due to their higher productivity and export value. Based on seed coat colour characteristics PBA Hallmark represents a darker seed coat (CIE L^* 43.7), and PBA Jumbo2 represents a brighter seed coat (CIE L^* 47.7).

Nitrogen (N_2) and carbon dioxide (CO_2) gases were selected as a source of oxygen-depleted atmospheres, where air was chosen as the control to compare the effects of an oxygen-depleted storage-modified atmospheric environment on grain quality traits. Additionally, two different storage temperatures, 35 and 15 °C, were selected to represent suboptimal and contrasting storage conditions, respectively. A storage period of 360 days was selected to represent one year of storage from the previous harvest until the next harvest cycle.

2.2. Experimental Design

A factorial storage experiment with 3 replicates was undertaken over 360 days, testing three storage atmospheres (N_2 , CO_2 and air), two storage temperatures (15 and 35 °C) and two red lentil cultivars (PBA Hallmark, and PBA Jumbo2). One hundred grams of lentil seeds were placed into aluminium laminated, heat-sealable bags and respective gases (N_2 , CO_2 and air) were added. N_2 (>99% purity) was produced through a nitrogen generator (Domnick Hunter, Corby, UK), CO_2 (99.9% purity) was sourced from a supplier (Supagas, Dandenong South, Victoria, Australia), and compressed air was generated through a pump (Hydrovane, Redditch, UK). All gases were manually added into a bag through a pipe connected to a regulator for the respective gas source. The pipe was inserted into the bag, and the gases were forcedly introduced into the bags, already filled with grains, from one corner at 45 Pounds per Square Inch (PSI) for approximately 20 s. The gases were then forced out from another corner to ensure complete displacement of air. Bags were sealed immediately using a heat sealer. Bags were stored at 15 °C in a cool chamber (Carel, Brugine, Italy) with 60% relative humidity and at 35 °C in an oven (Memmert, Schwabach, Germany) with 45% relative humidity during the entire 360 days. Multiple bags were prepared for each replicate of each treatment to enable regular sampling without the need to repeat atmospheric preparation. Bags were destructively sampled and selected at 30-day intervals, with grain analysed for seed coat colour, germination, and hydration capacities.

2.3. Quality Assessment Traits

2.3.1. Seed Coat and Cotyledon Colour

The degree of seed coat darkening was quantified using a Minolta Spectrophotometer (CM5, Hamburg, Germany) based on the Commission Internationale l'Elclairage (CIE) values L^* , a^* and b^* systems. The degree of darkening was calculated as CIE ΔE^*_{ab} (seed coat colour index) = $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ as described by Nasar-Abbas et al. [5]. Where ΔL^* , Δa^* and Δb^* are the difference between the original L^* , a^* and b^* values and each time series interval. CIE ΔE^*_{ab} was selected to represent darkening as it measures darkening in three dimensions with change in seed coat brightness (ΔL^*), redness (Δa^*) and yellowness (Δb^*). To record visual changes in seed coat colour (grains measured for the degree of seed coat darkening through spectrophotometer), images were taken using a Tagarano microscope (Tagarano, A/S, Horsens, Denmark) at $4.3\times$ magnification with a resolution of 1920×1080 as described by Assadzadeh et al. [32]. The instrument was calibrated according to the instrument requirements of Tagarano.

2.3.2. Germination Capacity

Germination capacity was calculated by germinating 100 seeds in a petri dish. All 100 seeds were placed on Whatman paper (No. 1) in a 90-mm Petri dish with 8 mL of distilled water and incubated at 20 °C with 60–70% humidity for 72 h. The total number

of seeds germinated at the end of 72 h was considered as the final germination capacity similar to that described by Nithya et al. [24] and Sathya et al. [33].

2.3.3. Hydration Capacity

The initial gravimetric weight (W_0) of 100 seed was measured and soaked in 60 mL of water at 20 °C for a period of 8 h. After 8 h, the seeds were drained and dried using double-layered tissue paper to remove excess water and reweighted (W_1). Hydration capacity was calculated as percentage water uptake calculated as $[(W_1 - W_0)/W_0] \times 100\%$, similarly as described by Kon [34] and Yousif et al. [35].

2.4. Statistical Analysis

The effect of storage time, temperature and modified atmospheres on key quality traits of cultivars were analysed using a four-factor analysis of variance (ANOVA) based on a factorial design model using GenStat (22nd edition) software [36]. To compare factor combination means over the storage period, the least significant difference (LSD) was calculated at the 0.05 level of significance. The estimated linear rate of change in traits over time for cultivars, influenced by storage temperature and the modified atmosphere, was analysed by a component variance analysis based on a linear mixed model using GenStat (22nd edition) software.

3. Results

3.1. Seed Coat Colour

The seed coat colour was significantly ($p \leq 0.01$) affected by a four-way interaction of storage atmosphere, temperature, cultivar, and storage time. The seed coat colour of grains stored in air at 35 °C exhibited the highest rate of darkening throughout the entire study period (Figures 1 and 2). Under these storage conditions, a significant increase in the seed coat colour index was observed after 30 days of storage in both cultivars, and this trend continued linearly until the end of the experiment. However, PBA Hallmark was observed to have a higher rate of darkening (9.039×10^{-3} CIE ΔE^*_{ab} increase per day) compared to PBA Jumbo2 (6.061×10^{-3} CIE ΔE^*_{ab} increase per day) (Table 1). The seed coat color index of both cultivars stored in N₂ and CO₂ atmospheres at 35 °C exhibited a minimal rate of increase throughout the study period. There was almost a three-times higher rate of darkening for both cultivars stored in air at 35 °C compared to N₂ and CO₂ atmospheres at the same temperature. Grains stored in either N₂ or CO₂ at 35 °C and stored at 15 °C regardless of modified atmospheres, maintained a change of no more than 1 CIE ΔE^*_{ab} unit over time (Figures 1 and 2) until the end of the experiment (with no more than an increase of 2.750×10^{-3} CIE ΔE^*_{ab} per day).

Table 1. Daily rate of change in quality traits of red lentil cultivars stored in different atmospheres and temperatures. The average rate of change in individual trait \pm margin of errors (sharing different letters) is significantly different at $p = 0.05$ within that trait according to variance component analysis.

Quality Traits	Units	Margin of Errors*	Cultivars	Temperature (°C)	Rate of Change in Traits per Day ($\times 10^{-3}$)					
					Storage Atmospheres					
					N ₂	CO ₂	Air			
Seed Coat Colour	CIE ΔE^*_{ab}	2.624	PBA Hallmark	15	2.194 ^a	2.472 ^a	3.094 ^a			
				35	2.633 ^a	2.750 ^a	9.039 ^b			
			PBA Jumbo2	15	2.541 ^a	2.427 ^a	2.638 ^a			
				35	2.563 ^a	2.394 ^a	6.061 ^c			
			Germination Capacity	%	8.741	PBA Hallmark	15	6.261 ^a	6.483 ^a	7.408 ^a
							35	6.940 ^a	6.787 ^a	−20.186 ^b
PBA Jumbo2	15	7.408 ^a				7.960 ^a	−8.290 ^a			
	35	7.975 ^a				8.483 ^a	−15.741 ^c			
Hydration capacity	%	1.461				PBA Hallmark	15	−11.563 ^a	−11.410 ^a	−20.825 ^b
							35	−12.174 ^a	−11.694 ^a	−24.534 ^c
			PBA Jumbo2	15	−10.772 ^a	−11.812 ^a	−23.483 ^c			
				35	−10.374 ^a	−12.519 ^a	−29.301 ^d			

* Half-width of 95% confidence interval.

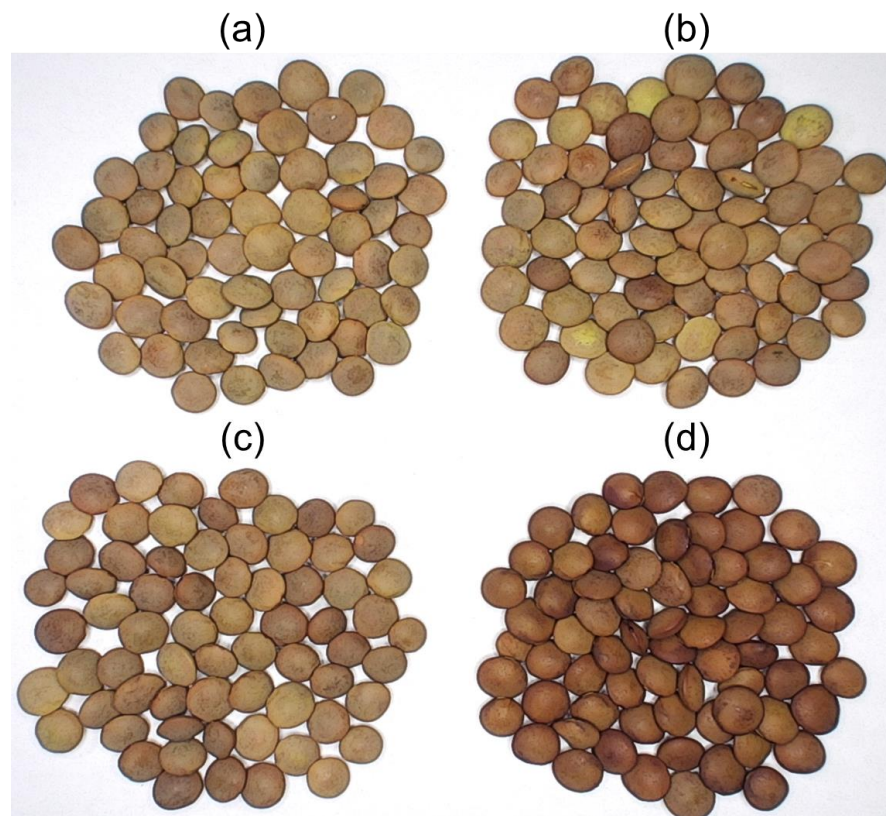


Figure 1. Impact of modified atmospheric storage condition on seed coat colour of red lentils (PBA Hallmark) at 35 °C before and after 360 days of storage. The seed coat colour (a) before storage, after 360 days of storage (b) in N₂, (c) in CO₂ and (d) in air, respectively.

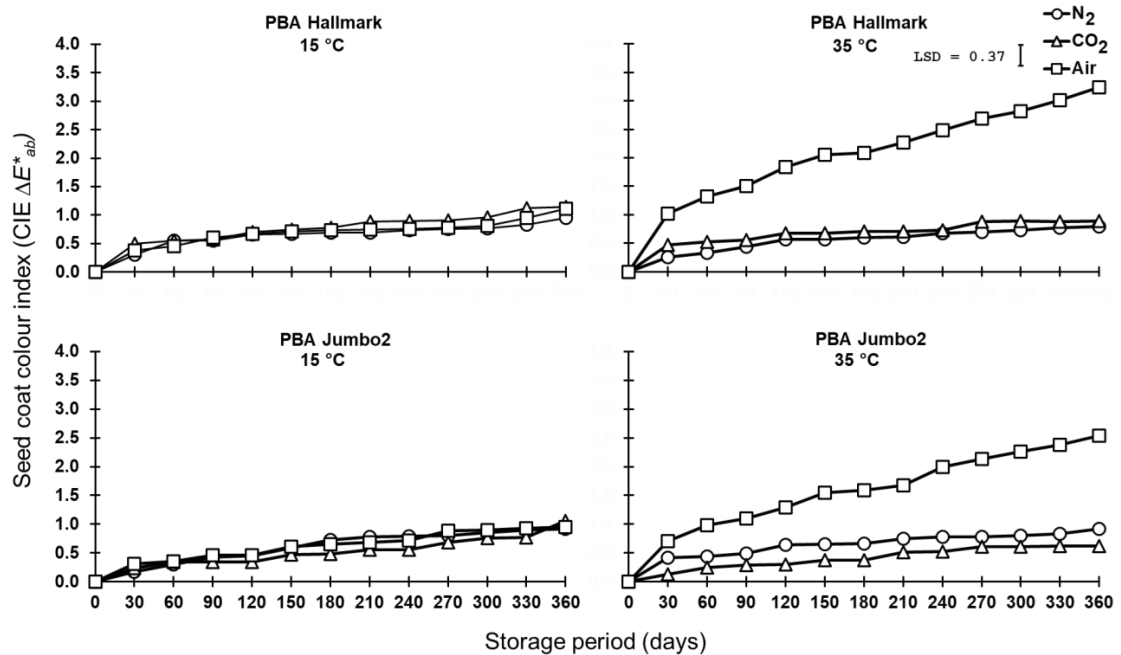


Figure 2. Impact of storage conditions on seed coat colour of red lentil cultivars stored at three atmospheres and two temperatures, measured every 30 days over a period of 360 days. Vertical bar represents the LSD = 0.37 ($p = 0.05$) for the interaction of atmospheres, temperatures, and cultivars over the storage period.

3.2. Germination Capacity

The germination capacity was significantly ($p \leq 0.01$) affected by a four-way interaction of storage atmosphere, temperature, cultivar, and storage time. The germination capacity of both cultivars significantly decreased after 60 days of storage, irrespective of the atmosphere and temperature treatments (Figure 3). However, the most significant reduction was observed when grains were stored in air at 35 °C. PBA Hallmark was observed to have a higher rate of reduction in germination capacity, with $20.186 \times 10^{-3}\%$ linear rate of reduction per day, whereas $15.741 \times 10^{-3}\%$ linear rate of reduction per day was observed for PBA Jumbo2. Despite this significant reduction, germination capacity for both cultivars remained above 90% throughout the study period. There was almost a three-times higher rate of reduction in germination capacity for PBA Hallmark and more than two times higher for PBA Jumbo2 stored in air at 35 °C compared to N₂ and CO₂ atmospheres at the same temperature. The rate of reduction in germination capacity was minimal (no more than $8.483 \times 10^{-3}\%$ linear rate of reduction per day) for both cultivars stored in either N₂ or CO₂ at 35 °C or stored at 15 °C, regardless of the atmosphere. Under the N₂ and CO₂ atmospheric treatments, both cultivars maintained more than 95% of their germination capacity throughout the study period.

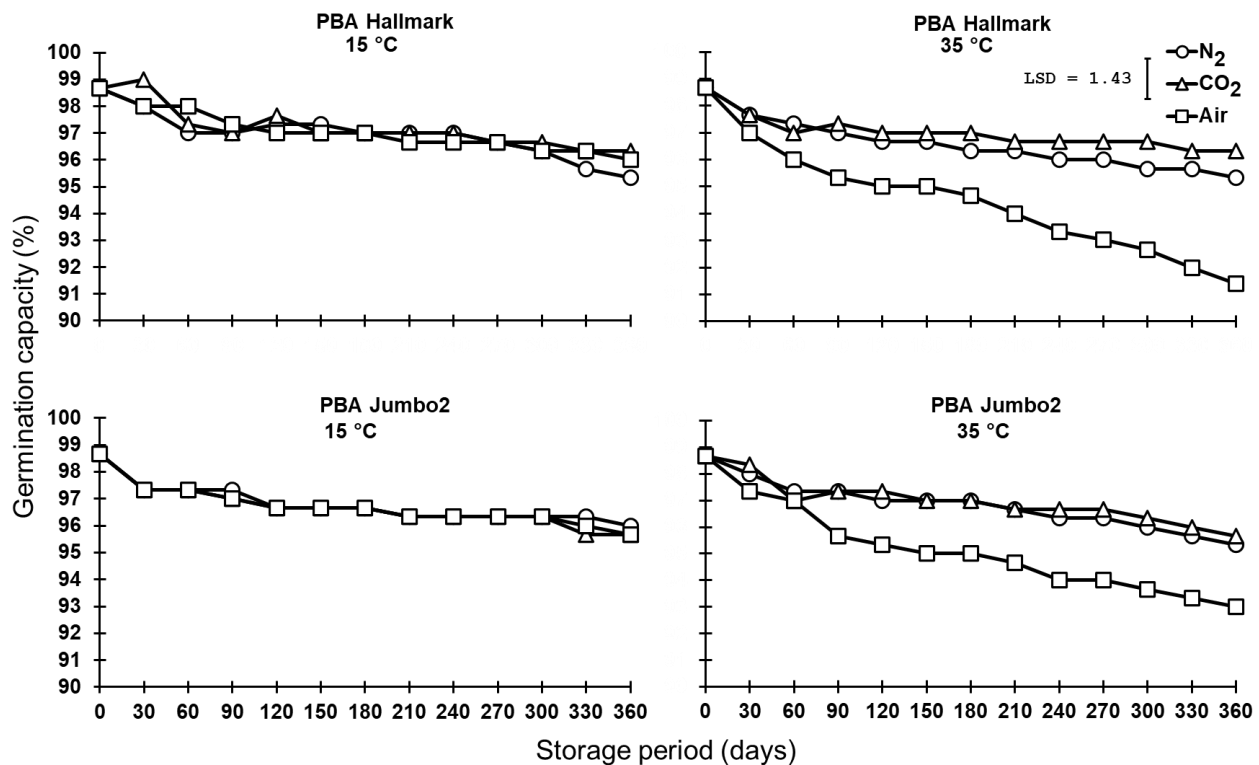


Figure 3. Impact of storage conditions on germination capacity of red lentil cultivars stored at three atmospheres and two temperatures, measured every 30 days over a period of 360 days. Vertical bar represents the LSD = 1.43, ($p = 0.05$) for the interaction of atmospheres, temperatures, and cultivars over the storage period.

3.3. Hydration Capacity

The hydration capacity was significantly ($p \leq 0.01$) affected by a four-way interaction of storage atmosphere, temperature, cultivar, and storage time. Throughout the experiment, the hydration capacities for both cultivars were significantly reduced in the air treatment at 15 °C and 35 °C and in the N₂ and CO₂ treatments at 35 °C (Figure 4). However, the highest rate of reduction was observed when lentils were stored in air at 35 °C. Significant reductions were observed after 60 days of storage for both cultivars in air at 35 °C, and this continued in a linear fashion over time until the end of the experiment.

However, the rate of reduction in the hydration capacity was observed to be higher for PBA Jumbo2 ($29.301 \times 10^{-3}\%$ linear rate of reduction per day) compared to PBA Hallmark ($24.538 \times 10^{-3}\%$ linear rate of reduction per day). Similarly, at 35 °C, there were no significant reductions observed until 210 days for both cultivars stored in N₂ and not until 300 days in CO₂ for both cultivars. The rate of reduction in hydration capacity in air at 35 °C was almost two times higher for both cultivars compared to N₂ and CO₂ atmospheres at 35 °C. No significant reduction was observed for cultivars stored in the N₂ and CO₂ at 15 °C treatments over time. A significant reduction was observed after 120 days of storage for lentil cultivars stored at 15 °C in air.

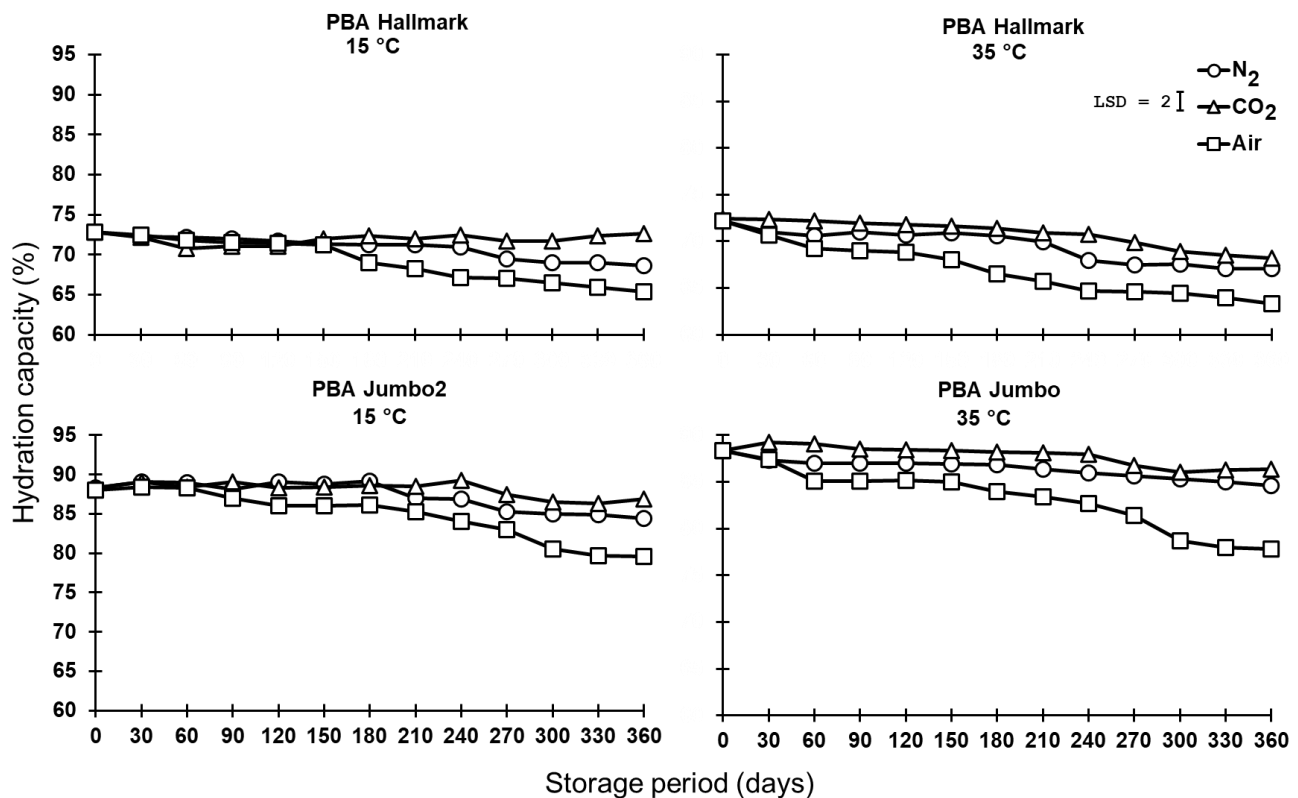


Figure 4. Impact of storage conditions on the hydration capacity of red lentil cultivars stored at three atmospheres and two temperatures, measured every 30 days over a period of 360 days. Vertical bar represents the LSD = 2, ($p = 0.05$) for the interaction of atmospheres, temperatures, and cultivars over the storage period.

Grain moisture was measured at 30-day intervals across all treatments. The change in grain moisture content was not significantly different across the treatments throughout the study period. PBA Hallmark maintained a moisture content of $10 \pm 0.05\%$ (Standard Deviation, SD) at 15 °C and $10 \pm 0.06\%$ (SD) at 35 °C when measured at all sampling times across treatments. Similarly, PBA Jumbo2 maintained a moisture content of $10 \pm 0.06\%$ (SD) at 15 °C and $10 \pm 0.06\%$ (SD) at 35 °C when measured at all sampling times across treatments.

4. Discussion

Storage temperatures exceeding 30 °C significantly increased the risk of seed coat darkening in pinto beans [13], reduced germination capacity in lentils [37] and reduced the hydration capacity in chickpeas [1]. On the other hand, degradation in these traits in these grains was shown to be prevented when stored at lower temperatures below 20 °C [13,24,37]. In semi-arid climatic regions where lentils could be harvested and stored at high temperatures for extended periods, without the option of aeration cooling to lower

the storage temperature below 20 °C, depleting the oxygen concentration (applying either N₂ or CO₂) in a sealed storage environment as early as 30 days after harvest may assist in maintaining key quality traits, such as seed coat colour, germination, and hydration capacity, in red lentils regardless of cultivars

The findings from this study indicated that depleting oxygen concentrations in lentil storage facilities slowed the rate of seed coat darkening at higher storage temperatures (35 °C), thereby maintaining seed coat colour and maximising economic returns. These results are similar to the findings reported in faba beans [5] and cowpeas [7], where seed coat colour was maintained above 20 °C for 1 year when grain was stored under N₂. Similar observations for apple pieces [38] and pear pieces [39] have been reported. Slowing the rate of seed coat darkening in oxygen-depleted atmospheric environments is likely to be related to the prevention of the oxidative breakdown of phenolic compounds, as reported for faba beans [15] and green lentils [2]. To better understand this mechanism in red lentils, a detailed investigation into the grain's chemistry throughout storage is necessary, with a particular focus on the breakdown of phenolic compounds.

Storing lentils in an oxygen-depleted environment is more likely to retain seed viability over time, which can lower input costs and reduce seeding rate requirements [20]. Furthermore, the improved nutritional quality of germinated pulse grains has been associated with higher germination capacity [23]. Thus, when red lentils are stored in an oxygen-depleted atmospheric environment, food manufacturers and consumers will benefit through higher sprout yields and the retention of nutritional benefits. Similarly, oxygen-depleted atmospheric storage environments retain an optimal hydration capacity, improving processing outcomes and maximising economic returns for lentil processors. In this study, low rates of reduction in germination and hydration capacity in oxygen-depleted atmospheric environments at higher temperatures were observed, which is similar to findings for chickpeas [40], soybeans [26] and carioca beans [30]. More than a 90% germination capacity was maintained for chickpeas when stored under a combination of 80% CO₂ and 20% N₂ for one year [40]. Similarly, in another study, soybeans maintained a germination capacity when stored under CO₂ compared to ambient air [26]. In carioca beans, hydration capacity was maintained when stored with N₂ for 360 days, as compared to an ambient environment [30]. Retaining optimal germination capacity when storing grains in an oxygen-depleted atmosphere may be associated with the inhibition of oxygen-dependent enzymatic activities, allowing greater mobilization of seed reserves for seedling root formation, as observed in soybeans [26]. In carioca beans, an optimal hydration capacity was likely retained due to the prevention of structural changes, such as lignification and thickening of cell walls [30]. To further validate this observation in red lentils, a comprehensive investigation into the relationship between limiting oxygen exposure and enzymatic reactions and structural changes in the grain is required.

Extended research is required to assess the impact of oxygen-depleted modified atmospheric storage conditions in commercial-scale storage facilities and validate their practicality in real-world scenarios. Air-sealed storage facilities equipped with pressure relief valves could potentially apply N₂ or CO₂ gases to create an oxygen-depleted atmospheric environment to upscale the experiment. Higher storage temperatures have been reported to degrade quality traits of pulse grains [13,37]; however, an oxygen-depleted atmospheric environment was demonstrated to maintain quality traits at high temperatures without requiring cooling systems. A comprehensive economic analysis of the costs involved in implementing oxygen-depleted storage would be highly advantageous for growers. This analysis would assist them in understanding the trade-off between the expenses associated with oxygen-depleted storage and those related to aeration cooling. With this information, growers would be better prepared to make informed economic decisions for extending the storage of lentil grains on-farm. In addition, further research is needed to determine the threshold concentrations of N₂ and CO₂ to store red lentils to reduce the risk of grain quality degradation at different temperatures, as only two temperatures were assessed in this study. Whilst both cultivars exhibited changes in grain quality that occurred at

different rates, it is necessary to assess a diverse range of cultivars and seed sources to fully understand the potential rates of quality decline over time. This knowledge will be valuable for breeding programs, facilitating the development of improved cultivars that can better maintain grain quality during storage. Studying a broader range of traits will add further evidence to potentially benefit storing red lentils in oxygen-depleted atmospheric environments.

5. Future Direction

The results of this study provide an initial proof of concept whereby storing red lentils under oxygen-depleted atmospheric environments may assist in maintaining grain quality during extended periods of storage. The current research serves as a foundation for future studies aiming to upscale the experiment to a commercial scale, exploring various cultivars and modified atmospheric conditions to assess the feasibility of implementing modified atmospheric storage systems. In addition, the observed variations in the response rates of tested lentil cultivars across treatments highlight the importance of future research aimed at investigating how different cultivars perform in different growing regions to validate the consistency of their response rates.

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