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Germination and Vigor of Maize Seeds: Pilot-Scale Comparison of Low-Oxygen and Traditional Storage Methods

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Abstract: Seed quality declines during storage depending on relative humidity, temperature, and oxygen concentration. Low-oxygen atmospheres significantly enhanced the germination and vigor of seeds during storage in laboratory-scale experiments. Low-oxygen atmospheres include self-modified atmospheres, where gas composition changes due to microbial respiration and oxidative processes, as well as modified atmospheres, where gas composition is initially altered from an external source without further adjustments. However, the potential of low-oxygen atmospheres to preserve the quality of maize (*Zea mays*) seeds in bags of 25–50 kg capacity, like those employed by seed companies and small-scale farmers, remains underexplored, hindering a broader adoption of this storage technology. Our study assessed the feasibility of applying low-oxygen atmospheres for seed storage on the pilot scale, i.e., hermetic containers of 25 kg capacity made of polyethylene and polyamide, under controlled conditions. We first evaluated the ability of the hermetic containers to maintain low oxygen levels over time. Then, we compared the germination and vigor of seeds stored in the hermetic containers under modified and self-modified atmospheres with those stored in traditional poly-paper bags under normal atmospheric conditions. The seeds had 14% moisture content (wet basis) and were stored at 25 °C and 10 °C. Maintaining low oxygen levels in polyethylene–polyamide bags was feasible. Moreover, at 25 °C, modified and self-modified atmospheres maintained higher germination values (95.8% and 94.4%, respectively) compared to traditional storage (68.3%), and both were as effective as refrigeration (97.6%). However, refrigeration was better for preserving seed vigor, with radicle emergence values of 85.2% in self-modified atmospheres and 78.9% in modified atmospheres, compared to 65.0% and 61.2%, respectively, at 25 °C. In conclusion, the advantages of modified atmospheres observed in laboratory-scale studies are achievable on a larger scale with a proper container design, advancing the prospects for the practical application of this technology for the seed industry and small farmers.

Keywords: physiological quality; *Zea mays*; hermetic storage; modified atmosphere; refrigeration

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1. Introduction

Maize (*Zea mays*) is one of the top three crops in the world, with over 1.22 billion metric tons produced in 2024 [1,2]. Losses of germination and vigor in maize seeds during storage, therefore, are of major concern [3–5]. Seed quality losses during storage depend primarily on relative humidity (or seed moisture content, m.c.) and temperature [6–8]. High moisture contents and temperatures accelerate deleterious seed aging reactions [8–11] and promote the growth of microorganisms [12–15], leading to rapid seed deterioration. To

avoid this, maize seeds should be stored below 12.5% m.c. (wet basis, w.b.) and refrigerated at 10–15 °C [3,16].

However, safe storage conditions for maize are not always achievable [3,4,16–18]. The seed industry, despite having proper drying technologies, often fails to achieve the safe storage moisture content in large seeds batches, and seeds may also rewet during storage in uncontrolled humidity chambers [3]. Small-farmer communities, on their part, destinate a part of their maize harvest as seed for the following planting season but lack proper infrastructure for seed drying and cooling [3,4,17]. Due to poor storage conditions, viability losses can reach 50% in these communities [16,17]. Exploring alternative technologies to improve seed preservation, thus, is essential for both the seed industry and small farmers.

Low-oxygen (hypoxic) atmospheres (i.e., between 1 and 3% (v/v) oxygen [19]) were more effective than the normal atmosphere (21% oxygen) in laboratory-scale experiments for preserving germination and vigor (hereinafter, all gas concentrations are expressed in % (v/v)) [20–25]. The hypoxic environment is thought to extend seed quality by impairing the formation of highly deleterious reactive oxygen species (ROS) in the seed cells (which disrupt the functions of fatty acids in membranes, proteins, and nucleic acids [26–29]) and the development of the main genera of seed microflora, which are primarily aerobic [12,19,26].

The advantages of low-oxygen atmospheres were observed in laboratory studies across several maize genotypes. Two yellow-dent hybrids (KM4500 and KM4580) stored at 14% m.c. (w.b.) and 25 °C for 6 months in low-oxygen atmospheres had 1.18- and 1.35-fold higher germination, respectively, than in a normal atmosphere ([20] and unpublished data of our group). Sweet corn (hybrid honey 236) seeds stored at 10% m.c. (w.b.) and 20 °C had 1.2-fold higher vigor after 360 days of storage in a low-oxygen atmosphere compared to a normal atmosphere [27]. Seeds of the yellow hybrid V-524 Tuxpeño stored at 15% m.c. for 90 days had 95% and 43% germination, respectively, under low-oxygen and normal atmosphere [25]. In addition to maize, the benefits of low-oxygen atmospheres were observed on the lab scale in a wide range of species including barley, broad beans, peas [21], pine tree [28], rye [22], Brassicaceae [23], groundnut [30], celery [24], and bitter gourd [29], suggesting that the mechanisms of seed quality protection of the low-oxygen atmospheres might be highly conserved among plant species.

Despite the promising prospect for low-oxygen atmospheres, the literature on scaling up from laboratory conditions to agronomic or industrial applications is limited [30–33], especially for maize seeds [4,5]. This is not surprising in view of the technical challenges of the scaling-up procedures. For example, while laboratory experiments often utilize 0.3–0.5 L hermetically sealed glass jars [14,20,25,34], the storage of 25–50 kg of seeds requires using 30–60 L flexible containers [23,31,35]. To preserve hypoxic environments throughout the storage period, these containers must be highly hermetic, usually requiring plastic films combining one or two layers of an oxygen-barrier material (such as polyamide or ethylene-vinyl alcohol, EVOH) to avoid the entrance of oxygen. Also, they must have highly effective seals at the edges and valves, and resistance to mechanical damage [35–37]. The injection and purging procedures in these containers, additionally, require higher gas volumes and larger times to achieve initial oxygen concentrations near 0% than in small jars, elevating the operative complexity and cost of these experiments [19].

The demonstrated benefits of low-oxygen atmospheres on the laboratory scale justify the need for further studies at a larger scale. This study aimed to determine whether the benefits of low-oxygen atmospheres observed on the lab scale for maize seed quality were achievable on the pilot scale, i.e., bags of 25 kg seed capacity and controlled storage conditions. For this, we first characterized the capacity of polyethylene and polyamide (a low-cost oxygen barrier material) bags of 25 kg seed capacity to maintain low oxygen concentrations over time. Secondly, we compared the germination and vigor of maize seeds stored in the previous containers with 14% m.c. (w.b., slightly above the safe m.c. of 12.5%), under modified and self-modified atmospheres, against seeds stored in normal atmosphere in traditional poly-paper bags, after 5 months. In the self-modified atmosphere bags, the oxygen is consumed due to the metabolic processes that occur within the hermetic

packaging (mainly the respiration of the seed microflora and, to a lesser extent, oxidative reactions of the seed macromolecules and some aerobic respiration of the seed), without any additional control [9,19,34]. In the modified atmosphere bags, in contrast, the oxygen is eliminated from the container at the beginning of storage using an external source, without further adjustment [38–40]. Two temperatures, 25 °C and 10 °C, were used in the comparison.

The main outcomes of this study are that (1) it is feasible to achieve low-oxygen atmospheres on the pilot scale using polyethylene and polyamide bags, and (2) self-modified and modified atmospheres are promising tools to preserve seed germination and vigor on the agronomic and commercial scales. We expect that our results, which are more representative of real-world scenarios, will enhance the prospects for the implementation of low-oxygen storage technologies among small farmers and the seed industry.

2. Materials and Methods

2.1. Preliminary Trial: Characterization of the Airtightness of Plastic Containers

The containers used for the modified and self-modified atmosphere were 65 cm × 47 cm polyethylene and polyamide plastic bags (approximate capacity: 30 L), equipped with a valve for product filling (Figure 1). Provided by a local company (ENVALIQ, Mar del Plata, Argentina), these bags were not originally designed for seed storage but rather for food products (dressings). We adapted them for oxygen monitoring by incorporating a rubber septum.

Figure 1. Plastic box with two plastic bags (transparent, for modified and self-modified atmosphere treatments) and one poly-paper bag (yellow, for normal atmosphere treatment), and a container with glycerol solution. The valve with the rubber septum can be seen in the corner of each bag.

Each of the two faces of the bag was made up of two films. The inner film (the one in contact with the seeds) was composed entirely of polyethylene. The outer film (the one in contact with the ambient air) was made of polyethylene and polyamide (in a ratio of 0.82:0.18, respectively). Each film was 70 µm thick approximately (therefore, each face of the bag was 140 µm thick approximately). The four films (the two inner and the two outer) were heat sealed between them only at their four edges.

The plastic containers would be considered appropriate if they maintained an oxygen concentration below 3% (v/v, on average) during the planned period for the main experiment (5 months, see Section 2.2). This decision was based on germination results obtained by our research group at the laboratory scale ([20] and unpublished data), where benefits of hermetic storage for 14% m.c. seeds were observed for both controlled atmospheres (where oxygen concentration is constantly monitored and actively maintained below 1%) and self-modified atmospheres, where the oxygen concentration gradually decreased until

hypoxia was achieved. The 3% threshold was considered reasonable as it represented an intermediate situation between both conditions.

Oxygen concentration was analyzed using a repeated measures design on 19 plastic containers. Each bag contained 3 kg of maize seeds with a moisture content of 11%. This low value was used to avoid alterations in the bag atmosphere due to respiration [41]. The bags were injected with carbon dioxide through a hose inserted into the valve and connected to an 8 m^3 compressed gas cylinder, allowing the purging of internal air. The oxygen concentration was measured during the injection/purging process until it reached a value below 1%. At that point, the hose was removed from the bag, and the valve was promptly closed with its hermetic cap. Subsequently, the bags were placed in a temperature-controlled chamber at 25 °C, and the oxygen concentration was measured at seven different time points (every 3 weeks for 15 weeks, at week 21, and at week 59). To measure the oxygen concentration inside the bags, gas samples were extracted with a needle through the septum on the cap and analyzed using a gas analyzer (see Section 2.2.5).

The oxygen concentration as a function of storage time (in days) up to week 21 was fitted with a simple linear regression model considering random effects of the bag on the slope and intercept. The inclusion of random effects accounted for the correlation between measurements made on the same bag [42]. Model assumptions were assessed using diagnostic plots and Akaike information criterion (AIC) and Bayesian information criterion (BIC) statistics [42]. The slope of the obtained line represents the average daily oxygen ingress rate into the used bags (% day^{-1}). In addition, to determine if the model effectively predicted oxygen concentrations in the bags beyond the monitored time, the oxygen concentration in the bags was extrapolated after 59 weeks of storage (412 days) with a 95% confidence interval and compared with the experimental value. All models and statistical analyses were conducted using R software version 4.1.3 [43].

2.2. Main Trial: Evaluation of Seed Quality Using Modified Atmosphere and Self-Modified Atmosphere Technology versus Traditional Technology

The germination and vigor of seeds with 14% m.c. were compared at the pilot scale after 150 days using hermetic storage technology with modified and self-modified atmospheres and contrasted with traditional technology (normal atmosphere) at 25 °C. This moisture content corresponds to an equilibrium relative humidity of 75% approximately for yellow-dent maize seeds [35]. We chose a 14% m.c. to reflect practical storage conditions often encountered by small-scale farmers without advanced drying facilities, and a storage temperature of 25 °C to simulate ambient conditions common in maize-growing regions [3,20]. Additionally, in the seed industry, while it is less common, such moisture content can occur in batches that fail to dry correctly [3] or that rewet during storage [20].

To compare with an optimal storage temperature [44,45], bags refrigerated at 10 °C were used. For hermetic storage, the plastic bags described in Section 2.1 were employed, while for storage using traditional technology, poly-paper containers (typically used by small farmers and the seed industry) were utilized. These containers have an inner layer of polypropylene coated with an outer layer of paper and, as they are permeable to gas passage, they determine a normal atmosphere around the seed. Their dimensions are 70 cm × 37 cm with a gusset of 10 cm and capacity of 25 kg of seeds.

2.2.1. Seed Material

The experiment was conducted with yellow-dent maize (*Zea mays* var. indentata) seeds, hybrid KM4580 (property of the company KWS SAAT SE & Co. KGaA, Balcarce, Argentina), which were produced in the province of Santa Fe, Argentina, and harvested in February 2022. The seeds were dried and stored by the seed company until March 2023, when they were brought into the Seed Quality Laboratory of IPADS (INTA-CONICET, Balcarce, Argentina). Upon arrival, they had a moisture content of 11% and a germination value of over 95%. They were kept refrigerated for three months at 4 °C in double hermetic bags to prevent moisture variation until they were used.

2.2.2. Experimental Design and Procedure

The study was carried out in three types of atmospheres (normal, modified, and self-modified) and two temperatures (25 °C and 10 °C), in triplicate. Initially, a 63 kg batch of seeds was conditioned from 11% to 14% m.c. following the procedure in Weinberg et al. [34]. At the end of the conditioning, the relative humidity and moisture content of the batch were measured (see Section 2.2.4) to verify they had reached the target. The original seed lot was divided into 18 equal parts of 3.5 kg of seeds and randomly assigned to 12 plastic bags and 6 poly-paper bags. Each bag was randomly assigned one of the two temperatures (25 °C or 10 °C).

The plastic bags were also randomized according to the type of atmosphere (modified or self-modified). The modified atmosphere was achieved by injecting nitrogen with a hose from an 8 m^3 compressed gas cylinder through the bag valve at the beginning of the experiment. This allows the purging of internal air until less than 1% oxygen remains inside the container. According to the definition, the self-modified atmosphere bags were not injected with nitrogen, but rather the concentration of gases resulting from respiration [14,41] was allowed to evolve.

Afterwards, the bags were placed in temperature-controlled chambers at 25 °C and 10 °C. The relative humidity in both chambers had been previously measured and found to be lower than the equilibrium relative humidity in the bags. Since poly-paper bags are permeable to water vapor, it was necessary to control the ambient relative humidity during storage at 25 °C to prevent the seeds from drying out and to avoid confounding effects. For this, each of the three poly-paper bags was placed inside a plastic box (43 cm × 34 cm × 26 cm, three plastic boxes were used in total), covered with a lid. A constant relative humidity of 75% was maintained inside each box using 56% w/w glycerol solutions [46] (Figure 1). Although hermetic plastic bags are considered impermeable to water vapor, they were also stored inside the plastic boxes to equalize storage conditions with the poly-paper bags (Figure 1). Each of the three plastic boxes, therefore, contained one poly-paper bag and two plastic bags (one for the self-modified atmosphere and one for the modified atmosphere treatments). The relative humidity in the three plastic boxes was monitored (see Section 2.2.4) daily throughout the experiment (Supplementary Figure S1). To ensure the normal atmosphere in the poly-paper bags, the boxes were ventilated weekly by opening their lids for two minutes.

On the other hand, seeds with 14% m.c. maintained their physiological quality for up to 9 months when refrigerated (unpublished data of our group). Indeed, the initial and final germination values were 98.5% ± 0.33% and 98.4% ± 1.26%, respectively ($p > 0.05$), while radicle emergence values (vigor) were 90.0% ± 4.9% and 86.4% ± 3.7%, respectively ($p > 0.05$). Therefore, in the 10 °C chamber, it was deemed unnecessary to control the relative humidity for the poly-paper bags since they were expected to maintain their initial quality, and even more if they dried out.

The oxygen and carbon dioxide concentrations in the plastic bags were measured at three stages of the experiment: initial, intermediate (75 days), and final (150 days). The moisture content of the seeds was also measured at the end of the experiment to verify if it remained constant throughout the experiment. After 5 months (150 days) of storage, the germination and vigor of each bag were evaluated.

2.2.3. Evaluation of Germination and Vigor

Germination is the percentage of seeds that produce normal seedlings under optimal laboratory conditions [47]. Vigor refers to the sum of attributes that determine rapid and uniform emergence of normal seedlings under a wide range of field conditions, and decreases earlier than germination [48]. Measuring vigor provides a more comprehensive description of the seed physiological quality, as batches with similar germination can differ in their vigor values [49].

Germination was determined according to ISTA [47]. Briefly, 150 seeds from each bag were sown in three trays (50 seeds each) in moist river sand and placed in plastic

bags to prevent drying. The trays were placed in a chamber with alternating temperatures (20 °C–30 °C, 16 h–8 h) and a photoperiod of 8 h light/16 h darkness, for 7 days. At the end, normal seedlings were counted, and results were expressed as a percentage of the total seeds sown.

Seed vigor was analyzed using the ISTA radicle emergence test [47], following Abadía et al. [20]. Briefly, 200 seeds from each bag were sown in eight trays (25 seeds each) between moist paper towels and incubated for 66 h at 20 °C. Seeds with a radicle equal to or greater than 2 mm were counted, and results were expressed as a percentage of the total seeds.

Given that both variables follow a binomial distribution, the data were fitted using a generalized linear model (GLM) with a logit link function [20,50,51]. For germination and radicle, the linear predictor of the models was described in terms of the main effects of atmosphere and temperature and their interaction. A significance level of $\alpha = 0.05$ was used in the deviance analyses and in the post-hoc Tukey's Honestly Significant Difference (HSD) test for multiple means comparison [52]. All statistical analyses were performed using version 4.3.1 of the R software [43].

2.2.4. Measurement of Relative Humidity, Moisture Content, and Temperature

The relative humidity of the seed bags was measured with iButton® temperature/humidity loggers DS1923 (Maxim Integrated, San José, CA, USA, accuracy range of ±5% relative humidity) after conditioning the seeds to 14% m.c. and at the end of the experiment. After opening the bags, 100 g of seeds were placed inside a closed jar in contact with a sensor and the relative humidity was recorded for 48 h. Afterwards, the sensor was removed from the jar, and the data were downloaded. To measure the relative humidity in the boxes containing the glycerol solutions, the sensor was placed inside the box.

The moisture content of the seeds was determined by drying in a forced-air oven [53]. The temperature of the storage chambers was monitored with iButton® temperature/ humidity loggers DS1923 (Maxim Integrated, San José, CA, USA, accuracy range of ±0.5 °C). The final moisture content of the seeds in each treatment was compared to the initial moisture content value using a two-sample *t*-test ($\alpha = 0.05$) [52], with version 4.3.1 of the R software [43].

2.2.5. Oxygen and Carbon Dioxide Measurement

Oxygen levels within the bags were determined with a Dansensor CheckMate3 gas analyzer (AMETEK MOCON, Rinsted, Denmark). This measurement involves the extraction of a 6 mL gas sample via a needle passed through the septum of the lid. The analyzer employs a zirconia oxygen sensor with an accuracy of ±0.01% absolute for concentrations under 1%, calibrated at the factory against oxygen concentrations of 100 ppm, 1000 ppm, 1%, 80% (nitrogen-balanced), and 20.9% (compressed dried atmospheric air). Additionally, the device uses a carbon dioxide infrared dual beam sensor with an accuracy of ±0.8%, calibrated at the factory for carbon dioxide levels of 0%, 25%, 60%, and 100% (nitrogen-balanced). The final oxygen and carbon dioxide concentrations in each treatment were compared to the normal atmospheric values (~21% and ~0.03%, respectively) using a one-sample *t*-test ($\alpha = 0.05$) [52], with version 4.3.1 of the R software [43].

3. Results

3.1. Preliminary Trial: Characterization of the Airtightness of Plastic Containers

The oxygen concentration during storage evolved in a similar manner in most of the 19 bags, increasing in an approximately linear fashion from the initial injection (below 1%), to less than 5% at 150 days. The exceptions were bags 2 and 6, where the oxygen concentration increased rapidly and reached higher final values (9.35% and 18.14%, respectively), possibly due to failures in the hermeticity of the edges and/or valves, as no breaks in the plastic were observed. These bags were excluded from further analyses.

With the initial injection and purging (week 0), a mean oxygen concentration of 0.65% was achieved in the bags. The closeness between the median and the mean indicated

a symmetric distribution of the data within each week. The increase in mean oxygen concentration was steady between measurements, i.e., 0.14% between weeks 6 and 9, 0.31% between weeks 3 and 6, and 0.41% between weeks 15 and 21 (6 weeks instead of 3). The greater increase of 0.81% that occurred between the beginning and week 3 is attributed to the desorption of oxygen from the seed matrix into the bag's atmosphere, as the seeds (initially in equilibrium with a normal oxygen concentration of 21%) were exposed to the hypoxic atmosphere [54,55]. The variability in oxygen concentration, reflected in the standard deviation, increased over time (from an initial 0.3% to 1% at the end). After 21 weeks of storage (5 months), the mean oxygen concentration was 2.93%, below the targeted nominal threshold of 3% (v/v). This behavior indicated that the assessed containers were suitable for the main experiment.

The linear model (Figure 2) adequately fitted the data, as seen by the alignment of the line with the experimental means. Observations from week 0 were excluded from the modeling to disregard the greater variability associated with the initial desorption of oxygen. The average daily rate of oxygen ingress into the selected container was 0.011% day^{-1}.

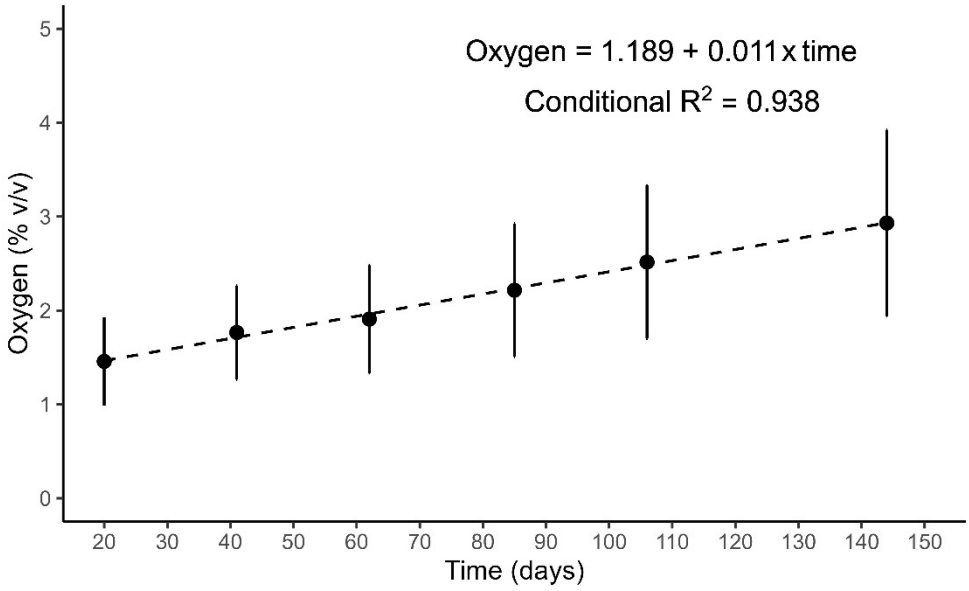

Figure 2. Oxygen concentration (% v/v) over time as predicted by the model (dotted line) after fitting experimental data (mean ± standard deviation, n = 17). The model equation includes only the fixed effects (slope p-value = 2.22 × 10^{-16}). Conditional R^2 represents the variance explained by the entire model, including both fixed and random effects.

To verify how the model predicted oxygen concentrations beyond the evaluated time interval (150 days), the predicted value was compared with the experimental value at week 59 (412 days). The oxygen concentration predicted by the model for this time was 5.98% ± 0.59% (mean ± standard error). The experimental value, on the other hand, was 6.04% ± 0.19% (mean ± standard error). Given the proximity of the experimental and predicted values, it was concluded that the model reasonably well predicts oxygen concentrations in the selected container for up to 400 days.

3.2. Main Trial: Evaluation of Seed Quality Using Modified Atmosphere and Self-Modified Atmosphere Technology versus Traditional Technology

The moisture content of the seed batch prior to storage was 14.3% ± 0.3% (average ± standard deviation), very close to the target (14%). The moisture of the seeds stored in plastic bags remained constant and close to the target during the experiment (Table 1), confirming that the polyamide and polyethylene film minimizes the exchange of water vapor between the inside and outside of the bag. In poly-paper bags, the moisture content

remained constant at 25 °C due to the controlled relative humidity of the chamber. In the refrigerated chamber, however, where the relative humidity was not controlled, the moisture content of the seeds in poly-paper bags dropped to 12.3%.

Table 1. Moisture content, oxygen, and carbon dioxide concentrations (mean ± standard deviation); and germination and radicle emergence (estimated marginal mean ± standard error) after 150 days of storage at pilot scale with different temperatures and atmospheres (n = 3).

Temperature (°C)	Storage Technology	Moisture Content (%)	Oxygen (%)	Carbon dioxide (%)	Germination (%)	Radicle Emergence (%)
25	Normal atmosphere (traditional)	14.2 ± 0.1	21	0.03	68.3 ± 2.7 ^{Bb}	31.0 ± 0.4 ^{Bb}
	Modified atmosphere	14.0 ± 0.1	0.05 ± 0.06 *	5.7 ± 5.0	95.8 ± 0.9 ^{Aa}	61.2 ± 2.2 ^{Ba}
	Self-modified atmosphere	14.0 ± 0.1	0.1 ± 0.1 *	10.8 ± 2.0 *	94.4 ± 1.1 ^{Aa}	65.0 ± 2.1 ^{Ba}
10	Normal atmosphere (traditional)	12.3 ± 0.5 *	21	0.03	97.6 ± 0.7^{Aa}	79.6 ± 1.8 ^{Aab}
	Modified atmosphere	14.3 ± 0.3	6.5 ± 6.0 *	1.1 ± 1.2	95.7 ± 1.2 ^{Aa}	78.9 ± 2.2 ^{Ab}
	Self-modified atmosphere	13.9 ± 0.1	19.1 ± 0.9	0.5 ± 0.1	96.9 ± 0.8 ^{Aa}	85.2 ± 1.6 ^{Aa}

Within moisture content, oxygen, and carbon dioxide, the * indicates significant differences ($p < 0.05$) with respect to the reference values. Reference values: moisture content: 14.3% ± 0.3% (mean ± standard deviation of the seed batch prior to storage); oxygen: 21% (normal atmospheric value); carbon dioxide: 0.03% (normal atmospheric value). Within germination and radicle emergence, same uppercase letters indicate not significant differences ($p > 0.05$) between temperatures, and same lowercase letters indicate not significant differences ($p > 0.05$) between atmospheres.

The oxygen concentration in the bags after injection was 1.7% ± 1.1% (average ± standard deviation) and slightly decreased towards the middle of the storage period to 0.02% ± 0.01%. At the intermediate time (75 days), bags with self-modified atmosphere at 25 °C had already reached hypoxia, with an oxygen concentration of 0.2% ± 0.4%. The carbon dioxide concentration by this moment was 13.7% ± 1.2%. These changes are compatible with the respiration expected for seeds stored at 14% moisture [14,34,41] and would also explain the further oxygen decrement observed in the modified atmosphere bags.

In both cases, the hypoxic atmosphere was maintained until the end of the experiment (Table 1). The final concentration of carbon dioxide in the modified atmosphere bags was slightly lower than in the self-modified atmosphere bags (Table 1). This was probably due to a greater impairment of aerobic respiration in the former, where oxygen was near 0% from the beginning of the experiment.

At 10 °C, modified atmosphere bags had a higher oxygen concentration than initially (6.5%, Table 1), indicating some failure in their hermeticity during the experiment. Bags with a self-modified atmosphere, on the other hand, had an oxygen concentration close to normal (19.1%, Table 1).

By the end of the experiment (150 days), the germination of seeds stored at 25 °C with modified and self-modified atmospheres had similar values (95.8% and 94.4%, respectively), significantly higher than those stored in the normal atmosphere (68.3%, Table 1). Furthermore, the germination of seeds stored in modified and self-modified atmospheres at 25 °C did not differ from the germination of seeds refrigerated at 10 °C. Among the refrigerated seeds, there were no differences in germination between atmospheres (Table 1).

Despite the similar germination values of the seeds stored at 25 °C and low-oxygen atmospheres and the seeds stored at 10 °C, there were differences in radicle emergence (vigor) among the different groups (Table 1). Refrigerated seeds had higher radicle emergence than seeds stored at 25 °C, regardless of the atmosphere. Within the refrigerated seeds, those stored in the self-modified atmosphere had slightly higher radicle emergence than those stored in the modified atmosphere ($p < 0.05$), while seeds stored in the normal atmosphere had an intermediate value ($p > 0.05$). Seeds stored at 25 °C in modified and

self-modified atmospheres, on the other hand, had similar radicle emergence values. As expected from the germination behavior, the seeds stored at 25 °C and normal atmosphere had the lowest vigor of all the groups.

4. Discussion

Our study has two main outcomes. First, it is feasible to achieve low-oxygen atmospheres in 25 kg capacity containers like those used for seed preservation and trade. Second, the benefits of low-oxygen atmospheres for seed germination and vigor on the lab scale are observable on the pilot scale under the evaluated conditions.

Polyethylene and polyamide bags successfully maintained the oxygen concentration below the desired 3% threshold for a period of 5 months (Table 1). By using dry seeds that were not actively respiring [41], the low oxygen concentration measured inside the bags was due to the high hermeticity of the packaging, which prevented the ingress of oxygen from the outside, rather than the consumption of oxygen by the seeds. The chosen packaging, therefore, is suitable for low-oxygen atmosphere storage, with the additional advantage of being more economical than other barrier materials such as EVOH [38].

The hermetic valve for gas injection and bag closure allowed the achievement of a very low initial oxygen concentration (0.65% on average in this experiment), as it permitted the rapid insertion and removal of the hose with minimal oxygen ingress. In containers featuring knot ties or zip-lock-like closures instead of valves (e.g., GrainPro, PICS™, Elite, ZeroFly®, AgroZ®, and Storezo [35]), achieving initial hypoxia is expected to be more laborious. Despite our satisfactory results, further improvement in the injection/purging procedure to achieve even lower initial oxygen concentrations will be important to prolong the hypoxic environment even further. Indeed, if an initial oxygen concentration closer to 0% was achieved, the model (Figure 2) predicts that the period of hypoxia could be extended by 75%, from 150 to 265 days (calculated as the quotient between 3% and the slope of the line). Additionally, the use of oxygen scavengers could mitigate the initial oxygen desorption from the seeds and potential oxygen leakages from the outside [36].

At 25 °C, both modified and self-modified atmosphere technologies were superior to traditional technology and equally effective in preserving germination potential (Table 1). Furthermore, during the studied period, modified and self-modified atmospheres enabled the same germination values at 25 °C as refrigeration (10 °C). However, modified and self-modified atmospheres for refrigerated seeds did not have additional benefits for germination compared to the normal atmosphere. For preserving radicle emergence, modified and self-modified atmosphere technologies at 25 °C were equally effective (Table 1). However, both technologies were less effective than refrigeration in this respect.

The benefits on seed germination and vigor observed at 25 °C on the pilot scale were consistent with previous observations of our group in laboratory experiments ([20] and unpublished data). On the lab scale, our germination models over time under anoxic and normal atmosphere conditions predicted germination values of 93.2% and 88.1%, respectively, for seeds with 14% moisture after 5 months of storage at 25 °C [20]. On the pilot scale, the difference in germination was even more pronounced in favor of the low-oxygen atmospheres (approximately 95% on average for both modified and self-modified atmospheres versus 68% in normal atmosphere, Table 1).

Regarding the evolution of gas concentrations in the main trial, the self-modified atmosphere bags reached hypoxia after 75 days of storage. This is in accordance with a previous work of our group at the laboratory scale using the same genotype (KM4580) and also with other authors, who reported between 30 and 60 days to reach hypoxia under similar storage conditions [14,34,41]. However, some variability in the times to reach anoxia can be expected when using different seed batches, since their higher or lower microbial loads may lead to respectively greater or lower respiration rates [14,41]. Also, different ratios of seed mass to container volume will likely affect the rate of oxygen consumption [14]. The final oxygen concentration in the plastic bags at 25 °C (0.05% and 0.1%, respectively, for modified and self-modified atmospheres, Table 1) was lower than

the initial (1.7% and 21%, respectively), consistent with the expected respiration for seeds stored at 14% m.c. [6,20]. The refrigerated bags with a self-modified atmosphere, on the contrary, had an oxygen concentration close to normal (19.1%, Table 1), suggesting a low respiratory activity at 10 °C. It is noteworthy that, in addition to maintaining the hypoxic environment, the plastic bags were effective in maintaining constant seed moisture as anticipated by previous reports [18,35,56,57], unlike the poly-paper bags (Table 1). In the latter, seeds stored at 10 °C dried out, as expected from the lower relative humidity in the refrigerated chamber. This property of the plastic bags would be especially useful in storage environments with high relative humidity to prevent seed moistening, with the consequent risk to germination and vigor [30].

The benefits of low-oxygen atmospheres for extending seed quality observed in our pilot-scale experiment in general support the previous limited literature. For maize, García-Lara et al. [5] observed that seeds stored with 12% moisture in a vacuum within hermetic plastic containers (sBag™ and Bioxilo™, flexible and rigid, respectively) at ambient temperature (which oscillated between 15 and 20 °C) had higher germination rates for 12 months, compared to 8 months using traditional technology. Williams et al. [4], also working with maize, found that the germination of seeds stored with 12–13% m.c. (w.b.) between 20 °C and 30 °C in hermetic PICS™ bags was slightly (1.12-fold) higher than in woven bags after 8 months. Similarly, Ludwig et al. [30] reported higher germination across various soybean seed varieties stored with 11.5% m.c. (w.b) in hermetic plastic bags under controlled low-oxygen atmospheres compared to normal atmosphere at various temperatures. Coradi et al. [31] demonstrated that storing soybean seeds in raffia containers lined with polyethylene or laminate material at room temperature had similar physical and physiological qualities to those stored in refrigeration. Furthermore, Capilheira et al. [32] found that hermetic storage of soybean, with or without carbon dioxide injection, slowed seed deterioration compared to storage in a normal atmosphere over a period of up to 180 days under uncontrolled environmental conditions. Alemayehu et al. [33] observed that germination of sesame seeds in traditional polypropylene and jute bags decreased from 90% to 62%, while it remained constant in hermetic PICS™ and GrainPro bags after 6 months of storage at ambient temperature (ranging between 18 and 22 °C).

The biophysical state and physiological (aging) and microbiological mechanisms that might be active under the experimental conditions are crucial for understanding how oxygen delays quality loss. At 14% m.c. (75% relative humidity) at either temperature (10 or 25 °C), the cytoplasm of maize seed cells is expected to be in a fluid state [10,58,59]. The fluid state facilitates substrate mobility, enzymatic reactions, and structural macromolecular changes [10,60,61]. Moreover, the presence of mobile ROS can accelerate auto-oxidation reactions [60]. Since temperature also influences reaction kinetics [62], deterioration phenomena are expected to occur more rapidly at 25 °C than at 10 °C, which is consistent with our results on germination and vigor. The greater germination and vigor of our maize seeds stored in low-oxygen atmospheres compared to normal atmosphere, therefore, would be compatible with the reduced production of ROS from molecular oxygen [63], leading to less oxidation of essential biomolecules and prolonged physiological quality. Furthermore, at relative humidity levels around 70%, genera such as *Eurotium*, *Aspergillus*, and *Penicillium* can germinate and develop [12,64]. However, since most filamentous fungi are aerobic, oxygen deficiency would restrict their development [12]. In our study, spores were easily observed on the surface of seeds stored in a normal atmosphere at 25 °C, giving them a dull appearance. In contrast, seeds stored at 25 °C in modified and self-modified atmospheres appeared clean and bright, suggesting lower microbial activity, like the refrigerated seeds, where the low temperature inhibited microbial development.

This study has practical conclusions for the agronomic and industrial scales of seed storage. Hermetic bags with modified or self-modified atmospheres are a superior option for preserving germination compared to traditional bags when storing seeds with 14% m.c. around 25 °C. This is particularly relevant for small-scale producers who reserve part of their corn harvest for seeding and do not have the capacity to dry it to 12.5% m.c. or

refrigerate it to 10 °C [3]. Since both offer similar outcomes, self-modified atmosphere technology is preferable to modified atmosphere, as it eliminates the need for initial injections into the packaging, avoiding costs and operational complexities. In the seed industry, although less common, this moisture content can occur when a batch fails to dry correctly or if the seeds become moistened during the application of phytosanitary products or during storage in uncontrolled humidity chambers [45]. In such cases, hermetic storage could improve the seed quality outcomes compared to traditional bag storage.

In the future, assessing the benefits of low-oxygen atmospheres on the pilot scale at lower moisture levels (below 10–12% m.c., w.b.) will be particularly valuable for those seeds that require long-term storage, such as high-value parental lines or carry-over seeds (i.e., those that were produced in a growing season and must be stored for planting not in the next, but in the subsequent season, typically for 18–20 months) [3,20]. Moreover, expanding the scope of the pilot-scale study to other species and corn hybrids will allow a better comprehension of the potential of low-oxygen technology for seed storage. Finally, a comparative economic study between the proposed technologies and the traditional one, considering the cost of packaging, injection processes, machinery adaptation for injection during bagging, and potential gains in terms of germination and vigor [65] is necessary. The environmental sustainability of the technology (including the possibilities for the reuse and recycling of packaging) must also be considered in the evaluation [35]. Altogether, this will help to determine the relative advantages of each approach and guide decisions in practice.

5. Conclusions

Twenty-five-kilogram seed capacity bags made of polyethylene and polyamide (a low-cost barrier) were sufficiently hermetic to maintain an oxygen concentration below 3% (v/v, on average). In the pilot-scale trial, seeds stored at 25 °C with 14% m.c. in those plastic bags with modified or self-modified atmospheres had on average 1.4-fold higher germination than seeds stored in a normal atmosphere in traditional poly-paper bags. The self-modified atmosphere, which eliminates the need for external gas supply and injection processes, and reduces operational costs compared to the modified atmosphere, is an especially attractive option. Moreover, in the studied period, the seeds stored in the low-oxygen atmosphere at 25 °C had the same germination potential as seeds refrigerated at 10 °C (around 95%), making low-oxygen atmospheres an attractive option when refrigeration is unavailable. Refrigeration, however, was the best alternative for preserving more sensitive attributes such as vigor. Future studies with low-oxygen atmospheres at lower moisture contents, as well as with other maize hybrids and species, are necessary to fully understand the potential of this technology for seed preservation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14081268/s1, Figure S1: Relative humidity inside the three plastic boxes. Dots represent the measurements, the solid line represents the mean of the relative humidity across the experiment in each box, and the dashed line represents the relative humidity target value (75%).

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