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ORIGINAL ARTICLE

CO_2 levels, technical breakage and quality of maize grains stored under different conditions[1]

Níveis de CO_2, quebra técnica e qualidade dos grãos de milho armazenados em diferentes condições

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HIGHLIGHTS:
CO_2 dynamics is associated with respiration of maize grains.
High CO_2 levels increase maize grain losses during storage.
High moisture contents increase respiration and technical breakage of stored grains.

ABSTRACT: The use of CO_2 sensors has been reported as an effective tool in the early detection of signs of deterioration, allowing good accuracy in decision-making about the quality of stored grains. The objective of this study was to quantify the CO_2 levels produced by maize grains stored at ambient temperature in a prototype silo, with initial moisture contents of 14, 16 and 18% w.b., and to evaluate the quality of the product over time and the technical breakage. Analyses of moisture content, ash, proteins, lipids, Hue Angle (color), germination and electrical conductivity were performed. Inside the silos, the amount of CO_2, relative air humidity and temperature were monitored every hour. Grain quality evaluations were carried out at four storage times (0, 30, 60 and 90 days). A completely randomized design in a 3 × 4 factorial scheme with six replicates was used. Grains with higher moisture contents showed higher technical breakage and losses associated with CO_2 emissions. Moisture content, protein, lipids, germination, bulk density, lightness and chroma decreased over time, while the electrical conductivity increased, resulting in greater damage to membranes and loss of quality of maize grains. Monitoring of CO_2 in the grain mass is a good tool to assess the quality of grain, and higher initial moisture content caused greater level of CO_2 emission and reduction in quality of maize grains.

Key words: *Zea mays* L., deterioration, carbon dioxide, respiration

RESUMO: O uso de sensores de CO_2 tem sido reportado como uma medida eficaz na detecção precoce de sinais de deterioração, permitindo uma boa acurácia na tomada de decisão sobre a qualidade de grãos armazenados. Neste estudo objetivou-se quantificar os níveis de CO_2 produzidos por grãos de milho armazenados em temperatura ambiente em silo protótipo, com teores de água iniciais de 14, 16 e 18% b.u., bem como avaliar a qualidade do produto ao longo do tempo e a quebra técnica. Foram realizadas análises de teor de água, cinzas, proteínas, lipídeos, Hue Angle (cor), germinação e condutividade elétrica. No interior dos silos foi monitorada a quantidade de CO_2, umidade relativa do ar e temperatura a cada uma hora. As avaliações de qualidade dos grãos ocorreram em quatro tempos de armazenamento (0, 30, 60 e 90 dias). Foi usado o delineamento inteiramente casualizado, em esquema fatorial 3 × 4 e seis repetições. Os grãos mais úmidos apresentaram maior quebra técnica e perdas associadas a emissões de CO_2. Os teores de água, proteína, lipídeos, germinação, massa específica aparente, L* e croma reduziram ao longo do tempo, enquanto que a condutividade elétrica aumentou, resultando em maior danificação das membranas e perda de qualidade dos grãos de milho. O monitoramento do CO_2 na massa de grãos é uma boa ferramenta para avaliar a qualidade dos grãos, e maior teor de umidade inicial causou maior nível de emissão de CO_2 e redução na qualidade dos grãos de milho.

Palavras-chave: *Zea mays* L., deterioração, dióxido de carbono, respiração

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Introduction

Maize has stood out among the grain crops produced in Brazil, with an estimated production of 125.83 million tons for the 2022/23 season, which is 11.2% higher than in the previous season (CONAB, 2022). However, these grains are processed and stored until the moment of their use, so their nutritional quality needs to be maintained. According to Coradi et al. (2020), several types of quantitative and qualitative losses, including deterioration of nutritional quality, can occur in grains during all post-harvest stages. Grain degradation rate depends on the activity of biotic and abiotic variables; biotic factors are pest insects and fungi, while abiotic factors are grain moisture content, air temperature, storage structure and gaseous environment, and levels of carbon dioxide (CO_2) and oxygen (O_2) (Likhayo et al., 2018).

The relationship between the biological activity that occurs in bulk grain mass and the concentration of CO_2 in the interstitial air of the stored grain has been the focus of study of several researchers in search of an improved and accurate grain monitoring system with potential for early detection of signs of grain deterioration (Garcia-Cela et al., 2018; Kaushik & Singhai, 2018; Valle et al., 2021). Fleurat-Lessard (2017) emphasized the need for an accurate and reliable monitoring system for early detection of respiratory activities in grains to attain an effective management of the risks of deterioration in stored grains. The operationalization of the CO_2 monitoring system can contribute as a tool to maintaining the quality of the stored grains throughout the year (Wenneck et al., 2022).

Aiming to improve the storage system, reduce losses after harvest and maintain product quality, the objective of this study was to quantify the CO_2 produced by maize grains stored at ambient temperature in prototype silos, with initial moisture contents of 14, 16 and 18% w.b., and to evaluate the quality of the product during storage associated with CO_2 levels and technical breakage.

Material and Methods

Maize grains were mechanically harvested with moisture content of 18% w.b. at Fazenda Tropical, in the municipality of Montividiu, GO, Brazil (17° 31' 41" S and 51° 10' 25" W and average altitude of 797 m). They were then transported to the Laboratório de Pós-Colheita de Produtos Vegetais of Instituto Federal Goiano, Campus of Rio Verde, GO, Brazil, to be subjected to drying with natural air, at temperature of 22.83 °C, relative air humidity of 36.53% and air flow of 30.57 m^3 min^{-1}. The moisture content of the grains was reduced to 14 and 16% w.b., and part of them remained with moisture content of 18% w.b.

The experiment was conducted in three prototype silos (14, 16 and 18% w.b.) made of metal plates with dimensions of 0.56 × 0.45 m, containing 50 kg of maize grains each, for 90 days of storage, between July and October 2021. The temperature and relative humidity of the ambient air of the site were monitored using a datalogger every one hour. Inside the prototype silos, the amount of CO_2, relative air humidity and temperature were monitored every one hour with a CO_2 monitor/datalogger (Extech $CO_2$10). The temperature of the grain mass was monitored three times a day using a portable digital thermometer (Instrutherm C/Rs-232 Th-060) with external probe. Grain quality evaluations were carried out at four storage times (0, 30, 60 and 90 days).

Germination (%) was determined according to the Rules for Seed Analysis (BRASIL, 2009), using four replicates of 50 grains, on three sheets of Germitest® paper, moistened with distilled water in a volume corresponding to two and a half times the mass of the dry paper. Then, they were placed in a B.O.D. chamber regulated at 25 ± 1 °C. Evaluations were carried out on the 8th day after setting up the test, considering root protrusion of 1 mm.

Electrical conductivity (μS cm^{-1} g^{-1}) was determined using the methodology described by Vieira & Krzyzanowski (1999). Four subsamples of 50 grains, weighed on a scale with 0.001 g resolution, were used in each replicate during the storage time. The samples were placed to soak in plastic cups with 75 mL of deionized water and kept in a B.O.D. incubation chamber at a controlled temperature of 25 °C for 24 hours. The solutions containing the grains were slightly shaken and immediately read in a digital conductivity meter.

Bulk density (kg m^{-3}) was determined using a container of known volume filled with the grains at a fixed fall height. After filling and weighing, bulk density was determined as the ratio between mass (kg) and volume (m^3) in a hectoliter scale, in three replicates.

Grain color was determined in a spectrophotometer (Color Flex EZ, Hunter LabReston, Canada), in duplicate. The results were expressed in Coordinate L*, Coordinate a* and Coordinate b*; L* values (lightness) can vary from black (0) to white (100), a* values from green (-60) to red (+60), and b* values from blue (-60) to yellow (+60) (Paucar-Menacho et al., 2008). The values of the coordinates a* and b* were used to calculate chroma (Eq. 1) and hue angle (Eq. 2).

$$\text{Chroma} = \sqrt{(a^*)^2 + (b^*)^2} \quad (1)$$

$$\text{Hue} = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

The moisture content of the grains (%, w.b.) was determined after drying in a forced air circulation oven, at a temperature of 105 ± 1 °C, for 24 hours, using three subsamples per replicate (BRASIL, 2009).

Crude protein was determined by the Kjeldahl method, which expresses the total organic nitrogen content (AOAC, 2002) using 0.25 g of the sample. The tubes containing the sample and sulfuric acid were placed in the digestion block with heating up to 400 °C until complete digestion of the sample (light green color). After digestion, the samples were distilled in a nitrogen distiller (Tecnal TE-0364, Piracicaba, SP, Brazil) and then titrated with 0.1 M hydrochloric acid.

Lipids were analyzed by the Soxhlet method (AOAC, 2002). 12 g of samples were placed in the Soxhlet extractor (Químis Q-328G26, Diadema, SP, Brazil), which was connected to flat-bottom flasks previously dried in a forced air circulation oven at 105 °C. 450 mL of hexane (A.R.) was then added to the extractor. The extraction time was 8 hours. The extracted residue was kept in a forced air circulation oven at 70 °C for 2 hours and at 105 °C for 1 hour, cooled in desiccator until reaching ambient temperature and then weighed.

To determine ashes, 2 g of sample were weighed in porcelain crucibles, with previously established mass. The crucibles with the samples were placed in a muffle furnace (Quimis Q-318S, Diadema, SP, Brazil) at 550 ± 15 °C, where they were kept until complete calcination of organic matter (about 5 hours). The samples were cooled in desiccator and weighed (AOAC, 2002).

Technical breakage was evaluated by monitoring dry matter loss in five samples of grains containing around 50 g each, which were wrapped with a permeable tissue (voile) in order to allow the passage of air through the product and distributed inside the grain mass in each of the prototype silos at different points. The samples were weighed every 15 days and, at the end of storage, the moisture content of the grains was determined and the technical breakage was considered as a function of the dry matter loss due to the respiration of the grains in this time.

The statistical analysis comprised the completely randomized design, 3 × 4 factorial scheme, with three initial moisture contents of the grains, four storage times and six replicates. The data were subjected to analysis of variance by the F test ($p \leq 0.05$) using the statistical package SISVAR® v. 5.7 (Ferreira, 2019). Subsequently, linear regression analysis was performed according to the results of the analysis of variance and the models were tested at $p \leq 0.05$ by the t-test, using the statistical package SigmaPlot 14.0® (Systat Software, Inc.).

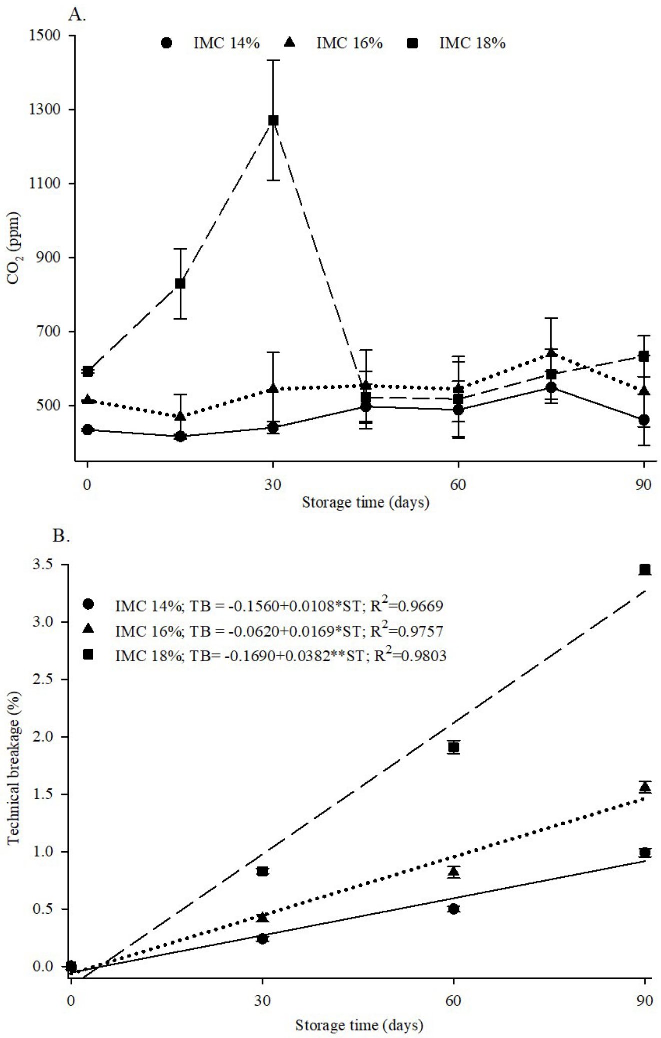
Results and Discussion

The average temperature and relative air humidity of the storage room were 22.18 °C and 42.13% in July, 25.57 °C and 43.44% in August, 29.65 °C and 40.66% in September and 28.31 °C and 61.79% in October, respectively. Bessa et al. (2015) highlight that the storage environment influences the conservation of grains.

During storage there was an increase of temperature in the mass of maize grains for the different moisture contents adopted as a function of time (Table 1), which may be related to the period of the year, since the final months of storage were warmer, despite the intensification of metabolic activity expected for higher moisture content in the product. Temperature and moisture content are the most common parameters used to monitor grain deterioration (Fleurat-Lessard, 2017).

The presence of a hot spot in the mass of stored grains is usually an indication of microbial growth, which causes degradation of the grains (Navarro, 2012). Table 1 shows that the temperature of the grain mass and the internal temperature of the silo indicated no hot spots during storage. However, the mean values of CO_2 levels and the technical breakage of maize grain mass along the storage time (Figure 1) point out the intense metabolic activity.

CO_2 levels increased during storage in all silos analyzed (Figure 1). The silo containing maize grains with moisture content of 18% w.b. had higher CO_2 emissions, revealing a more intense respiratory process, especially in the first 30 days of storage, exceeding mean values of 1,200 (ppm), which are within the range of 1,100-5,000 ppm, considered indicative of infestation by fungi or insects (Fleurat-Lessard, 2017).



*, ** - Significant at $p \leq 0.05$ and $p \leq 0.01$ by F test; IMC 14%, IMC 16% and IMC 18% - initial moisture content for 14, 16 and 18%, respectively

Figure 1. Mean values of CO_2 (A) and percentage of technical breakage (TB - B) of maize grains stored with three moisture contents over the storage time

Table 1. Mean values of internal temperature of the silos, internal relative air humidity of the silos and temperature of the mass of maize grains stored with three moisture contents throughout the storage time

Time (days)	Internal temperature in the silos (°C)			Internal relative air humidity in the silos (%)			Temperature of the grain mass (°C)		
	Silo1 (14%)	Silo2 (16%)	Silo3 (18%)	Silo1 (14%)	Silo2 (16%)	Silo3 (18%)	Silo1 (14%)	Silo2 (16%)	Silo3 (18%)
0	23.44	22.62	22.21	72.82	81.17	83.70	21.70	21.42	21.00
30	26.23	26.13	25.61	60.12	65.60	78.01	22.51	22.21	22.23
60	29.52	29.61	28.51	54.39	51.81	59.18	27.10	27.14	27.08
90	26.12	26.91	25.80	54.12	56.32	58.54	27.17	27.34	27.08

For the maize grains in silos with initial moisture contents of 14 and 16% w.b., the increase in CO_2 levels was smaller compared to the maize grains in silo with initial moisture content of 18% w.b., being within the range considered safe for storage of maize grains (Fleurat-Lessard, 2017) or beginning of fungal growth (Kaushik & Singhai, 2018). Higher moisture contents in the grain favors the respiratory activity, thus making the environment more favorable to the development of various microorganisms, due to greater water availability.

Valle et al. (2021) indicate an exponential increase in the respiration rate of maize stored at 25 °C and with moisture content above 16.5%. Gregori et al. (2013) reported increase of water activity in maize grains stored at different temperatures, as a result of increments in respiration rate and CO_2 concentration levels, revealing a greater accuracy in the identification of points of deterioration through CO_2 levels.

Zhai et al. (2015) describes that an increase in CO_2 concentration in the mass of stored grains may be associated with fungal proliferation and microbial deterioration of grains. The use of sensors of temperature and moisture content of the grains and CO_2 concentration allows a greater quality control of stored products, which helps predict quantitative and qualitative losses (Lutz & Coradi, 2022).

Figure 1B shows the mean values of technical breakage of the maize grain mass along the storage time. The technical breakage showed an increasing trend as a function of storage time. It is also observed that the values related to technical breakage showed a greater variation for higher moisture content, which can be observed by the slope of the curves in Figure 1B. As the initial moisture content increased, the time required to reach the acceptable limit of technical breakage of 0.5% decreased; 61, 33, and 18 days of storage were required for the initial moisture contents of 14, 16, and 18% w.b., respectively.

When correlating the data in Figure 1A with the technical breakage, it was observed that the value of 0.5% was reached before the peak of CO_2 for the highest initial moisture content, which occurred at about 30 days of storage. The observed values are consistent with a higher respiratory activity, which is more intense for the high moisture contents, leading to a faster degradation of dry matter. The acceptable level of dry matter loss is up to 0.5% (Suleiman et al., 2018), and losses above the recommended level were observed in this study. Garcia-Cela et al. (2020) found a relationship between the increase in water activity and temperature with the increase of respiration rate and, consequently, of CO_2 concentration, which resulted in greater dry matter loss.

Table 2 presents the summary of the analysis of variance with the values of mean squares for moisture content (MC), ash, protein, lipids, electrical conductivity, germination, bulk density and Hue Angle (color) of maize grains stored with three initial moisture contents (Silos) throughout the storage time (ST). There was no individual effect of the treatments on ash content and lightness, and lipid contents were affected by the storage time only. On the other hand, there were effects of interaction between storage time and silo for MC, protein, EC, G%, BD, L* and hue angle.

The behavior of moisture content throughout storage followed the same trend observed in the initial moisture contents, indicating a higher water activity for higher moisture contents during storage. However, maize grains were losing water along the storage time (Figure 2A), reflecting the combination between relative air humidity levels and temperatures recorded during the storage time (Table 1).

The trends observed in maize grains as a function of storage conditions reflect the hygroscopicity of agricultural products, as they are subject to the sorption process, that is, their moisture content tends to be adjusted to come into equilibrium with the relative air humidity and temperature of intergranular air (Corrêa et al., 2014).

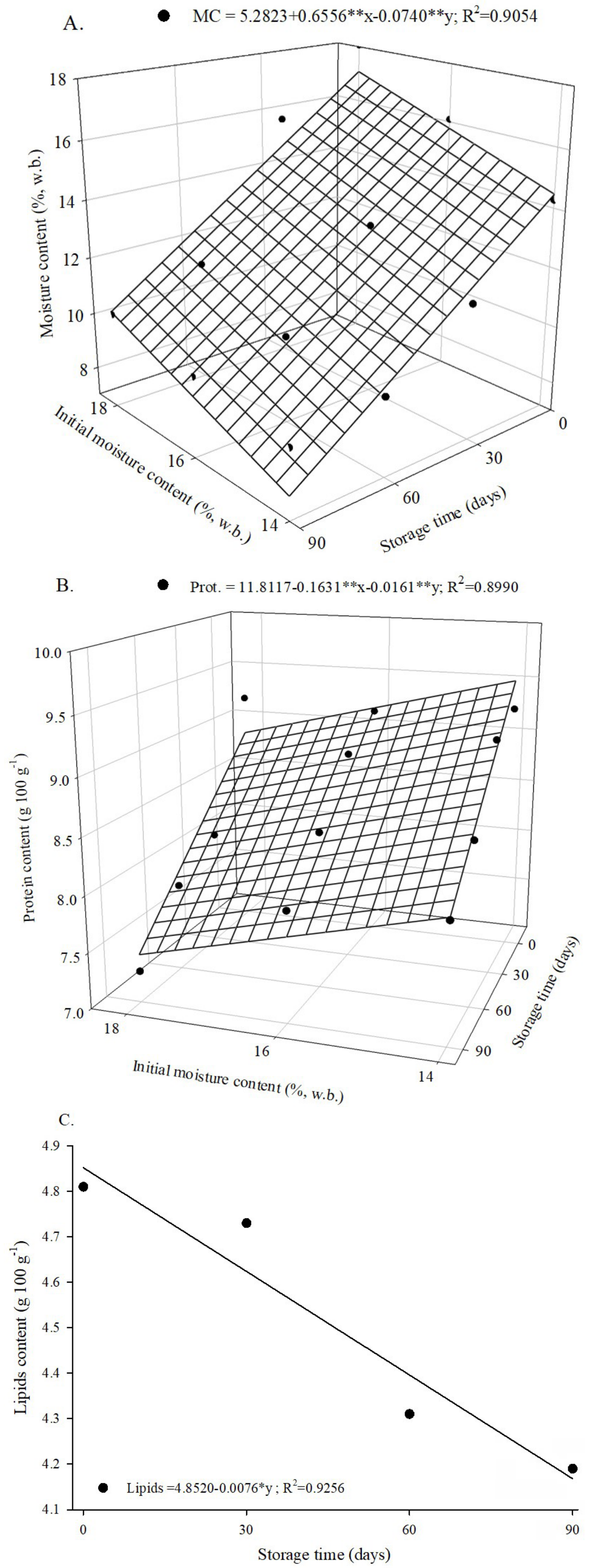
According to Suleiman & Rosentrater (2016), the moisture content of the grains is one of the main factors that influence their respiration, so the behavior of moisture content is acceptable compared to the CO_2 levels generated throughout storage. Even for the highest moisture content studied, there was an abrupt and continuous decrease in CO_2 levels, which reflects lower water availability for deteriorating reactions.

Protein contents showed a decreasing trend with the increase in initial moisture content and storage time, indicating a greater degradation of maize grains (Figure 2B). Schuh et al. (2011), when evaluating the physicochemical quality of maize grains, observed a reduction in protein contents along the storage. The same authors also report that this reduction is related to the moisture content of the grains, which intensifies

Table 2. Summary of the analysis of variance with the values of mean squares for moisture content (MC), ash, protein, lipids, electrical conductivity (EC), germination (G%), bulk density (BD), lightness (L*), chroma and Hue Angle (h*) of maize grains stored with three initial moisture contents (Silo) throughout the storage time (ST)

SV	DF	MC (g 100 g^{-1})	Ash (g 100 g^{-1})	Protein (g 100 g^{-1})	Lipids (g 100 g^{-1})	EC (µS cm^{-1} g^{-1})
Silo	2	42.26**	0.01^{ns}	6.11**	0.12^{ns}	1266.23**
ST	3	158.12**	0.003^{ns}	21.09**	1.65**	649.93**
Silo × ST	6	4.01**	0.01^{ns}	2.26**	0.03^{ns}	145.59**
CV (%)		0.91	9.4	3.09	4.09	3.23
Overall mean		12.43	1.50	8.48	4.51	29.91
		G% (%)	**BD (kg m^{-3})**	**L***	**Chroma**	**h***
Silo	2	2181.33**	2534.11**	214.73**	61.09**	51.52**
ST	5	7871.70**	1342.67**	8.94^{ns}	41.59**	10.69**
Silo × ST	10	149.88**	108.57**	5.44**	6.22^{ns}	0.61**
CV (%)		4.14	0.65	1.90	4.59	1.40
Overall mean		79.63	731.30	59.18	40.16	69.05

**, ns - Significant at $p \leq 0.01$ and not significant by F test; ST - Storage time; CV - Coefficient of variation; DF - Degrees of freedom; SV - Source of variation



*, ** - Significant at p ≤ 0.05 and p≤0.01; x, y - initial moisture and storage time

Figure 2. Moisture content (A) and protein (B) of maize grains stored as a function of initial moisture contents and storage time, and lipid contents as a function of initial moisture content (C)

respiration, and to the higher consumption of reserves, leading to a greater loss of chemical constituents. This is also observed in the behavior of lipid content (Figure 2C).

The mean values of lipid content ranged from 4.8 to 4.06 g per 100 g. Bessa et al. (2020), when evaluating lipid content in grains with moisture content of 11.75 and 13.84% w.b., observed that lipid contents decreased linearly throughout storage, with a reduction of 0.44% for each month of storage. The authors also report that such reduction can be caused by the respiration of the grains, which can consume lipids in the process. Although CO_2 levels did not show a clear trend throughout storage, the increment under higher initial moisture contents is a consequence of this increase in respiratory rate, with a reduction of 14.26% considering the two extremes of the storage time evaluated (Figure 2C).

The values of ash content showed a random behavior, with mean value of 1.50 g per 100 g. Similar results are reported in the literature, with mean value of 1.38 g per 100 g (Schuh et al., 2011). Figures 3A, B and C show the mean values of germination, electrical conductivity and bulk density of maize grains stored with different initial moisture contents and storage times.

The behavior of germination and bulk density was similar, whereas electrical conductivity showed the opposite behavior. The values of electrical conductivity increased with the increase in the initial moisture contents and storage time, revealing a greater deterioration, which led to a reduction in germination.

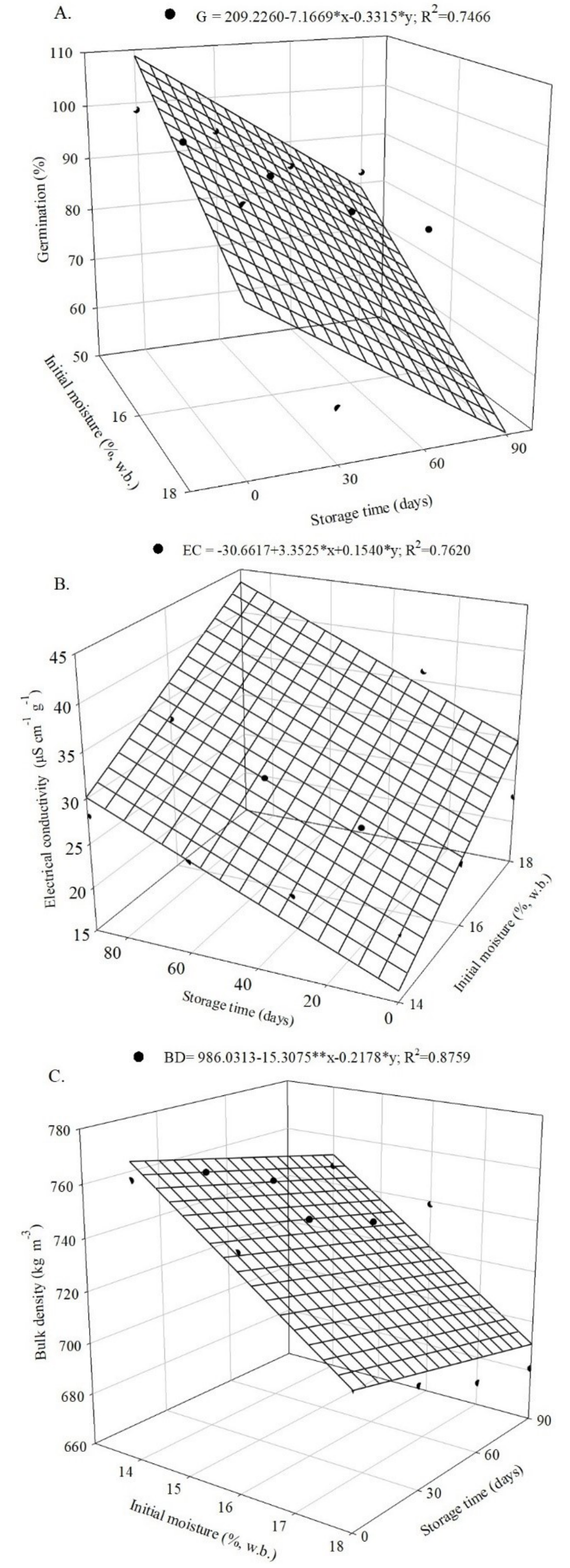
According to Alencar et al. (2009), electrical conductivity is related to the quantity and integrity of cell membranes, and unstructured and damaged membranes increase the value of electrical conductivity and, consequently, reduce seed vigor.

Santos et al. (2012) observed loss of germination, obtaining means below 20% in maize seeds with moisture content of 17.9% w.b. stored for 120 days, a value below that obtained in this study for maximum initial moisture content and storage time (50.39%).

Bulk density can be used as a quality parameter, because the higher the content of reserves, accumulated dry matter, the higher its value. In the present study, bulk density decreased with the increase in the initial moisture content and storage time, contributing to greater damage to the membranes, loss of reserves and, consequently, reduction in germination.

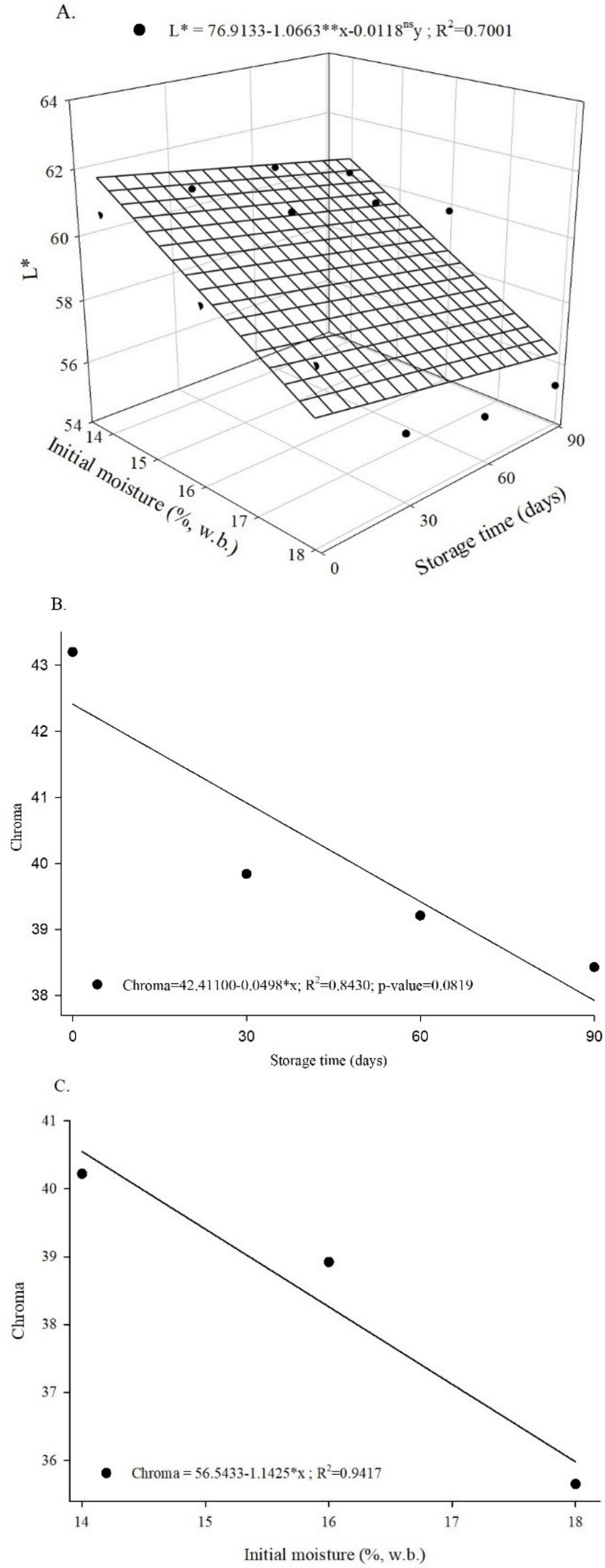
Among the color variables evaluated, a clear trend was observed only for the L* coordinate, which represents lightness (Figure 4A), and chroma (Figures 4B and C), which represents color saturation, with a reduction in their values along the initial moisture and storage time for L*, and separately for chroma. The behavior of the L* coordinate reveals a tendency of maize grains to become darker, a behavior that may also be associated with the increase in temperature observed over the storage time (Table 1), enzymatic browning or oxidative processes caused by the action of enzymes such as peroxidase (Oliveira et al., 2010).

The mean values of chroma decreased at the end of 90 days of storage. The variation in grain color may be associated with reduction in product quality (Alencar et al., 2009). Color analysis helps in characterizing the quality of agricultural products in post-harvest processes, with focus on the



*, ** - Significant at $p \leq 0.05$ and $p \leq 0.01$

Figure 3. Germination (A), electrical conductivity (B) and bulk density (C) of maize grains stored as a function of initial moisture and storage time



* - Significant at $p \leq 0.05$

Figure 4. Lightness (A) as a function of storage time and different initial moisture contents, and chroma (B) as function of storage time and (C) as function of initial moisture of maize grains

consumer, whose decision on the acquisition is based on the visual aspect of the product (Kumar et al., 2014).

Moisture content is one of the crucial factors in grain storage, as it conditions metabolic activity and CO_2 levels that reflect the intensity of deterioration. Due to the sensitivity in determining CO_2 levels with respect to temperature, the dynamics of carbon dioxide constitute a complementary and effective tool in identifying points of deterioration, thus allowing for better containment in the development of microorganisms and pest-insects, which intensify the consumption of dry matter, depreciating the final product quality. The mechanism of metabolic activity containment has a direct effect on reducing CO_2 levels, as well as a lower degree of deterioration of stored grains.

Conclusions

1. The highest initial moisture contents increased the CO_2 levels in stored maize grains, mainly in the initial phase and for the moisture content of 18% w.b., causing greater technical breakage and losses associated with CO_2 emissions.

2. Moisture content, protein, lipids, germination, bulk density, L* and chroma decreased over time, while electrical conductivity increased, resulting in greater damage to membranes and loss of quality of maize grains.

3. Monitoring CO_2 in the grain mass can help make decisions about storage and marketing strategies.

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