Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.5327/fst.29023

CO_2 monitoring associated with the quality of soybean grains stored in different moisture contentes

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Abstract

The objective of this study was to quantify the levels of CO_2 produced by soybeans stored at room temperature in a prototype silo, with initial moisture contents of 12, 14, and 16% (w.b.), as well as to evaluate the quality of the product over storage. Analyses of the moisture content, ash, proteins, lipids, color, germination, and electrical conductivity were performed. In the atmosphere in the silos, the amount of CO_2, relative humidity, and temperature were monitored every hour using a CO_2 meter data logger. Grain quality evaluations took place at four storage times (0, 30, 60, and 90 days). Analyses were performed in triplicate, and data were analyzed using analysis of variance and regression, adopting the 5% level of significance. The moisture contents, protein, lipids, germination, and color were reduced over time, resulting in a loss of grain quality. Soybeans initially stored with 16% (w.b.) of initial moisture content showed greater losses associated with the rise in the levels of CO_2. The rise in CO_2 emission in the internal atmosphere of the silo signals the beginning of grain deterioration and can be used as a decision-making tool, aiming at preventing and reducing losses in product quality over storage.

Keywords: *Glycine max* (L) Merrill; grain degradation; intergranular atmosphere; technical breakage.

Practical Application: An alternative tool to access the quality of stored soybean grains.

1 INTRODUCTION

Brazil is one of the largest grain producers due to its extensive variation in climate and soils. The cultivation techniques implemented allowed the country to produce up to three crops of grains using the same area, reaching high levels of productivity. These characteristics have increased crop production over the years, and in 2018/2019, it grew from 242.1 to 273.8 million tons for the 2020/2021 crop (CONAB, 2021).

According to the data obtained from CONAB (2023), grain productivity in the 2022/2023 harvest could reach 309.9 million tons. Most of the total volume is the result of soybean production, which represents a harvest of around 151.4 million tons, as shown in the 6th Grain Crop Survey 2022/2023.

Even with the increase in crop productivity, post-harvest losses during storage are still a significant challenge for many farmers (Suleiman et al., 2018). One of the obstacles in grain production is the quantity and quality of warehouses. Appropriate control must be maintained in order to avoid degradation of the material stored in these places (Schiavon et al., 2019).

The rate of deterioration depends on the activity of biotic and abiotic variables. Biotic factors are insects and microorganisms, while abiotic factors are grain moisture content, air temperature, relative humidity, storage structure, and intergranular atmosphere such as carbon dioxide (CO_2) and oxygen (O_2) levels (Likhayo et al., 2018).

One of the biggest problems that cause product deterioration is respiration, because the stored grain is a biologically living material. Thus, respiration is a characteristic of the grain itself and, together with the respiration of bacteria, fungi, and insects in the environment, may be responsible for much of the respiratory activity observed in storage (Garcia-Cela et al., 2020; Jian et al., 2019).

Storage temperature and grain moisture content are the main factors that have influence on grain respiration, and high moisture content and temperature during storage result in a shorter preservation time of the product (Suleiman & Rosentrater, 2016). The increase in temperature can lead to the sequencing of the grains and the loss of nutritional components (Liu & Zhu, 2017) and the production of CO_2 from the process transmitted from the grains and associated microorganisms can be related to the loss of dry matter of the final product (Garcia-Cela et al., 2019).

The operationalization of the carbon dioxide (CO_2) monitoring system can contribute as a tool to maintain the quality of grains stored throughout the year (Wenneck et al., 2022). The increase in CO_2 levels in grains is a reliable indicator of deterioration and, consequently, of taking corrective measures in real time (Neethirajan et al., 2010).

Received June 5, 2023.
Accepted July 28, 2023.
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Funding: IF Goiano, FACTO, CAPES, FAPEG, FINEP and CNPq.

To improve the storage system, reduce post-harvest losses, and maintain product quality, the objective of this work was to quantify the CO_2 produced by soybeans grain stored at room temperature in prototype silos, with initial moisture contents (IMCs) of 12, 14, and 16% (w.b.), as well as to evaluate the proximal composition and the technical breakage of the product during the storage.

2 MATERIALS AND METHODS

2.1 Soybean samples

Soybean grains were harvested mechanically with an IMC of 16% (w.b.) on Pai Manoel Farm located at BR-060, 422 km away from the municipality of Rio Verde, state of Goiás. Then, they were transported to the Post-harvest Laboratory of Plant Products of the Instituto Federal Goiano — Rio Verde campus, Goiás state, to be air-dried in the prototype silo until reaching the IMCs of 14 and 12% (w.b.), totaling three different IMCs. The mean of temperature and relative humidity of the drying air was 26.55°C and 73.22%, respectively, and the airflow used in the silos was 30.6 m^3 min^{-1} ton^{-1}.

2.2 Soybean storage

The experiment was carried out in three prototype silos made of metal plates with dimensions of 0.58×0.45 m containing 50 kg of soybeans in each one during 90 days of storage from April to June 2021. The temperature and relative humidity of the ambient air of the storage place were monitored with the aid of a data logger every hour. Inside the prototype silos, the amount of CO_2, relative humidity, and temperature were monitored using an Extech CO_2 meter/recorder model $CO_2$10 every hour. The temperature of the grain mass was monitored three times a day using a portable thermometer with an external probe. Grain quality assessments took place at four storage times (0, 30, 60, and 90 days).

2.3 Soybean grains evaluation

Germination (%) was determined following the Rules for Seed Analysis (Brasil, 2009b), using four replicates of 50 grains, on three sheets of Germitest® paper, moistened with distilled water at a volume of 2½ times the mass of dry paper. Then, they were placed in a biochemical oxygen demand (B.O.D.) regulated at 25±1°C. The evaluations were carried out on day 8 after the test was set up and a root protrusion of 1 mm was considered.

For electrical conductivity (μS cm^{-1} g^{-1}), the methodology described by Vieira and Krzyzanowski (1999) was adopted. Four 50-grain subsamples were used from each replicate during the time, and weighed on a 0.001-g resolution scale. The samples were placed for soaking in plastic cups with 75 mL of deionized water and kept in a B.O.D.-type incubator chamber at a controlled temperature of 25°C for 24 h. The solutions containing the grains were slightly shaken and immediately read on a CD 850 digital electrical conductivity meter.

To determine the moisture content of the grains (%, w.b.), the oven method was employed at 105±3°C for 24 h (Brasil, 2009a) using 10 g per sample. Crude protein was quantified through the Kjeldahl method, which expresses the total organic nitrogen content (AOAC, 2000) using 0.25 g of the sample. Lipids were analyzed using the Soxhlet method (AOAC, 2000). For ash determination, 2 g of the sample was weighed in porcelain crucibles, with previously established mass. The crucibles with the samples were placed in a muffle furnace (Químis Q-318S, Diadema, SP, Brazil) at 550±15°C and kept until the organic matter was completely calcined (about 5 h). The samples were cooled in a desiccator and weighed (AOAC, 2000).

The technical breakage was evaluated by monitoring the loss of dry mass in five-grain samples containing 50 g each, which were wrapped in a permeable fabric (voile) to allow air to pass through the product and distributed within 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 a function of the loss of dry mass due to the respiration of the grains in this period.

Grain color was determined in a spectrophotometer (Color Flex EZ, Hunter LabReston, Canada), in duplicate. The results were expressed in L*, a*, and b*; L values (lightness or brightness) 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 (Equation 1) and hue angle (Equation 2).

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

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

Statistical analysis comprised a completely randomized design (DIC), a 3×4 factorial scheme, with three IMCs of the grains, four storage times, and six replications. Data were subjected to analysis of variance by F test at 5% probability using the statistical package SISVAR® 5.7 (Ferreira, 2019), then linear regression analysis was carried out according to the results of the analysis of variance, and the models were tested at a 5% probability.

3 RESULTS AND DISCUSSION

The mean temperature and relative humidity in the storage room were 27.31°C and 67.91% for April, 25.87°C and 64.15% for May, and 24.64°C and 61.16% in June. However, no accentuated variations were observed in temperature in the mass of the grains stored at different moisture contents, with mean values of 26°C and 24°C at the beginning and end of the storage time.

The levels of CO_2 increased during storage in all assessed silos or IMC (Figure 1). The IMC with soybeans with 16% (w.b.) showed higher gas emissions due to the more intense respiratory process, particularly at the beginning of storage. At the end of storage, grains with different IMCs remained stable in the range of 450–550 ppm (Figure 1).

The levels of CO_2 emission increased during storage of soybeans with different moisture contents (Figure 1), mainly for the IMC of 16% (w.b.) until 30 days of storage (700 ppm). Moisture content is one of the main contributors to biochemical and metabolic reactions such as respiration in stored grains (Valle et al., 2021).

For IMC of 14 and 12% (w.b.), the increase in CO_2 levels occurred after 60 days of storage and in a lower proportion compared with the silo with IMC of 16% (w.b.).

Gregori et al. (2013) reported that the respiration rate and CO_2 concentration of maize stored in silos increased with increasing grain water activity independent of storage temperature. This higher CO_2 emission occurs due to the increase in the respiratory activity of grains, insects, and microorganisms.

Grains with an IMC of 16% (w.b.) showed higher CO_2 emissions with values above 500 ppm, indicating greater degradation at the end of storage. According to Ochandio et al. (2017), the high IMC of grain stored in bulk causes a high risk of degradation.

Figure 2 shows the percentage of technical breakage of stored soybeans. The silo with an IMC of 16% (w.b.) showed the greatest technical breakage during the storage time, resulting in greater mass losses due to chemical oxidation reactions during the respiratory process, which consume accumulated energy in the form of organic compounds such as sugars, starches, and others, effectively reducing the mass of the product (Neves & Savelli, 2017).

The silo with an IMC of 16% (w.b.) showed higher CO_2 emissions (Figure 1). It is also observed that the loss of grain mass due to the respiratory process increases proportionally with each grain storage time.

Aerobic carbohydrate respiration is a combustion process of breaking down dry matter into CO_2, water vapor, and energy (Coradi et al., 2020; Garcia-Cela et al., 2018).

Regarding the behavior of the percentage of breakage, the variations of IMC of 12 and 14% were relatively low when compared with an IMC of 16%, where the slope was 0.0325, equivalent to a loss of 0.0325% of dry mass per day of storage; at the end of the storage time, it was observed that the percentage of technical breakage for the IMC of 12, 14, and 16% was 0.0303, 0.1503, and 3.0641%, respectively. Therefore, the respiratory processes of grains were associated with dry matter loss (Raudienė et al., 2017).

Table 1 shows the summary of the analysis of variance with the mean square values for moisture content, ash, protein, lipids, electrical conductivity, color, and germination of soybean stored in three IMC (silos) over the storage time (ST). It was observed that there was no effect on ash contents and lipid contents showed an effect of storage time separately.

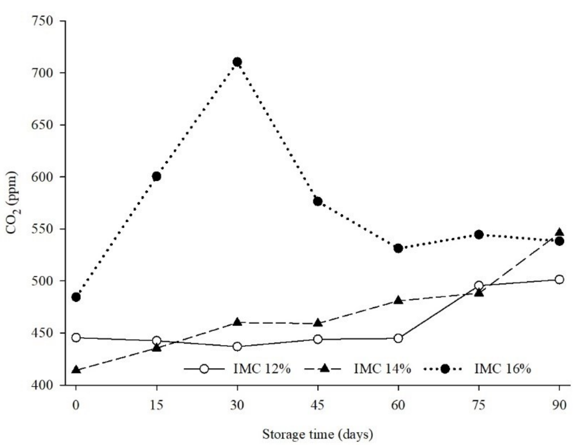
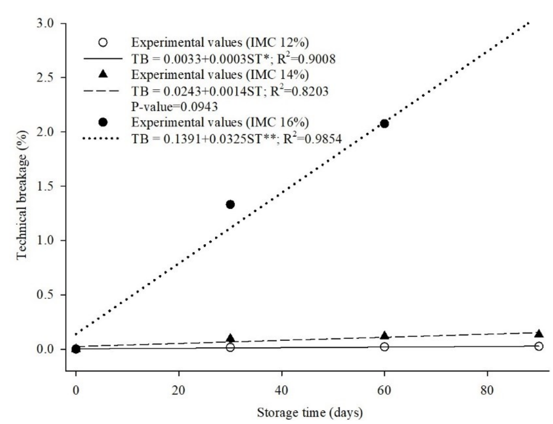


Figure 1. Mean values of CO_2 (ppm) inside the silos over the storage time of soybean grain in three IMCs.



*Significant at $p<0.05$; **significant at $p<0.01$.

Figure 2. Percentage of technical breakage of soybean grains in three initial moisture contents over storage time.

Table 1. Summary of analysis of variance with the mean square values for moisture content, ash, protein, lipids, electrical conductivity (EC), germination (Germ.), luminosity (*L), chroma, and hue angle (°Hue) of soybean stored in three initial moisture contents (silos) over the storage time (ST).

SV	DF	Moisture content	Ash	Protein	Lipids	EC
Silo	2	153.40**	0.01ns	4.59**	0.04ns	2912.54**
ST	3	66.75**	0.21ns	413.42**	99.63**	37776.50**
Silo×ST	6	2.19**	0.01ns	1.03**	0.01ns	426.50**
CV (%)		1.41	4.48	1.42	1.03	2.75
Average		12.87	4.54	32.05	17.28	116.29
SV	DF	Germ.	Luminosity	Chrome	°Hue	
Silo	2	1662.19**	167.38**	49.70**	37.44**	
ST	3	10393.88**	10.80**	64.48**	15.50**	
Silo×ST	6	228.88**	7.90**	5.09**	0.84**	
CV (%)		2.91	1.39	2.32	0.67	
Average		70.52	54.20	32.17	73.80	

*Significant at $p<0.05$ by the F test; **significant at $p<0.01$ by the F test; ns not significant: Silo: Soybean initial moisture content; ST: storage time; CV: coefficient of variation; DF: degrees of freedom; SV: source of variation.

The moisture content of the grains reduced during storage (Figure 3A), due to the exchange of water vapor between grains and air that promoted the hygroscopic balance of the soybean with the atmospheric air (Corrêa et al., 2014).

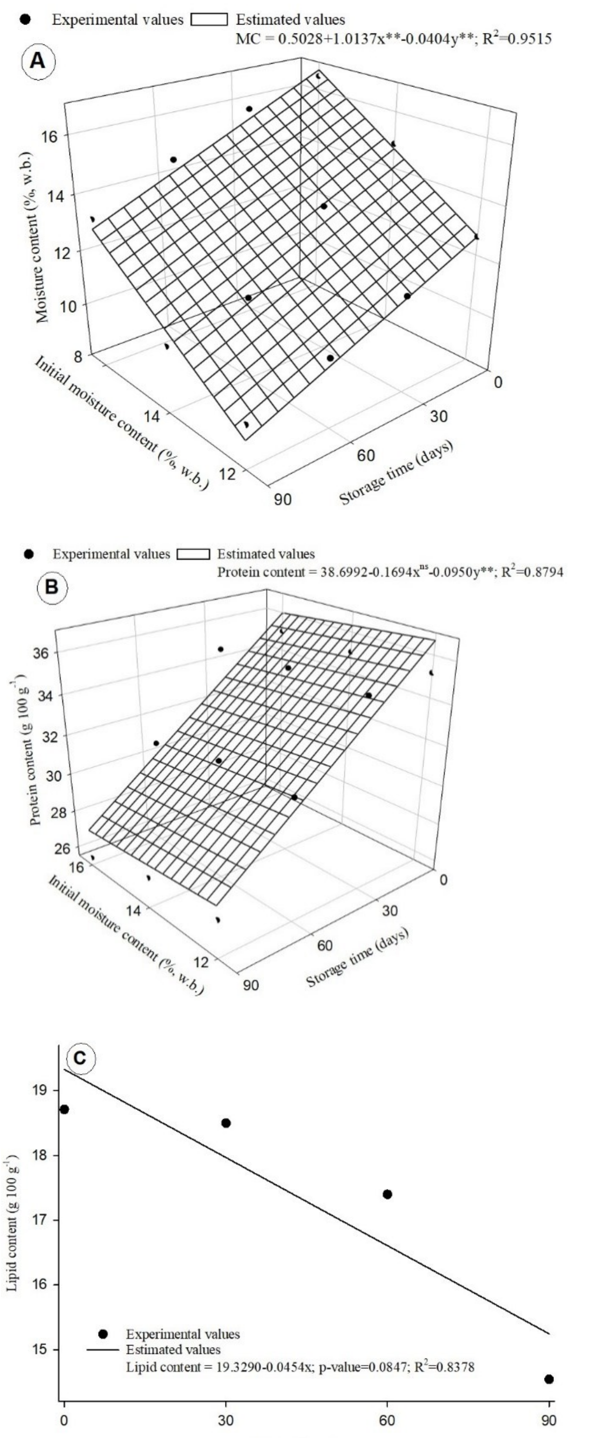
This reduction in moisture content may be associated with the reduction in CO_2 levels at the end of storage. According to Ziegler et al. (2021), the moisture content of the grains is one of the main factors that influence the athlete of the grains.

Protein levels reduced during storage, with greater intensity for grains stored with an IMC of 16% (w.b.), indicating greater degradation of grains in this condition (Figure 3B). Rani et al. (2013) reported that the protein content of black beans stored at different seed moisture contents (i.e., 12, 14, 16, 18, and 20% w.b.) reduced after 16 weeks of storage, with greater reductions associated with grains with high moisture contents.

The average values of lipids reduced over the storage (Figure 3C). The average values were 18.70 g 100 g^{-1} at the beginning of the storage, reducing to average values of 14.53 g 100 g^{-1} at the end of the 90 days. Ziegler et al. (2016) observed a reduction of approximately 3 g 100 g^{-1} in lipid content in soybeans stored at 16% (w.b.) of IMC and 25°C.

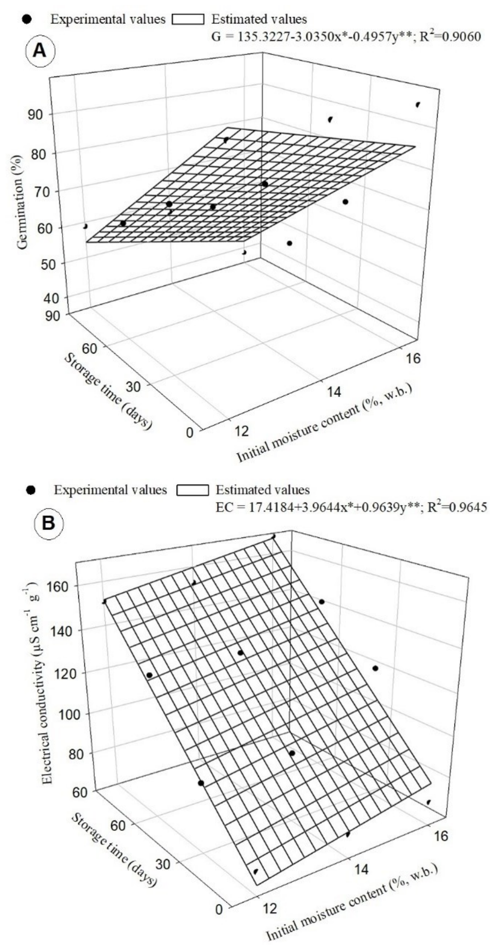
Soybean IMC and storage time did not affect the ash levels, which showed average value of 4.51 g 100 g^{-1}. According to Ferreira et al. (2017), no changes were observed in the ash content of black bean grains stored at 14 and 17% (w.b.) of moisture content during 12 months of storage.

The results shown in Figure 4 indicate that there was a reduction in germination in all treatments, with the greatest



x: initial moisture content, y: storage time; *significant at $p<0.05$; **significant at $p<0.01$.

Figure 3. (A) Mean values of moisture content, (B) protein content of stored soybeans as a function of initial moisture content and storage time and (C) lipid content as a function of storage time.



*Significant at $p<0.05$; **significant at $p<0.01$.

Figure 4. (A) Average values of germination and (B) electrical conductivity of stored soybeans grains as a function of IMC and storage time.

reductions observed in soybeans with a content of 16% (w.b.). The electrical conductivity averages increased during storage, and the highest values were found for soybeans stored with higher IMC.

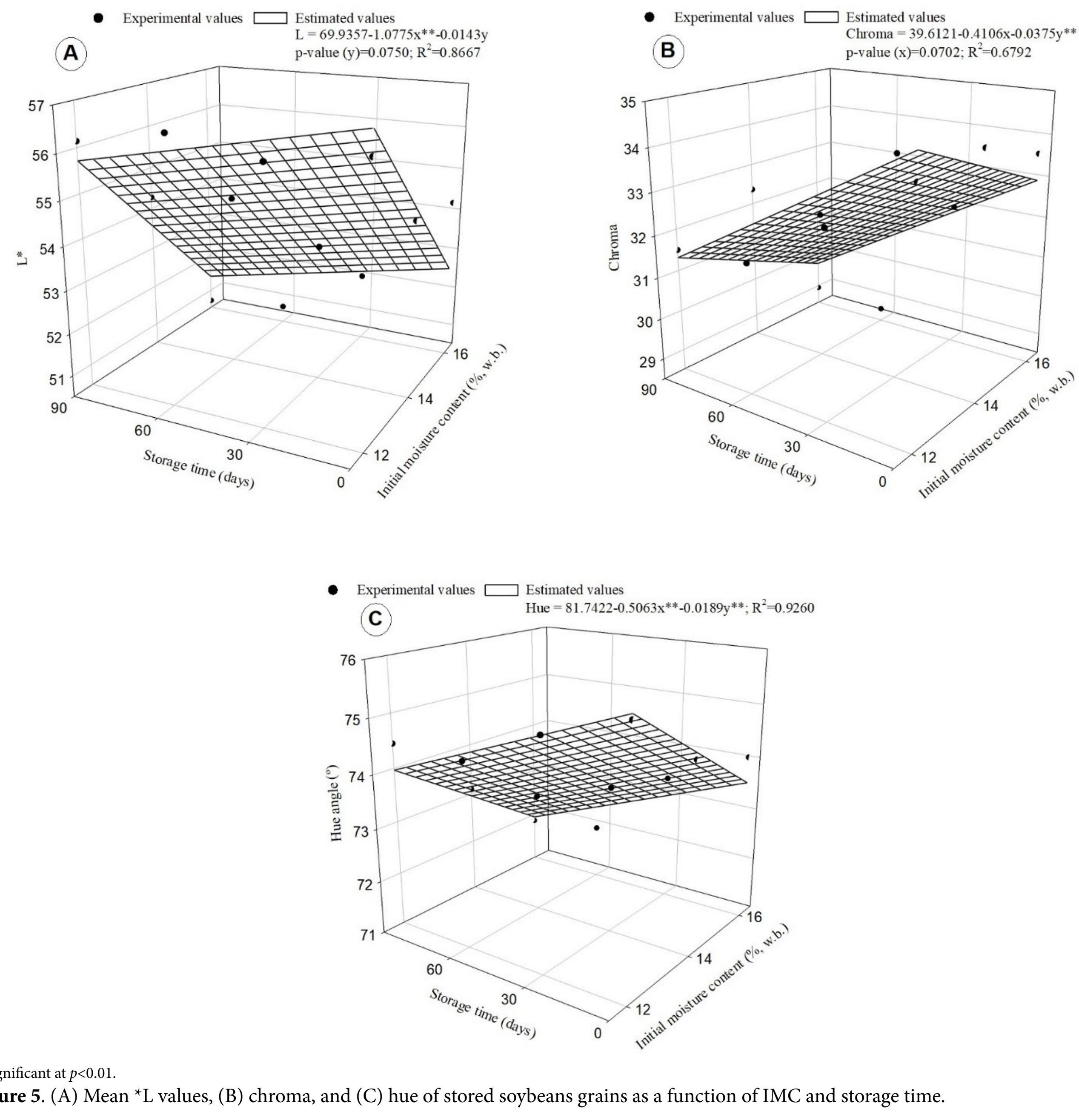
A reduction was found in soybean germination during storage, and mainly in grains with an IMC of 16% (w.b.). This reduction was greater than 58% (Figure 4A). Santos et al. (2012) observed the loss of germination, obtaining averages below 20% in corn seeds with a moisture content of 17.9% w.b. stored for 120 days.

The increase in electrical conductivity (Figure 4B) may indicate the presence of damaged grains and the higher the value of IMC, the more susceptible they were to the attack by insects and microorganisms. According to Costa et al. (2010), electrical conductivity is related to the quantity and integrity of cell membranes, and unstructured and damaged membranes increase the value of electrical conductivity.

The mean values of the color parameters (i.e., luminosity, chroma, and hue angle) (Figure 5) decreased during storage, and soybeans stored with an IMC of 16% (w.b.) had a greater reduction in luminosity (Figure 5A).

According to Alencar et al. (2009), the higher the moisture content of soybeans, the lower the luminosity, as the grains' respiration process intensifies with increasing IMC, combined with storage temperature, enhancing the grains' degradation process.

The means of the chroma (Figure 5B) reduced as the moisture content of the grains increased, after 90 days of storage, being intensified for the grains with an IMC of 16% (w.b.) that



**Significant at p<0.01.

Figure 5. (A) Mean *L values, (B) chroma, and (C) hue of stored soybeans grains as a function of IMC and storage time.

presented the lowest values. Tavares et al. (2016) reported that storage reduces the seed coat color of adzuki bean (*Vigna angularis* Wild.) seeds, which is a fact observed by the reduction of chroma values.

Regarding the hue angle (Figure 5C), a difference was also observed between the moisture contents, with the lowest values for IMC of 14 and 16% (w.b.), respectively. Over the storage time, the hue angle also reduced with greater intensity for grains stored with an IMC of 16% (w.b.).

4 CONCLUSION

Soybean grains stored with an IMC of 16% (w.b.) showed greater technical breakage and losses associated with CO_2 emissions. The moisture contents, protein, lipids, germination, and color reduced over time, resulting in a loss in grain quality.

Monitoring the levels of CO_2 over grain storage can provide information on product deterioration, which is not routinely detected through the measurement of grain mass temperature. The increase in CO_2 emission in the internal atmosphere of the silo signals the beginning of grain deterioration and can be used as a decision-making tool, aiming at preventing and reducing losses in product quality over storage.

ACKNOWLEDGMENTS

The authors would like Pai Manoel Farm for providing the grains.

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