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Microscopy of maize grains subjected to continuous and intermittent drying

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ABSTRACT. Drying is an important step in the post-harvest processes as a way of product conservation and quality preservation. In this context, this study aimed to evaluate the effect of continuous and intermittent drying of maize grains with different rest periods on the integrity of their micro- and macroscopic structures. Maize grains were harvested with a moisture content of 0.3399 ± 0.001 dry basis (db) and subjected to continuous and intermittent drying with 4, 8, 12, and 16 hours of rest period. An experimental fixed-bed dryer, with controlled drying air conditions at a temperature of 100 °C and air flow of 1.5 m^3 $min.^{-1}$ m^{-2} (12 m^3 $min.^{-1}$ m^{-3}), was used. Continuous drying was completed with a moisture content of 0.1628 ± 0.0003 db, whereas intermittent drying was interrupted with 0.2195 ± 0.0002 db and resumed after rest. The drying rate, integrity through grain images, the conformation of particles through scanning electron microscopy, and cell membrane integrity were evaluated. The drying rate increased with an increase in the rest period, the increase in rest period reduced the intensity of cracks, and the reduction in rest period led to higher dispersion and reduction in the size of starch granules and lower integrity of cell membranes.

Keywords: scanning electron microscopy; starch granules; drying rate; cracks, *Zea mays* L.

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Introduction

Visual observation of damage caused by heating during drying is associated with a low-quality product, and the absence of damage is not always an indication of a good quality product, as it can be manifested at a cellular level. According to Franco, Lima, Farias, and Silva (2019), the intermittent drying technique can minimize the quality problems of products. Scanning electron microscopy (SEM) has been frequently used to analyse superficial structures such as whole cells, tissues, and surfaces of various structures due to its high magnification capacity (Balastreire, Harum, & Blaisdell, 1982; Chakraborty, Pallen, Shetty, Roy, & Mazumder, 2020; Sharma & Bhardwaj, 2019).

In crops such as maize, in which sugars are accumulated in the form of starch, SEM allows visualizing structures agglomerated in the form of starch granules due to its trend to form helices (Chakraborty et al., 2020). The size and conformation of starch granules, as well as protein structures, can be visualized efficiently in flour samples (Holopainen-Mantila & Raulio, 2016). The form of starch granules can vary from oval to polyhedral, with a general variation in diameter from 2 to 30 μm (Chakraborty et al., 2020).

Silva, Kênia, Oliveira, and Pinho (2007) evaluated the effect of drying on the soybean crop by ultrastructural and physiological analysis and found that the reduction in cell volume was associated with the drying process due to ruptures in cell structures, the increased leakage of electrolytes, and the lower integrity of cell membranes.

The analysis of cellular ultrastructures assists in understanding the loss of quality during the drying process (Borém et al., 2013). The reduction in particle size observed through SEM is also associated with a lower fraction of starch (Feltre, Silva, Lima, Menegalli, & Dacanal, 2018).

Drying at higher temperatures generates a greater potential for the occurrence of cracks in maize endosperm, which are propagated by the cells around the starch granules (Balastreire et al., 1982). Possible damage due to high temperatures occurs at the end of the drying process and is due to the higher disproportionality between the energy supplied and the amount of water removed (Kumar, Karim, & Joardder, 2014). Thus, adopting an intermittent procedure promotes the better redistribution of water and improvements in terms of energy supplied and water removed, thus leading to a better distribution of starch granules and fewer cracks (Balastreire et al., 1982; Barbosa de Lima, Delgado, Neto, & Franco, 2016; Kumar et al., 2014).

The electrical conductivity test also helps determine the damage at the cell membrane level. The higher the electrical conductivity value, the higher the level of damage caused to cell membranes (Marcos-Filho, 2015). The increase in drying air temperature leads to higher damage due to an increase in electrical conductivity values (Coradi, Milane, Camilo, Andrade, & Lima, 2015; Ullmann, Resende, Chaves, Oliveira, & Costa, 2015).

Thus, this study aimed to evaluate the preservation of micro- and macroscopic structures of maize grains subjected to continuous and intermittent drying with different rest periods.

Material and methods

This research was carried out in August 2018 at the Laboratory of Post-Harvest Processes (LPPC) of the Faculty of Agrarian Sciences (FCA) of the Federal University of Grande Dourados (UFGD), Dourados, Mato Grosso do Sul State, Brazil, and the Central de Análises Laboratory of the Federal University of Technology – Paraná (UTFPR), Campus of Pato Branco, Paraná State, Brazil.

Maize grains from the cultivar Cargo TL were manually harvested at the FCA experimental farm with a moisture content of 0.3399 ± 0.001 dry basis (db). The initial and final moisture contents were determined using the gravimetric method, following the standard S352.2 of the American Association of Agricultural Engineers (ASAE), by subjecting 15-g triplicate samples to drying in a forced circulation oven at a temperature of 103 ± 1°C for 72 hours (ASABE Standards, 2009).

Maize grains were subjected to drying at a temperature of 100°C and air flow of 1.5 m^3 $min.^{-1}$ m^{-2} (12 m^3 $min.^{-1}$ m^{-3}) using an experimental fixed-bed dryer (Figure 1) equipped with a temperature and air flow control system. The experiment was conducted in a completely randomized design consisting of five rest periods (0, 4, 8, 12, and 16 hours), where zero corresponded to continuous drying and the other periods corresponded to intermittent drying, with four replications. Drying air flow was indirectly determined based on the speed (0.02 m s^{-1}), using a digital anemometer with a 0.01 m s^{-1} resolution. A grain volume of 0.035 m^3, with 626.84 ± 0.53 kg m^{-3} of bulk density, was adopted in each treatment for a drying chamber with 0.283 m^2 of base area fully perforated and a grain layer of 0.124 m in height.

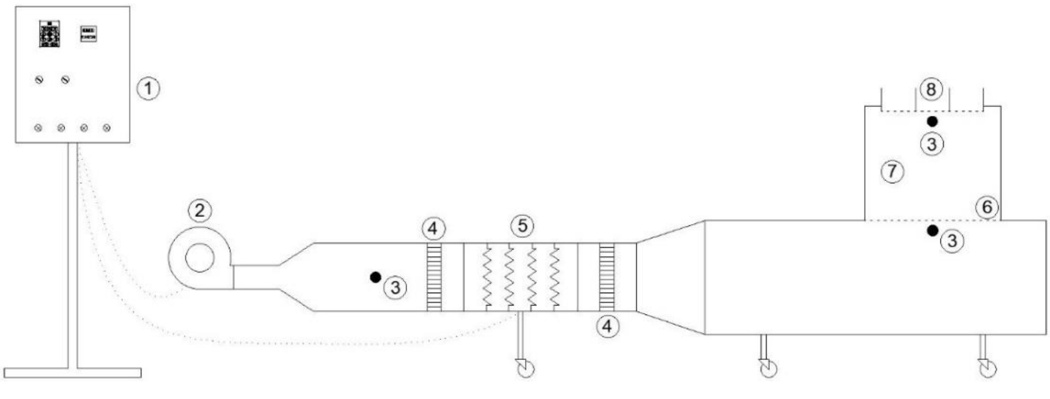


Figure 1. Experimental fixed-bed dryer used in the drying of maize grains. 1 – Temperature and air flow control panel; 2 – Centrifugal fan; 3 – Temperature measurement point; 4 – Air homogenizers; 5 – Set of electrical resistances; 6 – Perforated screen for thick-layer drying; 7 – Thick-layer bed drying; and 8 – Set of trays for thin-layer drying.

The grain mass was turned over at regular intervals throughout the drying process to avoid the formation of temperature and moisture content gradients. Moisture content was monitored based on the loss of mass by the grains using three fully perforated polyethylene packages containing 100 g of the product randomly placed in the middle of the grain mass. Continuous drying was finished with a moisture content of 0.1628 ± 0.0003 (db), whereas the process of the other treatments was interrupted with 0.2195 ± 0.0002 (db), being, subsequently, resumed until the grains reached a moisture content of 0.1628 ± 0.0003 (db).

During the rest, the maize grains were placed inside an expanded polystyrene box completely closed to simulate the silo conditions. This box had an initial volume of 100 L and was resized to present a dimension of 0.510, 0.300, and 0.160 m in length, width, and height, respectively, totalling a volume of 24.48 L (24.48 ×

10^{-3} m^3) (Figure 2), and 0.150-m thick insulation made of expanded polystyrene on all sides. It resulted in an equivalent layer of maize grains of 1.011 m, considering the thermal conductivity of the insulating material (0.024 W m^{-1} °C^{-1}) and the maize grains under the temperature and moisture content conditions during the rest (0.1618 W m^{-1} °C^{-1}) (Suleiman & Rosentrater, 2016; Leila et al., 2019; Chai & Chen, 2010).

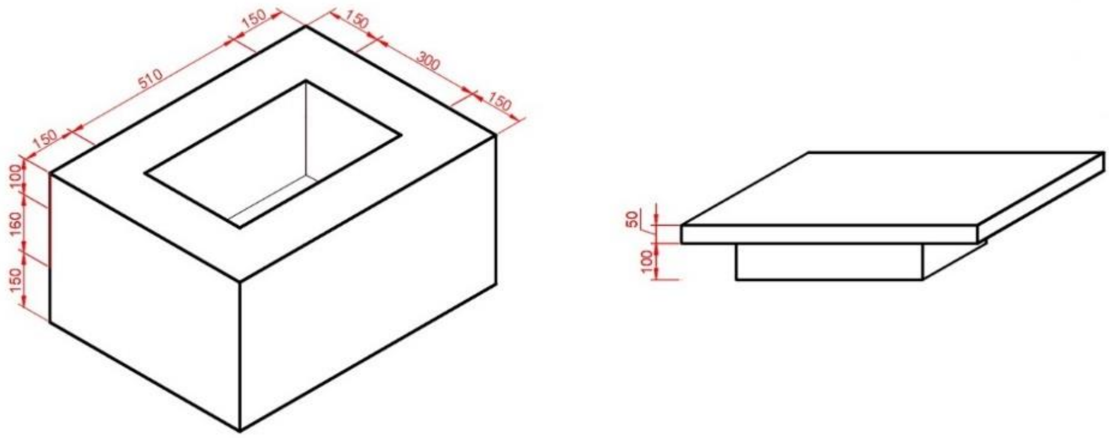


Figure 2. Characteristics and dimensions of the expanded polystyrene box used to hold maize grains during the rest period.

Drying rate

The drying rate was numerically determined by the ratio between the difference in moisture contents and the effective drying time, considering the initial and final moisture contents for several intervals along the drying process based on the following equation:

$$DR = (X_0 - X_i)/(t_i - t_0) = [(mw_0 - mw_i)]/[dm\ (t_i - t_0)]$$

where: DR is the drying rate (kg H_2O kg^{-1} dm h^{-1}), X_0 and X_i are the previous and current moisture contents, respectively (decimal, db), t_i and t_0 are current and previous total drying times, respectively (h), and dm, mw_0, and mw_i are the mass of dry matter, initial mass of water, and mass of water at time i, respectively (kg).

Image analysis

The images of grains were captured using a Canon EOS Rebel T6i photographic camera and an Olympus SZ40 110AL 2X WD38 stereoscopic magnifying glass in the two natural resting positions, that is, front and back.

Maize grains were ground before SEM observation in a Fortnox Star FT 60 Wiley knife mill with a 2-mm mesh sieve. The flour was dried in a forced circulation oven at 65 ± 1°C until reaching constant mass. Then, the samples were placed on copper films to improve electron scattering and placed under a Hitachi 3000 scanning electron microscope to capture images at various resolutions. The particle size was also determined under two magnifications of higher resolution (1,000 X and 2,000 X) by randomly selecting three particles according to the SEM analysis procedure of the Central de Análises Laboratory of UTFPR.

Electrical conductivity

Four replicates of 50 grains were used in each treatment and the mass of each replicate was measured on a digital scale with a 0.01-g resolution. The grains were placed in 100-mL disposable cups, which received 75 mL of deionized water. Then, the cups were placed inside a BOD-type chamber at a temperature of 25°C for 24 hours. Likewise, four cups containing 75 mL of deionized water were also placed in the chamber to determine the conductivity of the used water and deduct it from the reading value (Vieira & Krzyzanowski, 1999). The reading was taken after 24 hours using a conductivity meter and the values were converted from μS cm^{-1} to μS cm^{-1} g^{-1} by dividing the values by the grain mass before soaking.

Statistical analysis

The results were analysed through the observation and interpretation of microscopy images of maize grains and flour. Linear regression models were constructed for data of statistical nature and analysed according to their trend and characteristics related to the biological phenomenon under study using the software SigmaPlot 11.0®. Model validation was based on the significance of the regression by the F-test, coefficients of the model, and coefficient of determination (R^2) at a 1% significance level ($p < 0.01$). The analysis was carried out considering a completely randomized design with five treatments and four replications.

Results and discussion

The drying rate (Figure 3) increased substantially after the rest period in the intermittent drying. This behaviour is associated with the easier removal of water, which was redistributed while the product remained without receiving air flow. The drying rates for all rest periods had the same trend to increase when the drying process was resumed. Drying with the rest periods of 8 and 12 hours showed similar drying rates (0.0805 and 0.0775 kg H_2O kg^{-1} dm h^{-1}, respectively) when compared to continuous drying in maize.

The drying rate decreased continuously throughout the continuous drying (Figure 3) and, therefore, the energy expenditure to evaporate the same amount of water was higher. Moreover, the more energy is transferred to the grain, the higher the chances for the occurrence of some type of physical damage. Barbosa de Lima et al. (2016) recommended the use of intermittent drying, which increases the drying rate, as observed in this research (Figure 3), and reduces the effective drying time.

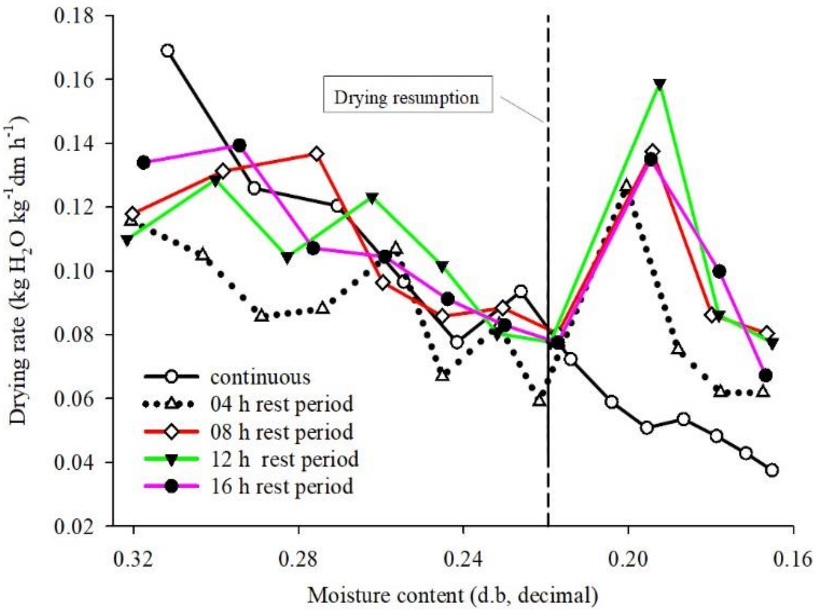


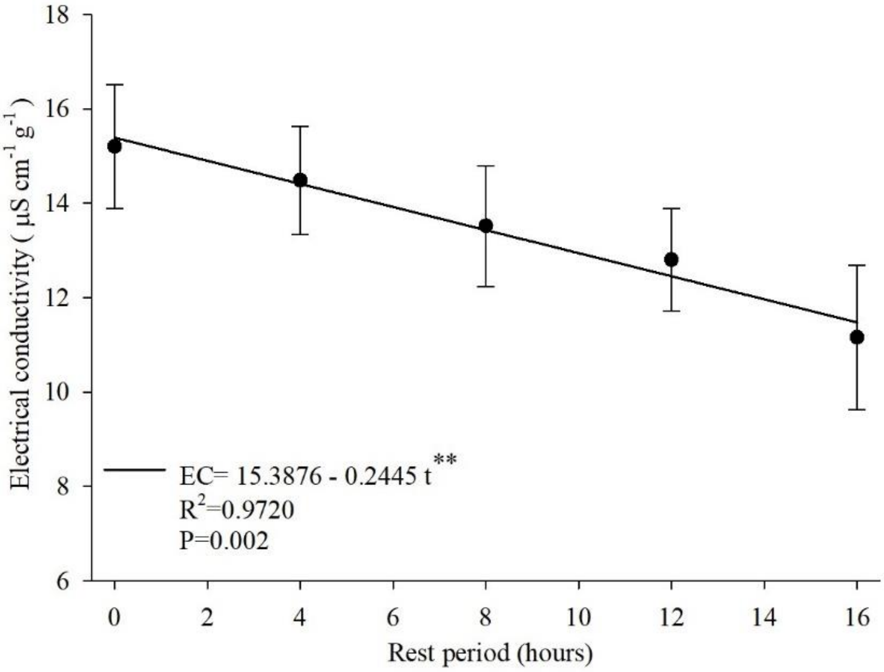
Figure 3. Drying rate as a function of time and moisture content of maize grains (db).

Electrical conductivity decreased linearly with an increase in the rest period of maize grains (Figure 4), and the variable t (time) for this model was statistically significant ($p < 0.01$). The leaching of electrolyte amounts in maize was reduced from 15.19 to 11.15 μS cm^{-1} g^{-1} when the rest period was increased from 0 to 16 hours, respectively, which corresponds to a 26.63% reduction. According to Nascimento, Queiroz, Marchi, and Aguiar (2012), electrical conductivity has been applied to determinate grain quality and not only seeds, as it is commonly used. This test evaluates the integrity of cell membranes and the higher its value, the higher the level of damage. Thus, continuous drying led to a higher level of damage, and the longest rest period caused the lowest impact, preserving the integrity of membranes (Barbosa, Silva, Medeiros, Centurion, & Vieira, 2014; Borém et al., 2014; Ullmann et al., 2015; Vergara, Capilheira, Gadotti, & Villela, 2018).

Figure 5 shows the physical aspect of maize grains after continuous and intermittent drying with different rest periods. The increase in rest period in the intermittent drying positively contributed to reducing the damage, mainly when comparing grains after 4 and 16 hours of resting. The level of damage can be observed by the presence and intensity of cracks, tending to decrease as the rest period increased. This trend is consistent with an improvement in the integrity of cell membranes since there were lower values for longer rest periods (Figure 4), as observed in the intensity of cracks.

The damage associated with drying is in essence irreparable and better detected by the membrane integrity test, as it allows direct reading of the contents leaked into the soaking solution after the electrical conductivity test. Structural changes could also be noticed by observing the visual aspect

(Figure 5) after continuous and intermittent drying. Therefore, the electrical conductivity values and the level of damage can show the storage potential of the product, which could be lower for grains from the continuous process.



** Significant at a 1% level of 1% by the F-test.

Figure 4. Electrical conductivity of maize grains after drying with different rest periods.

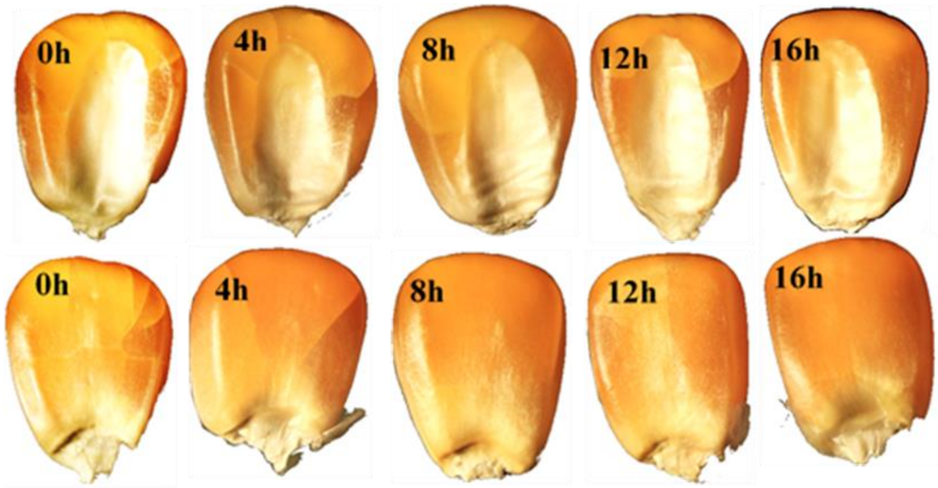


Figure 5. Physical integrity of maize grains subjected to different drying processes. The top and bottom of the image show the front and back sides of the corn grains.

The water concentration gradient formed within the grain during the drying process causes the evaporation rate of surface water vapour to be lower than the rate of replacement and movement of water through diffusion from the interior to the periphery of the product. It increases the tension forces inside the product, causing damage at a cellular level, which becomes visible in more severe cases. The intermittent drying technique improves the redistribution of water inside the product during the rest period, reducing the internal tensions and, consequently, leading to lower damage. According to Franco et al. (2019), intermittent drying minimizes the thermal damages (cracks) caused to the product by reducing the temperature on the grain surface during the process. Also, Wei et al. (2020) reported that drying stress on maize grains is mainly caused by the moisture gradient because of the difference between the convective heat transfer on the surface corn and the internal diffusion. The hard endosperm is the main component of the corn grain, where stress cracks are developed in the initial stage of the drying process, being more significant for continuous drying because of the moisture drying compared to intermittent drying, as shown in Figure 5.

The tempering process or rest period in the intermittent drying decreased the moisture content in the centre of the grain, and the difference in moisture between the centre and the surface decreased gradually, eliminating the moisture gradient after the tempering process (Zhao et al., 2018). Thus, moisture redistribution would be better for longer rest periods, preserving grain quality. Continuous drying using high air temperatures causes quality loss and damages (cracks) on the surface of the product (Franco et al., 2019), as observed in Figure 5.

The higher the imbalance between the evaporation and diffusion phenomena, the higher the probability of occurrence of damage on the surface of the grain due to its low elastic and plastic capacity, thereby reducing its capacity for expansion, especially when the moisture content is low (Barbosa de Lima et al., 2016).

Barbosa de Lima et al. (2016) also reported that the imbalance between the diffusion and evaporation phenomena is associated with the product. Thus, the maintenance of drying conditions and the high energy consumption eventually cause damage because of the high gradient generated on the surface. The authors recommended the use of intermittent drying, which increases the drying rate (Figure 3) and reduces the effective drying time. Structural damage has strong implications in cases of product transportation after the drying process, with significant effects on storage potential.

Abasi and Minaei (2014) observed a higher tendency of susceptibility to breakage in maize grains dried at temperatures of 40 and 70°C. Higher temperatures increased the drying rate, as well as the susceptibility to breakage, an effect similar to that caused by the use of continuous drying or shorter rest periods. Wang and Wang (2019) found a positive correlation between increased susceptibility to breakage and the presence of cracks. Shirmohammadia, Charraulta, and Blencowe (2018) also found that the resting and drying periods promoted changes in the mechanical properties of grains and reduced potential damage.

The increase in drying air temperature can be associated with changes in the structure, composition, and spatial arrangement of biopolymers during hot-air drying due to the presence of a moisture content gradient as the result of the removal of water from the grain, which causes breaks, cracks, and discontinuity in the structure depending on the used drying system (Abasi & Minaei, 2014).

Figure 6 shows the scanning electron microscopy (SEM) of the maize after its continuous and intermittent drying. The empty spaces between granular structures (starch) occur in a higher number in the continuous drying (Figure 6A) and decrease as the rest period increases in the intermittent drying. The SEM image showed that maize samples from the continuous (Figure 6A) and intermittent drying process, with 4 and 8 hours of rest period (Figure 6B and C), presented an aggregated structure with more irregular shapes than the samples that had 12 and 16 hours of rest period. In this case, the particles were more agglomerated and regular after drying (Figure 6D and E).

Lower agglomeration and particle size are associated with reduced starch fraction due to the appearance of cracks caused by the way through which water was removed from the grain when there was no rest or a short rest period was used (Feltre at al., 2018). According to Chakraborty et al. (2020), observations through SEM allow visualizing agglomerated structures in the form of grains, which are the starch, as observed in this study, whereas cracks or ruptures can be visualized as dark protrusions.

The dimensions of the randomly sampled cell structures (Figure 6) showed that the structures had a higher trend to maintain their integrity when rest was adopted during the drying process (Table 1). The average granule size was 9.82 ± 2.85 μm in the continuous drying and ranged from 14.53 ± 1.76 to 17.24 ± 2.25 μm in the intermittent drying (Table 1). The individual measurements ranged from 7.52 to 19 μm, being consistent with the range from 2 to 30 μm reported by Chakraborty et al. (2020). The values obtained in this study are an indication of the size of the starch granules for a typical stress crack. The analysis of variance indicated that the starch dimensions are dependent on the drying rest period at a 5% probability (Table 1). However, no difference was observed between the different periods of intermittent drying. Moreover, a correlation was found between the starch width and the stress cracks in the samples according to the intensity of cracks observed in Figure 5.

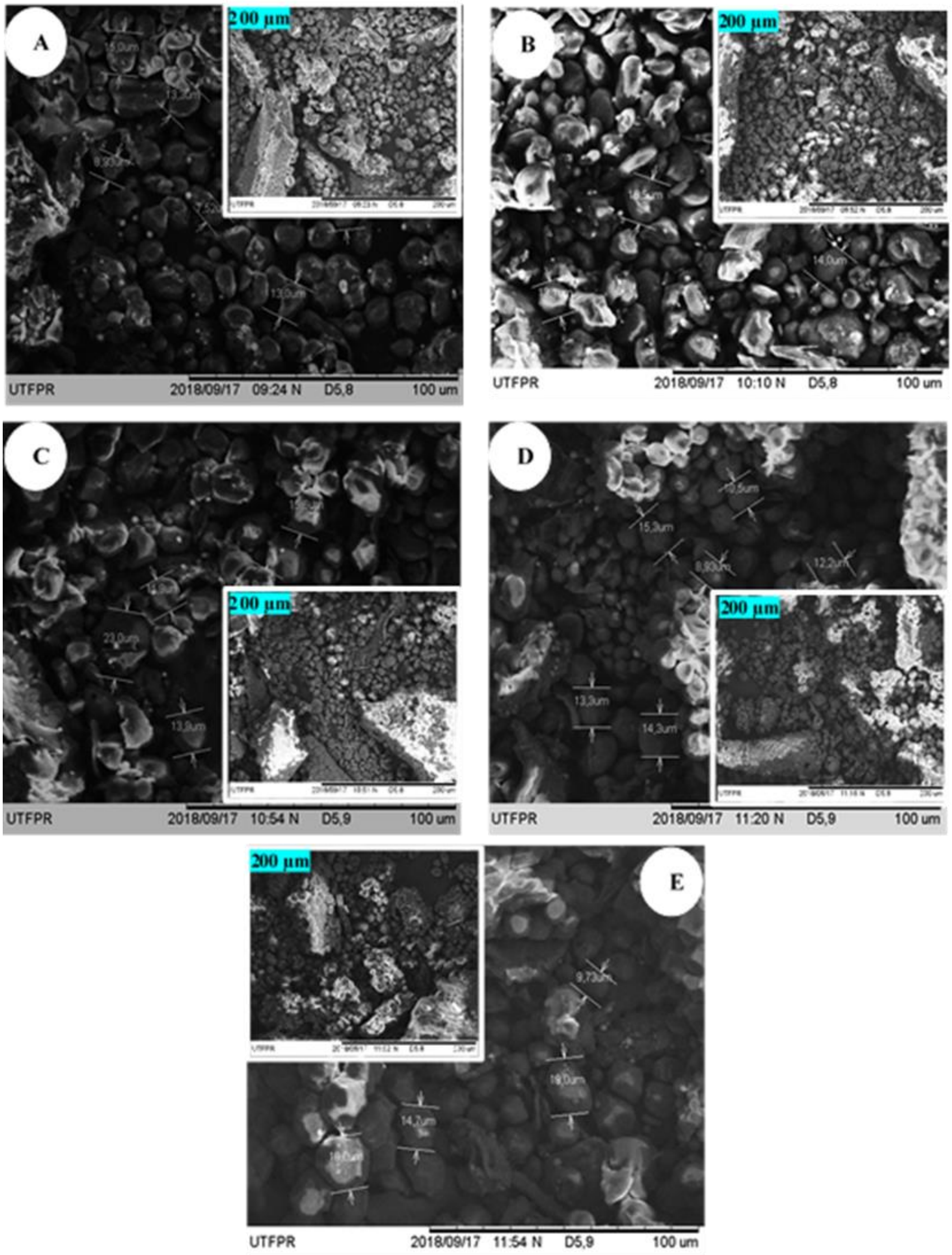


Figure 6. Scanning electron microscopy of maize flour after continuous and intermittent drying of maize grains with different rest periods. Images captured with resolutions of 500 X (200 µm, reduced) and 1,000 X (100 µm, normal). A) Continuous drying; B, C, D, and E) intermittent drying with 4, 8, 12, and 16 hours of rest period, respectively.

Table 1. Width of three starch granules (µm) of maize flour by scanning electron microscopy after continuous and intermittent drying of maize grains with different rest periods for a 1,000 X (100 µm) resolution.

Rest period	Starch granules width (µm) for a 1,000 X (100 µm) resolution			
	1	2	3	Average
0h (continuous)	13.00	8.93	7.52	9.82 ± 2.85 a
4h	13.10	16.50	14.00	14.53 ± 1.76 b
8h	15.60	13.90	16.80	15.43 ± 1.46 b
12h	13.30	14.30	15.30	14.30 ± 1.00 b
16h	18.00	14.70	19.00	17.23 ± 2.25 b
Average				14.26 ± 3.03
CV (%)				13.81

Means followed by different letters differ significantly from each other by the Scott-Knott test at a 5% level.

The occurrence of peaks in the drying rate (Figure 3) after the rest period in the intermittent drying positively influenced the reduction of cracks. Therefore, the integrity of starch granules was less affected, contrasting with the reduction in the continuous drying due to the loss of cell contents and shrinkage (Balastreire et al., 1982; Borém et al., 2013; Silva et al., 2007).

Balastreire et al. (1982) evaluated the fracture of the maize endosperm during drying and found higher separation between starch granules at high temperatures, which may be associated with the behaviour of the drying rate and the higher number and intensity of cracks observed in the absence of rest (Figure 5).

The images showed that the intermittent drying promoted structural improvements at a cellular level and the electrical conductivity test showed a trend of the integrity of the cell membrane. This behaviour is consistent with the results reported by Kumar et al. (2014), who stated that intermittent drying strategies improve product quality and may also promote higher energy efficiency, as the increase in the drying rate after resting represents a reduction in the effective drying time.

Conclusion

Intermittent drying promotes better integrity of cell membranes, evidenced by the low values of electrical conductivity. The intensity of cracks in the endosperm tends to decrease as the rest period increases. Rest periods of 8, 12, and 16 hours are the most favourable for a higher drying rate after rest and better structural integrity of maize grains. The arrangement of starch granules was influenced by the rest period, and larger and more agglomerated granules were observed in the intermittent drying. Starch granules were smaller in the continuous drying and larger in the intermittent drying with different rest periods.

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