SCANNING ELECTRON MICROSCOPY OF COFFEE BEANS SUBJECT TODIFFERENT FORMS PROCESSING AND DRYING

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ABSTRACT: The objective of the present work was to evaluate changes in the structure of coffee beans processed and dried in different ways. The experiment was conducted with two types of processing: dry and wet, and four drying methods: drying on ground and mechanical drying with air heated to 50/40 ° C, 60/40 ° C and 40/60 ° C, where the temperature was changed when the coffee beans reached 30% ± 2% (db), with supplementation of dehydrated to 11% ± 1% (wb). The mechanical drying system used consisted of three fixed bed dryers, which allows control of temperature and flow rate of drying. During the experiment, data were collected to assess the dynamics of drying.After treatment application, the coffee beans were subjected to studies in a scanning electron microscope (SEM). The observation of the coffee beans by SEM showed that the drying temperature of 50/40 ° C pulped coffees showed similar results to those dried in cafes, with a small contraction of the cells, without signs of rupture. It was observed that utilization of the drying temperature 40/60 ° C was the most damage caused to the structures of cells, regardless of the type of processing. It was found that the coffee prepared by dry showed higher destruction of the cellular components of the coffee pulped and, a longer exposure of coffee to the drying process.

Index terms: Coffee post-harvest, ultrastructural analysis, coffee drying curves.

MICROSCOPIA ELETRÔNICA DE VARREDURA DE GRÃOS DE CAFÉ SUBMETIDOS A DIFERENTES FORMAS DE PROCESSAMENTO E SECAGEM

RESUMO: *Objetivou-se, no presente trabalho, avaliar alterações na estrutura de grãos de café processados e secados de diferentes formas. O experimento foi realizado com dois tipos de processamento: via seca e via úmida; e quatro métodos de secagem: secagem em terreiro, e secagem mecânica com ar aquecido a 50/40°C, 60/40°C e 40/60ºC, onde a temperatura foi alterada quando os grãos de café atingiram 30%±2% (b.u.), com complementação da secagem até atingir 11%±1% (b.u.). O sistema mecânico de secagem utilizado constituiu-se de três secadores de camada fixa, o qual permite o controle da temperatura e fluxo de secagem. Durante o experimento foram coletados dados para a avaliação da dinâmica de secagem. Após a aplicação dos tratamentos, os grãos de cafés foram submetidos a estudos em microscópio eletrônico de varredura (MEV). A observação dos grãos de café em MEV mostrou que a temperatura de secagem de 50/40°C dos cafés despolpados apresentou resultados semelhantes aos cafés secados em terreiro, com pequena contração das células, sem sinais evidentes de ruptura. Pôde-se observar também que a utilização da temperatura de secagem 40/60°C foi a que mais causou danos às estruturas das células, independentemente do tipo de processamento. Verificou-se que os cafés preparados por via seca apresentaram maior desestruturação dos componentes celulares do que os cafés despolpados, bem como maior tempo de exposição desses cafés ao processo de secagem.*

Termos para indexação: *Pós-colheita do café; análise ultraestrutural; curvas de secagem do café.*

1 INTRODUCTION

Across different production segments, the search for quality is a major concern. Coffee is one of the few products whose value grows with improved quality. The quality of the coffee beverage is determined by the flavor and aroma, which are associated with chemical substances existing in the grains (Borém, 2008). Genetic, environmental, cultural and harvesting methods are important because they affect directly the quality of the coffee drink. Post-harvest, the drying parameters such as temperature and high drying rates and storage methods, also have significant contributions to the quality of the final product (ALPIZAR; Bertrand, 2004).

The processing of coffee also interferes significantly in its quality. There are two methods for processing coffee: the dry way and the wet. In dry processing, the fruits are subjected to drying intact without removal of the exocarp. In wet processing, can be produced: the cafes pulped, the result of mechanical removal of bark and partly the fruit mucilage; Cherry pulped coffees, sourced

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from mechanically peeled fruit with the remaining mucilage removed by fermentation, and cafes cherry desmucilados, result of mechanical removal of the bark as much mucilage (Borém, 2008).

The drying of the coffee bean is one of the stages of the production chain that relates to their quality. The coffee fruit when harvested at high water content, typically between 55% (wb) and 65% (wb) (Borém et al., 2006). To store them safely, we must reduce to 11% (wb) eliminating thus the risk to the high rates of respiration and growth of fungi and bacteria. Therefore, techniques must be used for efficient drying to maintain quality of the coffee bean and integrity of cell membranes, which favors the preservation of the aroma and flavor (Borém, 2004).

During drying, depending on the temperature used and rates of drying may occur processing chemical, physical and physiological grains, which may lead to a change or disruption of cell membrane selectivity (Ribeiro et al. 2003). There has been, in many works, the natural coffees are more sensitive to desiccation compared to cafes in parchment (Coradi et al., 2008; Taveira, 2009). This fact is of great importance for the handling of these coffees as new mechanical drying techniques, which use different temperatures during the drying process can collaborate in maintaining the quality of the coffee. Coradi et al. (2008) reported that the coffee pulped, in general, showed higher sensory quality when compared to natural coffees and a lesser degree of damage to cellular structures.

There are few studies related to the structural integrity of the coffee beans. Many researchers report that high temperatures reduce the mass of coffee quality. Studies report damage drying coffee beans, committing to obtain a drink of good quality. It is assumed that this is due to disorganization and disruption of cell membranes, which allow contact of chemicals with oxidative and hydrolytic enzymes, which are related to fermentation processes, compromising the flavor and aroma of the drink (Saath et al. 2010). The definition of the temperature and the time when such damage occurs in coffee beans is a current issue. Work aimed at understanding this phenomenon are of great importance.

Ultrastructural studies of tissues after drying, it has been found that the cell membrane is one of the first points of injury (Saath et al. 2010). Increased tolerance to desiccation of coffee seeds is observed due to the slow drying time for induction and operation of the protection mechanisms of the membranes. These analyzes, as well as the cell walls of the endosperm, may contribute to the understanding of the processes of quality loss during drying (Borém et al., 2008; MARQUES et al., 2008). These authors found that high rates of drying are harmful to the membranes of the coffee beans when these are with water content above 30% (db) for natural coffees and 20% (wb) for the pulped coffee when dried with temperature of 60 º C.

The scanning electron microscopy (SEM) comes to combining the research in pursuit of quality. Marques (2006) used the technique for ultrastructural analyzes of coffee beans subjected to various temperatures and periods of pre-drying. By the technique, it can be proved that the drying air temperature of 60 ° C a negative influence on the quality, because it causes breaks in cell membranes and hence the leakage of the plasma substance living cell, within cells and in intercellular spaces. However, it was not possible to evaluate the variation of drying air temperatures before and after the half-dry, on the quality of these coffees.

The study of cellular ultrastructures of coffee beans can help in understanding the processes of quality loss during processing and drying. In this context, the aim of this study was to analyze in natural and washed coffee, the effect of different drying methods in maintaining the integrity of the cell wall and the plasma membrane at the end of the drying process with alternating temperatures.

2 MATERIALS AND METHODS

The experiment was conducted with cherry coffee (*Coffea arabica* L. cv. Rubi), harvested at the Federal University of Lavras, UFLA. The harvested fruits were processed by dry (natural) and wet (pulped), separating only the cherry fruit. After processing, the coffee was dried in four different conditions: drying on ground and machine drying with heated air at 50/40 ° C 60/40 ° C and 40/60 º C, where the temperature was changed while the coffee beans reached 30% ± 2% (wb) with complementary drying up to 11% ± 1% (wb). After drying have been made to the ultrastructural analysis of the coffee beans in the Laboratory of Electron Microscopy and Ultra structural analysis of the Federal University of Lavras.

For coffee processing dry fruits were washed and separated hydraulically. After this procedure, a portion of the natural coffee was taken to the yard for drying and complete another portion subjected to pre-drying yard for two days before being transferred to the dryer.

For coffee processing wet, ripe fruits were peeled mechanically. After stripping, the coffee was subjected to fermentation in water to remove the mucilage, at ambient conditions, with average temperatures of 20 º C for 20h. After this period, the cafes on parchment were washed with water until complete removal of mucilage. When the mucilage was completely removed, a portion of the parchment coffee was taken to the yard for drying and complete another portion subjected to pre-drying yard one day before being transferred to the dryer.

For drying on ground after processing, the coffee remained under ambient conditions. These coffees were thinly scattered grain-to-grain and drying the course of its being folded layer was, according to the methodology proposed by Borém (2008). The temperature and relative moisture content of the environment during the drying period were monitored with a thermo-higrgraph.

After pre-drying, the plots were conducted for three fixed bed dryers, which allows controlling the flow and temperature (T) of the drying air accurately by an electronic panel. The grain layer reached a thickness of 20 cm. The air flow was controlled to 20 m³. Min-1.m-2, corresponding to a velocity of 0.33 ms-1.

The moment of transition from one temperature to another, in the case of treatment with heated air at 50/40 ° C 40/60 ° C and 60/40 ° C was determined as follows: the control of the water content of the grains during drying was done from an initial moisture content of the coffee from the yard, which made it possible to monitor the weight change in the respective samples. The water content of the coffee was determined by standard method ISO 6673 (INTERNATIONAL ORGANIZATION FOR STANDARDIZATION - ISO, 1999).

To determine the time of transition from air temperature, each tray containing the experimental plot was weighed every hour, and the water content was determined by mass difference by applying the equations below. When each drawer hit the mass on the water content of 30% ± 2% (wb) temperature was changed, remaining so until the coffee reaches 11% (wb).

$$Mf=Mi-((Mi\times PQ)/100)$$

$$PQ=[((Ui-Uf))/((100-Uf))]\times 100$$

where Mf: final mass (kg); Mi: initial mass (kg), FP: percentage breakdown (%); Ui: an initial moisture content (% wb); Uf: final water content (% wb).

After drying and cooling, the parchment coffee and natural remained stored in polyethylene bags at ambient temperature of 18 ° C.

The preparation and observation of samples in scanning electron microscopy were performed at the Laboratory of Electron Microscopy and Analysis Ultra structural (LME) , located in the Department of Plant Pathology / UFLA . The samples were cut longitudinally and immersed in fixative (Karnovsky modified) , pH 7.2 and stored in cold storage until the analyzes . They were then transferred to liquid cryoprotectant (30% glycerol) for 30 minutes and cut crosswise into liquid nitrogen. The sections were transferred to a solution of 1% osmium tetroxide in water for 1 hour and subsequently dehydrated in acetone series (25 %, 50 %, 75 %, 90% and 100% three times) and then taken to the critical point apparatus . Specimens were mounted on aluminum " stubs " , using a ribbon placed on a sheet of aluminum foil , coated with gold and observed by SEM LEO EVO 40 PVX . Were generated and recorded digitally , the variable increases , several images for each sample , the working conditions of 20 kV and working distance of 9 mm . The generated images were recorded and open in Photopaint Software package Corel Draw, which were selected and prepared the plates presented in this paper .

From the images generated could observe the contraction of the cells of the coffee beans, filling the intercellular spaces, cell extravasation lumen, forming points in droplets to characterize the effect of processing and drying used.

3 RESULTS AND DISCUSSION

The temperature maximum, minimum, average, and precipitation and humidity during the drying period, which extended from the 16th to 27th July 2009, are presented in Table 1.

Table 2 shows the average water content at the beginning and end of the mechanical drying and relative humidity before drying, 30% (wb) and at 30% (wb) and the total drying time for cafes processed by dry and wet.

The removal of the exocarp and mesocarp in wet processing of coffee helps in reducing the drying time of these cafes. It is observed in Table 2 that, even taking care to leave the natural coffee for a longer period of pre-drying in the yard, the initial water content of natural coffees were higher when compared to cafes pulped. However, this difference would be much higher if the heated air drying of natural coffee is initiated soon after harvest (Borém, 2008; Saath et al., 2010).

TABLE 1 - Averages of ambient maximum, minimum and average precipitation and humidity - Lavras - 2009.

Maximum temperature (°C)	Minimum temperature (°C)	Average temperature (°C)	Precipitation (mm)	Relative humidity (%)
27,10	14,05	19,41	0,006	69,65

TABLE 2 - average values of water content, humidity and drying total drying time for each drying treatment and processing - Lavras - 2009.

Drying treatment	Processing	Water level (% b.u.)		Drying Air humidity (%)		Drying time (h)	
		Start	End	Before 30% (b.u.)	After 30% (b.u.)	Before 30% (b.u.)	Total
50/40°C	Depulped	43,57	11,27	12,72	21,27	3,5	26
50/40°C	Natural	46,12	11,31	12,72	21,27	6	61
60/40°C	Depulped	43,52	10,80	7,88	21,27	3	19
60/40°C	Natural	46,61	11,22	7,88	21,27	5	58
40/60°C	Depulped	42,57	10,49	21,27	7,88	6	13
40/60°C	Natural	44,24	11,35	21,27	7,88	12	38
Terreiro	Depulped	42,95	11,02	-	-	-	156
Terreiro	Natural	46,13	11,21	-	-	-	264

According to Borém et al. (2006) and Ribeiro et al. (2003), the exposure time, the temperature and flow of air drying, the water content of the initial and final product, the ambient air temperature, and relative humidity are factors that affect the dynamics of drying, and have effect significant impact on the quality of agricultural products. Note, in Table 2, the drying treatment 40/60 ° C had a smaller total time when compared with other treatments mechanical drying. This fact stems from the ease of removal of free water when fruits are with higher water contents (Borém, 2008). It may also be noted that the largest total time of drying the coffee beans in treatment occurred in the yard due to the shorter exposure time of these cafes to high temperatures and higher relative humidity environment, these coffees were submitted (Table 1).

It is observed in Table 2, regardless of the drying treatment used, the higher drying times were found in natural coffees, which can be explained by the maintenance of the exocarp and mesocarp these cafes (MARQUES, 2006).

In Figure 1, one can observe the drying curves of coffee and pulped natural variation given by the water content of each drying treatment in function of time.

Analyzing the mechanical drying of coffee by drying curves (Figure 1), it is observed that the water content during the drying time for natural coffee, were always higher compared to the washed coffee. The total drying time of washed coffee was less than the natural coffee, for any drying treatment. The observed differences are justified by the processing employed. In pulped coffee, cut up the exocarp and mesocarp, endocarp getting the perisperm and endosperm. With the removal of these components eliminates all the water contained in the external coatings of the fruit, without which the starting fermented coffee drying with a water content lower than the natural (Table 2).

It is observed in Figure 1 that the drying treatments 50/40 ° C and 60/40 ° C for coffees natural curves were similar in their drying, differing only in total drying time, which was 58 hours to treat 60/40 ° C and 61 hours for treating 50/40 ° C.

The treatment of 40/60 ° C showed the shorter drying time, regardless of the type of processing, requiring 38 hours for natural coffee beans reach 11% (wb) and 13 hours for the pulped coffee beans.

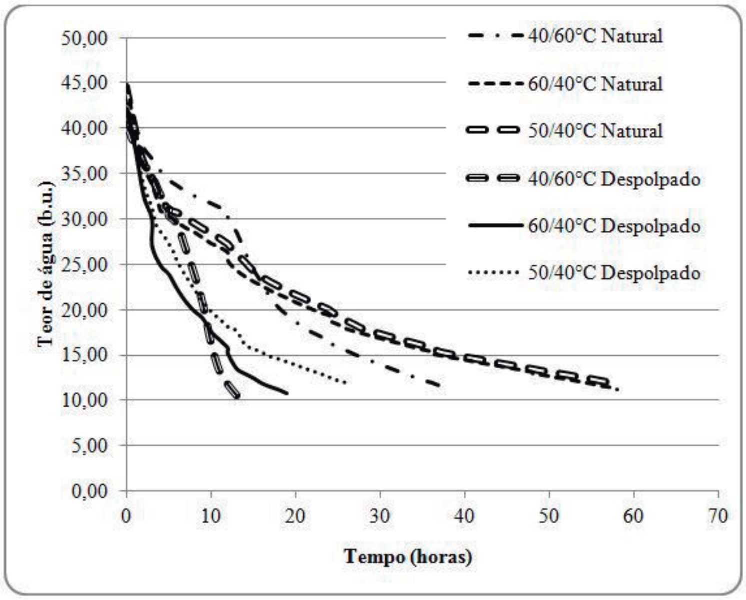


FIGURE 1 - Variation of water content of each drying treatment and mechanical processing as a function of time.

Although at the beginning of the drying process, in this treatment, the rate of water reduction was lower, as can be verified by the lower slope and drying curves after these cafes have reached the half-dry, where the change occurred drying temperature of 40 ° C to 60 ° C, its rate exceeded the drying treatment 50/40 ° C and 60/40 ° C, finishing the drying time under these treatments.

The high drying temperatures and high rates of loss of water degrade the structure of the coffee and cell membranes, leading to extravasation and oxidation in oils, increasing levels of fatty acids with increasing drying temperature (Marques 2006). As noted by other authors (Coradi et al., 2008; MARQUES, 2006; Reinato et al., 2007), the high drying temperatures have interference on the integrity of cell membranes. Coradi et al. (2008), in their studies, they observed that the final quality coffees was affected depending on the drying temperature and the kind of processing, increasing the drying temperature negatively influence the natural coffee, fermented and had little influence.

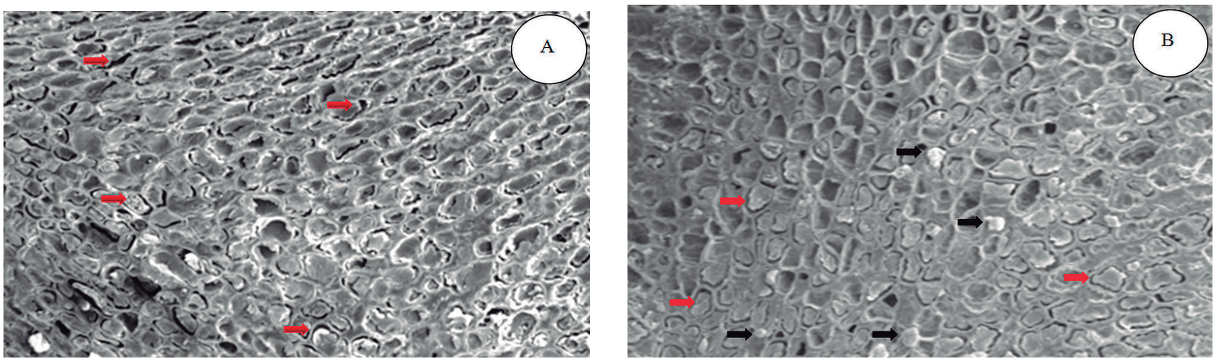
The results of the changes in the structure of cell membranes endosperm of natural and washed coffees, caused by drying effects are observed in the images shown in Figures 2, 3, 4 and 5, which were selected for a series of five images for each repetition.

Figures 2A and 2B shows the results of analyzes for SEM and pulped natural coffees, dried in the yard.

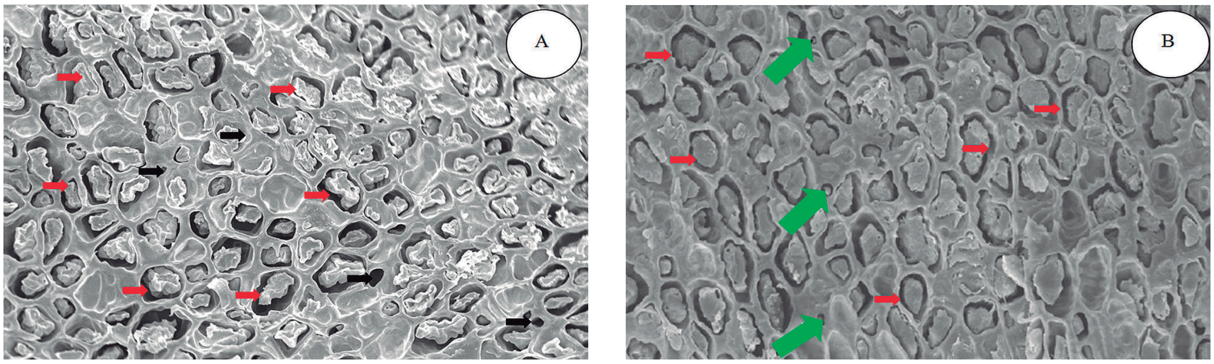
In Figures 3A and 3B shows the results of analyzes for SEM and pulped natural coffees, dried in dryers with temperatures of 50/40 ° C.

The result of this ultrastructural analysis of the endosperm fermented coffee (Figure 2A) and natural (Figure 2B) at the end of drying on ground coffees were similar to those for mechanical drying at room temperature in 50/ 40 ° C (Figures 3A and 3B). Observed for the pulped coffees dried in the yard, the contraction follows the same forms of mechanical drying at 50/ 40 ° C. In both drying methods the internal contents of the cells had contracted to but no signs of extravasation and cellular disruption , and that the space between the plasma membrane and the cell wall , the lumen , as well as intercellular gaps were presented . In natural coffees , dried in the yard, there was a greater contraction in relation to coffee pulped , dried well in the yard, agreeing Saath et al . (2010) , who studied the ultrastructure of coffee beans found that the shrinkage difference can be associated with other mechanisms due to the long exposure time of the coffee in the yard until the water content (11% wb). For natural coffee noted that the cellular material has some points in drop form .

In Figures 3A and 3B shows the results of SEM analysis for cafes and pulped natural, with water content of 11% (wb), dried with heated air 50/40 ° C. For natural coffee, an alteration in cell membrane structures with intercellular space filled, indicating greater disruption of cellular structures of these coffees according to the results of Marques (2006) and Saath et al. (2010) who studied the effect of drying temperature on the changes in the structure of cell membranes, observed the same phenomenon.



FIGURES 2A and 2B - Scanning electron micrographs of pulped coffee beans and natural, respectively, dried thoroughly in the yard until it reaches 11% (wb). (Arrows in red indicate cells contracted, black arrows indicate points in the form of drops).



FIGURES 3A and 3B - Scanning electron micrographs of grains of coffee pulped and natural respectively dried in dryers with temperatures in 50/40 ° C until reaching 11% (wb). (Arrows in red indicate cells contracted, black arrows indicate intercellular spaces empty, green arrows indicate intercellular spaces filled).

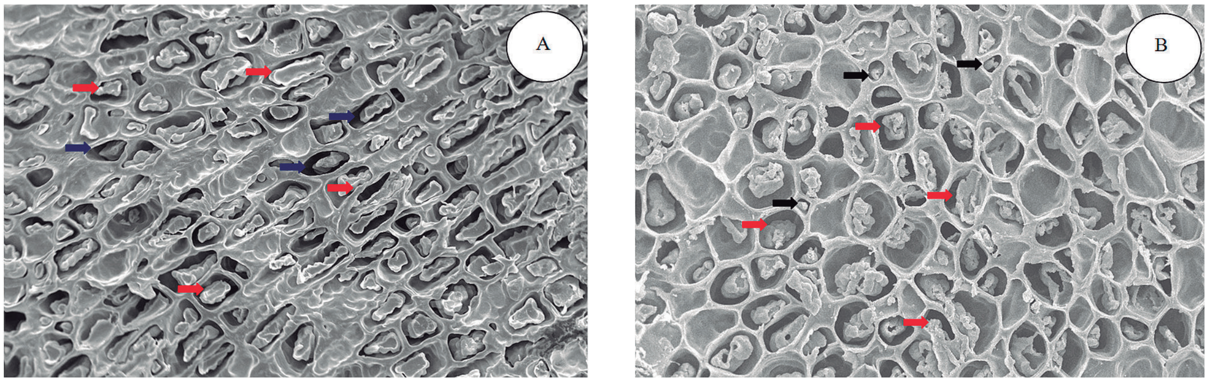
There is also the lumen space between the plasma membrane and the cell wall and intercellular spaces, voids are presented only for the coffee pulped, indicating a greater resistance to desiccation of these coffees when compared to natural coffees. Contractions were observed on the contents of cells of both types of processing, without, however, signs of cellular disruption and leakage of oil droplets.

Figures 4A and 4B shows the results of analyzes for SEM and pulped natural coffees, dried in dryers with temperatures of 60/40 ° C.

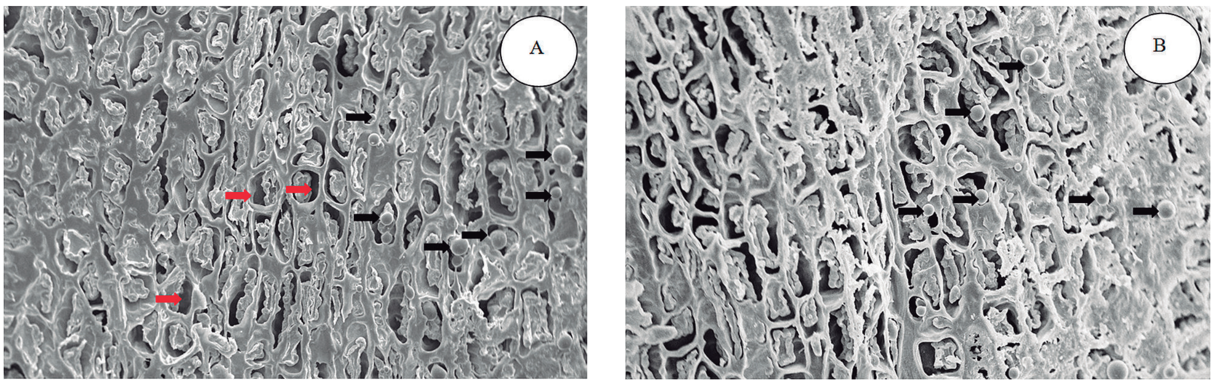
Figures 5A and 5B shows the results of analyzes for SEM and pulped natural coffees, dried in dryers with temperatures of 40/60 ° C.

In the analyzes of the endosperm with fermented coffee beans (Figures 4A and 4B) and natural (Figures 5A and 5B) during the drying temperature 60/40 ° C and 40/60 ° C, respectively, showed that the internal content cells were well contracted, and intercellular spaces filled with some cellular material, or the whole of the cytoplasm had been dehydrated and salient points in drop form on contraction of the cell wall. This means that damage to the membranes, the oil glands present in the internal perimeter of the ruptured plasma membranes, pouring its content, focusing on globular shape of the cellular material, as evidenced by Marques (2006).

For treatment 60/40 ° C and 40/ 60 ° C , the dehydration process proceeded under improper conditions affecting the integrity of the cells according to the results of Saath et al. (2010). Drying with heated air at a temperature of 60/40 ° C is noted that the coffee pulped showed no leakage of oil droplets as compared with the natural coffees , suggesting an increased tolerance to drying of coffee pulped at high temperatures. Moreover, it is noted that the temperature of 40/60 ° C contraction at some point along the drying was more abrupt as compared with the temperature 60/40 ° C , with changes in cell integrity , with leak oils , regardless of the type of processing used , suggesting that the most severe damage in cellular structures occurred after midnight drought.



FIGURES 4A and 4B - Scanning electron micrographs of pulped coffee beans and natural, respectively, dried in dryers with temperatures of 60/40 ° C up to 11% (wb). (Arrows in red indicate cells contracted; Arrows in black indicate intercellular spaces filled, blue arrows indicate the cell lumen).



FIGURES 5A and 5B - Scanning electron micrographs of pulped coffee beans and natural, respectively, dried in dryers with temperatures of 40/60 ° C up to 11% (wb). (Black arrows indicate oil droplets; red arrows indicate the cell lumen).

This corroborates with Saath et al . (2010) in which this author states that the greatest damage caused by the use of a temperature of 60 ° C occurred between water contents of 30% and 20% (wb).

The same author states that , when the water content is above 30% (wb) , all energy is transferred to the fruits used in the evaporation of water, keeping constant the temperature of the product , ie the product temperature remains the same the drying air and heat and mass transfers are offset , which confirms the minor damage caused by the use of temperature 60/40 ° C. However , the use of a temperature of 60 ° C after the half- dry compromised cellular integrity both in natural and in fermented coffee . According Borém (2004) is the phase of lower water content that occur the greatest risk of heating of the product.

In this phase , the rate of internal transport of water is less than the evaporator , thereby transferring heat from the air to the fruits is not compensated by transferring water vapor and, consequently , the temperature of the fruit increases. In this case, the increase in temperature causes tension within the cell , which can compromise the integrity of the plasma membrane , as observed changes in cell structure , and these disruptions expose the cell to oxidative processes . These oxidations are a strong indication that the quality of the coffee was not preserved , as noted by Coradi et al . (2008) in sensory analysis .

It is also known that the greatest damage was observed in the coffee processed by dry independent of the used drying treatment, suggesting that increased exposure to such coffees drying process dramatically affects their cellular structures.

4 CONCLUSIONS

The SEM studies showed that the fermented coffee dried at room temperature in 50/40 ° C and yard, had a higher integrity of cell membranes and vesicles, with no signs of a break, with small cell contraction and intercellular empty spaces, suggesting a better drying condition.

With natural coffee dried at a temperature of 40/60 ° C and 60/40 ruptured and coalescence of vesicles rupture of membranes and extravasation of cellular contents, formation of drops of oil and filling the intercellular spaces.

In natural coffees there was more damage with ruptures and leakages phones evident, regardless of the method of mechanical drying.

5 THANKS

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