

## Antioxidant enzymes preserving coffee quality in refrigerate environment

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### ABSTRACT

Post-harvest phases may cause biochemical and physiological changes with direct effects on coffee (*Coffea* sp.) grains quality during its storage. The aim of this study was to determine the relevance of antioxidant isoenzyme expression on the coffee grains quality undergoing different types of processing and storage conditions. *Coffea arabica* L. ripe fruits was harvested, part of them was dry processed and another part was wet processing. Immediately, the coffee was dried. After drying, some of the grains were hulled and the other part was maintained unhulled. After that, the grains were stored under refrigerate controlled conditions (10 °C and 50% relative humidity) or at 25 °C without relative humidity control for a period of 12 months. Enzymatic expression of catalase, esterase, peroxidase, and alcohol dehydrogenase enzymes was evaluated in the coffee grains in these storage conditions by electrophoretic analysis. The results were compared to the sensorial and physiological profiles of the samples. It was found that expression of enzymes of the antioxidative process is associated with changes in the quality of coffee grains. Natural coffee obtained by dry processing is more sensitive to biochemical changes than wet-processed coffee. A refrigerated environment has a beneficial effect for preserving the coffee grains showed by higher expression of catalase, peroxidase, and alcohol dehydrogenase enzymes after six months of storage in these conditions. The protection of the endosperm by the presence of the pericarp in natural coffee or the endocarp in fully-washed coffee is beneficial to maintenance of quality and is related to expression of antioxidant enzymes.

Keywords: alcohol dehydrogenase, catalase, *Coffea arabica* L., esterase, peroxidase

## Enzimas antioxidantes preservan la calidad del café en ambiente refrigerado

### RESUMEN

Las etapas de la poscosecha pueden causar alteraciones bioquímicas y fisiológicas con efectos directos en la calidad de los granos de café (*Coffea* spp.) durante su almacenamiento. El objetivo de este trabajo fue determinar la relevancia de la expresión de isoenzimas del proceso antioxidante, en la conservación de la calidad de los granos sometidos a diferentes tipos de procesamiento y condiciones de almacenamiento. Los frutos de *Coffea arabica* L. fueron cosechados en estado de maduración cereza y secados, luego de ser beneficiados por vía seca y por vía húmeda. Después del secado, parte de los granos fue trillada y otra parte conservada en coco o en pergamino. Luego, los granos fueron almacenados en condiciones

controladas de aire refrigerado (10 °C y humedad relativa del 50%) o a 25 °C sin control de la humedad relativa, durante un periodo de 12 meses. La expresión enzimática de las enzimas catalasa, esterasa, peroxidasa y alcohol deshidrogenasa fue evaluada en los granos de café a lo largo del almacenamiento por medio de un análisis electroforético. Estos resultados fueron comparados con el perfil sensorial y fisiológico de las muestras. Se constató que la expresión de las enzimas del proceso antioxidante, está asociada con alteraciones en la calidad de los granos de café. El café natural obtenido por beneficio mediante secado, es más sensible a las alteraciones bioquímicas del beneficiado por vía húmeda. El efecto beneficioso de la refrigeración del aire de almacenamiento en la preservación de la calidad del café, es evidenciado por la mayor expresión de las enzimas estudiadas después de seis meses. La expresión de las enzimas del proceso antioxidante, está asociada al efecto protector del pericarpio y endocarpio.

Palabras clave: alcohol deshidrogenasa, catalasa, *Coffea arabica* L., esterasa, peroxidasa

## INTRODUCTION

In post-harvest, coffee (*Coffea* spp.) grains are subject to numerous physical, physiological and biochemical changes, which may have a negative effect on sensorial quality (Borém *et al.*, 2013; Saath *et al.*, 2014; Selmar *et al.*, 2014; Taveira *et al.*, 2014; Ribeiro *et al.*, 2016). These events could cause grain deterioration, induce the production of reactive oxygen species (ROS), enzyme degradation and inactivation, reduction in respiratory activity, and loss of cell membrane integrity (Vidigal *et al.*, 2009; Sharma *et al.*, 2012; Coelho *et al.*, 2017).

Some environmental stresses lead to production of ROS that could cause oxidative damage to the tissues depends on the delicate equilibrium between the production and their scavenging (Dussert *et al.*, 2006). The progress of the grains deterioration under stress conditions, as occurs during storage, can be detected by changes in expression of enzymatic systems (Dussert *et al.*, 2006; Sharma *et al.*, 2012). Therefore, biochemical analyses can provide deterioration markers and could contribute to ensure preservation of coffee grains quality. The changes in the activity of certain enzymes, depending on the intensity, time of exposure and the type of stress (Moussa and Abdel-Aziz, 2008; Han *et al.*, 2009).

Coffee fruits are beneficiate by mechanical processing operations for removing the pericarp, in the case of natural coffee, and the endocarp in fully-washed coffee. These operations can cause cracks or latent damage that will later show up, during storage. The mechanical damage lead to destruct cell membranes and provoke cell disorganization

with furthering reduction in quality (Selmar *et al.*, 2008).

Usually, coffee grains are stored after processing and drying. The large-scale storage carried out in conventional models using jute bags in an environment without temperature and relative humidity control. Therefore, grains are exposed to stress conditions. An alternative to reduce or avoid the effect of stress on grains quality is the storage in a refrigerate environment which has shown positive results (Araujo *et al.*, 2008; Rigueira *et al.*, 2009; Abreu *et al.*, 2017). Nevertheless, the role of antioxidant enzymes in this storage condition not have been fully elucidate.

The aim of this paper was to determine the relevance of antioxidant isoenzyme expression on the coffee grains quality undergoing different types of processing and storage conditions.

## MATERIALS AND METHODS

### *Plant material*

Fruits of *Coffea arabica* L. cv. Catuaí Amarelo harvested from an experimental farm of Fundação Procafé in the municipality of Varginha, MG, Brazil.

### *Fruit processing*

Fruits were harvested selectively in the ripe stage of maturation (cherry) and then it were washed to remove floaters fruits malformed or attacked by coffee borer and other impurities before processing. Part of the selected fruits were immediately submitted to drying (natural coffee) and another part was subjected to mechanical pulping and

removal of mucilage through fermentation in water for 24 hours (fully-washed coffee) and then, drying.

#### *Grain processing*

The grains (seeds) were dried on suspended polyethylene screens and turned 12 times per day until reaching 30% moisture content wet basis (wb) for natural coffee and 25% wb for fully-washed coffee. In the half-dried state, the grains were transferred to fixed-bed mechanical driers where the temperature was maintained at 35°C and constantly monitored using a mercury thermometer until the grains reaching 11% wb moisture content.

After drying, a part of the grains was hulled in the pulper (Palini & Alves, model PA-DESC), connected to the dryer, for cleaning, hulling, and ventilation, and the other part was not hulled.

#### *Grains storage*

The coffee grains were packed in Jutex® polypropylene bags and stored for 12 months in two environments: in a climate-controlled cooling chamber (10 °C, 50% RH) and in a storage room at constant 25 °C, without control of relative humidity. At evaluation time, the coffee grains stored without hulling were hulled manually to avoid damage.

#### *Isoenzymatic analysis*

Before and after three, six, and twelve months of storage, samples were collected from all the treatments, and it were kept in a deep freezer (-80 °C) until performing electrophoretic analysis of isoenzymes.

The extraction of the esterase (EC 3.1.1.2), catalase (EC 1.11.1.6), and alcohol dehydrogenase (EC 1.1.1.1) enzymes was performed with buffer 0.2 M Tris HCl, pH 8.0 and 0.1% β-mercaptoethanol at the rate of 320 µl per 100 mg of grains. The mixture was homogenized in a vortex and kept for 60 minutes at 4 °C in a refrigerator, followed by centrifuging at 14 000 rpm for 60 minutes at 4 °C.

In the case of peroxidase (EC 1.11.1.7) enzyme the extraction was made in phosphate buffer (0.034 M disodium phosphate, 0.2 M

sucrose, 2.56% PVP40, 3M DTT, 5.7 mM L-ascorbic acid, 2.5 mM sodium borate, 1% PEG 6000, 0.002% β-mercaptoethanol) and 2 g PVP 40 (polyvinylpyrrolidone) antioxidant.

Electrophoresis analysis was performed in a polyacrylamide gel system with a 7.5% separator gel and 4.5% concentrator gel. The gel/electrode system used was Tris glycine, pH 8.9. An aliquot of 50 µl of the samples supernatant was applied in the gel, and the electrophoresis was carried out at 120 V for 5 hours. The gels were revealed for the enzymes esterase, catalase, alcohol dehydrogenase, and peroxidase, as described by Alfenas (2006). Evaluation of the enzymatic patterns was carried out in a qualitative manner according to the presence and intensity of the bands, using a transilluminator.

The results of isoenzymes analysis were compared to sensorial analysis of the coffee and physiological profile of the grains, evaluated by the germination test.

#### *Sensory analysis*

The sensory analysis followed the protocol set by the Specialty Coffee Association (SCA) for sensory evaluation of specialty coffees (Lingle, 2011). According to this methodology, scores are attributed to fragrance/aroma, acidity, body, flavor, aftertaste, sweetness, uniformity, clean cup, balance and overall impression.

Moderately light roasting was carried out in 100g of coffee beans (> 16 mesh). Temperature was monitored to assure that the roasting time was not shorter than 8 min or longer than 12 min. The roasting sample pattern of the first storage period was stored in order to standardize the roasting process and to prevent it from influencing the evaluation of the tasters. Such pattern was used as reference during the roasting process in each evaluation period. All samples were roasted at least 12 hours prior to tasting. The final sensory evaluation results encompassed the sum of all attributes.

#### *Germination test*

Performed in four replicates of 50 grains without the parchment, on germitest-type paper sheets moistened with distilled water,

in a germinator regulated at 30 °C. The evaluation was performed at 30 days after sowing, expressing the results in percentage of normal seedlings, based on the recommendations of the RAS (Brasil, 2009).

## RESULTS AND DISCUSSION

Electrophoretic analysis showed that in natural coffee the expression of catalase was higher in comparison to fully-washed coffee (Figure 1). In other studies, similar results are informed and coinciding with poor physiological quality in wet-processed coffee grains compared to dry-processed (Taveira *et al.*, 2012). Over the storage period, reduction in expression of the catalase enzyme was observed, especially in natural coffee stored at 25 °C. These results may be associated with the level of deterioration since in these treatments low physiological and sensorial performance is observed throughout storage when compared to grains that are wet processed (Figure 2, Figure 3). The results indicated that storage of natural coffee under 25 °C can propel or accelerate the grains deterioration process.

In relation to the effect of hulling, the mechanically hulled coffee exhibited lower

expression of catalase compared to the unhulled grains over the storage period. Upon associating this result with the physiological profile of the grains, it can be noted that the germination of the hulled and stored grains was null, as of the first period of evaluation. In addition, in the sensorial profile, a greater reduction was also noted in the quality of the hulled coffees stored in the two types of processing (Figure 3).

Thus, the lower expression of catalase can be explained by the fact that these grains are already in an advanced state of deterioration, probably by the presence of mechanical damage caused by hulling, affecting enzyme synthesis (Sharma *et al.*, 2012). As for grains stored without hulling, the presence of the pericarp may have favored preservation of quality throughout storage (Selmar *et al.*, 2008; Rendón *et al.*, 2014). It should be emphasized that these grains did not exhibit mechanical damage since it were hulled manually, before the evaluation.

For the fully-washed coffee grains, differences in expression of the catalase enzyme were not observed, possibly because wet-processed coffee is less sensitive to changes caused by the steps of post-harvest processing compared to natural coffee (Taveira *et al.*, 2012; Malta *et al.*, 2013; Saath *et al.*, 2014).

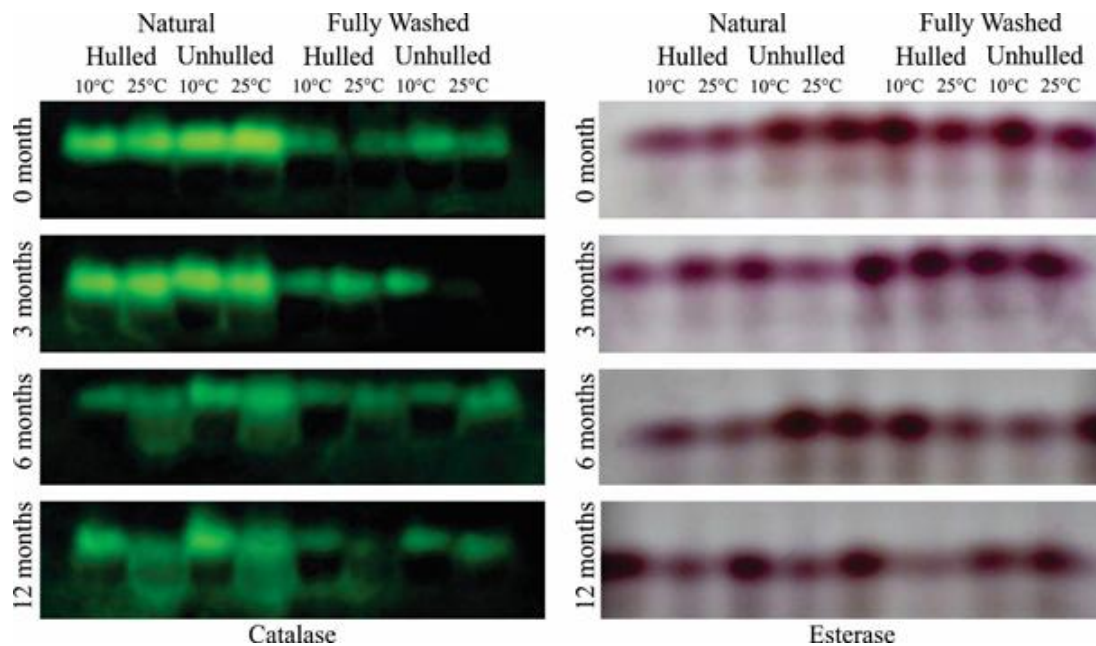


Figure 1. Electrophoretic profile of the catalase and esterase isoenzymes in coffee grains that were dry processed (natural coffee) or wet processed (fully-washed coffee), hulled or unhulled, and stored for 12 months under different conditions.

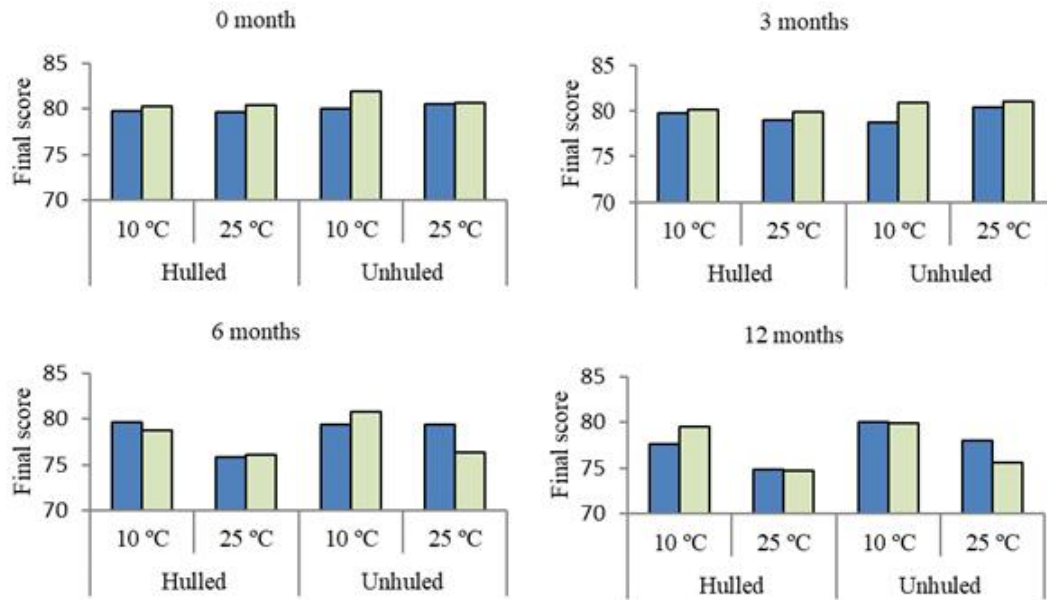


Figure 2. Sensorial profile of coffee grains that were wet processed (fully-washed coffee, in blue) and dry processed (natural coffee, in green), hulled or unhulled, and stored for 12 months under different conditions.

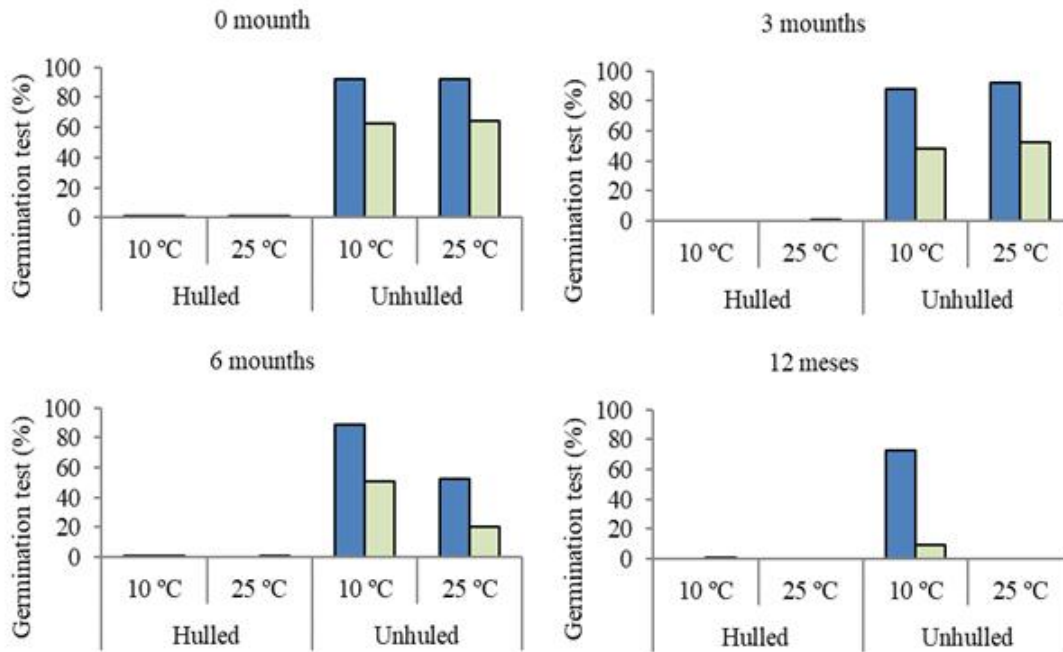


Figure 3. Physiological profile of coffee grains that were wet processed (fully-washed coffee, in blue) and dry processed (natural coffee, in green), hulled or unhulled, and stored for 12 months under different conditions.

In relation to storage conditions (10 °C, RH 50%, 25 °C without control RH), the biggest differences were observed as six months of storage, with higher enzymatic expression of catalase in the grains stored at 25 °C, without control of RH, in comparison to

coffee grains stored in a cooled environment (10 °C, 50% RH). However, this situation was more evident in the dry-processed grains, which was also observed in the results of sensorial analysis (Figure 3). It is known that inadequate storage can lead to

stress conditions for grains, favoring the deterioration process and changing enzyme expression (Dussert *et al.*, 2006). Temperature and relative humidity conditions are extremely important for evolution of the deterioration process in coffee grains during storage (Nobre *et al.*, 2007; Borém *et al.*, 2008; Abreu *et al.*, 2017).

In general, for the esterase enzyme, higher enzyme expression was observed in coffee grains before storage (time zero of evaluation) or after the first months of storage, with reduction in expression of this enzyme throughout storage (Figure 1), just as was observed in evaluation of sensorial quality (Figure 2). In the same way, in other species had been observed higher intensity of the bands and activity of the esterase enzyme before storage and it decline as the deterioration process advanced throughout storage (Padiha *et al.*, 2001; Santos *et al.*, 2004).

Differences were observed in the electrophoretic profile of the esterase enzyme in relation to the type of processing, with higher expression in unhulled natural coffee at the beginning of storage compared to hulled grains. The result of enzyme expression observed in unhulled grains stored at 10 °C after six months of storage demonstrated that lower air temperature and the presence of the pericarp protecting the grains has a beneficial effect.

As for the wet-processed grains (fully-washed), at the beginning of storage, a similar pattern of esterase expression among the treatments can be observed. However, after six months of storage, a reduction in enzymatic expression was observed in hulled coffee grains stored at 25 °C in relation to the other treatments. In contrast, there was no change in enzyme expression when the fully-washed coffee was stored in cooled air (10 °C), regardless of whether the coffee was hulled.

The grains unhulled and stored at a 10 °C showed higher expression of the esterase enzyme during the storage, also exhibited better physiological performance (Figure 2). The results are in agreement with the reports of Brandão *et al.* (2002) that reported a decrease in the number of bands and intensity

of the esterase enzyme with a loss of viability of coffee seeds.

In relation to the peroxidase enzyme, an increase in enzymatic expression was observed throughout storage time (Figure 4). This enzyme is associated with oxidation by peroxides, promoting cell detoxification upon removing hydrogen peroxide. This is an important defense mechanism in plant organisms (Moussa and Abdel-Aziz, 2008; Han *et al.*, 2009).

Before storage, lower expression of peroxidase was observed in fully-washed coffee that was mechanically hulled in comparison to the other treatments.

In fully-washed coffee grains, higher enzymatic expression of peroxidase was observed after sixth month of storage under cool conditions (10 °C and 50% RH) in relation to grains stored at 25 °C. However, in natural coffee, higher expression of the enzyme occurred in unhulled (dry cherry) grains, stored at 10 °C and 50% RH. In both types of processing, the grains that had higher peroxidase expression also had better physiological performance (Figure 2, Figure 3).

Similar results were informed by others authors, that observed higher enzymatic expression of peroxidase in grains with superior physiological quality since the loss of its activity can make the seeds more sensitive to the effects of O<sub>2</sub> and free radicals on unsaturated fatty acids of the membrane (Taveira *et al.*, 2012; Saath *et al.*, 2014). This situation leads to degeneration of membranes and physiological damage to grains.

In the case of alcohol dehydrogenase (ADH) enzyme, a reduction on its expression was observed (Figure 4), coinciding with reduction in physiological quality (Figure 2) and sensorial quality (Figure 3) of the grains during the storage period in all the treatments. The result confirmed the association between accumulation of acetaldehyde and progression of the deterioration process during storage (Zhang *et al.*, 1994). ADH enzyme acts in the anaerobic metabolism of plants, promoting a reduction of acetaldehyde to ethanol (Buchanan *et al.*, 2015).

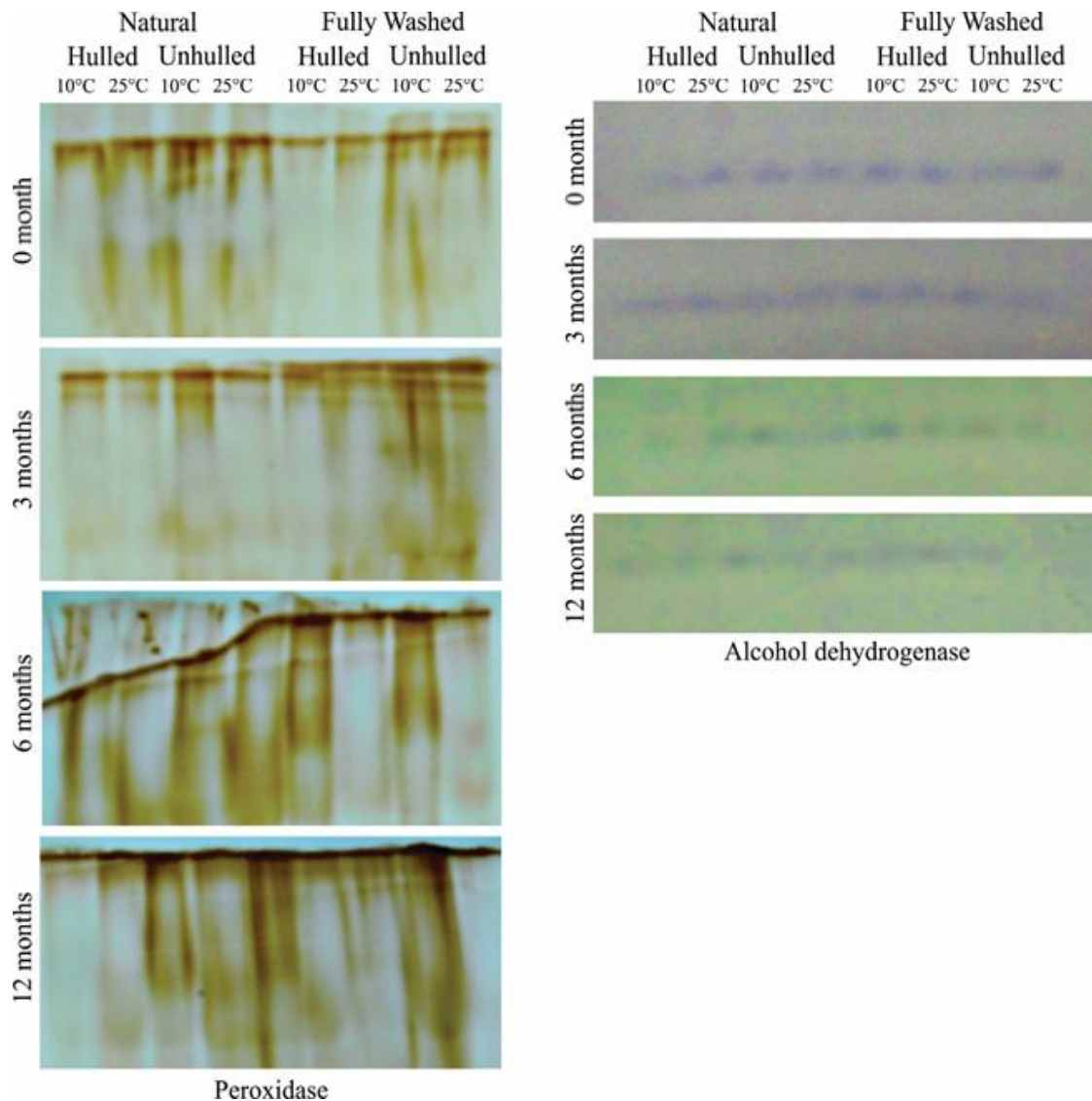


Figure 4. Electrophoretic profile of the peroxidase and alcohol dehydrogenase enzymes in dry-processed (natural coffee) and wet-processed (fully-washed) coffee grains, hulled or unhulled, and stored for 12 months under different conditions.

Upon comparing the wet-processed grains and dry-processed grains, it can be observed that natural coffee, with lower physiological quality in relation to fully-washed coffee (Figure 2), also exhibits lower expression of alcohol dehydrogenase (Figure 4). The effects of the type of processing are observable after sixth month of storage. This shows the greater sensitivity of natural coffee to storage conditions and, therefore, to the deterioration process.

Electrophoretic analysis revealed that after 6 months of storage, the ADH enzyme showed

an increased expression in grains stored under 10°C and 50% RH. In addition, after 12 months of storage, the enzymatic expression was higher in grains unhulled and stored under conditions more favorable to maintaining the quality. In both situations, the grains that had higher expression of ADH are also those that had better physiological results (Figure 2) and better sensorial quality (Figure 3). These results corroborate those found by other authors, that observed reduction in enzymatic expression of ADH together with a decline in grains physiological performance (Vidigal *et al.*, 2009).

## CONCLUSIONS

Expression of antioxidant enzymes is associated with deterioration of coffee grains during the storage. In addition, coffee grains with dry processing are more sensitive to biochemical changes in the deterioration process than those submit to wet processing, evidenced by higher expression of the catalase and alcohol dehydrogenase enzymes in wet-processes grains. Besides, a refrigerated environment has a beneficial effect for preserving the coffee grains showed by higher expression of catalase, peroxidase, and alcohol dehydrogenase enzymes after six months of storage in these conditions. The protection of the endosperm by the presence of the pericarp in natural coffee or the endocarp in fully-washed coffee is beneficial to maintenance of quality and is related to expression of antioxidant enzymes.

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## Conflict of interest

The authors not declared conflict of interest.

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