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# Protein profile in arabica coffee seeds in electrophoresis gel: importance of freeze-drying

**Nathália Aparecida Bragança Fávaris[1], Sttela Dellyzete Veiga Franco da Rosa[2]*, Madeleine Alves de Figueiredo[1], Stefânia Vilas Boas Coelho[1], Ana Luiza de Oliveira Vilela[1], Lilian Padilha[2]**

**ABSTRACT:** Coffee seeds are sensitive to desiccation and are used or stored with different moisture content values, which may affect the results of quality assessment. The aim of this study was to evaluate the changes in protein profile in electrophoresis gel in coffee seeds with different moisture content values under freeze-drying and without freeze-drying. Two lots of arabica coffee seeds were used, one of newly-harvested seeds and another of stored seeds. The seeds were dried to the moisture content values of 12, 15, 20, 25, 30, 35, and 40%. The physiological quality of the seeds was assessed through the germination test, electrical conductivity, and the profiles of the enzymes SOD, CAT, PO, GOT, MDH, and EST and of heat-resistant proteins. In general, there is an effect on expression of these enzymes in accordance with the presence of free water in the seeds. Moist seeds have little to no enzyme expression. The freeze-drying process allows preservation of coffee seed quality and does not change the functionality of the enzymes studied. The isoenzyme profiles of the antioxidant process in arabica coffee seeds are affected by the initial moisture content of the seeds. The freeze-drying process of the seeds ensures greater sensitivity in detection of the expression of these isoenzymes.

Index terms: *Coffea arabica* L., enzyme expression, isoenzymes, quality assessment.

**RESUMO:** Sementes de café são sensíveis à dessecação e utilizadas ou armazenadas com diferentes umidades, podendo interferir nos resultados da avaliação da qualidade. O objetivo do trabalho foi avaliar as alterações no perfil proteico em gel de eletroforese em sementes de café com diferentes umidades e submetidas ou não à liofilização. Foram utilizados dois lotes de café arábica, sendo um de sementes recém-colhidas e outro de sementes armazenadas. As sementes foram secadas até as umidades de 12, 15, 20, 25, 30, 35 e 40%. A qualidade fisiológica das sementes foi avaliada pelo teste de germinação, condutividade elétrica e os perfis das enzimas SOD, CAT, PO, GOT, MDH e EST e de proteínas resistentes ao calor. De forma geral, ocorre uma interferência na expressão dessas enzimas em função da presença de água livre nas sementes. Sementes úmidas apresentam baixa ou nenhuma expressão enzimática. O processo de liofilização permite a preservação da qualidade das sementes de café e não altera a funcionalidade das enzimas estudadas. Os perfis de isoenzimas do processo antioxidativo em sementes de café arábica é influenciado pela umidade inicial das sementes. O processo de liofilização das sementes garante maior sensibilidade na detecção da expressão dessas isoenzimas.

Termos para indexação: *Coffea arabica* L.; expressão enzimática; isoenzimas; avaliação da qualidade.

***Corresponding author**
E-mail: sttela.rosa@embrapa.br

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[1]Universidade Federal de Lavras, Caixa Postal 3037, 37200-000 – Lavras, MG, Brasil.

[2]Empresa Brasileira de Pesquisa Agropecuária, Embrapa Café, Brasília, DF, Brasil.

## INTRODUCTION

Brazil is the world leader in coffee production (IOC, 2021) at 46.88 million bags (bags of 60 kg of hulled coffee) in a planted area of approximately 1.8 million hectares (CONAB, 2021). In this context, the use of high-quality seeds is of utmost importance in obtaining arabica coffee (*Coffea arabica* L.) seedlings. This constitutes a great challenge for coffee growers, due to the limited longevity of coffee seeds, which show losses in germination capacity (Abreu et al., 2014) and in vigor (Coelho et al., 2015) during storage.

Arabica coffee seeds are classified as intermediate in relation to desiccation tolerance to moisture content near 10%, and as low tolerance in relation to storage under low temperatures (Ellis et al., 1990). Nevertheless, the ideal moisture content for conservation of coffee seeds has not yet been completely defined, and there is considerable divergence among the results obtained in research. In this respect, there are reports that the best moisture content for storage of coffee seeds is from 9% to 11% (Hong and Ellis, 1992; Dussert et al., 1999). In other studies, satisfactory results were obtained in coffee seeds with moisture content values from 31% to 48% (Rosa et al., 2005). Currently, other authors found that coffee seeds can be stored either dried or wet (Penido et al., 2021). Thus, coffee seeds are commonly stored, used, or assessed with different moisture content values.

The assessment of physiological quality of coffee seeds is officially performed through the germination test (Brasil, 2009) and can be complemented by vigor tests or by analyses of biochemical changes and analyses of expression of enzyme systems (Dussert et al., 2006; Sharma et al., 2012; Coelho et al., 2015; Coelho et al., 2017a; Coelho et al., 2017b; Abreu et al., 2018; Coelho et al., 2019).

Deterioration of seed quality is related to oxidative stress, which results in the formation of free radicals. Oxidative stress, among other deleterious effects at cell level, causes enzyme degradation and inactivation, an increase in respiratory activity, and loss of permeability of the cell membrane (Sharma et al., 2012; Coelho et al., 2017a; Coelho et al., 2017b; Abreu et al., 2018). The main enzymes that act together in the antioxidant process are catalase (CAT), superoxide dismutase (SOD), and peroxidase (PO) (Barbosa et al., 2014). The glutamate oxaloacetate transaminase (GOT) enzyme and other proteins are likewise prominent, for they are also used to assess the degree of seed deterioration.

Determination of the protein profile is commonly used in seed studies and divergent results are common, as such results may be affected by the moisture content at which the seeds are analyzed. There are various reports of contrasting results when wishing to associate physiological performance and the expression of proteins or enzymes acting in diverse metabolic processes (Abreu et al., 2014; Santos et al., 2014; Coelho et al., 2015; Coelho et al., 2017b; Coelho et al., 2017a; Figueiredo et al., 2021). In this respect, there are signs that different moisture content values at which the seeds are analyzed may be one of the causes of this divergence.

Freeze-drying / lyophilization is a desiccation method by sublimation under low temperature and high vacuum (Jadhav and Moon, 2015). In general, this method allows preservation of the entire structure and chemical composition, and it is often used in compounds sensitive to deterioration, such as antioxidants of the tocopherol type, ascorbic acid, carotenoids, phenolic compounds, proteins, lipids, fungi, bacteria, and seeds (Rudy et al., 2015). Therefore, it is a useful alternative of preparation for determination of the protein profile of seeds with different moisture content values.

Due to the divergent results regarding the ideal moisture content for use or storage of coffee seeds and a possible effect of water on the assessment of enzyme activity of these seeds (which may affect quality assessment), the aim of this study was to investigate the changes in the protein profile in electrophoresis gel of coffee seeds with different moisture content values, under freeze-drying or without freeze-drying.

## MATERIALS AND METHODS

### *Location and plant material*

The experiment was conducted in the Central Seed Laboratory of the Department of Agriculture of the *Universidade Federal de Lavras* (UFLA) in Lavras, Minas Gerais, Brazil. Fruit from the arabica coffee cultivar Topázio MG 1190 was used. The fruit was harvested in the cherry stage from the middle branches of the plant in the middle part of the branches at the experimental field of EPAMIG at Três Pontas, MG. After that, the fruit was washed and mechanically hulled; the mucilage was removed from the seeds by fermentation in water for 24 h and then pre-dried on screens, in the shade, for removal of surface water.

### *Seed drying and preparation*

The seeds were dried in the presence of activated silica gel in seed germination type (*gerbox*) boxes, arranged in a single layer on metal screens. The containers were sealed and kept in BOD type chambers regulated to a temperature of 25 °C without light. Water loss during drying was monitored by continual weighing on a precision balance with 0.001 g resolution until achieving the moisture content values of interest in this study: 12, 15, 20, 25, 30, 35, and 40% wet basis (wb). Two lots of these seeds were used: one that was newly harvested and another that was kept in impermeable plastic bags for nine months in cold storage at 10 °C and 50% relative humidity. The aim of this storage was to obtain a seed lot with different physiological quality in relation to the lot of newly-harvested seeds.

After drying, the seeds, without parchment, were assessed for physiological quality (germination and vigor) and underwent biochemical analyses.

### *Physiological assessment*

#### Germination test:

Four replications of 50 seeds for each treatment were used. They were sown on *germitest* type germination paper moistened in distilled water in the amount of 2.5 times the weight of the dry paper. The rolls were then kept in a seed germinator at a constant temperature of 30 °C for 30 days (Brasil, 2009). Determinations were made of percentage of root emergence at 15 days and of normal seedlings at 30 days after sowing (Brasil, 2009). After the end of the germination test, the normal seedlings were maintained, and the percentage of seedlings with expanded cotyledonary leaves at 45 days after sowing was determined, as well as the dry weight of the shoots and roots.

#### Dry weight of seedlings:

The dry weight of the seedlings was determined at 45 days after sowing. The shoots were separated from the roots and the plant material was placed in paper bags, which were dried in a forced-air circulation oven at 60 °C for 4 to 5 days or until reaching constant weight. Dry weight was determined on a precision balance, with the results expressed in $mg.seedling^{-1}$.

#### Electrical conductivity test:

Four replications of 25 seeds were first weighed and then placed to soak in containers with 37.5 mL of deionized water (Krzyzanowsky et al., 1991) and kept for 24 h in BOD at a constant temperature of 25 °C. After that period, the electrical conductivity of the solution containing the seeds was read, in $\mu S \cdot cm^{-1}.g^{-1}$.

### *Protein expression in electrophoresis gel*

For biochemical analysis of the protein profile, part of the seeds of the lots, with different moisture content values, was freeze-dried, and the other part was analyzed without freeze-drying, that is, seeds were analyzed at their initial moisture content values. The purpose of freeze-drying was dehydration of the seeds with different initial moisture content to values near 4% wb.

### Freeze-drying of the seeds:

The seeds were kept at a temperature of -86 °C up to the time of freeze-drying. An Integrated Speed Vac System freeze-dryer, Liobras model L101, was used, operating under vacuum and a mean pressure of -150 µHg for 120 h. After freeze-drying, the seeds were macerated in liquid nitrogen in the presence of polyvinylpyrrolidone (PVP), and the ground material was stored from 4 °C to 6 °C in impermeable packaging up to the time of analyses. The non-freeze-dried seeds were also macerated in liquid nitrogen in the presence of PVP, and the ground material was stored in an ultra-freezer at a temperature of -86 °C.

### Expression in electrophoresis gel:

The methodology proposed by Alfenas (2006) was used for extraction, the electrophoretic run, and revelation of the enzymes of the antioxidant process: superoxide dismutase (SOD), catalase (CAT), peroxidase (PO), glutamate oxaloacetate transaminase (GOT), malate dehydrogenase (MDH), and esterase (EST), and of the heat-resistant proteins.

### *Statistical analysis*

For the physiological assessments, a completely randomized experimental design was used in a 2 × 7 factorial arrangement (2 seed lots × 7 moisture content values), with four replications. Analysis of variance was performed with the assistance of the R software (R Core Team, 2021) and the means were compared by the Scott-Knott test at 5% probability. The results obtained for the heat-resistant proteins and for the isoenzymes were interpreted taking the presence/absence and the intensity of bands in the gel into consideration.

## RESULTS AND DISCUSSION

The physiological quality of the newly-harvested seeds and of the seeds stored for nine months are shown in Table 1. In general, the two seed lots had different levels of physiological quality, confirming the use of a seed lot of better quality and another of inferior quality.

Before drying, the seeds of the newly harvested lot and of the lot stored for nine months had germination of 85% and 65% and moisture content of 41% and 41%, respectively. In the drying process from 40% moisture to the limit of 12% moisture, the stored seeds exhibited similar germination percentages, ranging from 53% to 71%. In the newly-harvested seed, the wettest seeds (40% moisture) obtained assessments of greater physiological quality than the seeds at the other moisture content values (Table 1).

In relation to dry weight of the seedlings, no significant differences were observed among the seeds at different moisture content values within each lot. However, in the newly-harvested seeds, the dry weight of the shoots and roots were greater compared to the stored seed lot, indicating greater physiological quality of the coffee seeds soon after harvest (Table 1).

In the electrical conductivity test, the results varied significantly in the two lots among the different seed moisture values, and there was an increase in the amounts of leachates as the seeds were dried, both in the newly-harvested seeds and in the stored seeds. This increase in electrical conductivity in seeds with lower moisture content indicates the destructuring of the membranes as the seeds lose water, which results in greater leaching of solutes in soaking seeds. In spite of that, the different values of electrical conductivity were not reflected in reduction in the physiological performance of the seeds, as observed by the germination results (Table 1).

The freeze-drying of coffee seeds for 120 h led to dehydration to the moisture content of 4% wb, possibly removing all the free water. It should be noted that the purpose of using two seed lots was to investigate the effects of moisture on enzyme expression, given the different levels of quality and even physiological performance within each seed lot. Comparing the physiological results or results of the protein profile between the two seed lots was not the purpose of the present study.

Table 1. Physiological quality of seeds of the cultivar Topázio MG 1190 with different moisture content values without storage or stored for nine months.

| Moisture (%) | RE (%) | NS (%) | CL (%) | SDM (mg.seedling$^{-1}$) | RDM (mg.seedling$^{-1}$) | EC (µS.cm$^{-1}$.g$^{-1}$) |
|---|---|---|---|---|---|---|
| Newly-harvested seed lot | | | | | | |
| 12 | 95 a | 75 b | 72 b | 42.62 a | 7.82 b | 34.89 a |
| 15 | 92 a | 74 b | 72 b | 44.76 a | 8.90 a | 36.71 a |
| 20 | 96 a | 78 b | 72 b | 41.50 a | 7.89 b | 31.63 b |
| 25 | 95 a | 77 b | 71 b | 42.27 a | 7.96 b | 31.55 b |
| 30 | 95 a | 78 b | 73 b | 42.01 a | 8.11 b | 29.37 c |
| 35 | 92 a | 79 b | 75 b | 41.65 a | 8.33 b | 26.36 d |
| 40 | 94 a | 85 a | 80 a | 40.47 a | 7.77 b | 23.59 d |
| CV (%) | 5.89 | 16.86 | 19.37 | 3.87 | 8.78 | 16.87 |
| Seed lot stored for 9 months | | | | | | |
| 12 | 86 a | 61 a | 54 a | 38.75 a | 6.64 b | 31.72 a |
| 15 | 78 b | 58 a | 55 a | 35.50 b | 7.58 a | 29.88 a |
| 20 | 88 a | 71 a | 68 a | 38.99 a | 6.76 b | 30.66 a |
| 25 | 90 a | 60 a | 57 a | 38.32 a | 6.10 b | 25.11 b |
| 30 | 93 a | 68 a | 61 a | 37.82 a | 6.29 b | 24.65 b |
| 35 | 96 a | 53 a | 53 a | 40.56 a | 6.89 b | 23.67 b |
| 40 | 89 a | 65 a | 63 a | 39.19 a | 7.46 a | 19.44 b |
| CV (%) | 2.84 | 5.59 | 7.19 | 7.59 | 5.64 | 8.08 |

Mean values followed by the same letter in the column do not differ from each other by the Scott-Knott test at the level of 5% probability. Abbreviations: RE – root emergence; NS – normal seedlings; CL – seedlings with expanded cotyledonary leaves; SDM – shoot dry matter; RDM – root dry matter; EC – electrical conductivity.

In the coffee seeds of the two seed lots, variations in the intensity and/or in the appearance of new isoforms were observed in the enzyme profiles of the antioxidant process, performed with or without freeze-drying of the seeds, according to the different moisture content values (Figure 1 and Figure 2). Comparison of enzyme expression clearly shows that there was greater intensity of bands after freeze-drying, as well as the appearance or suppression of some isoforms in relation to the seeds that were not freeze-dried and that had moisture content from 12% to 40%. This mainly occurred for the enzymes SOD and PO at moisture content greater than 25%-30%.

The expression of SOD after storage for nine months is shown in Figure 1 both for seeds without freeze-drying (A.1) and for freeze-dried seeds (A.2). A greater presence of isoforms can be seen in the SOD enzyme, which was expressed only in the freeze-dried seeds. In contrast, there were no changes in the intensity of SOD activity as a result of the levels of moisture in the seeds. In the physiological assessment of these seeds, there were likewise no differences in physiological performance, assessed in the germination test (Table 1). The activity of this enzyme is related to the antioxidant response for neutralizing the reactive oxygen species (ROS) in the loss of physiological quality of the seeds (Berjak, 2006). SOD is the first enzyme to act on reactive oxygen species, converting the superoxide radical into peroxide of hydrogen and oxygen under stress conditions (Gill et al., 2015). It is the first line of defense when connected with a series of events necessary for complete detoxification (Wattanakulpakin et al., 2012).

Just as observed in stored seeds, in general, in newly-harvested seeds after freeze-drying, there was no differential expression of SOD (Figure 2). In these seeds with better physiological quality, a greater presence of isoforms was

likewise found, regardless of the seed moisture content, when the seeds were freeze-dried. In addition, greater expression of SOD is also observed for seeds with 20% moisture, both in the freeze-dried and non-freeze-dried seeds (Figures 2A.1 and A.2). In other studies, such as Coelho et al. (2017a), SOD activity was likewise not greatly associated with physiological quality of coffee seeds.

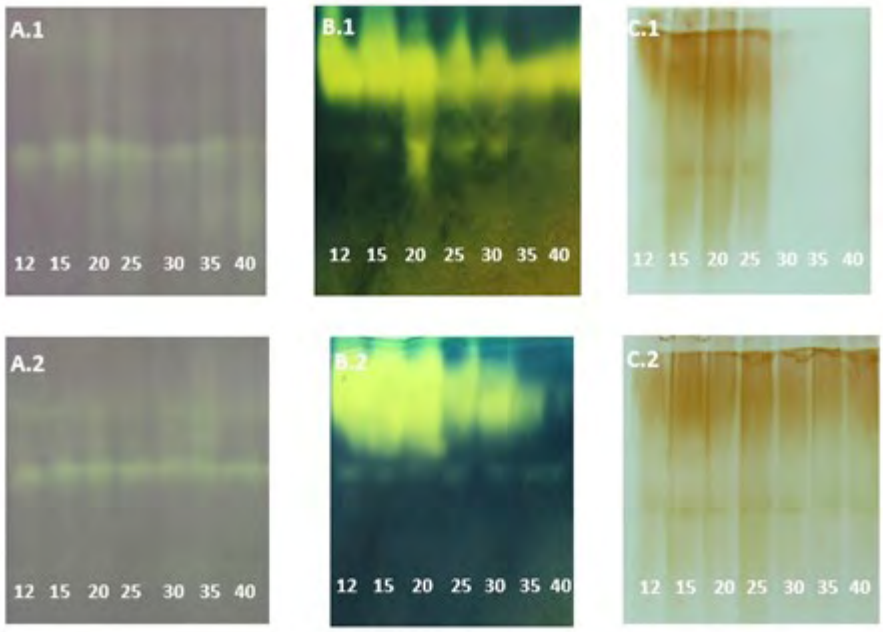


Figure 1. Electrophoretic expression of the enzymes superoxide dismutase (A), catalase (B), and peroxidase (C) in stored seeds of the cultivar Topázio MG 1190 with different moisture content values that were not freeze-dried (1) or freeze-dried (2).

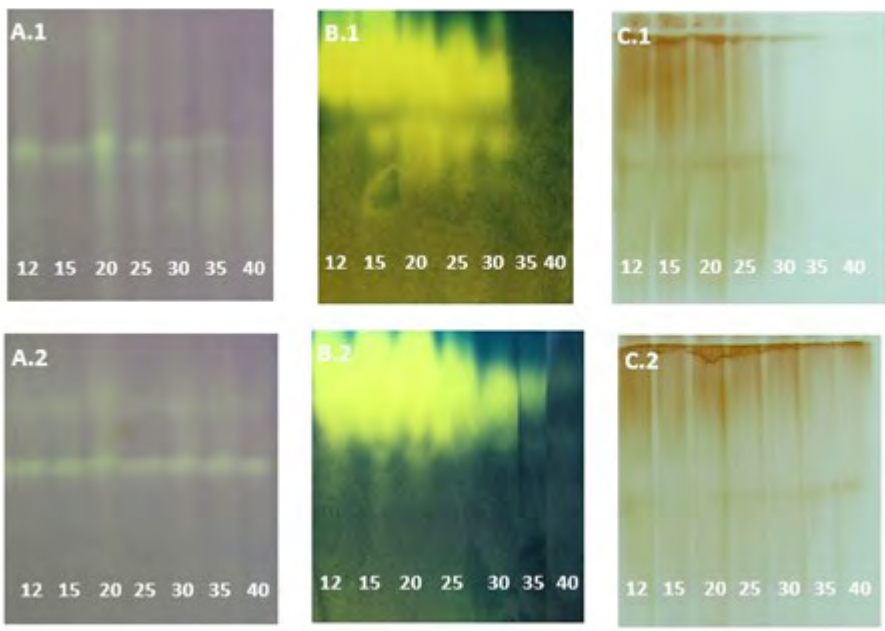


Figure 2. Electrophoretic expression of the enzymes superoxide dismutase (A), catalase (B), and peroxidase (C) in newly-harvested seeds of the cultivar Topázio MG 1190 with different moisture content values that were not freeze-dried (1) or freeze-dried (2).

Unlike SOD, expression of the CAT and PO enzymes was clearly affected by seed moisture content. In stored seeds, changes were observed in the electrophoretic pattern of CAT in accordance with freeze-drying (Figures 1 B.1 and B.2). In the freeze-dried seeds, greater intensity of activity of this enzyme is observed in seeds with moisture contents from 12% to 30%. In addition, there was more limited appearance of isoforms for seeds with moisture content values of 12%, 35%, and 40%. This isoform had not been observed in the non-freeze-dried seeds, and the intensity of the bands in this case was similar for all the moisture content values. This may indicate that in the non-freeze-dried seeds, the different moisture content of each sample affected the expression of the isoforms of CAT (Figure 1 B.1).

Lower expression or absence of CAT isoforms in newly-harvested seeds, whether freeze-dried or not, were found at moisture content values greater than or equal to 30% (Figures 2 B.1 and B.2). In non-freeze-dried seeds, the presence of different isoforms of the CAT enzyme was observed at moisture content from 15% to 30% wb (Figure 2 B.1). CAT is responsible for converting hydrogen peroxide into water and oxygen (Dubey, 2011), acting as a cell protector against deterioration. The greater activity may be associated with the reduction or increase in the mechanism of prevention against oxidative damage. In coffee seeds, CAT has been extensively researched as a possible indicator of physiological quality. Nevertheless, the results are divergent, sometimes showing positive correlation and sometimes negative with physiological performance (Abreu et al., 2014; Santos et al., 2014; Coelho et al., 2015; Coelho et al., 2017a; Figueiredo et al., 2021). In these studies, seeds with different moisture levels were assessed for physiological quality or protein profile.

Freeze-drying the seeds grants greater sensitivity in detection of PO enzyme expression, both in low quality seeds stored for nine months and in newly-harvested seeds. When the seeds (stored or newly-harvested) were assessed without freeze-drying, there was no detection of PO expression when they had moisture content values greater than 30% (Figures 1 and 2 C.1). In contrast, in the freeze-dried seeds (Figures 1 and 2 C.2), the accumulation of peroxide can be observed at all the moisture content values evaluated. The PO enzyme is located in the cell wall and in the vacuole, with the function of reduction and exhibition of the effects of oxygen on the seed defense mechanisms (Dussert et al., 2006). In the present study, the seeds of the two lots had similar physiological performances at all the moisture content levels, and the assessment of enzyme expression was more consistent with the quality of the freeze-dried seeds compared to the non-freeze-dried seeds.

The expression or the quantification of enzymes has been used in several studies on coffee seeds, aiming to better understand the causes of loss of quality in different stress situations. Coelho et al. (2015) evaluated the effects of moisture content of coffee seeds after slow and rapid drying on physiological quality and enzyme activity. These authors observed greater esterase activity after rapid drying in seeds of better physiological quality.

In expression of the GOT enzyme, just as of MDH, as shown in Figures 3 and 4 for stored or newly-harvested seeds, there was greater intensity of the bands in freeze-dried seeds. This greater activity in freeze-dried seeds mainly occurred at moisture levels from 20% to 12% for GOT and above 20% for MDH. There was greater intensity of bands; however, all the isoforms of both enzymes, GOT and MDH, were present with or without freeze-drying of the seeds.

The changes in the electrophoretic profile of GOT are affected by the deterioration process (Tunes et al., 2010; Tunes et al., 2011). GOT plays an important role in seed germination since it acts in oxidation of amino acids, supplying energy for the Krebs cycle or for reduction in alpha-ketoglutarate for new amino acids directed to embryo growth and protein synthesis (Vieira et al., 2009; Tunes et al., 2010).

In relation to the MDH enzyme, it can be affirmed that the moisture of those seeds that were not freeze-dried affects the intensity of its expression in the gel. Seeds with moisture of 35% or 40% express a smaller number and lower intensity of isoforms, which was not observed when these seeds were freeze-dried (Figures 3 B.1 and B.2). Uniformity of moisture in the seeds appears to be fundamental for correct assessment of MDH. This enzyme is associated with the seed breathing process, and high respiratory activity is related to greater deterioration. MDH is present in all the treatments evaluated. By the pattern observed for freeze-dried seeds, the level of moisture of the seeds does not have an important effect on the activity of this enzyme. It is an enzyme of the respiratory pathway and has an important function in catalysis of the malate and oxalate reaction in the last reaction of the Krebs cycle (Taiz and Zeiger, 2013; Santos et al., 2017).

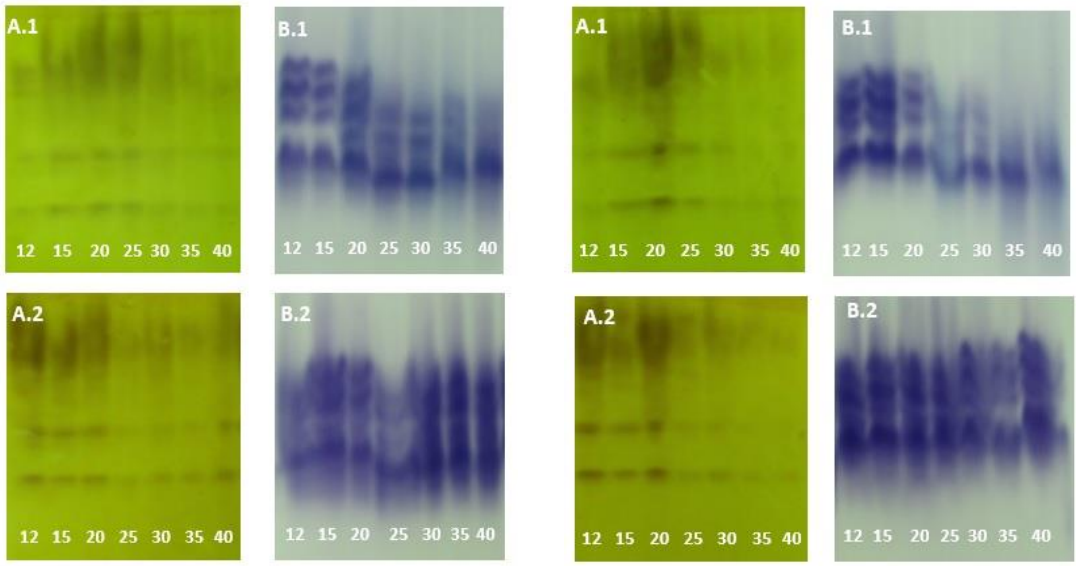


Figure 3. Electrophoretic expression of the enzymes glutamate oxaloacetate transaminase (A) and malate dehydrogenase (B) in stored seeds and newly-harvested seeds of the cultivar Topázio MG 1190 with different moisture content values that were not freeze-dried (1) or freeze-dried (2).

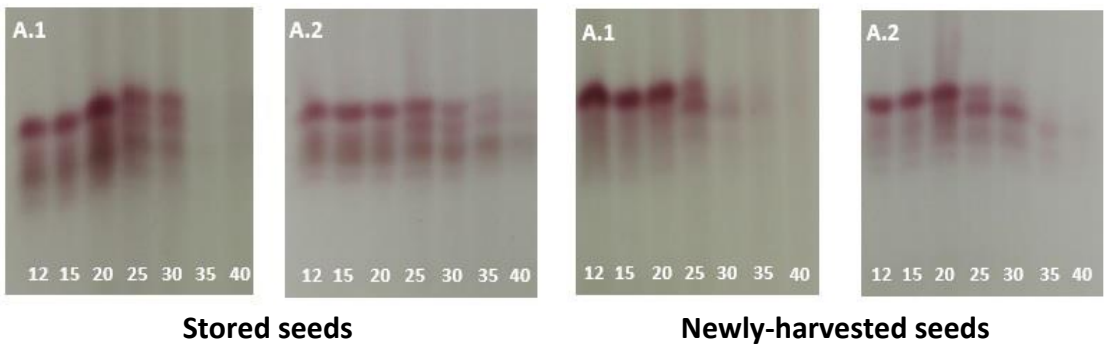


Figure 4. Electrophoretic expression of the enzyme esterase in stored seeds and newly-harvested seeds of the cultivar Topázio MG 1190 with different moisture content values that were not freeze-dried (1) or freeze-dried (2).

EST is a good indicator of seed deterioration, with an important catalytic function in cell detoxification, acting in hydrolysis of esters and in the metabolism of lipids (Russel et al., 2011). In this study, as the moisture of the stored seeds decreases, there is an increase in EST activity, regardless of the freeze-drying process (Figures 4 A.1 and A.2). Nevertheless, after freeze-drying, there is expression in the seeds with moisture greater than 35%, which does not occur when the seeds were assessed at the initial moisture levels. Similar results were obtained by Coelho et al. (2017b) upon finding an increase in EST activity in dry coffee seeds.

Regardless of freeze-drying, in newly-harvested seeds there is low expression and even absence of the EST enzyme band as of the moisture content of 30% before freeze-drying and above 35% after this process (Figures 4 A.1 and A.2). It is noteworthy that for this enzyme, seeds that were freeze-dried had greater sensitivity in detection of expression, with expression at the highest levels of moisture. This was observed mainly in the stored seed lot, which does not occur in seeds without freeze-drying (Figure 4). The EST enzyme present in the cell membrane participates in ester hydrolysis reactions, and may act on the metabolism of lipids (Coelho et al., 2015). Saath et al. (2014) concluded that

this enzyme indicates deterioration in coffee beans; however, in the present study, similar expression was observed between the newly-harvested seeds and stored seeds. The pattern of EST expression changed according to seed moisture content (Figure 4).

The response in expression of heat-resistant proteins was different from that of the other proteins analyzed in this study. There was expression of the proteins in the stored or newly-harvested seeds at all the moisture levels (Figure 5). After freeze-drying, more deteriorated seeds (stored lot) showed reduction in the activity of this enzyme at moisture levels above 25% wb (Figure 5 A.2). However, in newly-harvested seeds, expression of the heat-resistant proteins was observed at all moisture levels (Figure 5 A.2). In contrast, in non-freeze-dried seeds, greater expression was observed at moisture levels of 30% and 25% wb (Figure 5 A.1).

The heat-resistant proteins are mainly associated with protection against drought or cold stresses (Battaglia and Covarrubias, 2013; Amara et al., 2014), and their accumulation is always related with the physiological quality of coffee seeds (Stavrinides et al., 2020). Abreu et al. (2014) found that the electrophoretic profile of heat-resistant proteins was intense in seeds dried more slowly and that there was reduction in their synthesis as the moisture content of coffee seeds decreased. In addition, these proteins are more structurally flexible under aqueous conditions and more stable at lower moisture levels. They may exhibit various isoforms in an organism, able to carry out several functions, such as acting in the stability of membranes, in redox balance, and in the homeostasis of proteins and nucleic acids (Hand et al., 2011). These proteins are highly soluble in water, are rich in glycine and other hydrophilic amino acids, have few hydrophobic residues, and may be located in the cytosol. These characteristics suggest that they play a structural role and protect against desiccation (Tunnacliffe et al., 2010).

The effect of moisture on the expression of isoenzymes in coffee seeds was shown in this study. The expression of antioxidant enzymes has been used in studies on coffee seeds to understand the causes of loss of quality in different stress situations. In this context, controversies in interpretation of the results are common, especially when the seeds analyzed have different moisture levels (Coelho et al., 2015; Coelho et al., 2017a; Coelho et al., 2017b; Abreu et al., 2018; Coelho et al., 2019; Figueiredo et al., 2021). In a study on the effects of moisture content after slow drying and rapid drying on physiological quality and the activity of EST, PO, SOD, and CAT, Coelho et al. (2015) also observed the effect of moisture and of seed drying speed on the activity of enzymes that protect against oxidation processes. These authors found that both the seeds of lower vigor dried to 5% and the seeds of higher vigor with 30% moisture without drying had high and similar activity of enzymes involved in this process. Conflicting results were also observed in the expression of isoenzymes in studies on the tolerance of coffee seeds to cryopreservation. Figueiredo et al. (2021) found an increase in EST expression in more highly dehydrated seeds that had the best physiological quality. In addition, these authors found greater activity of the CAT enzyme in seeds dried to 17%, which maintained their high initial quality after immersion in liquid nitrogen. As already cited, this enzyme plays a

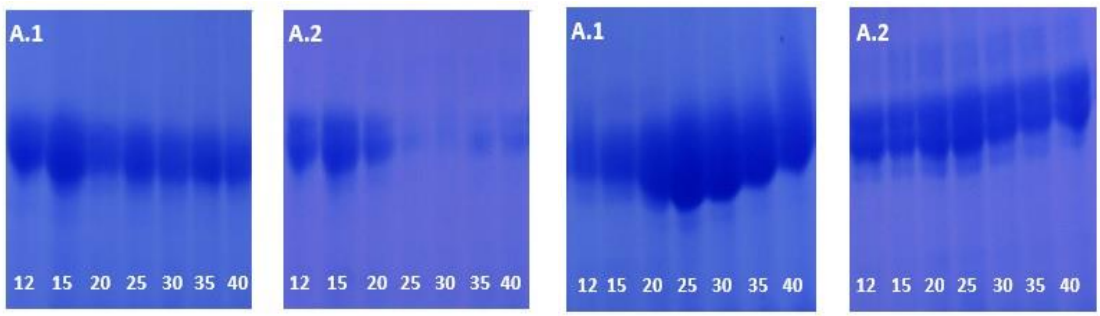


Figure 5. Electrophoretic expression of the heat resistant proteins in stored seeds and newly-harvested seeds of the cultivar Topázio MG 1190 with different moisture content values that were not freeze-dried (1) or freeze-dried (2).

fundamental role in neutralization of hydrogen peroxide in the cells (Sharma et al., 2012). In contrast, other studies report a considerable effect of moisture in seeds on the isoenzyme expression of CAT, as well as other antioxidant enzymes (Coelho et al., 2017a; Coelho et al., 2017b; Abreu et al., 2018; Coelho et al., 2019; Figueiredo et al., 2021).

Lower divergence in the results of expression of antioxidant isoenzymes are observed when coffee seeds have the same moisture content. Taveira et al. (2012) and Saath et al. (2014) studied the antioxidant isoenzyme profile in coffee beans under different drying methods and concluded that the changes that occurred in these enzymes are associated with the deterioration process and may be used to differentiate the quality of coffees under different post-harvest management practices.

In the present study, the freeze-dried seeds of both seed lots had more intense enzyme expression and/or enzyme expression with the appearance of new bands in the electrophoresis gels. All the seeds analyzed in the present study had moisture content below 4% wb at the time of extraction and electrophoretic run, due to the freeze-drying process. The non-freeze-dried seeds, which were analyzed at moisture levels from 12% to 40% wb, had varied expression in intensity or number of bands, and there is evidence that the presence of free water, which is the water removed in the freeze-drying process, affects the results.

It is important to emphasize that the absence of bands or of enzyme expression in the wet seeds could therefore lead to a mistaken interpretation, since these enzymes were expressed when the seeds were dehydrated.

The expression of antioxidant enzymes and their protective action in seeds under stress is well documented in the literature. One of the primary causes of seed deterioration is lipid peroxidation, which begins with the generation of free radicals, an atom or a molecule with unpaired electrons. Seeds have a complex antioxidant system that defends against the damaging consequences of reactive oxygen species, which may be enzymatic or non-enzymatic. However, the action of this antioxidant system does not occur in any and every situation, since lipid peroxidation, which triggers the production of free radicals, is dependent on seed moisture. Lipid peroxidation occurs in all cells, however in cells that have high moisture, water acts as a buffer between the free radicals and the macromolecules that are the target of attack, therefore reducing the level of damage (McDonald, 1999; Berjak, 2006). According to these authors, peroxidation may be the primary cause of deterioration of seeds at moisture content below 6%; and above 14%, peroxidation can once more be stimulated by the activity of hydrolytic and oxidative enzymes, such as lipoxygenases, becoming more active as moisture increases. Thus, in seeds with high moisture, antioxidant enzymes are not expected to have protective action, but rather other mechanisms act in this protection. Nevertheless, in quantification or analysis of the expression, such as electrophoretic analysis, the enzymes are isolated, and what is expected as a result is the product of their reaction on a specific substrate, without the interference of other molecules, including water molecules.

Finally, in studies on the physiological performance of coffee seeds under any type of stress, care must be taken upon choosing to use analysis of expression or quantification of enzymes, especially when the seeds have different moisture content. As shown in this study, determination of the protein profile is directly affected by coffee seed moisture content.

## CONCLUSIONS

The freeze-drying process allows preservation of the quality of coffee seed samples and does not change the functionality of the enzymes SOD, CAT, PO, GOT, MDH, and EST and of the heat resistant proteins. The profiles of isoenzymes of the antioxidant process in arabica coffee seeds of the cultivar Topázio MG 1190 is affected by the initial moisture content of the seeds. The freeze-drying process of the seeds ensures greater sensitivity in the detection of the expression of these isoenzymes.

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