

ACTIVITY OF SOME ISOENZYMATIC SYSTEMS IN STORED COFFEE GRAINS

Atividade de alguns sistemas isoenzimáticos em grãos de café armazenados

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ABSTRACT

Considering the worldwide consumption of coffee, it is natural that throughout the history many people have dedicated the research to markers that contribute somehow on gauging its quality. This research aimed to evaluate the biochemical performance of arabica coffee during storage. Coffee in beans (natural) and in parchment (pulped) dried in concrete terrace and in dryer with heated air were packed in jute bags and stored in not controlled environmental conditions. Enzymatic activities of superoxide dismutase, catalase, peroxidase, polyphenoloxidase, esterase and lipoxygenase in coffee grains were evaluated at zero, three, six, nine and twelve months by means of electrophoresis. Independently of the drying method, the activity of isoenzymatic complexes highlighted deteriorative processes in stored grains of coffee. The treatments 60/40° C and 60° C used to reduce the water content imposed a greater stress condition, accelerated metabolism of natural coffee in the storage with decreased activity of defense mechanisms due to latent damage in these grains. Natural coffees are more sensible to high drying temperatures and its quality reduces faster than pulped coffee in the storage.

Index terms: *Coffea arabica* L., post-harvest processes, coffee quality.

RESUMO

Considerando o consumo de café em nível mundial, é natural que, ao longo da história, muitos tenham dedicado à pesquisa a marcadores que contribuam de alguma forma, na aferição de sua qualidade. A pesquisa teve como objetivo avaliar o desempenho bioquímico de grãos de café arábica durante o armazenado. Cafês em coco (natural) e em pergaminho (despolpado) secados em terreiro de concreto e em secador com ar aquecido foram embalados em sacos de juta e armazenados em condições de ambiente não controlado. As atividades enzimáticas da superóxido dismutase, catalase, peroxidase, polifenoloxidase, esterase e lipoxigenase nos grãos dos cafês foram avaliadas aos zero, três, seis, nove e doze meses por meio de eletroforese. Independente do método de secagem, a atividade dos complexos isoenzimáticos evidenciou os processos deteriorativos dos grãos de café armazenados. Os tratamentos 60/40° C e 60° C utilizados para reduzir o teor de água promoveram uma condição de tensão maior no metabolismo de café natural, diminuiu nestes grãos a atividade de mecanismos de defesa no armazenamento (danos latentes). Os cafês naturais são mais sensíveis a altas temperaturas de secagem e sua qualidade diminui mais rapidamente do que o café despolpado no armazenamento.

Termos para indexação: *Coffea arabica* L., processos pós-colheita, qualidade dos cafês.

INTRODUCTION

The lack of orientation during harvest and post-harvest phases brings impairments to the coffee fruits. Many alterations that occur with coffee during storage (Corrêa et al., 2003; Coradi, Borém; Oliveira, 2008) are consequences of the drying temperature effects of the grain mass (Borém et al., 2008; Isquierdo et al., 2009; Saath et al., 2010); these damages are not immediate and many times they only are perceptible and measurable during storage (Saath et al., 2012). Such alterations have direct influence on the appearance and biochemical components

of the grains, which are responsible by the characteristic aroma and flavor of the coffee beverage (Bytof et al., 2007; Giomo; Borém, 2011).

Oxidative reactions, of enzymatic nature or not, involving phenolic compounds and enzymes (Chalfoun; Parizzi, 2008; Abrahão et al., 2010) may affect negatively the grains during post-harvest (Borém et al. 2008; Saath et al., 2012). When a multienzymatic complex acts on some chemical constituents, it causes some reactions that may result on the rupture of cell wall and membranes, besides acting over chemical compounds of the grain and beverage, promoting undesirable taste and odor (Salva; Lima, 2007;

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Santana et al. 2008). This occurs because enzymes act like biological catalysts of several metabolic pathways involved on the quality of coffee beverage (Carvalho et al., 1994; Carvalho et al., 1997). For example, when cell membranes are damaged, polyphenoloxidase is released and activated, which oxidizes chlorogenic acid and its isomers, increasing the astringency of the fruit (Pimenta, Chagas; Costas, 1997; Carvalho, Gosmann; Schenkel, 2001). Besides contributing with characteristic flavor and aroma of coffee beverage, due to its reducing properties and chemical structure, chlorogenic acids act on the neutralization of free radicals and chelation of transition metals (Abrahão et al., 2010).

Enzymes present the highest variety and specialization among the protein products. However, their catalytic activity depends on the integrity and native conformation of the protein, thus, the structure of enzymes is essential for their activities (Nelson; Cox, 2006). Commonly present similar sequences of aminoacids and in many cases they share the same evolutionary origin (Malone et al., 2006), in the cells of a tissue, in the diverse ontogenetic stages and in different tissues (Pinto et al., 2001). However, the intensity of bands and the isoenzymatic profile are specific of a determined tissue and development stage of the plant. This occurs because through division the cells have the ability to lose or acquire specific biochemical characteristics (Taiz; Zeiger, 2004).

On seeds post-harvest the activity of the enzymes have been associated to mechanisms of protection and/or deterioration of seeds in different levels (Rosa et al. 2005; Carvalho, et al., 2006; Henning et al. 2009). This occurs because they are given the ability to prevent, tolerate or repair attacks of free radicals (Brandão Júnior, Vieira; Hilhost, 2002; Veiga et al., 2007; Vidigal et al., 2009). For example, the catalase acts on the protection of seeds because it has the capacity to transform oxygen peroxide in water (Taiz; Zeiger, 2004; Buchanan, Gruissem, Jones, 2005); the presence of lipoxigenase favors a higher speed of seedling emergence (Oliveira et al., 2006; Nakada et al., 2010).

On storage, biochemical alterations in the seeds or grains may be associated to the lack of synchronism in the activities of enzymatic complex (Lima et al., 2004; Nakada et al., 2010), evidenced by undesirable changes on the quality of the product (Santos, Chalfoun, Pimenta, 2009). Thus, it is important to highlight that only the joint action of the protein system and the isoenzymatic complex protect the membranes from peroxidative damages (Taiz; Zeiger, 2004), neutralizing the effects of oxygen (O₂), thus avoiding excessive oxidations (Faria et al., 2003; Freitas et al., 2006; Santos, Menezes; Villela, 2005; Veiga et al.,

2010). Considering the hypothesis that due to the damage level of membranes the oxygen acts more intensively promoting the oxidation of compounds, affecting the bioavailability of the chemical content of the grains, and that these oxidative processes may be intensified under stress conditions, the current work aimed to evaluate the biochemical performance of arabica coffee grains during storage after different post-harvest processes.

MATERIAL AND METHODS

After finishing the drying and being in hygroscopic equilibrium with the environment, coffee in beans (natural) and in parchment (pulped) were packed in jute bags and stored in not controlled environment conditions. Temperature and relative humidity of air were monitored by means of a thermohygrograph during the period of twelve months. Samples removed at zero, three, six, nine and twelve months of storage were evaluated by means of biochemical analyzes in order to characterize the effects of storage on the activity of isoenzymes of the grains. The adopted statistical design was entirely randomized in factorial scheme 2x4x5, being two processing methods – natural coffee (NC - T_A) and pulped coffee (PC - T_B); four drying methods – terrace (T₁) as control, constant temperature at 40° C (T₂) and at 60° C (T₃) and alternated temperature of 60/40° C (T₄); and five storage periods (zero, three, six, nine and twelve months), with three repetitions. The water content of coffee was determined through the standard method ISO 6673:2003 (ISO, 2003). For electrophoresis of isoenzymatic profiles, the preparation of material, electrophoresis running and gel revelation for the enzymes superoxide dismutase (SOD), catalase (CAT), peroxidase (PO), polyphenoloxidase (PPO), esterase (EST), lipoxigenase, the methodology described by Alfenas (2006) was followed.

RESULTS AND DISCUSSION

The observed bands varied both in quantities as in the intensity of isoenzymatic complex activity. Thus it is possible to affirm that oxidation of biochemical compounds occurred due to the level of damage in the cell membrane of the storage grains. The isoenzymatic activity decrease may be attributed to latent damages caused by thermal and mechanical damages during coffee processing. In addition, the interaction among climatic elements of the storage environment and the grains previously damaged, provide increased exposure of membrane systems to oxygen effects (O₂). The activity of protein systems also showed variations of the studied isoenzymatic complexes in the evaluated coffee grains.

In the revealed profile of superoxide dismutase (SOD), it was observed an intense activity in the grains dried in terrace and with heated air at 40° C, independently of the processing method, for all the storage periods. However, the grains obtained from drying at 60/40° C and 60° C, the number of bands and enzyme activity of SOD enzyme decreased during storage, being more expressive in natural coffee (Figure 1). The variation of activity may be an effect of latent damages provided by the drying speed, in function of the used temperature, because (Saath et al., 2010) the presence of bark on coffee in beans offered a higher resistance to energy and mass exchanges within the grains during drying, providing a higher stress to the cell structure of the grains. They had increased the metabolic activity, affecting the efficiency of SOD against reactive forms of oxygen.

As observed (Figure 1.1), in general during the evaluated period SOD was active in the defense against reactive forms of oxygen. This fact, according to Rosa et al. (2005) and Nakada et al. (2010), has canceled the action of superoxides (O_2^-), catalyzing reactions of transference of two electrons to produce hydrogen peroxide (H_2O_2). However, mainly in coffee in beans, in function of the

accelerated metabolism in these grains there was a higher consumption of the energy from reserves, affecting the efficiency of SOD against the action of O_2^- , what was evidenced by the reduction of the activity of the enzyme at twelve months. The reduction of activity allowed the formation of free radicals in the grains. This hypothesis corroborates the results described by several authors for coffee seeds (Brandão Júnior, Vieira; Hilhost, 2002; Faria et al., 2003; Lima et al., 2004; Rosa et al., 2005; Freitas et al., 2006; Veiga et al. 2007; Veiga et al., 2010). The conditions of temperature and relative humidity of air are extremely important for the evolution of grain deterioration, which is a continuous process and may be avoided (Santos, Menezes; Villela, 2005; Santos, Chalfoun; Pimenta, 2009). The extent of changes that occur in this process depends mainly of the period and the environmental conditions of storage, which may result on alterations that may be characterized by the activity of enzymatic complexes, such as the enzyme esterase (EST), since it acts on the hydrolysis of esters and on the metabolism of lipids (Brandão Júnior, Vieira; Hilhost, 2002; Santos, Menezes; Villela, 2005).

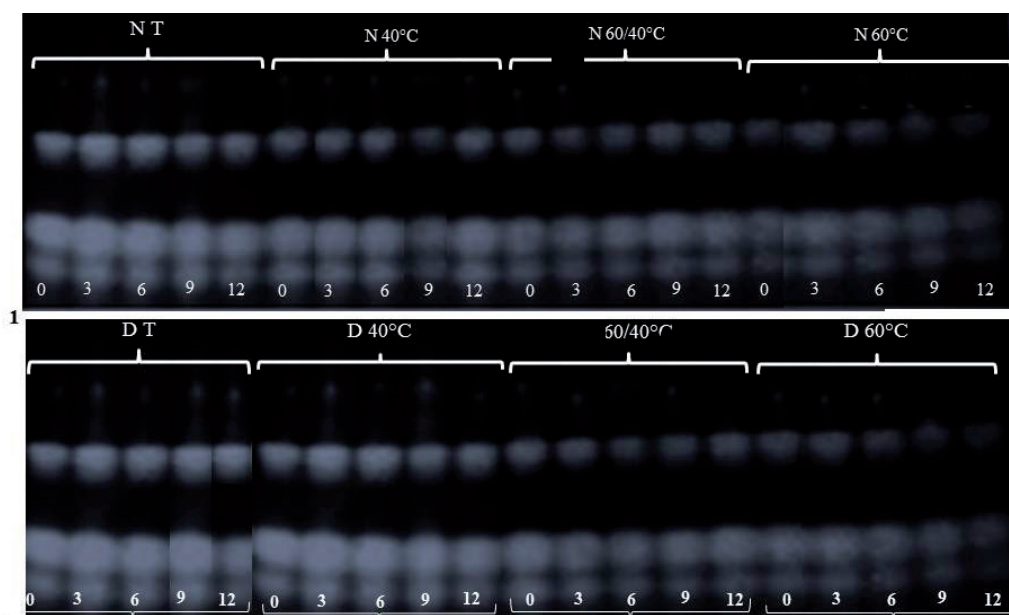


Figure 1 – Enzymatic patterns, throughout storage, revealed for the enzymes superoxide dismutase (1), esterase (2), catalase (3) and lipoxigenase (4) of natural and pulped coffee grains submitted to different drying methods: Natural Coffee: drying terrace (NT), drying in temperature 40° C (N40° C), drying in temperature 60° C (N60° C) and alternated temperature of 60/40° C (N60/40° C); and Pulped Coffee: drying terrace (DT), drying in temperature 40° C (D40° C), drying in temperature 60° C (D60° C) and alternated temperature of 60/40° C (D60/40° C).

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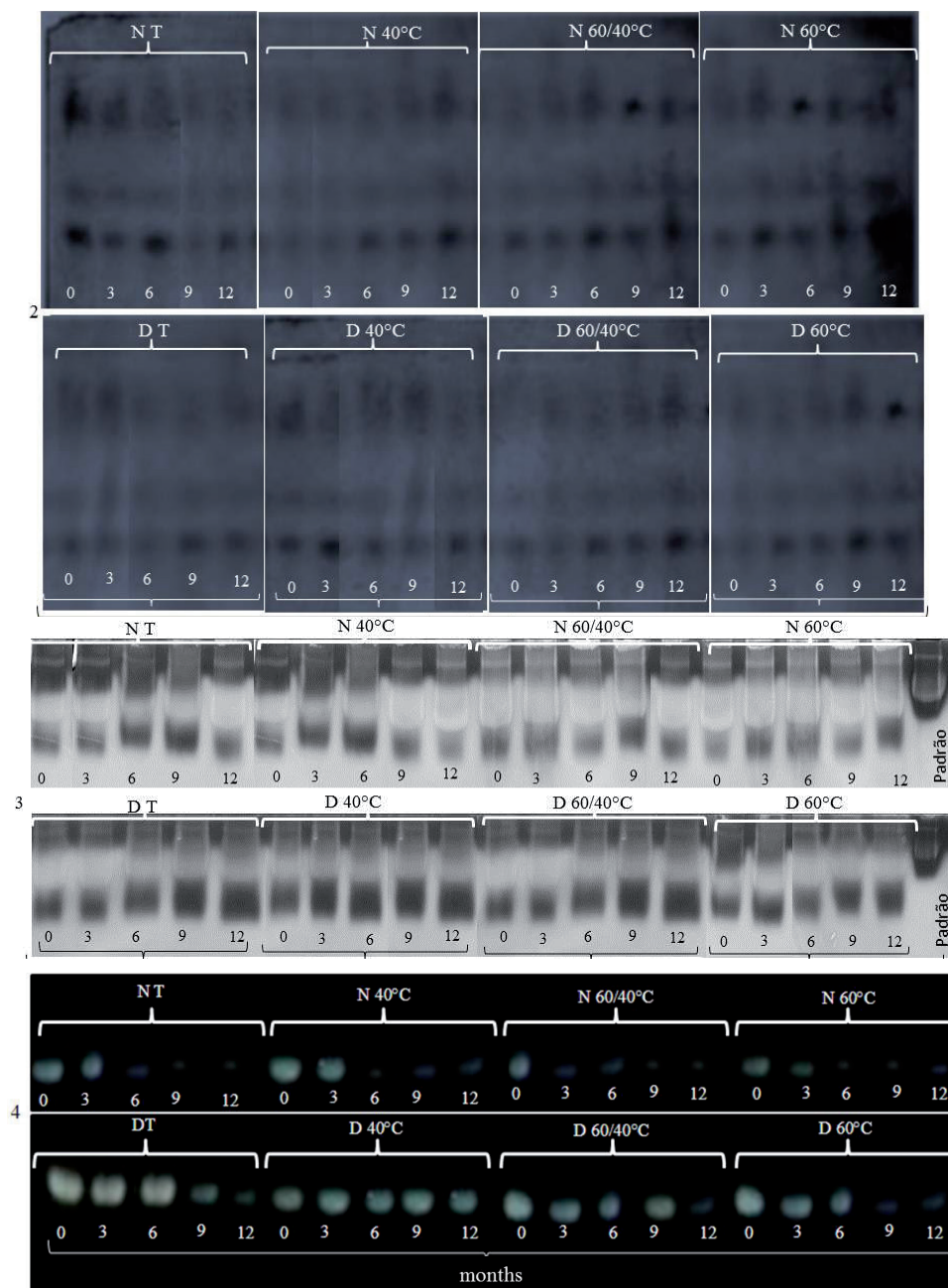


Figure 1 – Continued.

On the esterase electrophoresis gel, the expressed activity was higher in the natural grains of coffee mainly in the submitted to higher drying temperatures (Figure 1.2). Alterations in the patterns of EST evidence the occurrence of deteriorative events that may contribute for the reduction of grain quality as the storage period

increases, because esterase is an enzyme involved in reactions of esters hydrolysis, being directly related to lipids metabolism. In function of the storage time, similarities were verified between the profiles of grains dried in terrace at environment temperature and in dryer at 40° C (Figure 1.2). However this activity is

more intense in the grains of natural coffee, and under temperature of 60/40° C and 60° C there was a gradual increase of the enzymatic activity as the storage time increased (Figure 1.2). Probably this occurs due to a more accelerated metabolism, suggesting (MALONE et al., 2006) that most of the reserve material had already been metabolized. This increased activity is associated to the oxidation of lipids, characterizing the degenerative process of cell membranes of stored coffee. This occurs because, according to Santos, Menezes and Villela (2005), Malone et al. (2006) and Nakada et al. (2010), EST is involved both on hydrolysis of esters and on lipids metabolism, like the total phospholipids of membrane.

Esterase is accumulated before the deteriorative process in order to prevent the action of free radicals (Malone et al., 2006), and under stress conditions its activity, besides characterizing the seed deterioration, may help in the germination process since the fatty acids released from the lipids of this group of hydrolytic enzymes are used on the β -oxidation as energy source for germination (Nakada et al., 2010; Veiga et al., 2010). In this case, the activity is an indicative of higher loss of quality in the grains of coffee in beans.

It is known that the enzymatic complex present in the coffee grain is responsible for the decomposition of reserve substances in simpler compounds, that enzymes act in synergism, and in function of the action of SOD occurs the formation of hydrogen peroxide (H_2O_2), which is transformed in water (H_2O) by catalase (CAT), that acts for the conservation of the grain quality (Taiz; Zeiger, 2004; Buchanan, Gruissem; Jones, 2005). In relation to the enzyme CAT (Figure 1.3), independently of the processing and drying method, as well as the storage time, they presented intense enzymatic activity in stored coffee. However, in the grains of natural coffee dried at 60/40° C and 60° C, the reduction of the activity intensity of such enzymes was observed throughout the storage (Figure 1.3). In the other treatments, CAT presented intense and homogeneous enzymatic activity, evidencing the action of this enzyme on the removal of H_2O_2 produced by other enzymes, thus protecting the cell of this toxic compound during the coffee storage. It was observed that in the grains submitted to drying at 60/40° C and 60° C the damages caused by high temperatures reflected negatively in the enzyme CAT, found by the lower activity, which may be associated to the level of deterioration. Nakada et al. (2010) found similar results for stored seeds of cucumber. It is possible that the produced H_2O_2 has been being more consumed in oxidative processes, like the lipid peroxidation, than eliminated of metabolism through the action of the enzyme, since the efficiency of SOD had reduced against the action of superoxides (Figure

1.1). For Leymarie et al. (2012) it is possible that this decrease in H_2O_2 was partly related to the slight increase of CAT activity, because these events in the embryonic tissues are associated with the completion of germination in seeds. In the other hand, Parmoon et al. (2013) observed that Catalase and Peroxidase activity was reduced during aging because of the increase in H_2O_2 and free radicals in the stressed cell cytoplasm during oxidative stress, leading to a reduction of seed viability.

The high activity of lipoxygenase of coffee in parchment in the storage period was highlighted, except at twelve months, when only the treatment 40° C kept the intensity of the activity. For the coffee in beans submitted to different drying methods, this activity was observed until the third month of grains storage (Figure 1.4). They presented a slight reduction of activity from the sixth month on. The activity reduction may be associated to unbalanced isoenzymatic complexes, which compose the system of cell membranes. Lipoxygenase has as function the peroxidation of lipids, where the membrane lipids are more willing because (Nakada et al., 2010) they have a large surface and predominance of unsaturated lipids highly sensible to degradation, what may have caused the unbalance on the viscosity and permeability of membranes. After its action, free radicals are formed, what is related to the seeds deterioration. However, Oliveira et al. (2006) detected higher speed of soy germination when this enzyme was present helping in the mobilization of lipids; Nakada et al. (2010) verified the better performance of cucumber seeds until the storage period of six months, in which the lipoxygenase also was higher, and Freitas et al. (2006) that the increase of accelerated aging period promoted decreases in the activity of the enzyme in cotton seeds.

According to the enzymatic patterns that express the activity of enzymes polyphenoloxidase - PPO (1) and peroxidase - PO (2), differences were verified regarding its activity in natural and pulped grains of coffee (Figure 2).

On post-harvest processes occurred the progressive deterioration of grains, with damages on the membranes, resulting in the lower activity of PPO, what may be verified by the intensity and number of bands in the electrophoresis Figure 2.1. For coffee, the molecular weight of bands varies from 14 to 148 kDa (Oliveira et al., 1976). It is important to highlight that the complex of enzymes polyphenoloxidase is associated to the quality, because it is linked to cell membranes (Carvalho, Gosmann; Schenkel, 2001; Santana et al. 2008). Thus, under stress conditions, independently of the molecular weight of each band, PPO has its activity reduced in function of intra and extra cell phenolic substrates (Carvalho et al., 1997; Carvalho, Gosmann; Schenkel, 2001; Santana et al., 2008).

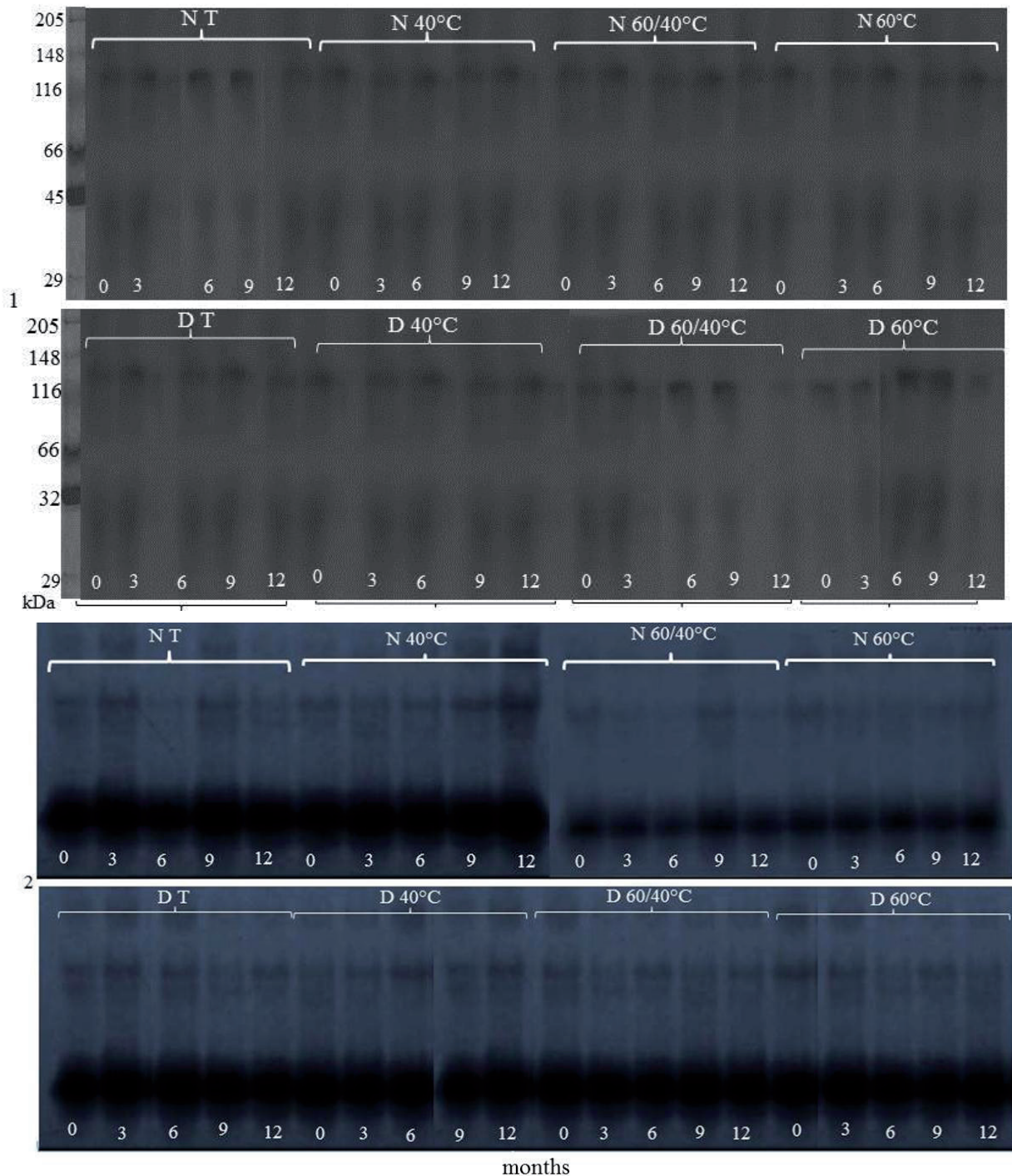


Figure 2 – Enzymatic pattern throughout storage, revealed for the enzymes polyphenoloxidase (1) and peroxidase (2), of natural and pulped grains of coffee, submitted to different drying methods. Natural Coffee: drying terrace (NT), drying in temperature 40° C (N40° C), drying in temperature 60° C (N60° C) and alternated temperature of 60/40° C (N60/40° C); and Pulped Coffee: drying terrace (DT), drying in temperature 40° C (D40° C), drying in temperature 60° C (D60° C) and alternated temperature of 60/40° C (D60/40° C).

In the studied grains of coffee a well defined band was verified, those with molecular weight of 116 kDa. Even this band was present in all the treatments, there are evidences that it presents higher activity on pulped coffee when compared to the natural, mainly in the drying at 60/40° C and 60° C (Figure 2.1). Considering the hypothesis that damages to the coffee endosperm affect the cell structure of the grain (Saath et al., 2010; Saath et al., 2012), they also warms the activity of PPO, contributing to the deterioration of stored grains of coffee. This occurs because according to Carvalho, Gosmann and Schenkel (2001) and Santana et al. (2008), the enzyme action is reduced by the interference of phenolic compounds. Studying the relation between the PPO activity in seeds and the quality of coffee beverage, (Carvalho et al., 1997) reported that the lower the activity and molecular weight of the band, the worse the quality of coffee beverage. However Abrahão et al. (2010), Borém et al. (2008) and Saath et al. (2012) attribute the lower sensorial quality to the higher content of phenolic compounds. This is due to the action of PPO over phenolic compounds, which provide to the coffee the astringent and undesirable flavor when in high contents (Santos et al., 2009).

On the other hand, the enzyme PO on coffee grains is related to alterations of aroma, texture and color during storage, which loss of activity may make the grain more sensible to the effects of O₂- and free radicals. This occurs because the isoenzymatic complex of peroxidases use H₂O₂ to catalyze the oxidation of organic and inorganic compounds, including polyphenols. In this enzyme gel, bands present homogeneous intensity in the evaluated period, except in the grains of natural coffee obtained of drying at 60/40° C and 60° C (Figure 2.2). This result suggests that the condition in the storage inhibited the activity of antioxidant enzymes more sharply in the grains of natural coffee.

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the water content imposed a greater stress condition and accelerated the metabolism of these grains, thus reducing the efficiency of defense mechanisms and prevention against loss of quality. This observation corroborates the information provided by researchers who reported the role of the enzyme PO in the metabolism of seeds as mechanism of defense and prevention of quality loss due to the fact that it uses peroxides as acceptor of hydrogen (Brandão Júnior, Vieira; Hilhost, 2002; Rosa et al., 2005; Santos, Menezes; Villela, 2005; Beltrã; Oliveira, 2007; Veiga et al., 2010; Taveira et al., 2012). PPO and PO are correlated to the seed quality, considering they are directly present in the cell membrane complex. As the activities of enzymes is a complex set, their declines is associated with some level of deterioration, damage due to long period of stress (Udosen, Nkang; Sam, 2012; Xin et al., 2014).

Since the peroxidation of lipids is an event associated to damages on the grains membranes, alterations (Figures 1 and 2) may denote the occurrence of deteriorative events which may contribute to the reduction of quality as the factor time increases. Moreover, the grain deterioration is a continuous process of physiological and biochemical causes related to the seed metabolism, which may lead to the denaturation of enzymes (Taiz; Zeiger, 2004), affecting the efficiency of protection on the membrane phospholipids (Carvalho, et al., 2006; Henning et al. 2009), thus generating toxic compounds (Dussert et al., 2006), allowing the higher production of leached (Santos et al., 2004; Vidigal et al., 2009; Veiga et al., 2010). This is in accordance to Yang, Chen and Gu (2011), who suggests that stress can promote oxidation the enzymes in the seeds stored.

CONCLUSIONS

Natural coffees are more sensible to high drying temperatures and its quality reduces faster than pulped coffee in the storage; the drying at 60/40° C affects negatively the quality of natural coffees; alterations in the isoenzymatic systems SOD, EST, CAT, PO and PPO are associated to deterioration processes of coffee grains.

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