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Total lipid and fatty acid profiles of *Coffea arabica* endosperm and embryo tissues and their relationship to seed desiccation sensitivity

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Abstract

As seed chemical composition may lead to different responses during drying and other post-harvest operations, the objective of this study was to determine the effect of drying rate (slow and rapid) and the concentration and composition of lipids in the embryo and endosperm tissues of *Coffea arabica* seeds on desiccation tolerance/sensitivity. The total concentration of lipids and the composition of fatty acids were evaluated in isolated embryos and endosperms, and in whole (intact) seeds. Embryos had a higher total lipid concentration (23%) than endosperm tissue (8%). Linoleic acid was the predominant fatty acid, with concentrations of 48% in the endosperm and 34% in the embryo tissues. Although rapid or slow drying did not change the fatty acid profile in the embryos or endosperms, the palmitic and linoleic acid concentrations varied significantly between the embryo and endosperm. The predominance of linoleic acid in the endosperm supports the hypothesis that the endosperm tissue may be an important source of damage to the seed and may advance seed deterioration. This indicates that unsaturated fatty acids may have a greater effect on sensitivity to desiccation than the percentage of total lipids.

Keywords: coffee seeds, drying, lipids, salt solutions, silica gel

Introduction

Brazil is the world's largest producer and exporter of coffee and the crop is the fifth most exported agricultural product. The area planted with coffee in the country totals 2.16 million hectares and it was estimated that between 60.00 and 61.65 million 60 kg-bags of coffee were harvested in 2018 (CONAB, 2019). Due to the considerable socioeconomic importance of coffee, it is essential to conserve the genetic diversity of the crop. However, setting up seed banks is not feasible for the *Coffea* genus, which produces intermediate-

to-recalcitrant seeds. Therefore, it is not possible to store its seeds in the conventional manner used for orthodox seeds (Roberts, 1973; Ellis *et al.*, 1990; Abdelnour-Esquivel, 1992; Dussert *et al*., 1997; Barbedo *et al*., 2013).

The limited longevity of coffee seeds has been attributed to several factors, including sensitivity to drying, different responses to desiccation of different seed parts, and reserve compounds (Rosa *et al*., 2011). According to some studies, whole seeds and isolated embryos respond differently to the drying process (Dussert *et al*., 1997, 2000; Coelho *et al.*, 2015). Further, the relative proportion of the reserve compounds (proteins, carbohydrates and lipids) depends on the tissue of the plant in which they are located. (Mello *et al*., 2010). Variations in the relative proportion of these compounds may lead to different responses to drying, with a direct effect on seed quality (Rosa *et al*., 2011).

It is known that the presence of lipid reserves in seeds is a key survival factor against drying damages, as well as exposure to ultra-low temperatures and to storage (Smith and Berjak, 1995). Lipids are the main components in *Coffea arabica* L. seeds, with around 0.14 g g^{-1} dry matter (14%) (Trugo, 2003; Oliveira *et al.*, 2006; Toci *et al.*, 2006). Wilson and McDonald (1986) proposed a model in which lipid peroxidation and subsequent formation of by-products is related to seed deterioration. During storage, seeds that have lipids as their main reserve substance are subject to slow and constant attack from oxygen, forming hydroperoxides, other oxygenated acids and free radicals (Schwember and Bradford, 2010; Walters *et al*., 2010; Abreu *et al*., 2012). Furthermore, according to Smith and Berjak (1995), Pérez-Galvés and Mínguez-Mosquera (2004) and Mello *et al*. (2010), not only the proportion of this reserve component, but also its degree of unsaturation, determined by the number of double bonds, has a strong influence on desiccation tolerance and hence storability.

Thus, although the total lipid concentration in coffee seeds has already been measured, there are no reports on the distribution of these lipids individually in the endosperm and embryo tissues. In addition, relative determination and identification of fatty acids is highly important since the structure of the fatty acids determines the physical properties of the lipids and their consequent influence on sensitivity or tolerance to seed desiccation.

Understanding the mechanisms involved in the coffee seed deterioration process is a strategic requirement to make seed conservation feasible. Thus, the aim of this study was to determine the appropriate seed drying rate (slow or rapid) and the concentration and structural composition of lipids in embryos and endosperms of *C. arabica* in relation to seed physiological quality.

Materials and methods

The study was conducted at the Central Seed Laboratory of the Agriculture Department, the Food Science Laboratory of the Food Science Department of the Universidade Federal de Lavras, and in the Laboratory for Coffee Quality Analysis of Empresa de Pesquisa Agropecuária de Minas Gerais.

Harvest and processing

Fruits of *Coffea arabica* L. cv. 'Catuaí Amarelo' were harvested at the mature berry stage in the Fundação Procafé in Varginha, MG, Brazil. The fruits were mechanically pulped and the mucilage removed from the seeds through fermentation in water ("fully washed") and, finally, seeds were pre-dried in the shade for removal of surface water.

Seed drying

To understand how water loss occurs in the different parts of the seeds, the drying curve was determined separately for isolated embryos and endosperms, as well as for whole seeds, using rapid drying and slow drying (150 whole seeds and the same number for each part evaluated). Rapid drying was performed in a hermetic container with 80 g of activated silica gel. Slow drying was achieved by the use of 5% lithium chloride (50 g L^{-1} H_2O; 95% relative humidity) and after 105 hours, this was replaced by a saturated solution of magnesium chloride (32% relative humidity). The containers were kept in B.O.D. chambers at a temperature of 25°C.

Water loss from the whole seeds and the separated parts (endosperms and embryos) was monitored by weighing on a precision balance (± 0.001 g) throughout the drying period, until the minimum desired moisture content was achieved, which occurred at 130 hours for rapid drying and 200 hours for slow drying. During the drying process, seed samples were taken at moisture contents of 30, 20, 10 and 5%, and embryo samples at moisture contents of 60, 50, 40, 30, 20, 10 and 5% for physiological evaluations.

Seed moisture content was determined by the laboratory oven method at 105°C for 24 hours, with two subsamples from each treatment (BRASIL, 2009). The results are expressed as mean percentage (wet basis).

Evaluation of physiological quality

The germination test was performed on four 50-seed subsamples distributed on germitest germination paper moistened with water equivalent to two and a half times the weight of the dry substrate, which were then placed to germinate at a temperature of 30°C. Evaluations of the number of normal seedlings were performed at 15 and 30 days after sowing (Brasil, 2009).

The tetrazolium test was performed both on embryos dried after they were separated from the seeds, and on embryos removed from seeds after the intact seeds were dried. In both cases, 10 embryos were used, which were stained with 0.5% tetrazolium solution in the dark for three hours at 30°C. After evaluation, the results were expressed in percentage of viable embryos.

Structural composition of fatty acids

Fatty acids were extracted according to Folch *et al*. (1957) using three replicates of 1 g of ground whole seeds, endosperms and embryos before and after they underwent slow and rapid drying. Fatty acids were determined in a gas chromatograph Shimadzu GC-17A, flame ionisation detector (FID), and the compounds were separated and identified in a Carbowax® capillary column (30 m × 0.25 mm). For chromatographic separation, 1 µL of sample was injected using a 10 uL (microliters) syringe (Hamilton®) with system

split = 5. Nitrogen gas was used as a carrier, with linear velocity programmed for 37.8 cm second^{-1}. The temperatures of the injector and of the detector were controlled at 220 and 240°C. The initial column temperature was 200°C (maintained for two minutes), increasing at a rate of 4°C per minute to 240°C, for a total of 20 minutes of analysis. The flow of carrier gas in the column was 1.0 mL minute^{-1}, and compounds were identified by retention time in the corresponding standard column.

Determination of total lipid concentration

Total lipids were quantified in fresh seeds (control) and in seeds subjected to slow drying and rapid drying. Three grams of the samples were weighed and then divided into three 1 g replicates, which were placed in a filter paper cartridge. After that, the cartridge was connected to a Soxhtel apparatus together with a flat-bottomed flask, previously dried at 105°C and weighed. 200 mL of diethyl ether was added and placed under continuous extraction for eight hours. At the end of the extraction period, the cartridge was remove, and the ether distilled. After distillation, the residue that contains the oil was placed in a laboratory oven at 105°C for one hour, cooled in a desiccator and weighed on a precision balance.

Statistical Analysis

Germination data were subjected to the Shapiro Wilk normality test, and when an absence of normality ($P < 0.05$) was found, they were square root-transformed. Normal (or normalised) data were subjected to ANOVA and when significance was observed by the F-test, means compared by the Scott-Knott or Tukey test at the level of 5% probability.

Results

The drying method affected the rate of drying of the intact *C. arabica* seeds and the isolated tissues (figure 1). The intact seeds, with an initial moisture content of 38%, dried to 5% moisture content after 100 hours of rapid drying. In the slow drying treatment, the same moisture content was attained after approximately twice that time (190 hours). For isolated embryos, the difference between the treatments was even greater with less than five hours of drying in silica gel (rapid drying) decreasing the moisture content from 68 to 10%. For slow drying, 113 hours were necessary to attain the same moisture content (10%).

Embryos separated from seeds and subjected to rapid and slow drying were evaluated for viability using tetrazolium salt solution (figure 2). In the rapid drying treatment, a small reduction in moisture content, from 70 to 60%, led to a decline in viability, from 98 to 45%. At 40% moisture after rapid drying, the embryo viability had declined to 7%, whereas at the same moisture content attained through slow drying, viability remained at 100%.

In addition to the tetrazolium test on embryos separated from seeds and subjected to drying, the tetrazolium test was also conducted on dried seeds. Comparison of the results of the tetrazolium test on embryos with the germination test (normal seedlings) showed

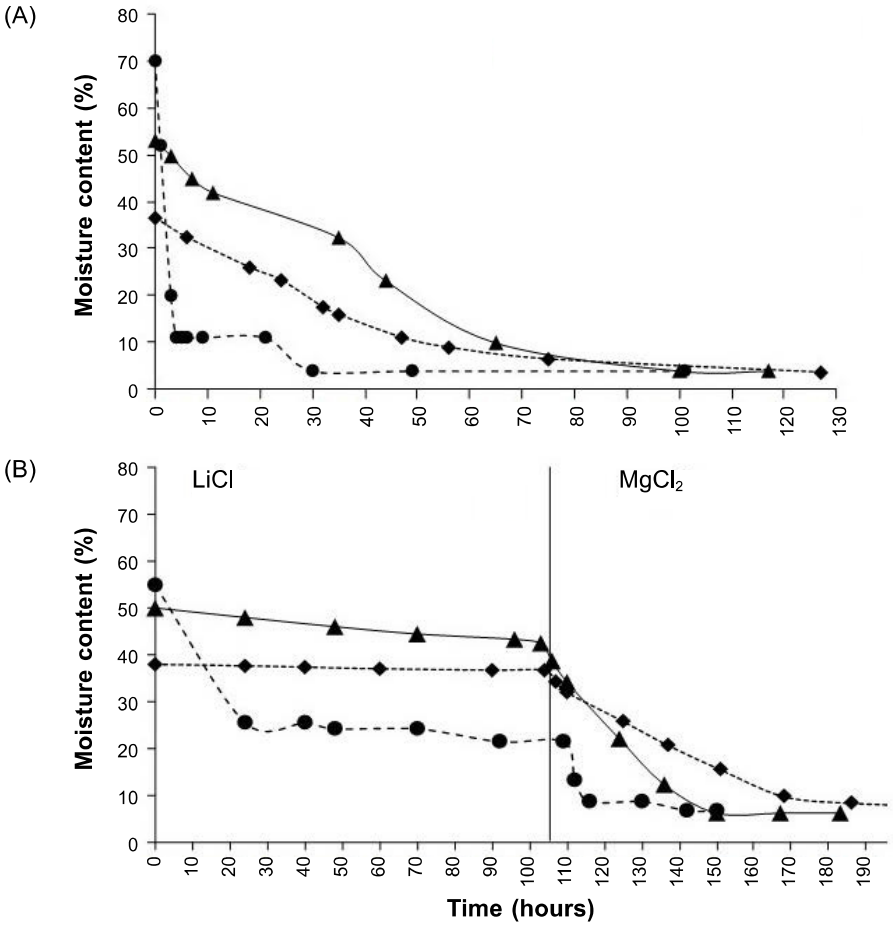

Figure 1. Drying speed of whole coffee seeds (♦), endosperms (▲), and embryos (●) after rapid drying in silica gel (**A**) and slow drying in saline solutions (**B**).

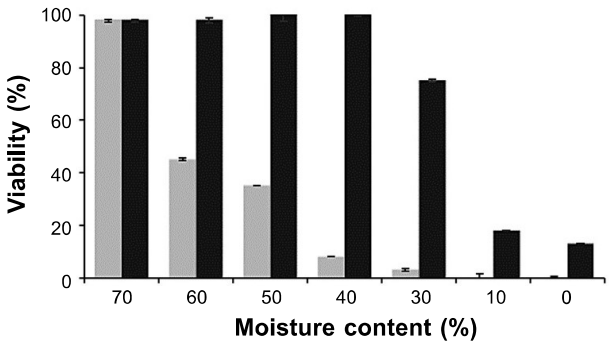

Figure 2. Viability in the tetrazolium test of coffee embryos separated from seeds and subjected to rapid drying in silica gel ( ) and slow drying in saline solutions ( ).

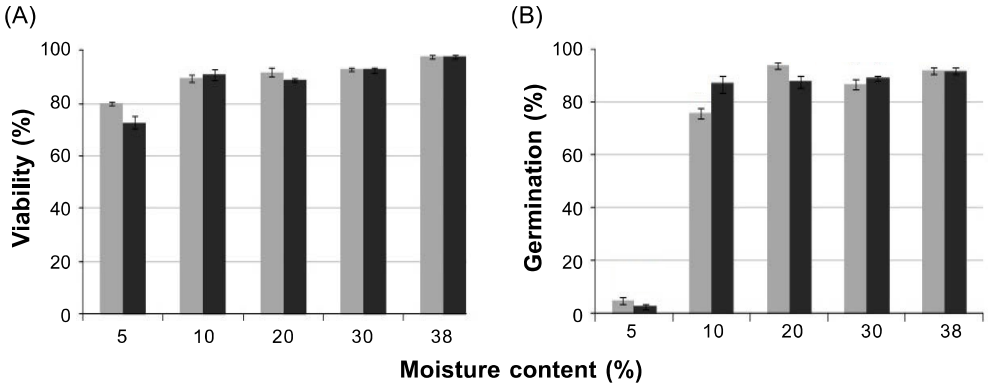

Figure 3. Viability of coffee seeds in the tetrazolium test (**A**) and percentage of normal seedlings in the germination test (**B**). Light grey bars for rapid drying in silica gel and dark bars for slow drying in saline solutions. There were no significant differences in viability between the rapid- and slow-dried seeds at each moisture content.

that after rapid drying to 5% moisture, 80% of the embryos were viable (figure 3A), but at the same moisture content, only 5% of the seeds gave rise to normal seedlings (figure 3B). In a similar manner, in the slow drying treatment, 73% of the embryos dried to 5% moisture were considered viable, whereas only 3% of the seeds gave normal seedlings.

The total lipid content of whole seeds was 12% (table 1). Regarding the distribution of total lipids in the different parts of the seed, a greater concentration was found in the embryo tissues, 25%, which corresponds to approximately three times the value determined in the endosperm (9%).

The total lipid content of the isolated embryos increased after slow drying (27%) but was lower (21%) in the rapidly dried embryos, indicating a consumption or degradation of lipids presumably due to stress arising from the high rate of water removal. In whole seeds, the total lipid concentration, initially 12%, decreased after slow and rapid drying, to 5 and 7%, respectively.

The fatty acid profile of seeds that were not dried (control) and after the slow and rapid drying processes are shown in table 2 for endosperms, embryos and whole seeds. The linoleic and palmitic fatty acids were predominant in the endosperm and in whole seeds. In the embryos, palmitic acid was predominant, followed by linoleic acid.

Table 1. Total lipids quantified by gas chromatography in embryos, endosperms, and whole coffee seeds before and after different drying treatments. Slow drying in saline solutions and rapid drying in silica gel.

	Total lipids (percentage of dry matter)		
Treatment	Embryo	Endosperm	Whole seed
Seeds without drying	25 b ± 0.14	9 a ± 0.58	12 b ± 0.19
Slow drying	27 a ± 0.93	6 b ± 0.07	5 a ± 0.07
Rapid drying	21 c ± 1.42	8 a ± 0.50	7 a ± 0.01

± Standard deviation of the means.

Table 2. Fatty acid fractions quantified in separate parts of coffee seeds before and after slow and rapid drying. Myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and eicosadienoic (C20:2) acids.

Treatments/ Parts of the seeds		Fatty acids (Percentage of dry matter)						
		C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:2
Seed without drying	Whole seeds	1 ± 0.14	35 ± 1.27	5 ± 0.21	8 ± 0.21	49 ± 1.27	2 ± 0.07	1 ± 0.07
	Endosperms	1 ± 0.15	35 ± 0.37	5 ± 0.28	8 ± 0.15	48 ± 0.75	2 ± 0.17	1 ± 0.05
	Embryos	1	41	7	14	34	1	2
Rapid drying	Whole seeds	0 ± 0.11	18 ± 2.36	3 ± 0.77	4 ± 0.25	24 ± 3.04	1 ± 0.17	1 ± 0.17
	Endosperms	1 ± 0.14	35 ± 0.49	5 ± 0.28	8 ± 0.14	48 ± 0.98	2 ± 0.14	1 ± 0.07
	Embryos	1	40	6	13	36	2	3
Slow drying	Whole seeds	0 ± 0.25	18 ± 0.45	3 ± 0.20	4 ± 0.11	25 ± 0.32	1 ± 0.05	1 ± 0.26
	Endosperms	0 ± 0.17	34 ± 1.53	6 ± 0.66	8 ± 0.05	48 ± 2.49	2 ± 0.15	2 ± 0.28
	Embryos	1	40	8	14	32	1	3

± Standard deviation of the means.

Discussion

Coffee seeds, endosperms and embryos after rapid drying in silica gel and slow drying over saline solutions showed significantly different physiological behavior. Rapid drying was more damaging to the viability of coffee embryos than slow drying (figure 2 and 3). In contrast, Pammenter *et al*. (2000) found that seeds or excised embryonic axes that undergo rapid drying can better survive low moisture contents compared with those that undergo slower drying performed in an environment with high relative humidity. Several authors attributed the greater survival due to the fact that in recalcitrant seeds, the embryos/embryonic axes are metabolically active and during slow drying, they remain for a long time at intermediate levels of moisture leading to more accumulated damage (Pammenter *et al*., 1991, 1998, 2000; Berjak *et al*., 1993; Marcos-Filho, 2005; Berjak and Pammenter, 2013). An explanation for our contrasting results, for embryos separated from coffee seeds and subjected to rapid drying in silica gel and slow drying in saline solutions. For some cases it is known that rapid drying does not allow sufficient time for deleterious reactions to process viability (Pammenter *et al*., 1998). According to Oliver and Bewley (1997), a higher tolerance to desiccation can also be observed in slow drying, due to the time allowed for the induction and operation of the protection mechanisms. Quick drying prevents recovery processes and more time is needed for repair upon rehydration.

The viability of *C. arabica* seeds in the tetrazolium test after rapid and slow drying indicate that endosperms are more susceptible to damage from drying than embryos (figure 3). Similar results were found by Coelho *et al*. (2015), who compared the results of viability in the tetrazolium test with the results of the germination test in seeds

subjected to subzero (°C) temperatures. Dussert *et al*. (1997, 2000) also found damage in the endosperms in *C. arabica* seeds that underwent stress from exposure to subzero (°C) temperatures. The embryos separated from the frozen seeds produced normal seedlings when cultivated *in vitro* after thawing. When whole seeds were subjected to the germination test, a low germination percentage (evaluated by formation of normal seedlings) was observed in several studies (Dussert *et al*., 1997, 1998, 1999, 2000, 2001, 2003, 2012; Dussert and Engelmann, 2006).

The results of quantification of total lipids obtained for the whole seed (12%) are in agreement with determinations made by other authors, with concentrations ranging from 11 to 20% (Nogueira and Trugo, 2003; Oliveira *et al*., 2006; Toci *et al.*, 2006). The quantity may vary according to the genetic characteristics (cultivar or variety) and depending on the environment (Carvalho and Nakagawa, 2012). In our study, a small increase was observed in the percentage of total lipids after slow drying (27%). An increase in the total lipid concentration was also found in embryos of *Hevea brasiliensis* Muell. Arg. during storage (Bonome, 2006), which was attributed to the process of recalcitrant and intermediate seed development after seed detachment from the mother plant, through continuation of the process of cell differentiation and deposition of reserves. The same process may have occurred during slow drying of the embryos studied here since gradual removal of moisture occurred, and this removal was not sufficient to halt the initial metabolism at the beginning. In rapid drying of the embryos, a fall in total lipid concentration (21%) was observed, indicating consumption or peroxidation of lipids due to stress arising from the high rate of water removal.

In whole seeds, the total lipid concentration, initially 12%, decreased after slow and rapid drying, to 5 and 7%, respectively (table 1). In a similar way, Corte *et al*. (2010) found a reduction in lipid concentration in naturally aged and artificially aged *Melanoxylon brauna* Schott seeds. Lipids are considered the constituents most susceptible to chemical degradation and directly influence the drying and preservability of the stored product (Rupollo *et al*., 2004). Gutkoski *et al*. (2009) and Abreu *et al*. (2013) investigating corn and sunflower seeds, respectively, concluded that there was a reduction in the amount of lipid during storage, regardless of the storage conditions.

Lipid peroxidation due to the presence of free radicals leads to DNA denaturation, affects transcription of RNAs and translation of proteins, and causes destruction of cell membranes, oxidation of amino acids (Popovic *et al*., 2006; Tian *et al*., 2008; Schwember and Bradford, 2010) and a series of reactions that produce potentially toxic products (Marcos Filho, 2005). As a consequence, there is a decrease in germination, vigour and development of seedlings.

In *C. arabica* the linoleic and palmitic fatty acids were predominant in the endosperm and in whole seeds (table 2). In the embryos, palmitic acid was predominant, followed by linoleic acid. Although the fatty acid composition varies among species, it is known that lauric, palmitic, stearic, oleic, linoleic and linolenic acids generally occur in greater quantity (Schery, 1972) and may compose up to 60% of the weight of some seeds (Buckeridge *et al*., 2004). Even though a change was not detected in the fatty acid fractions of the isolated embryos and endosperms subjected to drying, the quantities of linoleic and palmitic acid varied significantly between these structures. The linoleic

acid fraction was predominant, constituting 48% of the total lipids in the endosperm and 34% in the embryo. The palmitic acid fractions constituted 35% of total lipids in the endosperm and 41% in the embryo. Dussert *et al*. (1997, 2000) showed that sensitivity in *C. arabica* seeds subjected to stress through drying and freezing was expressed more in the endosperm since the embryos separated from these seeds formed normal seedlings when cultivated *in vitro*. This may be due to the fact that more saturated fatty acids (as is the case of palmitic acid found in a higher proportion in the embryos) contribute to preservation of membrane functionality after desiccation (Smith and Berjak, 1995; Pérez-Galvés and Mínguez-Mosquera, 2004; Mello *et al*., 2010).

In contrast, unsaturated fatty acids (as is the case of linoleic acid that is predominant in the endosperm) have a high propensity to autoxidation, and the higher the degree of oxidation, the higher the damage to membrane-associated proteins, altering its permeability (Smith and Berjak, 1995; Pérez-Galvés and Mínguez-Mosquera, 2004). Therefore, the intensity and the speed of the deteriorative process in the seeds may be related to the chemical composition of the seeds. Thus, the seeds with higher lipid content will have a greater predisposition to the deterioration process, especially in those with higher proportion of unsaturated fatty acids (Freitas, 2009; Abreu *et al.*, 2013; Brigante, 2013).

A positive correlation between the number of double bonds of the linoleic fatty acid and the formation of peroxides was obtained by Neff *et al*. (1992), and a negative correlation between linolenic acid concentrations and the physiological quality of soybean and sunflower seeds was observed by Oliveira (2004), Abreu *et al*. (2013) and Brigante (2013). The results of germination and of accelerated ageing obtained in the studies mentioned above suggest that seeds with a greater concentration of unsaturated fatty acids are more susceptible to peroxidation and are more predisposed to deterioration when exposed to unfavourable conditions. These results also are in accordance with Trawata *et al*. (1995a, b), who found lower viability and vigour in seeds with higher contents of linolenic acids stored at ambient temperature. Thus, differences in lipid composition and fatty acids support the hypothesis that an important source of damage and promotion of the deterioration process may reside in the endosperm of coffee seeds.

In the present study, no significant variation was observed among the lipid fractions after slow or rapid drying (table 1), either in embryos or in endosperm, thus indicating that the drying methods evaluated did not affect the lipid distribution and composition when seeds were subjected separately to drying. Nevertheless, there was a reduction in three fatty acids when whole seeds were dried; after slow drying and fast drying, the concentration of palmitic fatty acid varied from 35 to 18% of total lipids, linoleic acid from 49 to 25%, and oleic acid from 8 to 4%.

For Flood and Sinclair (1981), the reduction in fatty acid contents was a sign of the peroxidative degradation process that may be responsible for loss in viability of the seeds. Smith and Berjak (1995) suggested that a probable consequence of lipid peroxidation may be reduction in the concentration of unsaturated fatty acids. Abreu *et al*. (2013) and Brigante (2013) also identified a reduction of these acids in sunflower cultivars studied under different storage conditions. These assertions corroborate the results found in this study because drying seeds to 5% moisture content resulted in a decline in the germination capacity of coffee seeds.

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