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Oil composition and physiological quality of niger seeds after drying

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ABSTRACT. With the depletion of fossil fuel resources accelerating the search for renewable energy sources, studies on agricultural products containing a significant amount of oil in their compositions have intensified. The objective of this work was to investigate the fatty acid profile and the physiological quality of the oil extracted from niger seeds dried at different temperatures. The seeds were dried at 40, 50, 60, and 70°C, until their moisture content reached $8.5 \pm 1\%$ (wet basis). The physiological properties of niger seeds were determined by assessing their germination, germination speed index, mean germination time, cold test, accelerated aging with a saturated solution, emergence percentage, and emergence speed index. The quality of the oil was evaluated using gas chromatography, based on the fatty acid profile. We concluded that drying air temperatures of 40 and 50°C did not compromise the physiological quality of niger seeds. However, the 70°C drying temperature seriously impaired seed quality. The drying temperatures that were used in this study did not substantially alter the fatty acid profile of niger oil.

Keywords: Guizotia abyssinica Cass.; postharvest; fatty acid profile; oilseed.

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Introduction

Recently, concern about the energy matrix has been growing worldwide. Much of the energy consumed globally is derived from fossil fuels, a source that may be exhausted in the future. Therefore, obtaining energy from renewable sources has been one of the main targets of current research.

Many crops contain significant quantities of vegetable oils, which could serve different purposes, either to meet the demand for biofuels or to meet the needs of the pharmaceutical, cosmetic, and food industries. In addition to containing a high concentration of oil, it is necessary for the oil in the seeds to be high quality and have high oxidative stability, as in the case of the niger seed (*Guizotia abyssinica* Cass.).

Niger seeds stand out, nationally, because they can be used to feed sheep and to supply cattle with silage (Getinet & Sharma, 1996). This species also has potential for phytomass production when used as a soil cover (Carneiro et al., 2008). While it both protects and improves the properties of the soil, it is also a good source of extra income for the growers.

As with some crops, niger seeds are susceptible to climatic conditions, depending on the harvest season, making natural drying in the field infeasible. In light of this, niger seeds need to be artificially dried to reduce their water content, maintain their quality and ensure their safe storage.

After the drying process, it is necessary to perform certain tests, such as the germination test, to evaluate the quality of the seeds and to verify whether they underwent any kind of physiological alterations (Carvalho & Nakagawa, 2012). However, according to Coimbra, Martins, Tomaz, and Nakagawa (2009), the germination test alone is not sufficient to detect differences in the physiological quality across different seed lots. Nonetheless, these differences can be easily observed by using vigor tests, which include the calculation of germination speed index, mean germination time, accelerated aging, cold test, field emergence, etc. These factors are important variables to analyze the seeds and obtain quality seedlings.

As for the oleaginous species, changes in the chemical composition of vegetable oil are expressed by variations in the molar ratio between the different fatty acids present in its structure. Thus, analyzing fatty

acid composition is the first procedure in carrying out a preliminary evaluation of oil quality and can be achieved by gas chromatography (Mittelbach, Roth, & Bergmann, 1996).

In relation to the composition of fatty acids, the oil from niger seeds grown in Ethiopia resembles those of safflower and sunflower seeds, with a high linoleic acid content of up to 85% (Riley & Belayneh, 1989). The oils extracted from niger seeds can be obtained in different regions of Ethiopia, which is their country of origin. Usually, these are rich in linoleic acid (54 - 73%) and contain different levels of oleic (5 - 27%), palmitic (8 - 10%), and stearic acids (5.5 - 8.1%) (Dutta, Helmersson, Kebedu, Alema, & Appelqvist, 1994; Dagne & Jonsson, 1997).

A decrease in moisture content during the drying process may significantly impair seed quality as well as physical, physiological, and chemical properties depending on the method and drying conditions. Numerous studies have been performed to analyze the drying process of seeds of various oleaginous species, e.g., soybean (Silva, Diniz, Oliveira, & Pinho, 2007), pequi (Aquino et al., 2009), crambe (Costa et al., 2012), and sunflower (Sacilik, Tarimci, & Colak, 2007).

Considering the bioenergetic potential of the crop, the importance of the drying process between the post-harvesting stages, as well as its influence on the physiological quality of seeds and on the composition of their oil, the objective of this work was to study the fatty acid profile of the oil extracted from niger seeds, as well as the physiological quality of the seed, after being dried at different temperatures.

Material and methods

This study was carried out at the Laboratory of Physical Properties of Agricultural Products, the Laboratory of Preprocessing and Storage of Agricultural Products, the Laboratory of Seeds, the Laboratory of Synthesis and Molecular Characterization at the Federal University of Grande Dourados (UFGD), Dourados, Mato Grossodo Sul State, and the Laboratory of Fuels at the Federal University of Mato Grosso do Sul (UFMS), Campo Grande, Mato Grosso do Sul State, Brazil.

The niger seeds used in this study were manually harvested. They had an initial moisture content of approximately 19% on a wet basis (w.b.). Seeds were processed with the aid of metal sieves to remove impurities and other foreign materials. After the impurities were removed, the seeds were dried inside an experimental dryer at temperatures of 40, 50, 60, and 70°C.

A fixed-layer dryer was used to dry the seeds. This was equipped with a system capable of accurately controlling the flow and temperature of the drying air, and a series of sensors connected to a control panel in order to obtain a fine adjustment and monitor the drying conditions.

During the drying process, each tray containing 0.200 kg of seed samples was periodically weighed until it reached the final drying point, corresponding to a water content of $8.5 \pm 1\%$ (w.b.).

The water content of the niger seeds was determined by the gravimetric method in an oven at $105 \pm 1^{\circ}$ C for 24h, for three replicates (Brasil, 2009).

Effect of air temperature during drying on the physiological quality of the seeds

Before each physiological test, the seeds were treated with a fungicide (Maxim[®]) to minimize potential errors caused by pathogen contamination and to allow the product to reach its full potential within the established conditions.

The physiological properties of the niger seeds at each drying temperature were determined by the following tests.

Germination test

The germination test was carried out using a Gerbox-type box, in which 50 seeds were conditioned on a Germitest[®] paper moistened with distilled water proportional to 2.5 times the mass of the dry paper. The test was conducted under continuous light in a Biochemical Oxygen Demand (B.O.D.)-type germination chamber, at a regulated temperature of 25°C. This process was carried out four times for a total sample size of 200 seeds for three replicates (Brasil, 2009). The seeds were evaluated seven days after sowing, and the percentage of germination was recorded, taking into account whether normal seedlings (i.e., a developed shoot and root system). Along with the germination test, the germination speed index (GSI) was calculated, according to Equation 1, and the result was expressed as seeds day⁻¹ (Maguire, 1962). The mean germination time (MGT) was also determined according to Equation 2 (Edmond & Drapalla, 1958).

$$GSI = \frac{G1}{N1} + \frac{G2}{N2} + \dots + \frac{Gn}{Nn}$$
(1)

Physiological quality and oil composition of niger seeds

 $MGT = \frac{[(N1 G1)+(N2 G2)+...+(Nn Gn)]}{(G1+G2+...Gn)}$ (2)

where: G1, G2, ... Gn = number of germinated seeds calculated on a given counting day, and N1, N2, ... Nn = number of days after sowing.

Cold test

This test was carried out in paper rolls moistened with a water volume equivalent to 2.5 times the mass of the dry paper, using two sheets of Germitest[®] paper for sowing and one sheet to cover the seeds. After sowing, the rolls were wrapped in a plastic bag and transferred to germination chambers set at 5°C for five days. After this cooling period, the rolls were transferred to another germination chamber set at 25°C and maintained in continuous light, which was the same methodology that was used for the germination test (Gordin, Marques, Masetto, & Scalon, 2012). The percentage of normal seedlings was determined after seven days.

Accelerated aging test using a saturated solution

For each treatment, this test was carried out in individual chambers containing a suspended metal screen, on which a layer of niger seeds was uniformly distributed (1 g). Within each compartment, 100 mL of distilled water and 20 g of sodium chloride (NaCl) were added to maintain 76% relative humidity. The boxes were then placed in the oven at 41°C for 24h (Gordin, Scalon, & Masetto, 2015). After this period, the seeds underwent a germination test (percentage).

Field emergence of seedlings

Field emergence of the seedlings was obtained by sowing niger seeds into plastic trays filled with dystroferric Red Latosol and washed sand at a volumetric ratio of 1:1, which were moistened whenever necessary. Four replicates of 50 seeds per treatment were maintained in a greenhouse covered with Sombrite[®], which reduced luminosity by 30%, in an experimental area of the Faculty of Agricultural Sciences, FCA/UFGD. The results were expressed as the percentage of normal seedlings, and the evaluation ended as soon as seedling emergence stabilized. Concomitant to the emergence test, the emergence speed index (ESI) was calculated daily by counting the number of emerged seedlings (Maguire, 1962).

Effect of drying air temperature on the chemical composition of the oil

The oil was extracted from the seeds (approximately 20 g) using the direct method with hexane (100 mL). The seeds were ground using a portable mixer to increase the surface area in contact with the solvent. Thereafter, the seeds and solvent were added to an Erlenmeyer flask where they remained for 24h, during which oil extraction occurred. Filtration was then performed using filter paper, which enabled the contents to be transferred to another flask. The oil was separated from the solvent at a reduced pressure in a rotary evaporator.

The process of biodiesel production

Niger biodiesel was obtained via alkaline transesterification of the niger oil using potassium hydroxide (KOH) as a basic catalyst (1.5% relative to the oil) and using a 6:1 molar ratio of methanol to oil. Potassium methoxide was prepared by dissolving the catalyst in methanol at 45° C and pouring the solution into an Erlenmeyer flask containing preheated oil at 45° C. The reaction mixture was stirred constantly for 90 min. To separate the biodiesel from the glycerin and other undesirable components, the mixture was transferred to a decanting funnel, where it remained for approximately 12h. The biodiesel was separated by decanting the byproducts and washing three times with a combination of ultrapure water and saturated sodium chloride. The pH of the biodiesel was subsequently adjusted to a value that was close to neutrality. After this step, in order to eliminate water residues, the biodiesel was filtered in the presence of sodium sulfate (Na₂SO₄).

Fatty acid profile

Gas chromatography with a flame ionization detector (GC-FID) was the separation technique used to determine the fatty acid profile of the niger oil samples.

A Varian CP-3800 chromatograph with an automatic injector and FID and a BPX 70 (SGE) 30 m x 0.25 mm (length x internal diameter) column with a 0.25-µm-thick film were used.

The chromatography parameters are shown in Table 1. To identify the peaks corresponding to C4 to C22, the chromatograph was first injected with the chromatographic patterns of fatty acid methyl esters (FAMEs).

Injector and detector parameters		Oven parameters	
Injection volume	1 µL	Heating rate (°C min. ⁻¹)	4
Injector temperature (°C)	200	Isotherm (min.)	10
Detector	FID	Total run time (min.)	52
Injection mode	Split	Temperature (°C)	80
Split ratio	1:100	Carrier gas	Helium
Detector temperature (°C)	250	Flow	1 mL min. ⁻¹

Table 1. Analysis parameters for gas chromatography with a flame ionization detector.

Statistical analysis

The experiment evaluating the physiology of niger seeds after drying was carried out three times using a completely randomized design and four drying conditions (40, 50, 60, and 70°C) with at least three repetitions. The data were analyzed by ANOVA *F*-test using SISVAR software. Mathematical models were fitted through a non-linear regression analysis, by the Gauss-Newton method, using the Statistica 7.0 software. In order to verify the degree of fit of each model, the magnitude of the determination coefficient (R^2 , decimal), the relative mean error (P, %), the significance of the equation and the understanding on the development of the biological phenomenon were considered. On the other hand, a descriptive analysis was used to assess the oil quality data. The value of the relative mean error was calculated according to Equation 3:

$$P = \frac{100}{n} \sum_{i=1}^{n} \left(\frac{|Y - \hat{Y}|}{Y} \right)$$
(3)

where: n = number of experimental observations; Y = the experimentally observed value; and $\hat{Y} = the value calculated by the model.$

Relative mean error lower than 10% was considered as one of the criteria to select the models, according to Mohapatra and Rao (2005).

Results and discussion

Physiological quality of niger seeds

Drying temperatures of 40, 50, and 60°C led to the highest mean germination values for niger seeds (Figure 1). In contrast, at 70°C, the mean value in the experiment was lower than those at the other temperatures, probably because of the rupture of cell membranes in the seeds, caused by the effect of drying air temperature. According to Marcos-Filho (2015), depending on the drying conditions, there may be a reduction in seed physiological quality, due to the intensification in the deterioration process. This occurs because of the tensions exerted on the most superficial layers of the seeds, where high temperatures cause high drying rates, thus forcing a rapid removal of water from the product.





Costa et al. (2012), Ullmann, Resende, Chaves, Oliveira, and Costa (2015), and Hartmann Filho, Goneli, Masetto, Martins, and Oba (2016a) evaluated the influence of different drying temperatures on the physiological quality of crambe fruits, sweet sorghum and soybean, respectively, and observed the same germination behavior. These authors report, as also suggested by Afrakhteh, Frahmandfar, Hamidi, and Ramandi (2013), and observed by Menezes, Cicero, Villela, and Bortolotto (2012a), and Menezes, Pasqualli,

Barbieri, Vidal, and Conceição (2012b), that this result is usually caused by the high rate of water removal from the seed, stimulated by the high temperature of the drying air, which creates a high pressure gradient between the exterior and the interior of the seed, resulting in seed coat cracks and the formation of microfissures on the cotyledon, negatively influencing seed quality.

As observed for the germination of niger seeds (Figure 1), the germination speed index was higher for the 40, 50, and 60°C (Figure 2) drying temperatures. These results corroborate the results of several studies, including Oliveira, Resende, Smaniotto, and Campos (2016), who evaluated different drying temperatures for corn seeds; Resende, Almeida, Costa, Mendes, and Sales (2012), who evaluated adzuki bean drying; and Ullmann et al. (2015), who evaluated sweet sorghum seeds.



Figure 2. Germination speed index (GSI) of niger seeds as a function of drying temperature.

In niger seeds, the 70°C drying temperature caused a lower GSI, which is associated with less vigorous seeds. Menezes et al. (2012a) report that the increment in drying temperatures increases the percentage of seeds with cracks, which, associated with other effects of drying, negatively affect the germination speed index.

For mean germination time (Figure 3), seeds subjected to drying air temperature of 70°C obtained the highest mean values. The higher the value, the longer the time required for germination to occur. Such elevated temperature may have caused reduction in the speed of metabolic reactions, affecting the processes that are essential to trigger germination (Carvalho & Nakagawa, 2012), thus reducing germination speed, percentage and also increasing the mean time of germination.



Figure 3. Mean germination time (MGT) of niger seeds as a function of drying temperature.

The mean germination time is a good index for evaluating the speed at which a species occupies a given area (Ferreira et al., 2001). Thus, higher values of MGT are not desirable because they are associated with lower vigor.

Lower drying air temperatures, particularly 40 and 50°C, led to higher percentages of normal seedlings in the cold test, compared with the other drying temperatures (Figure 4). The 60°C drying temperature

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resulted in intermediate values, which were also observed in measures of germination percentage (Figure 1). High temperatures caused more serious damage to the seeds than lower temperatures, and this result is similar to that observed by Saravia, Peres, and Risso (2007) and Hartmann Filho, Goneli, Masetto, Martins, and Oba (2016b), when drying rice and soybean seeds, respectively. These results demonstrate the efficiency of the cold test as a physiological evaluation, which is often used as an auxiliary parameter in the selection of seed quality.



Figure 4. Percentage of normal seedlings of niger seeds obtained with the cold test as a function of drying temperature.

With an increase in drying temperature, there is an immediate decrease in the vigor of the seeds subjected to the accelerated aging test (Figure 5).





The accelerated aging test, as well as the cold test, showed a lower mean percentage of normal seedlings than was observed for germination test. Thus, it is possible to suggest that, since the integrity of the seeds had been hampered, there was a reduction in their capacity to withstand the adverse conditions applied in the test.

Menezes et al. (2012b) and Sarath, Goneli, Masetto, and Oba (2016), evaluating the physiological quality of rice and peanut seeds, respectively, through accelerated aging and cold tests, observed that as the drying air temperature increased, seed vigor was reduced, due to the deleterious effects of the higher temperatures.

Lower drying temperatures led to higher experimental means for the percentage of normal seedlings that emerged (Figure 6), compared with the other treatments, thus demonstrating reduced damage to the cell membrane system of niger seeds. The emergence test has also shown satisfactory results in other works that evaluated seed vigor (Santos et al., 2010; Delarmelino-Ferraresi, Villela, & Aumonde, 2014; Ullmann et al., 2015).



Figure 6. Percentage of emerged seedlings of niger seeds obtained through the emergence (E) test as a function of the drying temperature.

On average, the 40 and 50°C drying temperatures led to higher experimental emergence speed index values (Figure 7). On the other hand, the 60 and 70°C drying temperatures resulted in reduced experimental values, with small variation between them. There was no significant effect on the experimental results, so no model could be fitted to satisfactorily represent the behavior.



Figure 7. Emergence speed index (ESI) of niger seedlings as a function of drying temperature.

The emergence speed index (Figure 7) is slightly more sensitive than the other tests, since the difference between the damage caused by the 50°C and 60°C drying temperatures was less explicit in the other tests. This test is of considerable importance to the producer, because it aims to reproduce the behavior of the seed in the field.

According to Schuh, Antunes, Ferrari Filho, Dionello, and Bender (2013), orthodox seeds, such as niger seeds, are more tolerant to desiccation, especially in slow drying processes, which reduce the physiological damages caused in the seeds. In addition, these seeds have protection mechanisms that allow them to preserve cell membranes and macromolecular structures, besides reserving substances that reactivate their physiological functions when they are rehydrated (Guimarães, 1999).

Thus, lower temperatures, for causing slower water removal, have lower influence on the physiological potential of the seeds, preserving their germination and vigor (Schuh et al., 2013; Alves et al., 2015; Cardoso, Alves, & Alves, 2015).

Fatty acid profile of niger oil

Eleven different fatty acids were identified in the composition of niger oil for every drying temperature studied, and only those expressing at relatively higher quantities were assessed. The other acids (butyric (C4:0), palmitoleic (C16:1), arachidic (C20:0), and α -linolenic (C18:3n3)) were identified with concentrations below 0.5%.

In general, the profile of the fatty acids in niger oil did not vary in expression as the drying temperature increased (Figure 8). In this vegetable oil, the main component is linoleic acid, with mean concentration higher than 70%. Both linoleic and α -linolenic fatty acids cannot be synthesized by humans (Kschonsek, Stimming, Libuda, Kersting, & Böhm, 2016; Song et al., 2016). Therefore, based on its high nutritional quality, niger oil could be an important component in the human diet, providing a wide range of health benefits.



Figure 8. Fatty acid profiles of niger biodiesel at different drying temperatures.

High concentrations of palmitic, oleic and stearic fatty acids were also detected (Figure 8). The values detected here are similar to those reported by other studies using seeds from Ethiopia, which evaluated the fatty acid composition and concentration of the oil from niger seeds (Dutta et al., 1994; Dagne & Jonsson, 1997; Ramadan & Mörsel, 2003). These data indicate that there was no loss of oil quality from after artificial drying or from the niger crop having been cultivated in the novel climate conditions of Mato Grosso do Sul, as compared with its region of origin.

Oleic, linoleic, palmitoleic, and α -linolenic fatty acids, for having unsaturated chains, are easily oxidized to hydroperoxides, which in subsequent reactions are transformed into a wide variety of low-molecular weight compounds, causing unpleasant aroma and taste in the product (Gutkoski & El-Dash, 1999). According to Costa Neto, Rossi, Zagonel, and Ramos (2000), oxidation is accelerated by high temperature and is the main factor responsible for the modification of the physicochemical and sensory characteristics of the oil. However, it is notable that, even at the highest drying temperature (70°C), there was no damage to the fatty acids contained in niger oil.

Maintaining such quantity of unsaturated fatty acids with the change in drying temperature is desirable because, at high temperatures, the drying time is shorter and it leads to optimization of the process as a whole, maximizing its operational performance.

Conclusion

Drying air temperatures of 40 and 50°C do not compromise the physiological quality of niger seeds. On the other hand, the 70°C drying temperature seriously impaired the quality of the seeds.

It is possible to use high temperatures to dry niger seeds without compromising the quality of their oil.

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