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Crambe seeds quality during storage in several conditions

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Crambe abyssinica **seeds are spherical and surrounded by a structure called the pericarp. The basic function of the pericarp is to protect the grains against abrasion and shocks, to function as a barrier against microorganisms and to allow the seeds to be stored for long periods of time. The aim of this study was to evaluate the *C. abyssinica* seed quality stored under different environmental conditions without the pericarp. Crambe seed with 6.5% w.b. moisture content were used. Measurements for the electrical conductivity, water uptake, germination percentage and index of germination velocity (IGV) were performed at the beginning of the experiment (zero months) and every two months for a period of a year. The seed were stored under three environmental conditions: Room temperature (26±3°C, 55±12% relative humidity [RH]), a cold room (5±1°C, 79±5% RH) or a climate-controlled chamber (18±1°C, 53±7% RH). The climate-controlled chamber maintained the best quality in the crambe seed, with better germination percentage and IGV than the other conditions. The storage conditions promoted decrease in the crambe seed quality. It was possibly visualize that there was a loss of dry matter during storage, especially lipids adhered in Kraft paper bags.**

Key words: *Crambe abyssinica*, seeds quality, storage environment.

INTRODUCTION

Currently, businesses, as well as state and federal agencies, have prioritized the search for alternative raw materials for biodiesel production and are continuously evaluating the effect of their attributes, such as oil content, yield, production system and crop cycle. *Crambe abyssinica* is a winter crop that has great potential to become a raw material for biodiesel in addition to being a good candidate for crop rotation.

Crambe (*C. abyssinica*) is a member of the Brassicaceae family that is native to Mediterranean regions and is considered a potential crop for the production of biodiesel due to its cultivation characteristics and high oil content. Studies conducted with crambe grown in Mato Grosso do Sul - Brazil (FMS Brilliant variety) have shown a 40% oil content in the seed (Souza et al., 2009).

Crambe shows important characteristics, such as a low production cost, a short growing cycle, tolerance to drought and low temperatures; it is one of winter crops, but can be grown in other times and it can be planted at later times when there is too much risk to other crops in

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the Midwest region of Brazil (Pitol et al., 2010).

The fruits have been reported to show low bulk density, approximately 328 kg m^{-3} (Reuber et al., 2001), which is the major problem when setting up a production chain due to the high transportation and storage costs. Peeling (pericarp removal) of the crambe fruit could significantly reduce the operational costs of the crop. However, there is no adequate information on the seed quality of crambe when it is stored without the pericarp.

C. abyssinica seeds are spherical and surrounded by an integument structure called the pericarp (Ruas et al., 2010). The pericarp, which remains attached to the seeds after harvest, representing 25 to 30% of the total weight of the fruit, has high lignin content (40%) and is approximately 41% cellulose (Gastaldi et al., 1998).

Several studies have been conducted to study the influence of storage conditions on seed quality of crops, including annual ryegrass (Eichelberger et al., 2003), annatto (Corlett et al., 2007), arnica (Melo et al., 2007), sorghum-sudan (Toledo et al., 2007), *Coffea arabica* (Vieira et al., 2007) papaya (Berbert et al., 2008), cotton (Queiroga et al., 2009), castor (Fanan et al., 2009) and soybean (Forti et al., 2010).

The main purpose of storage, which actually begins before the harvest when the seeds reach their physiological maturity and continues until the time of sowing, is to maintain seed quality and minimize deterioration, as the quality of the seed is determined during development and cannot later be improved, even under ideal storage conditions (Baudet, 2003). Seed deterioration is irreversible and cannot be stopped, but it is possible to reduce the rate of deterioration through proper handling and efficient environmental storage conditions (Baudet, 2003).

Vieira et al. (2004) have suggested that the determination of electrical conductivity of seeds was a sensitive test for the evaluation of vigor because in the deterioration process, one of the first events is the loss of membrane integrity. Seeds with low vigor tend to show disorganization of the cellular membrane structures, allowing for the increased leaching of solutes, such as sugars, amino acids, organic acids, proteins, phenolic substances and inorganic ions, including K^+, Ca^{2+}, Mg^{2+} and Na^{2+} (Vanzolini and Nakagawa, 2005; Dias et al., 2006). Therefore, the objective in this study was to evaluate the crambe seed quality when stored under three different environmental conditions without the pericarp.

MATERIALS AND METHODS

Crambe (*Crambe abyssinica*) seeds (cultivar FMS Brilhante) produced at the Fundação MS were used in this study. The fruit was harvested in August 2008 using a harvesting machine adapted for the process, and the pericarp was mechanically removed. The experiment was conducted at the Laboratory of Postharvest of Plant Products and Laboratory of Seeds at the Instituto Federal de Educação Ciência e Tecnologia Goiano - Câmpus Rio Verde, GO, Brazil (IF Goiano-Câmpus Rio Verde). The seeds were conditioned in three replicates in Kraft paper bags, unifoliate, with an initial seed moisture content of 6.5% (wet base, w.b.) that was determined gravimetrically using an incubator at 105±3°C for 24 h (Brazil, 2009). Approximately 0.4 kg of seed was used in the paper bag, which were kept under three conditions: room temperature (26±3°C, 55±12% relative humidity [RH]), a cold room (5±1°C, 79±5% RH) and a climate-controlled chamber (18±1°C; 53±7% RH). During storage, the RH and temperature were recorded by a digital datalogger.

The seeds were stored from August 11th, 2009, to August 11th, 2010, and the samples were evaluated at 0, 2, 4, 6, 8, 10 and 12 months, in three repetitions, for water absorption, bulk density, electrical conductivity, germination and the index of germination velocity (IGV).

To determine the water uptake, the samples were subjected to hydration in distilled water for a period of 12 h. Absorption was performed in a chamber with a controlled temperature at 25 ± 2°C and plastic cups (100 - ml capacity) containing 75 ml of distilled water with 15 g of seeds (a 5:1 mass ratio). The samples were gently agitated so that all of the seeds were completely submerged. After hydration, the samples were removed from the cups and placed on a filter paper to blot for two minutes and then weighed to 0.01 g. The moisture content after absorption was obtained by the following equation:

$$U^* = \frac{M_e - M_s}{M_s} \quad (1)$$

where, U^* = moisture content of the product (decimal dry base, d.b.), M_e = mass after water absorption (kg) and M_s = mass of the dry product (kg).

The bulk density (ρ_{ap}), expressed in kg m^{-3}, was determined with an electronic hectoliter scale with a 0.01 g resolution using a 90 ml container. The electrical conductivity (EC) of the crambe seeds was measured without the pericarp using the method described by Vieira and Krzyzanowski (1999). Fifty seeds were used for four replicates of each treatment and weighed accurately to two decimal places (0.01 g). The samples were soaked in plastic cups (100 ml capacity) containing 75 ml of water and kept in a BOD chamber with a controlled temperature at 25 ± 2°C for 24 h. Solutions containing the seeds were lightly agitated for uniformity of the leaching and immediately measured using a portable digital conductivity meter (model CD-850 “INSTRUTHERM”). The results were divided by the mass of 50 seeds and expressed in μS cm^{-1} g^{-1}.

The germination test was conducted with four subsamples of 30 seeds from each treatment. The fruits were packed in Gerbox boxes on blotting paper moistened with distilled water, which was equivalent to 2.5 times the dry substrate mass, to achieve adequate moisture and uniformity of the test. The samples were kept in a Mangelsdorf germinator set at a constant temperature of 25 ± 2°C. The evaluations were performed every two days from the second day after sowing until 32 days were completed according to the criteria established in the Rules for Seed Analysis (Brazil, 2009). The average germination percentage was calculated, and the IGV was calculated as follows: $IGV = n_1.d_1^{-1} + n_2.d_2^{-1} + n_3.d_3^{-1} ... n.d_n^{-1}$; where n_1 is the number of seeds germinated on the first day of counting; n_2 is the number of seeds germinated on the second day of counting; n_3 is the number of seeds germinated on the third day of counting; n_n is the number of seeds germinated on the n^{th} day of counting; d_1 is the first day; d_2 is the second day; d_3 is the third day; and d_n is the n^{th} day (Maguire, 1962).

The experiment was designed according to a subdivided plot scheme, with the three storage conditions (room temperature, cold room and climate-controlled chamber) plots and the evaluation months as the subplots. The averages were compared by Tukey’s test at 5% significance.

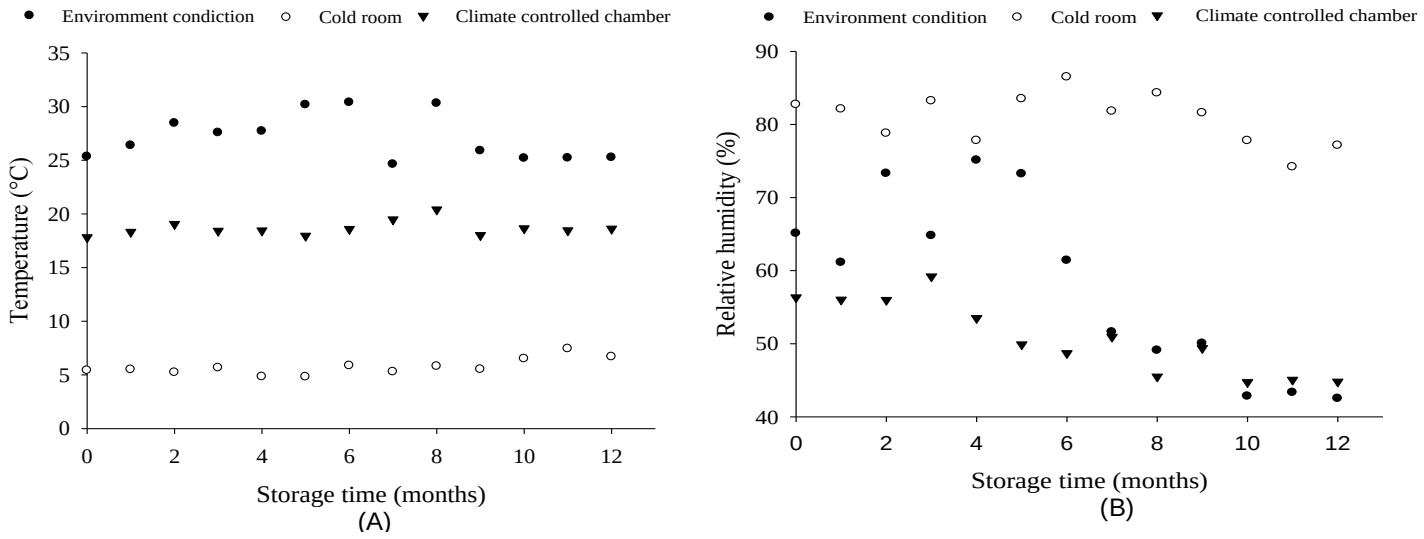

Figure 1. (A) Average temperature during storage in the three conditions: Room temperature (26±3°C), a cold room (5±1°C) and a climate-controlled chamber (18±1°C). (B) Average relative humidity during storage: room temperature (55±12%), a cold room (79±5%) or a climate-controlled chamber (53±7%).

RESULTS AND DISCUSSION

Figure 1 shows the average monthly values of the temperature and the relative humidity of the air in the three storage chambers for crambe seeds without the pericarp. The cold room provided the highest relative humidity due to the low temperature of the environment, and showed the lowest changes over time. The environment condition showed the greatest changes in the temperature and relative humidity, which can be attributed to the changes in thermal and moisture regimes associated with the change of seasons. The climate-controlled chamber showed the lowest relative humidity due to the cooling system, which removed water vapor from the chamber. A summary of the analysis of variance for the variables analyzed during the storage of crambe seeds without the pericarp under three environmental conditions is shown in Table 1.

Table 2 shows the moisture content values of the crambe seeds without the pericarp. Seeds stored under room temperature and in the cold room showed large variations in their moisture content during storage. The changes in the air conditions caused constant alterations in the moisture content of the seeds stored in bags permeable to water vapor.

It was observed at the end of the storage period that there was a decrease in the moisture content of the seeds stored in the cold room, due to the moderately low temperature and relative humidity under this condition (Figure 1), which caused the seeds to attain equilibrium with the environmental conditions in question. This result was similar to those observed by Catunda et al. (2003) for the storage of passion fruit seeds under three different conditions for 10 months.

Table 3 shows the variations in the bulk density of the crambe seeds during their storage under the three different environmental conditions. Although the values were influenced by the time and storage conditions, they did not, however, exhibit a defined behavior. The values ranged from 513.63 to 573.79 kg m^{-3}. Therefore, in this research, verified that seeds without pericarp presented the bulk density more than Reuber et al. (2001) for crambe fruit approximately 328 kg m^{-3} (with pericarp). Thus, it appears that the seed without pericarp present decreasing the storage and transportation costs.

According Pitol et al. (2010), one of the alternatives to solve the transportation problem of seeds is the peel of the fruit of crambe (removal of the pericarp) before shipping, so the product has bulk density peeled around 740 kg m^{-3}.

Water uptake by the seeds was not different under the three storage conditions (Table 4); however, over storage time, significant differences did arise for this parameter. Costa et al. (2012a) when storing fruits of crambe (crambe with pericarp) under the same conditions and storage period of this study, which concluded at the end of 12 months of storage, the water absorption is not different from baseline for the three conditions storage.

Water uptake and distribution in the seeds, which are regulated by the cellular water potential, occur as much by capillary action as by diffusion in the high-to-low water potential direction. According to Ullmann et al. (2010), water absorption is a good parameter for the evaluation of mechanical damage, as their values are linked to damage caused in the integument and seed structure. It is possible that no physical damage occurred with the removal of the pericarp, which remained attached to the crambe seed after harvest, and this could have influenced the values of water absorption during storage.

Regarding electrical conductivity, there was an increase

Table 1. Moisture content, bulk density, water absorption, electrical conductivity, germination rate and index of germination velocity during storage of crambe seeds without the pericarp under different environmental conditions for 12 months.

Variables analyzed	Source of variation	Mean squared	CV (%)
Moisture content	Environment	34.92**	9.30
	Months	6.91**	9.59
	Environment x Months	4.84**	
Bulk density	Environment	388.32*	1.27
	Months	1393.69**	2.04
	Environment x Months	419.13**	
Water absorption	Environment	0.00033^{NS}	4.69
	Months	0.011*	3.18
	Environment x Months	0.0010^{NS}	
Electrical conductivity	Environment	60153.47**	6.04
	Months	34343.84**	4.94
	Environment x Months	4464.97**	
Germination	Environment	1891.74**	12.17
	Months	1885.80**	16.28
	Environment x Months	247.60**	
IGV	Environment	53.75**	15.68
	Months	34.99**	21.09
	Environment x Months	4.71*	

**Significant at 1% by F test. *Significant at 5% by F test. ^{NS}Not significant.

Table 2. Moisture content in crambe seeds without the pericarp (% w.b.) subjected to storage under room temperature, cold room or climate-controlled conditions for 12 months.

Environments	Storage period (months)						
	0	2	4	6	8	10	12
Room temperature	6.54^{aAB}	5.11^{aBC}	7.04^{aA}	6.32^{bAB}	6.01^{bABC}	4.59^{bC}D	3.12^{bD}
Cold room	6.54^{aBC}	5.36^{aC}	7.25^{aB}	8.93^{aA}	8.74^{aA}	10.08^{aA}	6.55^{aBC}
Climate -controlled chamber	6.54^{aA}	5.80^{aA}	5.69^{aA}	5.12^{cAB}	5.86^{bA}	4.20^{bB}	3.83^{bB}

Means followed by a same lower case letter in the columns and uppercase in lines do not differ by Tukey's test at 5% probability.

Table 3. Bulk density (kg.m^{-3}) of crambe seeds without the pericarp stored at room temperature, in a cold room or in a climate-controlled chamber for 12 months.

Environments	Storage period (months)						
	0	2	4	6	8	10	12
Room temperature	513.63^{aB}	547.46^{bA}	530.63^{bAB}	545.58^{aA}	534.52^{aAB}	549.59^{aA}	540.73^{abAB}
Cold room	513.63^{aD}	573.79^{aA}	560.99^{aAB}	532.78^{aCD}	537.53^{aBCD}	531.46^{aCD}	557.63^{aABC}
Climate -controlled chamber	513.63^{aB}	540.95^{bAB}	544.58^{abA}	548.70^{aA}	542.40^{aA}	535.02^{aAB}	525.70^{bAB}

Means followed by a same lowercase letter in the columns and uppercase in lines do not differ by Tukey's test at 5% probability.

in the amount of electrolytes released by the seeds during storage. The EC tended to increase more under room temperature and in the climate-controlled chamber, confirming the influence of time and storage conditions in the amount of solutes leached into the solution. In general, seeds stored in the refrigerated chamber showed lower electrical conductivity values than those kept under the other storage conditions, indicating that refrigeration of the seeds results in the least amount of electrolyte leakage (Table 5). These results agree with those obtained by Pontes et al. (2006) for *Caesalpinia peltophoroides* seeds stored for a period of 240 days at 5

Table 4. Water absorption of crambe seeds without a pericarp (decimal db) stored at room temperature, in a cold room or in a climate-controlled chamber for 12 months.

Environments	Storage period (months)						
	0	2	4	6	8	10	12
Room temperature	1.09	1.05	1.10	1.06	1.01	1.13	1.10
Cold room	1.09	1.05	1.11	1.05	0.99	1.13	1.09
Climate -controlled chamber	1.09	1.03	1.10	1.10	1.05	1.09	1.06
Averages	1.09^{AB}	1.04^{C}	1.10^{A}	1.05^{BC}	1.04^{C}	1.11^{A}	1.08^{ABC}

Means followed by a lowercase letter in the columns and uppercase in lines do not differ by Tukey's test at 5% probability.

Table 5. Electrical conductivity of crambe seeds without the pericarp (μS cm^{-1} g^{-1}) stored at room temperature, in a cold room or in a climate-controlled chamber 12 months.

Environments	Storage period (months)						
	0	2	4	6	8	10	12
Room temperature	329.57^{aE}	433.69^{aCD}	382.65^{aD}	465.45^{aC}	541.33^{aB}	589.49^{aAB}	600.36^{aA}
Cold room	329.57^{aC}	365.75^{bBC}	325.46^{bC}	386.56^{bB}	439.22^{bA}	406.15^{bAB}	414.45^{bAB}
Climate -Controlled chamber	329.57^{aD}	367.41^{bBCD}	350.22^{abCD}	392.28^{bABC}	413.82^{bAB}	443.18^{bA}	427.58^{bA}

Means followed by a lowercase letter in the columns and uppercase in lines do not differ by Tukey's test at 5% probability.

Table 6. Germination and index of germination velocity (IGV) of crambe seeds without the pericarp stored at stored at room temperature, in a cold room or in a climate-controlled chamber for 12 months.

Environments	Germination (%)						
	Storage period (months)						
	0	2	4	6	8	10	12
Room temperature	72.87^{aA}	66.67^{aAB}	65.00^{aAB}	49.44^{bBC}	36.39^{bCD}	35.00^{bCD}	22.50^{bD}
Cold room	72.87^{aA}	74.72^{aA}	75.83^{aA}	46.11^{bBC}	27.50^{bC}	55.83^{aAB}	45.00^{aBC}
Climate -controlled chamber	72.87^{aAB}	72.78^{aAB}	82.22^{aA}	68.89^{aAB}	60.83^{aAB}	68.89^{aAB}	55.56^{aB}
				IGV			
Room temperature	8.53^{aA}	7.73^{aAB}	8.33^{aA}	5.3^{bABC}	4.02^{bBC}	3.50^{bC}	2.46^{bC}
Cold room	8.53^{aAB}	9.70^{aA}	9.77^{aA}	5.14^{bC}	2.75^{bC}	6.03^{bABC}	5.60^{aBC}
Climate -controlled chamber	8.53^{aA}	9.94^{aA}	10.83^{aA}	8.6^{aA}	7.52^{aA}	9.33^{aA}	7.22^{aA}

Means followed by a lowercase letter in the columns and uppercase in lines do not differ by Tukey's test at 5% probability.

or 20°C with 70 or 62% relative humidity, respectively. In addition, Borba Filho and Perez (2009) have stored the seeds of white (*Tabebuia roseoalba*) and purple (*T. serratifolia*) ipê for 300 days at laboratory room temperature (21 to 31ºC, 40 to 78% RH), in a refrigerated chamber (4 to 6ºC, 38 to 43% RH) and in a cooled chamber (14 to 20ºC, 74 to 82% RH). They reported that the highest values of electrolytes were leached from the seeds kept in the laboratory environment, which became apparent after only 60 days of storage.

Table 6 shows the values for the germination percentage and index of germination velocity (IGV) of the crambe seeds stored for 12 months in all three environments. For each month of storage, the germination potential was higher in the seeds stored in the climate-controlled chamber. Crambe seeds had a higher percentage of germination at month zero, the beginning of the storage period, under all storage conditions. As this may indicate an absence of dormancy, it is important to note that these seeds started the storage period 12 months after their harvest. The dormancy mechanism is common in the seeds of several species after harvesting (Brazil, 2009). A study on crambe seeds dormancy would be necessary to consider the possibility that the germination potential increased with the time interval following harvest. Costa et al. (2012b) and Faria et al. (2012) have studied the viability of crambe seeds subjected to different drying conditions and moisture content and have observed a low germination percentage. In addition, Oliva (2010) found than low germination after drying crambe seeds and storing them for 8 months in unifoliate paper bags.

During storage, the percentage of germination increased in cold room and Climate controlled chamber until the fourth month, from which time, the values decreased and fluctuated (Table 6). Crambe seeds are protected by the pericarp when the fruits are left intact after harvest. It is possible that the process of removal pericarp affected the seed quality because after four months, the seeds stored in Kraft paper bags showed oily patches. It was possibly visualize that there was a loss of dry matter during storage, especially lipids adhered in Kraft paper bags, which are one of the major reserve substances found in crambe seeds.

The seeds rich in lipids have limited longevity due to their specific chemical composition. For example, sunflower seed storage demands special attention due to high oil content, otherwise processes may occur that lead to loss of germination ability and seed viability (Christensen, 1971)

Different longevity of seed storage as well as storage conditions exerts significant influence on seed germination (Nkang and Umoh, 1997). The results of Sharma (1977) clearly pointed out to declining trends in total oil content and seed germination during storage of oilseed species. Seed aging during storage is an inevitable phenomenon, but the degree and speed of decline in seed quality depend strongly, beside storage conditions, on plant species stored and initial seed quality (Elias and Copeland, 1994) as well as on seed genetic traits (Malenčić et al., 2003).

It was possibly visualize that there was also a higher incidence of fungal infection in the seeds stored in the cold room. This fact is due to the higher values of water activity in the seeds under this condition (higher than 0.75, Figure 1), showing high levels of water throughout the whole storage period.

The index of germination velocity (IGV) was also higher in the seeds stored in the climate-controlled chamber, as observed for the germination percentage. This index increased significantly until the fourth month of storage, from which time the values decreased and fluctuated (Table 6).

Santos and Paula (2005) have shown that “branquilo” seeds exhibited a decrease in the percentage and rate of germination when stored for five months in paper bags, indicating that, in addition to the storage environment, a reduction in germination may also be associated with the packaging used.

Conclusions

Based on the results presented, we conclude that: The climate-controlled chamber retained the best quality of crambe seeds lacking the pericarp and provided a higher percentage of germination and higher IGV values than for the other two environments, and the storage conditions promoted decrease in the crambe seed quality. It was possibly visualize that there was a loss of dry matter during storage, especially lipids adhered in Kraft paper bags.

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