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Chemical composition, germination and sanity of landrace maize seeds stored

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Abstract

The preservation of quality, sanity and nutritional value of the seeds during the storage period depends not only on the conditions of production and harvesting, but the storage and maintenance of appropriate storage conditions of the product. The aim of this study was to evaluate the relationship between chemical composition, germination and sanity in seeds of landraces maize stored under different conditions. Lots of corn seeds of the varieties Oito Carreiras, Cabo Roxo and Lombo Baio were used. The seeds were stored for nine months in paper bags at temperature of 10°C in a laboratory / cold chamber (condition 1) and in plastic packaging atmosphere at room temperature (condition 2). Germination test, sanity and chemical composition analysis were performed. The seed quality is affected by changes in the chemical composition during storage. Fungi incidence is related to the chemical composition of the seeds is changed during the storage period.

Introduction

Corn landrace varieties present high genetic variability and are adapted to the specific regions where they grow (Araújo and Nass, 2002). Thus, the landrace corn has been a viable alternative to cropping systems that have low levels of technological investments and financial capacity. This specific type of corn is traditionally cultivated by rural communities, especially on small farms with family agriculture, which have adopted movements with the objective to return to the agricultural practices, combined with low cost of production through exploration of rustic genetic materials. Its utilization is also important to preserve their genetic variability in natural growing conditions (Sandri and Tofanelli, 2008).

Seed quality is one of the main factors to be considered in the implementation of the corn crop. Studies on germination and vigor are extremely important as well as the physiological and sanitary quality and chemical composition. Under the seeds quality control, the sanity test has been used to define the profile of the quality of a lot. Also can elucidate the seedling evaluation and causes of a low germination and vigor complementing the germination test (Brasil, 2009a).

Many factors can influence the chemical composition of corn seeds, such as origin, variety, processing and attack of pests and diseases. The chemical composition of the seeds is determined primarily by genetic factors and varies between different species and cultivars of the same species. The environmental conditions at the time of seed formation and cultural practices (fertilization, sowing times) may cause changes in the chemical composition of the seeds (Nedel, 2012). Corn seeds are easily attacked by insects which take over your chemical composition, and inadequate storage conditions may provide the fungi development. The ability to preserve the quality and sanity during the storage period depends not only on the conditions of production and harvesting, but also the storage conditions and product maintenance (Elias, 2002).

Despite the considerable increase in knowledge regarding the analysis of seeds, many varieties of landrace seeds require basic information concerning the ideal conditions for germination and vigor. These varieties have great genetic variability and demand studies regarding the assessment of physiological and sanitary quality, and also in the analysis of the chemical composition of its seeds including the storage period. Whereas these issues are rarely addressed together,

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the aim of this study was to analyze the relationship between chemical composition and physiological and sanitary quality of seeds of landraces of maize stored under different conditions.

Material and Methods

Materials

The seeds of landrace maize (about 3 Kg each) Oito carreiras, Lombo baio and Cabo roxo from the Association of Landrace Seed Keepers of Ibarama, RS (29°25'10"S; 53°08'05"W, altitude: 317 m) were used. These seed batches were produced in the same area and under the same conditions of temperature of environment (climate humid subtropical).

Storage conditions

Maize seeds were stored for nine months under two conditions: condition 1 (C1): in paper bags kraft, brown (18 cm length x 42 cm height x 6.5 cm width) at a temperature of 10°C and condition 2 (C2): in plastic packing (34,5 cm length x 10,5 cm diameter) at room temperature, both in Santa Maria / RS (29º42'24"S; 53º48'42"W altitude: 116 m) humid subtropical climate.

Determination of physiological and sanitary quality

The physiological quality of the seeds of all varieties was assessed every three months the seeds were subjected to the two storage conditions for a total of nine months. The sanitary quality was assessed every three months for a total of nine months for the varieties Oito carreiras and Lombo baio, and a total of six months for the variety Cabo roxo (this test could not be completed in the latter variety because there was an insufficient number of seeds). The following tests were conducted:

Germination test

Conducted at 25°C, with eight replications of 50 seeds distributed in moistened paper roll with amount equivalent to 2.5 times the weight of the substrate water. Seeds counting were performed seven days after sowing and the results expressed as a percentage of normal seedlings (Brasil, 2009b).

Sanity test

Conducted with eight replicates of 50 seeds and distributed in plastic boxes lined with two sheets of sterilized and moistened with sterile distilled water filter paper. Incubation was performed in a chamber with temperature control of 22 ± 3°C and 12 hours photoperiod for 7 days (Brasil, 2009a). In this period the assessment and identification of fungi with the aid of optical stereoscopic microscope and specialized bibliography were performed.

Determination of physicochemical properties

At the beginning and the end of the storage period we determined:

Dry matter

At 105°C; ash in oven at 550°C.

Crude protein

By the micro-Kjeldahl method (N x 6.25) according to the techniques described in AOAC (1995).

Lipid

Was determined using chloroform and methanol as described by Bligh and Dyer (1959) and also used as a preliminary step of preparing the sample for the determination of fatty acid profiles.

Total dietary fiber, soluble fiber and insoluble fiber

Were quantified according to the enzymaticgravimetric method N°. 985.29 and N°. 991.42 (AOAC, 1995), which analytically determines the levels of insoluble dietary fiber and total and quantifies, for difference, the soluble fiber content of the sample. The enzymes used in the enzymatic methods were α-amilase (Termamyl 2X®), protease (Alcalase 2.4 L FG®) e amyloglucosidase (AMG 300 L®), all produced by Novozymes Latin American Limited, Araucária, PR, Brazil.

Non-fiber carbohydrate

Was calculated by difference and obtained as described in Mayer *et al.* (2007). Analytical data were obtained in duplicate and their final values were calculated to dry basis.

Fatty acid

The extracted lipids were used for derivatization of triglycerides into fatty acid methyl esters (FAME) according to the method of Hartman and Lago (1973). Lipids were derivatizated using KOH (0.4M) and sulphuric acid (1M) methanolic solutions with heating by 10 min in a water bath at 100°C for each solution. The FAME were extracted with hexane and determined by gas chromatography Agilent Technologies 6890N series, equipped with a capillary column (Supelco SP2560, Sigma-Aldrich) (100 m x 0.25 mm id x 0.2 μm thick film) and a flame ionization detector (FID). The heating program was started with the column 170°C for 2 minutes, and gradual increase of 3°C per minute to a final temperature of 240°C

Table 1. Germination (%) and incidence of fungi (%) in maize seed variety Oito carreiras, Lombo baio and Cabo roxo submitted to different periods and storage conditions (C_1: paper bag, temperature of 10°C and C_2: plastic packing room temperature)

		Oito carreiras		Lombo baio		Cabo roxo	
Variable	Months	Condition 1	Condition 2	Condition 1	Condition 2	Condition 1	Condition 2
Germination	0	78 a A*	78 a A	91 ab A	91 a A	89 ab A*	89 a A
	3	81 a A	81 a A	93 a A	88 a B	91 a A	88 a A
	6	72 b A	71 b A	86 b A	87 a A	85 b A	75 b B
	9	62 c A	56 c B	72 c A	66 b B	-	-
Aspergillus spp.	0	13 b A	13 c A	1 b A	1 b A	0 b A	0 c A
	3	83 a A	88 b A	8 a A	5 b A	83 a A	88 a A
	6	78 a B	96 ab A	9 a A	4 b B	0 b B	17 b A
	9	82 a B	100 a A	1 b B	29 a A	-	-
Fusarium spp.	0	20 c A	20 ab A	75 a A	75 a A	77 a A	77 a A
	3	52 a A	26 a B	69 a A	60 b A	52 b A	26 b B
	6	35 b A	13 ab B	24 b A	26 c A	37 b A	40 b A
	9	22 bc A	6 c B	29 b A	14 c B	-	-
Penicillium spp.	0	95 a A	95 a A	97 a A	97 ab A	95 a A	95 a A
	3	95 a A	94 a A	93 a A	96 ab A	61 b B	94 a A
	6	97 a A	92 a A	87 b B	92 b A	98 a A	92 a A
	9	92 a A	41 b B	94 a B	100 a A	-	-

*Averages followed by the same lowercase letter in the column and same capital letter in the line do not present relevant difference at 5% of probability level by Tukey's test.

and remained so for 7 minutes. Nitrogen was used as the carrier at 0.9 $mL.min^{-1}$ gas. The injected sample volume (split mode) was 1µL. The temperature used for the detector (FID) was 280°C. The fatty acids were identified by comparison with retention times of reference standards (Supelco 37 FAME mix ref. 47885-U, Sigma, Bellefonte, USA). The retention times and areas were automatically computed by Agilent ChemStation software.

Statistical analysis

Completely randomized with treatments arranged in a 2 x 4 (condition x storage time) factorial design was used. Comparisons between treatment means were performed using Tukey test at 5% probability and when significant effect observed was performed regression analysis. The variables whose results were expressed as percentage had their data converted to sine.

Data from the germination tests were subjected to analysis of simple correlation (r) between the data healthiness and chemical composition, separately for each variety. Pearson correlation analysis between the data only healthiness and chemical composition was also performed. The significance of the r values were determined by t-test at 5%.

Results and Discussion

Evaluation of physiological and sanitary quality of seeds.

The germination of maize has been reduced significantly during the nine months, on both storage conditions and at the three varieties studied (Table 1). In Cabo roxo variety, there was significant difference between the two storage conditions (Table 1). Storage in Condition 1 showed the highest values of normal seedlings in relation to storage in Condition 2. In Oito carreiras variety there was no significant difference in the two storage conditions, although for the variety Lombo baio (Table 1) the storage condition 1 (paper bag, 10°C) showed the highest values of normal seedlings in relation to storage in the first condition 2 (plastic packing at room temperature).

According to Baudet (2012) a factor that should be taken into account in a significant decrease in

Table 2. Chemical composition of landrace maize seeds submitted to different periods (0 and 9 months) and storage conditions

Chemical composition	Storage	Condition 1	Condition 2
Dry matter	Initial	86 b*	86 b
	Final	88 a	87 a
Ash	Initial	1.45 b	1.45 b
	Final	2.44 a	2.03 a
Crude protein	Initial	10.13 a	10.13 a
	Final	9.75 b	9.37 b
Lipids	Initial	5.79 a	5.79 a
	Final	4.50 b	5.04 b
Saturated fatty acids	Initial	21.34 a	21.34 a
	Final	17.25 b	17.80 b
Monounsaturated fatty acids	Initial	32.43 b	32.43 b
	Final	33.56 a	33.69 a
Polyunsaturated fatty acids	Initial	42.56^{ns}	42.56^{ns}
	Final	45.96^{ns}	46.29^{ns}
Dietary fiber	Initial	18.57^{ns}	18.57^{ns}
	Final	17.54^{ns}	17.19^{ns}
Soluble fiber	Initial	1.35^{ns}	1.35^{ns}
	Final	1.19^{ns}	1.14^{ns}
Insoluble fiber	Initial	17.23^{ns}	17.23^{ns}
	Final	16.34^{ns}	16.04^{ns}
Non-fiber carbohydrates	Initial	64.05^{ns}	64.05^{ns}
	Final	65.63^{ns}	66.48^{ns}

* Averages followed by the same lowercase letter in the column do not present relevant difference at 5% of probability level by Tukey's test. ^{ns} = not significant

Table 3. Pearson correlation matrix between sanity and germination, between germination and chemical composition and between sanity and chemical composition in landrace maize seeds at the end of the storage period (9 months)

Varieties	Test	*Aspergillus* spp.	*Fusarium* spp.	*Penicillium* spp.
Oito carreiras	Germination	-0.998*	0.649	0.776
Cabo roxo	Germination	0.302	0.394	-0.336
Lombo baio	Germination	-0.721	0.999*	-0.189

* significant at 5%

germination of stored seeds is the natural decay which is an irreversible process. The physiological manifestations during the process of deterioration may cause a decline in germination rate; the decrease of potential conservation during storage; decreased resistance to the action of micro-organisms; reducing the percentage of germination (Marcos Filho, 2005; Carvalho and Nakagawa, 2012).

Besides, Martins and Lago (2008) emphasize that humidity and temperature have great influence on seed conservation by influencing the biochemical reactions that regulate metabolism involved in the process, factors that are determined by packaging and storage condition Thus, the types of package is subject to the influence of variations in external environmental conditions, leading to loss of quality of stored seeds.

Among the fungi detected in sanity test (Table 1) there was a high incidence of *Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp. These results are similar to those observed by Noal (2013) which found a higher incidence of these fungal genera in seven landraces of maize from the city of Ibarama (RS, Brazil), though different from surveyed in this work. According to Lucca Filho (2012), the damage caused by micro-organisms transmitted by seeds are quite variable, being dependent on the pathogen involved, the cultivated species, climate conditions during the development of culture, among others For this author, the main fungi involved in the loss of stored products belonging to various species of the genus *Aspergillus* and some *Penicillium* species and have their activity regulated by environmental conditions during storage and the conditions of the seed lot, especially their status physical, water content and initial inoculum.

Evaluation of the chemical composition of maize seeds

Through data analysis of chemical composition variables in maize seeds, we observed no significant difference in the two storage conditions (Table 2). At the end of the storage period there was an increase in the percentage of ash and monounsaturated fatty acids and a decrease in protein content, fat and saturated fatty acids, regardless of storage conditions used. On the other hand, no significant difference in the percentage of polyunsaturated fatty acids, fiber (total, soluble and insoluble) and carbohydrates (Table 2).

According to the authors Carvalho and Nakagawa (2012) and Nedel (2012) quantitative chemical composition of the seed is genetically defined and can be influenced by conditions to which the plants were originated. Therefore variations depending on the species, the variety of flowering physiology, nutrition and environmental conditions are observed The chemical composition of stored seeds changes in intensity and variable speed as the deterioration progresses This process involves, for example, changes in reserve substances, respiratory rate, synthesis and activity of enzymes, all with direct influence on the chemical composition of the seeds

Table 4. Pearson correlation matrix between germination and chemical composition in landrace maize seeds at the end of the storage period (9 months)

Varieties	Test	ASH	PROT	LIP	DF	SF	IF	SFA	MFA	PFA	NFC
Oito carreiras	G	-0.981*	0.998*	0.988*	0.995*	0.999*	0.995*	0.815	-0.806	-0.677	-0.998*
Cabo roxo	G	-0.975*	0.847	0.850	0.999*	0.993*	0.999*	0.975*	-0.851	-0.981*	-0.894
Lombo baio	G	-0.999*	-0.905	0.999*	0.993*	0.996*	0.991*	0.972*	-0.987*	-0.973*	-0.999*

G: Germination; ASH: Ash; PROT: Protein; LIP: Lipids; DF: Dietary fiber; SF: Soluble fiber; IF: Insoluble fiber; SFA: saturated fatty acids; MFA: Monounsaturated fatty acids; PFA: Polyunsaturated fatty acids; NFC: Non-fiber carbohydrates. * significant at 5%

(Marcos Filho, 2005).

Relationship between germination, healthiness and chemical composition

Through correlation between Person germination and fungi of the genera *Aspergillus, Fusarium* and *Penicillium* (Table 3) matrix it was observed that there was a significant and negative correlation between germination and the fungus *Aspergillus* in Oito carreiras variety. Thus, the high incidence of *Aspergillus* spp. probably has caused the decrease in the percentage of normal at the end of the storage period (Table 1) seedlings. In the variety Cabo roxo, after six months of storage, there is no significant correlation between germination and fungi present (Table 3). However, the variety Lombo baio, there was a decrease in the percentage of normal seedlings and incidence of *Fusarium* fungi at the end of storage period, verified by positive and significant correlation between the two variables (seed germination and *Fusarium* spp.). On the other hand, there was an increased incidence of *Aspergillus* spp. while the percentage of normal seedlings has decreased. However, this correlation was not significant.

The results of this study are consistent with the observations of Carvalho and Nakagawa (2012) which stated that the considered storage fungi such as *Aspergillus* spp. and *Penicillium* spp. are present in freshly harvested seeds, usually at very low percentages and are able to survive in an environment with low humidity, proliferating in succession wit fungi field, causing the deterioration of seeds. Furthermore, these microorganisms attack the seed interacting on the metabolic processes accelerating the deterioration and eventually may produce toxins that damage membranes and inhibit the germination of seeds (Freitas, 2009; Baudet, 2012).

Regarding the chemical composition and germination, it is observed that at the three varieties of landraces maize was a significant and negative correlation between germination and the ash content (Table 4). Thus, it is concluded that after storage period (9 months) as it decreases the percentage of normal seedlings, there is increased consumption of organic matter reflected in increased ash content and therefore reducing the seed quality.

As for protein, it was observed that, in the variety Oito carreiras there was a significant positive correlation between the protein content and germination rate (Table 4). Thus, at the end of the storage period, protein content decreased as well as the percentage of normal seedlings. According to Marcos Filho (2005) during the process of seeds deterioration changes in proteins such as a decrease in the content and protein synthesis occur; increase in the content of amino acids; decrease in the content of soluble proteins; denaturation of proteins caused by high temperatures, causing the loss of ability to perform its functions.

In this work, it was also possible to verify that there was a significant positive correlation between the percentage of normal seedlings and the lipid content in the varieties Oito carreiras and Lombo baio. Thus, reduction in lipid content decreased as the percentage of normal seedlings (Table 4). The results of this study are similar to those obtained by Gutkoski

Table 5. Pearson correlation matrix between sanity and chemical composition in landrace maize seeds at the end of the storage period (9 months)

Varieties	Fungi	ASH	PROT	LIP	DF	SF	IF	SFA	MFA	PFA	NFC
Oito carreiras	Asp.	0.839	-0.999*	-0.977*	0.540	0.983*	-0.630	-0.796	0.839	0.666	0.671
	Fus.	-0.074	0.607	0.758	0.343	-0.450	0.999*	-0.0004	-0.074	-0.996*	-0.996*
	Pen.	-0.253	0.740	0.863	0.168	-0.603	0.988*	0.179	-0.252	-0.994*	-0.995*
Lombo baio	Asp.	0.433	-0.947	-0.734	0.272	-0.604	0.529	-0.526	0.822	0.558	0.957*
	Fus.	-0.934	0.907	0.999*	-0.859	0.986*	-0.968*	0.967*	-0.988*	-0.976*	-0.893
	Pen.	-0.166	-0.588	-0.207	-0.333	-0.033	-0.056	0.060	0.342	-0.023	0.614

Asp.: *Aspergillus* spp.; Fus.: *Fusarium* spp.; Pen.: *Penicillium* spp.; ASH: Ash; PROT: Protein; LIP: Lipids; DF: Dietary fiber; SF: Soluble fiber; IF: Insoluble fiber; SFA: saturated fatty acids; MFA: Monounsaturated fatty acids; PFA: Polyunsaturated fatty acids; NFC: Non-fiber carbohydrates. * significativo a 5%.

et al. (2009), where there was a reduction in lipid content during the storage of dried corn seeds and stored in granary with forced natural air. Likewise, Abreu *et al.* (2013), working with sunflower seeds, concluded that oil content in the seeds declined over time regardless of storage condition

Regarding to fatty acids some facts were observed: a significant and positive correlation between the percentage of normal seedlings and the content of saturated fatty acids (except for Oito carreiras variety); negative and significant correlation between the percentage of monounsaturated fatty acids and seed germination rate in Lombo baio variety and a significant and negative correlation between the content of polyunsaturated fatty acids and the percentage of normal seedlings (except for Oito carreiras variety) (Table 4). Thus, after a period of storage has decreased as the percentage of normal seedlings was higher content of monounsaturated fatty acids and polyunsaturated, depending on the maize variety studied. These results are in agreement with Baudet (2012), who stated that during seed deterioration, changes in chemical composition, for example, oxidation of lipids and the increase in fatty acids are observed.

Regarding the fiber content (Table 4), in the landraces of maize seeds is possible to say that at the end of the storage period, as it decreased the percentage of normal seedlings (observed by the germination test) decreased the fiber content total, soluble and insoluble. Performed papers by Martinez *et al.* (2011) who analyzed the chemical changes in soybean seeds with germination, have found that the levels of these fibers increased after seed germination. Ghavidel and Prakash (2007) demonstrated that after germination, total and soluble dietary fiber fractions in legume seeds increased and the insoluble fraction was significantly reduced.

As for carbohydrates, there was a negative correlation between carbohydrate content and germination at the end of the storage period, with significant values at the variety Cabo Roxo (Table 4). According to Taiz and Zeiger (2013) during the germination process occurs the starch breakdown into soluble sugars by the activity of α-amylase and maltase enzyme. These sugars are consumed in the respiration process of the seed causing a reduction in the total carbohydrate and dry matter loss. Furthermore, carbohydrates seeds are directly consumed by their own metabolism and associated micro-organisms, so there is a decrease of its contents during storage periods. Changes in the levels of soluble carbohydrates lead to restrictions on the availability of substrates for respiration, causing a drop of germination and seed vigor (Marcos Filho, 2005).

Regarding healthiness and chemical composition of maize seeds there was a significant and negative correlation between the fungus *Aspergillus* spp. and the content of proteins and lipids in the variety Oito carreiras (Table 5). Thus, at the end of the storage period, there was consumption of proteins and lipids by the fungus *Aspergillus* spp. and, consequently, a decrease in germination percentage of these seeds

(Table 1). The incidence of *Fusarium* spp. correlated negatively and significantly with the content of polyunsaturated fatty acids and carbohydrates (Oito carreiras variety); soluble fiber, fatty acids mono and polyunsaturated (Lombo baio variety). Moreover, the incidence of *Penicillium* spp. correlated negatively and significantly with the content of polyunsaturated fatty acids and carbohydrates (Oito carreiras variety) (Table 5).

Among the chemical compounds present in the seed, lipids are more susceptible to decay, either by reducing their total content and/or the susceptibility to structural changes (Elias, 2002). Thus the seeds with higher fat content will have greater predisposition to the deteriorating process, mainly those with higher content of unsaturated fatty acids (Freitas, 2009).

According to Marcos Filho (2005), during the seeds deteriorate a decrease in sugar content can occur causing loss of ability to use carbohydrates and affecting their mobilization of tissue reserves for the embryo. These changes in carbohydrate levels lead to restrictions on the availability of substrates for respiration, causing a drop of germination and seed vigor. Moreover, among the major damage caused by fungi in seeds are the changes in chemical composition, odor, flavor, color; heating and production of toxins; loss of organic matter and decreased germination rate (Elias, 2002).

In the present study, the results show that the seed quality is affected by changes in the chemical composition during storage. Fungi incidence is related to the chemical composition of the seeds is changed during the storage period.

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