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Influence of the addition of organic acids in the fungal contamination and in the conservation of grains of sorghum hermetic stored

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Abstract

In Brazil sorghum is produced mainly in small and middle size properties, which seldom have appropriated technologies for drying and storage. One of the difficulties faced in the storage of grains without an efficient previous drying it is in the microbial contamination of the grains and its consequences in the conservability. Aiming to study these aspects, sorghum grains, produced in the area of Pelotas, RS, harvested with about 20 % of moisture, they were stored in the Laboratory of Post-harvest, Industrialization and Quality of Grains, without drying for 12 months in metallic bin, of 120 kg, covered internally with varnish, constituting of the following treatments: T1) with initial aerobic conditions and without incorporation of acids; T2) with modified initial aerobic conditions and without incorporation of acids; T3) with initial aerobic conditions and with incorporation of 1 % of the mixture of acetic and propionic acids; T4) with initial modified aerobic condition and with incorporation of 1 % of the mixture of acetic and propionic acids. Since the moment of the installation of the experiment up to twelve months of storage, the moisture, basic chemical composition (carbohydrate, crude protein, ether extract and acidity of the ether extract and ashes), dry weight, germination and microbial contamination of the grains was evaluated at every four months. The results indicate that: the initial reduction in the rate of oxygen and the incorporation of 1 % of the mixture of acetic and propionic acid, in the proportion 1:1, in hermetic system, improve the conservability of grains; *Aspergillus* and the bacterial are favored by these conditions, while the *Fusarium* and *Penicillium* are controlled efficiently; for the storage of seeds the addition of acids in hermetic form is not recommended due to the loss of the germination of these seeds.

Key words: sorghum, storage, acetic acid, propionic acid.

Introduction

Sorghum is the fifth largest cereal production after rice, corn, wheat and barley and it represents an important source of energy and protein for human consumption and animal feed, as well as of raw material for industry. USA, Nigeria, India and Mexico are the largest producers in the world (FAO, 1998). In Brazil, in the crop of 2004/2005, 1,520,539 ton of sorghum grains were produced, with a averageproductivity of 1,929 kg/ha, and the largest producing state is Goiás and Rio

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Grande do Sul produces about 70 % of the sorghum grains produced in the south area of the country (IBGE, 2002).

The amount of losses varies among countries, geographical areas and different crops, depending on the regional climatic conditions, the amount of grains harvested and of the level the post harvest technology available. In underdeveloped countries of Asia, Africa and Latin America, the post harvest losses are approximately 30 % of the annual crop (Schneider, 1991).

For this reason, storage alternatives exist to minimize the qualitative and quantitative losses of grains, preferably without the use of pesticides, one of these techniques is the hermetic storage of grains, that is of easy use and low cost. The hermetic storage has been used for many years in the preservation of grains (De Lima, 1990; Quezada et al., 2006). The basic principle of these systems is the complete elimination of the oxygen, with an increase in CO_2, in the storage atmosphere, which is developed by the breathing of insects, fungi and grains (Varnava et al., 1995; Brunet et al., 2000). The insects die when the oxygen of the storage atmosphere is reduced to 3 % or less and the fungal interrupts its development when the level of oxygen is reduced to 1 %.

Aspergillus and *Penicillium* are the largest causes of deterioration losses of grains in storage (Dejene et al., 2004). The consumption of nutritious reserves, color changes, alterations in the structure of carbohydrates, lipids, proteins and vitamins, the production of toxins, the reduction of the germination, the development of unpleasant scents, heating and alteration of the physical characteristics of grains are the main damages caused by the attack of microorganisms in stored grains (Dejene et al., 2004; Silva et al., 2004).According to Elias (2002), the substitution of drying by the use of acids in storage, as method of conservation of grains, even for not very long periods, it represents an efficient alternative, especially for small and medium producers, that do not have adequate drying and storage facilities. On the other hand, the delay of the drying, in cooperatives, industries, medium and big producers, makes it possible to rationalize the use of the facilities, without increasing the losses or even reducing them, and diminishing the idleness of the drying structures and transports, According to Baird-Parker (1980), organic acids are used as conservants, in grains, essentially due to its antimicrobial properties.

This way, the present work sought to study the conservability of grains of sorghum stored in hermetic system with harvest humidity, without the need of drying, without and with incorporation of 1 % of the mixture of acetic and propionic acids, in the proportion 1:1, weight the weight, with and without alteration of the initial aerobic conditions, with special interest in the characterization microbiological and evaluation of the quality in the different treatments.

Materials and methods

Materials - Sorghum grains (*Sorghum bicolor* L. Moench), produced in Pelotas, RS - Brazil.

Organic acids - Acetic and propionic acid p.a.

Storage unit - Metallic bin of 0,85m of diameter were used, covered internally by a varnish film.

Methods

Harvesting and Pre-cleaning - The grains were harvested mechanically and submitted to the operation of pre-cleaning, in an air flow and sifting machine.

Experiment - Sorghum grains with moisture content of approximately 20 %, as harvested, without drying, for 360 days were stored in hermetic bin.

Treatments (T): T1 – with initial aerobic conditions and without incorporation of acids; T2 - with modified initial aerobic conditions and without incorporation of acids; T3 - with initial aerobic conditions and with incorporation of 1 % of the mixture of acetic and propionic acids; T4 - with initial modified aerobic condition and with incorporation of 1 % of the mixture of acetic and propionic acids.

The organic acids were incorporate to the mass of grains, mixed in the proportion 1:1 weight to weight (w/w). After the incorporation the grains were stored in bin, with 50 kg of grains in each.

The experiment was led in a hermetic bin under an initial aerobic condition and an altered condition. In the installation of the experiments there is a natural initial atmosphere inside the unit of storage, the alteration of the initial aerobic condition was obtained by the combustion of cotton soaked in alcohol, in 1/5 of the head space volume of the storing unit not occupied by grains.

The conservability of the stored grains was evaluated at 4, 8 and 12 months, and at the moment of the installation of each experiment (time zero). The study of the grains conservability was accomplished with the evaluation of the following physico-chemical and chemical analyses: protein (AACC, 2000.); ash (AOAC, 1990); moisture according to the Rules for Analysis of Seeds official methodology of seeds of Brazilian Ministry of the Agriculture (Brazil, 1992); total lipids and free fat acid (AOCS, 1996) and carbohydrate by difference; dry weight – weight of 280 cm^3 of dried grains

Microbiological analysis and germination of the grains was led according to the official methodology of seeds of Ministry of the Agriculture (Brazil, 1992).

The statistical analysis was accomplished with the use of the program SANEST (Zonta and Machado, 1982). The experiments were analyzed individually, through the factorial outline 2 x 2 x 4 (acid x aerobic conditions x time of storage).

Results and discussion

During the hermetic storage of the grains, the temperature varied between 10.2 and 23.6 ºC, characterized as the annual climatic periods of winter, spring, summer and autumn, with the average temperatures of 13.3; 19.2; 23.0 and 14.1 ºC, respectively.

Table 1 presents the grains moisture. Variations in losses and increments did not show significant differences, results similar to the found by Tomé et al. (2000) in bean, Garcia et al. (2000) in corn, Rupollo et al. (2004) in oat, Daglish and Wallbank (2005) in the sorghum storage.

The maximum reduction of the $O_2.CO_2^{-1}$ ratio in the atmosphere of the intersticial space is an important conservability factor, considering the gradual reduction of the intrinsic metabolism and reduction of infestation and of associated aerobic microflora (Varnava et al., 1995).

The Table 2, 3 and 4 show the conservability of the organic compounds of the stored grains, through the analysis of the carbohydrates, protein and ether extract contents.

For specific variations of each organic fraction, the smallest losses in protein, lipids and apparent increments in carbohydrates correspond to the best conservation effects in the stored grains.

According to the Table 2 and 3 the organic composition was characterized with apparent increments, in carbohydrates, and losses in protein and lipids not varying significantly among the treatments.

Carbohydrate variation was inferior to 1.42 %, being the smallest increases verified in the stored grains with addition of organic acids. This variation in apparent increments of the carbohydrate fraction is reflect of the intrinsic metabolism of the grains, of microorganisms and of the associated pest, besides that they are susceptible to enzymatic and not enzymatic chemical transformations during the storage.

Table 2 shows that the losses of protein content, at 12 months, were smaller in the samples stored with incorporation of the mixture of acids, with average values of 4.72 and 4.52 % respectively for storage in normal and altered aerobic conditions and, that in the grains stored without incorporation of acids presented average values of 9.44 and 8.65 % respectively, for the same storage conditions.

In the same way, it was verified that for the

Table 1. Effect of the alteration of initial aerobic condition and of the incorporation of organic acids on the moisture content (%) of sorghum grains harvest with 20 % moisture, stored in hermetic system, without drying, during one year[1].

Experiment/ Treatment[2]	Storage (months) 0	4	8	12
NA-0	20.39 Aa	20.45 Aa	20.55 Aa	20.52 Aa
MA-0	20.39 Aa	20.48 Aa	20.57 Aa	20.45 Aa
NA-1	20.39 Aa	20.38 Aa	20.41 Aa	20.44 Aa
MA-1	20.39 Aa	20.37 Aa	20.42 Aa	20.39 Aa

[1] The values (%) represent the simple arithmetic average of 3 repetitions, in wet basis (w.b.). Different capital letters, in the line, and different small letters, in the column, differ significantly from each other, to 1 % of significance, for Duncan Test.

[2] Experiment/Treatment - NA - initial normal aerobic conditions. - MA – modified initial aerobic condition. - 0 - without incorporation of organic acids - 1 - with incorporation of 1 %, (w/w), of the mixture of acetic and propionic acids in the proportion of 1:1.

Table 2. Effect of the alteration initial aerobic condition and of the incorporation of organic acids on the content (%) of rude protein of sorghum grains harvest with 20 % moisture, stored in hermetic system, without drying, during one year[1].

Experiment/ Treatment[2]	Storage (months) 0	4	8	12
NA-0	10.17 Aa	9.61 Ba	9.32 Ca	9.21 Da
MA-0	10.17 Aa	9.67 Ba	9.40 Ca	9.29 Da
NA-1	10.17 Aa	9.94 Ba	9.78 Ca	9.69 Da
MA-1	10.17 Aa	9.96 Ba	9.79 Ca	9.71 Da

[1] The values (%) represent the simple arithmetic average of 3 repetitions, in wet basis (w.b.). Different capital letters, in the line, and different small letters, in the column, differ significantly from each other, to 1 % of significance, for Duncan Test.

[2] Experiment/Treatment - NA - initial normal aerobic conditions. - MA – modified initial aerobic condition. - 0 - without incorporation of organic acids - 1 - with incorporation of 1 %, (w/w), of the mixture of acetic and propionic acids in the proportion of 1:1.

Table 3. Effects of the alteration initial aerobic and of the incorporation of organic acids on the content (%) ether extract of grains of sorghum, harvested with 20 % moisture, stored in hermetic system, without drying, during one year[1].

Experiment/ Treatment[2]	Storage (months) 0	4	8	12
NA-0	3.76 Aa	3.46 Ba	3.28 Ca	3.14 Ca
MA-0	3.76 Aa	3.49 Ba	3.28 Ca	3.20 Ca
NA-1	3.76 Aa	3.58 Ba	3.46 Ca	3.49 Ca
MA-1	3.76 Aa	3.60 Ba	3.48 Ca	3.42 Ca

[1] The values (%) represent the simple arithmetic average of 6 repetitions, in dry basis (d..b.). Different capital letters, in the line, and different small letters, in the column, differ significantly from each other, to 1 % of significance, for Duncan Test.

[2] Experiment/Treatment - NA - initial normal aerobic conditions. - MA – modified initial aerobic condition. - 0 - without incorporation of organic acids - 1 - with incorporation of 1 %, (w/w), of the mixture of acetic and propionic acids in the proportion of 1:1.

Table 4. Effects of the alteration initial aerobic and of the incorporation of organic acids in content (%) of ashes of grains of sorghum, harvested with 20% moisture, stored in hermetic system, without drying during one year[1].

Experiment/	Storage (months)			
Treatment[2]	0	4	8	12
NA-0	1.53 Ca	1.71 Ba	1.81 Aa	1.86 Aa
MA-0	1.53 Ca	1.70 Ba	1.80 Aa	1.85 Aa
NA-1	1.53 Ca	1.62 Ba	1.68 ABa	1.71 Aa
MA-1	1.53 Ca	1.61 Ba	1.67 ABa	1.70 Aa

[1] The values (%) represent the simple arithmetic average of 3 repetitions, in wet basis (w.b.). Different capital letters, in the line, and different small letters, in the column, differ significantly from each other, to 1 % of significance, for Duncan Test.
[2] Experiment/Treatment - NA - normal initial aerobic. - MA - initial modified aerobic condition. - 0 - without incorporation of organic acids - 1 - with incorporation of 1 %, (w/w), of the mixture of acetic and propionic acids in the proportion 1:1.

Table 5. Effects of the alteration of initial aerobic condition and of the incorporation of organic acids on the content (%) of free fat acids sorghum grains, harvested with 20 % moisture, stored in hermetic system, without drying, during one year[1].

Experiment/	Storage (months)			
Treatment[2]	0	4	8	12
NA – 0	3.83 Da	14.27 Ca	25.73 Ba	28.69 Aa
MA - 0	3.83 Da	11.30 Ca	22.33 Ba	26.53 Aa
NA – 1	3.83 Da	4.80 Cb	15.51 Bb	19.16 Ab
MA - 1	3.83 Da	4.28 Cb	13.55 Bb	17.21 Ab

[1] The values represent (%) simple arithmetic average of 6 repetitions, in acid oleic. Different capital letters, in the line, and different small letters, in the column, differ significantly from each other, to 1 % of significance, for Duncan Test.
[2] Experiment/Treatment - NA- normal initial aerobic. – MA- initial modified aerobic condition. - 0 - without incorporation of organic acids - 1 - with incorporation of 1 %, (w/w), of the mixture of acetic and propionic acids in the proportion 1:1.

Table 6. Germination of sorghum grains, stored in hermetic system, harvested at different moisture and dried, with the alteration of the initial aerobic condition and incorporation of organic acids[1].

Experiment/	Storage (months)			
Treatment[2]	0	4	8	12
NA – 0	60	25	0	0
MA - 0	60	20	0	0
NA – 1	60	0	0	0
MA - 1	60	0	0	0

[1] The values represent percentage of grains germinated in the conditions of the setting of the Blotter Test (Brasil, 1992).
[2] Experiment/Treatment – NA – normal initial aerobic. - MA - initial modified aerobic condition. - 0 - without incorporation of organic acids - 1 - with incorporation of 1 %, (w/w), of the mixture of acetic and propionic acids in the proportion 1:1.

protein (Table 2) the smallest losses were observed when the mixture of acids was used during the storage, with average values of 7.18 and 9.04 % respectively for storage in normal aerobic and altered conditions, when compared with storage without addition of the mixture of acids that had average losses of 16.49 and 14.89 % respectively for normal aerobic and altered conditions. The largest losses (%) during storage happened in the lipid fraction. According to Salunkhe et al. (1985), the lipids are concentrated mostly in the germ and are more susceptible to chemical degradation, to the attack of pest and to microbial contamination during storage. For the three appraised characteristics, it was observed smaller protein losses and lipids, and smaller carbohydrates increments at 12 months of storage with the application of the mixture of acids, showing good efficiency in the conservability of these grains. When altered aerobic condition was used, the conservation effect was superior in relation to the normal aerobic condition.

The intrinsic properties of the organic acids applied increase the permeability of the cellular membranes, with a relative increment of intracellular carbonic anhydride, and they also increase the conservation of the grains when stored in hermetic conditions.

The Tables 4 and 5 show, respectively, the results of the analyses of the mineral composition with the evaluation of ashes and acidity of lipid fraction, expressed in oleic acid.

The mineral composition of the stored grains (Table 4) as the organic compounds did not show significant differences among the different treatments for similar periods of storage. Grains stored for 12 months, with the incorporation of the mixture of acids, presented the best conservability with apparent increments of 11.8 and 11.1 % of ashes, respectively, for normal aerobic and altered conditions. In the same period, grains stored, without the incorporation of acids, presented similar apparent increments of ashes, showing average indexes of 20.9 and 21.6 % respectively for normal aerobic and altered conditions.

The apparent increments of mineral composition observed during storage reflect the intensity of the degradation of the organic compounds. The metabolic activity of the grains and of the associated microorganisms consume the organic matter, producing CO_2, water, heat and other products, without altering the total mineral content. In that way, the determination of ashes assumes larger values proportionally to the organic matter consumed (Dejene et al., 2004; Dejene et al., In Press).

The acidity of the lipids, presented in the Table 4, showed significant variations, influenced by the different treatments and storage periods studied. The incorporation of acids to the stored grains for 12 months presented a average efficiency of 40 %, in relation to the same condition, without the incorporation of acids, showing increments corresponding to 349.4 – 400.3 % and 592.7 – 649.1 %, respectively, in the altered and initial normal aerobic conditions. The increase of the acidity of the lipids, besides evidencing the intensity of the deteriorative processes of the grains, resulted in undesirable nutritional, sensorial and technological characteristics. Similar behaviors have been observed in other works (Rombaldi, 1988; Rupollo et al., 2004). The intensity of variation of lipid acidity is correlated with the degradation, being constituting important parameter of evaluation of the conservation of stored grains (Puzzi, 2000).

The intensity and the interference of reduction of the O_2/CO_2 ratio, in the maintenance of physiologic integrity of the grains stored under hermetic conditions with high moisture content, may be observed in Table 6, through the analysis of the loss of your germinative potential.

Stored grains for 4 months, without the use of a mixture of acids, showed a reduction of initial germinative potential from 60 % to 20-25 %, loosing it completely before 8 months of hermetic storage. The incorporation of the mixture of acids intensified these effects, reducing the germinative capacity of the grains before 4 months of storage, characterizing an inadequacy of those conditions for the maintenance of the physiologic quality of grains.

In the same proportion and intensity the grains stored with high moisture content in hermetic condition showed that its germinabililty is reduced. These effects are evidenced by the maintenance of organic constituents due to the destruction of the enzymatic processes.

The reduction of the pH, the destruction of tissues and the acidification of the cellular content are factors of inactivation of the enzymatic systems. This phenomenon characterizes the biostatic and biocide action of the organic acids on associated microorganisms and the loss of the germinative power in the stored grains (Baird-Parker, 1980). According to Silva et al. (2005), the acid penetrates in the grains and kills the embryo, reducing the breathing and other metabolic activities.

The microbiological analysis, accomplished through the Blotter Test methodology (Brazil, 1992), show following contaminants: *Alternaria*, *Aspergillus*, *Curvalaria*, *Fusarium* and *Phoma* represented the principal fungal contaminant. Other microorganisms with smaller counts were also dectected as *Colletotrichum*, *Helminthosporium*, *Phomopsis* and *Penicillium*.

The gender *Aspergillus* sp. presented the largest potentials of contamination in the hermetic conditions studied, favored by the high moisture content of the grains. According to other authors the species *Aspergillus* and *Penicillium* are the largest causes of the losses by deterioration of the grains in the storage (Dejene et al., 2004). The selective biological characteristics of *Aspergillus* sp. to grow in hermetic conditions of storage of sorghum grains, with the incorporation of the mixture of acids, besides the influence of the temperature it is due to its intrinsic capacity of metabolize the acids used as conservants.

The incorporation of the mixture of acetic and propionic acid, in the proportion 1:1, associated with the alteration of the initial aerobic condition, characterized by their efficiency in controlling the contamination by fungi in the hermetic conditions studied.

The antimicrobial efficiency of the hermetic conditions, in the storage of grains with high moisture content, resulted from the spontaneous and artificial alteration of the intergranular atmosphere, with the development high contents of CO_2 (Baird-Parker, 1980; Pelhate, 1980; Elias, 2002; Rupollo et al., 2004; Silva et al., 2005).

Conclusions

The results obtained in this work allowed the following conclusions:

a) The incorporation of 1 % of acetic and propionic acids mixture, in the proportion 1:1 (w/w), and the alteration of the initial aerobic conditions of storage improves the efficiency of the hermetic systems to conserve grains with harvesting moisture content of approximately 20 %;

b) *Aspergillus* sp. and bacteria are favored for the conditions of hermetic storage, with addition of acids and alteration of the initial aerobic conditions, while fungi , *Fusarium* sp. and *Penicillium* sp. are controlled efficiently;

c) Storage of seeds in this storage system is not recommended with addition of acids in the hermetic form because of the loss of the germination of these seeds.

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