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Pathogens incidence and quality of extracted crude oil from soybean seeds stored under different conditions

Incidência de patógenos e qualidade do óleo bruto extraído de sementes de soja armazenadas em diferentes condições

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Abstract

Objective of the present work to investigate the influence of different temperature conditions in the environment and moisture content in the seeds over six months of storage on the incidence of fungi in soybean seeds, as well as the quality of the crude oil extracted from the seeds. Soybean seeds were used with initial moisture contents of 12.0; 13.0; and 14.0% (w.b.) which were placed in plastic bags of polypropylene, over a period of 180 days in two different environments: lab environment (uncontrolled conditions: 27 ± 0.6 °C), air-conditioned environment (20 ± 1.2 °C). The health of the seeds was evaluated by identifying and counting the percentage of infected kernels. For assessing the quality of crude oil extracted from the seeds were carried out oil content, acidity index and peroxide value. The experiment was a triple factorial 2 x 3 x 4, completely randomized design with three replications. Soybean seeds, disinfected and not disinfected were analyzed separately. Data were analyzed using analysis of variance and regression, the means were compared using Tukey's test at 5% significance. The initial moisture content of 14.0% (w.b.) provides greater fungal incidence rate at the end of storage in soybean seeds not disinfected. The atmospheric temperature during storage interferes with the quality of the crude oil extracted from the soybean seeds. The temperature of 27 °C causes more increase in acid number soybean oil during storage.

Additional keywords: health; *Glycine max* (L.); oil content.

Resumo

Objetivou-se no presente trabalho verificar a influência de diferentes condições de temperatura no ambiente e os teores de água nas sementes, ao longo de seis meses de armazenamento sobre a incidência de fungos em sementes de soja, bem como na qualidade do óleo bruto extraído das sementes. Foram utilizadas sementes de soja com teores de água iniciais de 12,0; 13,0; e 14,0% (b.u.), as quais foram acondicionadas em sacos plásticos de polipropileno, durante o período de 180 dias, em dois ambientes distintos: ambiente de laboratório (condições não controladas: 27 ± 0,6 °C) e ambiente climatizado (20 ± 1,2 °C). A sanidade das sementes foi avaliada por meio da identificação e da contagem da porcentagem de grãos infectados. Para a avaliação da qualidade do óleo bruto extraído das sementes, foram realizadas as análises do teor de óleo, índice de acidez e índice de peróxido. O experimento foi montado em esquema fatorial triplo 2 x 3 X 4, em delineamento inteiramente ao acaso, com três repetições. As sementes de soja, desinfestadas e não desinfestadas, foram analisadas separadamente. Os dados foram analisados por meio de análise de variância e regressão, e as médias foram comparadas utilizando o teste de Tukey, a 5% de significância. O teor de água inicial de 14% (b.u.) proporciona maior índice de incidência fúngica ao final do armazenamento nas sementes de soja não desinfestadas. A temperatura do ambiente, ao longo do armazenamento, interfere na qualidade do óleo bruto extraído das sementes de soja. A temperatura de 27 ºC ocasiona maior incremento no índice de acidez do óleo de soja ao longo do armazenamento.

Palavras-chave adicionais: *Glycine max* (L.); sanidade; teor de óleo.

Introduction

Soy is a major source of protein and vegetable oil in the world. In Brazil it is predominantly used for processing grain in oil and protein, having about 20% of lipid content, which are susceptible to the qualitative deterioration process, as degradation of these substances, when improperly stored, causing serious prejudices to the food industry.

According to Faroni et al. (2009), oil quality is influenced by the quality of the crude material used in the extraction. Several factors may influence in obtaining oil of good quality, such as the characteristics of grains, climate conditions during the grain development and its storage. One of the principal forms of oil deterioration is the oxidation that occurs when atmospheric oxygen is dissolved in oil and reacts with its constituents (unsaturated fatty acids), the higher degree of unsaturation more reactive with oxygen will be. This oxidation is responsible for the appearance of some strange flavors and odors in foods, making their sensory characteristics objectionable by consumers and besides damaging the nutritional quality and possibly producing toxic substances. The peroxide and acidity values are among the methods for assessing the oxidation levels of oils and fats (Araújo, 2008).

Oxidation is a process accelerated by high temperature, being responsible for the modification of oil physicochemical and sensorial characteristics. Oil becomes viscous, dark, increased its acidity and develops unpleasant odor, commonly named rancidity (Costa Neto & Rossi, 2000).

According to Lacerda Filho et al. (2008), the maximum moisture content in soybean seeds, suitable for storage between 6 months and 1 year, in natural environment, is 12% (w.b.), however, in Brazil, by normative forces of standardization and commercial classification; soybean storage can be performed with moisture contents of up to 14% (w.b.). The storage quality greatly influences in the quality of seeds and though the deterioration process is inevitable and irreversible, it is possible, mitigate it or delay it through the proper and efficient management of environmental conditions (Baudet, 2003).

Temperature conditions can also influence in the quality of seeds during storage. Not only temperature, as well water availability, nutritional status, insects or microbial attack, air pollutants and light conditions, are factors that influence the longevity both seeds and microorganisms (Caldwell, 2005).

Soy can be attacked by various pathogens microorganisms that cause diseases such as fungi, bacteria, nematodes and viruses. Among them, fungi are especially noted due to the largest number and to prejudice that can occur in both yield and quality of these seeds. Furthermore, soybean seeds are an important transmission vehicle (Henning, 2004). The moisture content of seeds at harvest and the mass temperature during storage can determine the intensity of these injuries (Chen, 2000).

The health of seeds is extremely important because approximately 90% of crops used for food, both human and animal, are propagated by seeds (Henning, 2005) and their inoculum that are present may result in increased disease in field and its introduction in pathogen free areas (Brand et al., 2009).

Thus, in view of the above, the aim of this study was to investigate the influence of different conditions of temperature and moisture contents along six months periods of storage at fungal incidence in soybean seeds, and the quality of the extracted crude oil as well.

Material and methods

The study was conducted in the Laboratory of Postharvest of Plant Products and Phytopathology at the Instituto Federal de Educação, Ciência e Tecnologia Goiano (IF Goiano – Campus Rio Verde), located in the municipality of Rio Verde, GO, Brazil.

Soybean seeds were used, CD 242 RR cultivar, acquired with 11% moisture content (w.b.). In order to obtain the initial moisture contents of 12.0; 13.0; and 14.0% (w.b.), seeds were pre-wetted in B.O.D chamber maintained at 20 °C and 85% relative humidity. For following the mass gain, seeds were weighed in balance with a resolution of 0.01 g until reach the desired moisture contents. After re-wetting, samples were homogenized and packed in polypropylene plastic bags with 1.0 kg capacity during six months period. The packages were kept in two distinct environments: lab environment, uncontrolled conditions, with average temperature of 27 ± 0.6 °C in the period and in air-conditioned environment with 20 ± 1.2 °C temperature. During the storage period, the temperature of the environments was monitored using a digital recorder.

Polypropylene plastic packages were subjected to permeability tests, using the Permatran-W Model 1/ 50G, MN-USA, three packages with two replications were used. The mean value was 7.499 (g m^{-2} day^{-1}) at 38 °C, which is considered semipermeable (Baudet, 2003).

Throughout storage seed samples were taken every 60 days (0, 60, 120 and 180 days). For evaluating the quality of the oil extracted from seeds that were not disinfected oil content analysis, acidity and peroxide values were performed. The incidence and identification of fungi was carried out in disinfected and not disinfected soybean seeds.

The oil content was determined by the official methodology, described by the Instituto Adolfo Lutz (2008). About 100 g of soybean seeds were divided into four homogeneous portions (4 x 25 g) and transferred to Soxhlet, an extraction apparatus. About 250 mL of hexane were added (mass ratio: 1:10

volume) and maintained under constant heating for 8 hours. The solvent was distilled under reduced pressure on a rotary evaporator and the oil percentage content, expressed on dry base (% d.b.) was determined in relation to the seed mass.

The acidity value was determined by the official methodology, described by described by the Instituto Adolfo Lutz (2008). From 1 to 2 g of each sample oil were placed in a 125 ml Erlenmeyer and 30 mL of ethyl ether and ethyl alcohol solution (1:1) were added, stirring until complete oil dilution; adding three drops of phenolphthalein acid/base indicator (0.1%), and the titration with 0.02M KOH solution was proceeded until the pink color appearance, stable for 30 seconds.

The KOH solution was standardized using dry potassium biphthalate as primary standard.

The official methodology was used for peroxide value as described by the Instituto Adolfo Lutz (2008). About 1g of each oil sample was placed in a 125 ml Erlenmeyer, 6 mL of glacial acetic acid and chloroform solution (3:2) was added, and 0.1 mL of a saturated solution of potassium iodide with stirring for about 2 minutes. Next, 6 mL of distilled water and 0.1 mL of 1% starch solution 1% were added, and titration was carried out with 0.005M sodium thiosulphate solution until the mixture become transparent.

Sodium thiosulfate solution was standardized using potassium dichromate at acid medium.

For fungal incidence, soybeans, disinfected and not disinfected with 1% hypochlorite were distributed in gerbox, containing two sheets of filter paper, previously sterilized and moistened with distilled and sterile water. Each plot was consisted of 20 seeds distributed in gerbox, totaling 120 seeds per treatment.

To the soybeans health analysis was used the method of filter paper ("blotter test"), as described in Regras para Análise de Sementes (Brasil, 2009). Three replicates with 16 sub-replicates of 25 seeds was carried out that have been previously washed in 1% sodium chloride for 3 min and placed on two sheets of filter paper in plastic boxes of gerbox type. The sheets of filter paper were previously dry sterilized in forced air circulation stove at 160 °C for 2 h and moistened with distilled water and autoclaved. The identification of pathogens was carried out after seven days of incubation at 22 ± 2 °C and under light for 12 h. In the end of this period, seeds were individually examined, with the aid of a stereoscope microscope with until 60x increasing, when needed; microscopically preparations for the identification of fungi were performed. Zero scores were attributed to three in accordance with the inoculum density in seeds, as follows: 0 - seeds free of fungi; 1 - seed exhibiting small visible fungal colonies only with magnifying glass; 2 - seeds exhibiting large visible fungal colonies without the use of magnifying glass and covering an area less than 50% of seed surface; 3 - seeds with a high degree of deterioration with fungal colonies covering an area more than 50% of seed surface.

The experiment was conducted according to triple factorial 2 x 4 x 3 scheme [two storage conditions: lab environment (uncontrolled conditions: (27 ± 0.6 °C), air-conditioned environment (20 ± 1.2 °C), four storage times: 0, 60, 120 and 180 days and three initial moisture content: 12.0%; 13.0 % and 14.0% (w.b.) in a completely randomized design with three replications. Soybean seeds, disinfected and not disinfected were separately analyzed. Data were measured using variance and regression analysis. Models were selected based on the significance of the equation, by F test, the significance of coefficients of regression using the "t" test adopting at 5% significance level and the coefficient of determination (R^2).

Results and discussions

Regarding to the initial moisture content 12; 13 and 14 °C there was a similar reduction for two studied environments along the storage time, coming to the end of 180 days with 11.2; 12.3 and 13.2% (w.b.) for controlled condition; and 11.3; 12.2 and 13.2% (w.b.) to uncontrolled condition. This reduced moisture content can be related to the package permeability in which the seeds were stored, as it allows water vapor exchange with the environment. Seeds and grains are hygroscopic, subject to the sorption processes, i.e. the moisture content is always in equilibrium with the relative humidity and air temperature. Siqueira et al. (2012) state that oleaginous products have unstable bonds with water, thus are more hydrophobic than the no oleaginous grains, facilitating the water removal during the drying process. Due to this hydrophobicity, small increments of water can cause serious damage to seeds, because it will be more free water, prone to adverse reactions and facilitating infection of microorganisms.

In Figure 1 the mean monthly ambient temperature of laboratory are presented (uncontrolled condition) and conditioned throughout the soybean seeds storage time. It is observed that the average temperature in air-conditioned environment during storage was 20 °C, and the maximum average was 21.7 °C, and the minimum average was 18.9 °C. In the laboratory environment (uncontrolled condition), the average temperature was 27 °C, in August, the minimum average of 26.4 °C and the maximum average recorded was 27.4 °C.

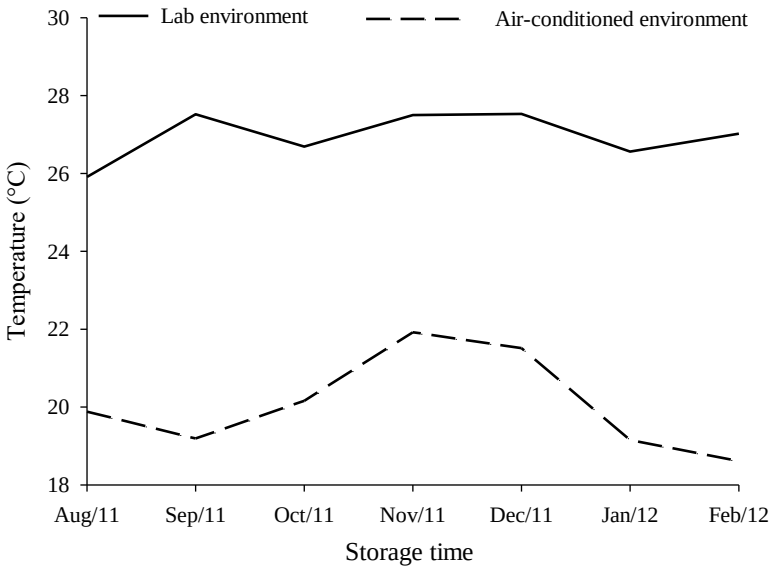

Figure 1 - Monthly average temperature of laboratory environments (uncontrolled condition) and conditioned throughout the soybean seeds storage time.

In Table 1, there is the summary of the analysis of variance to acidity and peroxide values and fungal incidence in the disinfected and not disinfected seeds.

It is noted that the results of the analysis of variance by F test at 1% significance, that the moisture content (MC) and storage time (ST) interaction was significant for oil content characteristic and the interaction of temperature (T) and storage time (ST) for acidity value characteristic. For the peroxide value, there was 5% significance by F-test on the moisture content (MC) and storage time (ST) interaction.

The temperature has an effect on the peroxide value, indicating oxidation reaction. The initial moisture content influenced the oil content and peroxide value, but did not affect the acidity value. The storage time influenced on all variables: oil content, acidity and peroxide.

Table 1 - Summary of variance analysis for the acidity and peroxide values and for the incidence of fungi in disinfected and not disinfected soybean seeds.

Sources of variation	DF	Average square				
		Oil content	Acidity value	Peroxide value	Disinfected	Not Disinfected
T	1	1.43^{NS}	0.41^{NS}	6.62*	0.42^{NS}	0.22^{NS}
Initial MC	2	37.90^{**}	0.34^{NS}	6.51*	6.48^{NS}	1.57^{NS}
ST	3	9.50^{**}	3.07**	40.57**	440.75**	62.69*
T X MC	2	3.07^{NS}	0.36^{NS}	0.47^{NS}	0.57^{NS}	0.23^{NS}
T X ST	3	2.62^{NS}	0.51**	4.80*	0.84^{NS}	0.27^{NS}
MC X ST	6	11.49^{**}	0.11^{NS}	2.48^{NS}	0.39^{NS}	5.69 **
T X MC X ST	6	1.63^{NS}	0.11^{NS}	1.80^{NS}	0.33^{NS}	0.11^{NS}
CV %		11.76	28.63	34.13	9.83	7.32

** 1% Significant * 5% Significant and ^{NS} Not significant by F test; T: temperature; ST: storage time; initial MC: initial moisture content.

For soybeans that have been disinfected, there were differences during the storage only for the time factor. For soybean seeds that have not been disinfected, beyond the storage time factor, the moisture content * time interaction was 1% significant by F test.

The disinfection of seeds for plating performing is important for a possible inference in the relation infestation and infection. If the incidence was higher in seeds not disinfected, it may be due to the presence of external fungi in seed, if after disinfection still has high incidence, owing to fungi internally present in seeds.

In Figure 2 the experimental values of the extracted oil content of soybean seeds are presented, for the different levels of water during the storage time.

It is noted that in the early storage, to 12% (w.b.) moisture content, it has obtained lower oil content index, differing from the 13 and 14% (w.b.),

which had the highest values. There was no difference between the oil content within 60 days. There was a reduction in the oil contents in all moisture contents studied within 120 days, in which the moisture content of 14% (w.b.) had a higher content compared to the others. The oil content showed a little decline at the end of the 180 days of storage for stored seeds with 12% (w.b.). For seeds stored with initial moisture content of 13% (w.b.) decreased after storage. But the extracted oil content of soybean seeds stored at 14% (w.b.) had an increase at the last 180 days, which is, the moisture content with higher oil income. The oil content did not follow a clear line of trend depending on the storage time. Alencar (2006) observed that in general the lipid content of soybeans showed no change during the storage period, except for the grains stored with 14.8% moisture content (w.b.) at temperatures of 30 and 40 ºC. Hou & Chang (2005) analyzing the chemical composition of soybeans stored under different conditions, found increased lipid content when grains were stored at 30 °C and 84% RH. However, the authors did not justify such increase.

In Figure 3, experimental and estimated values of oil acidity value of soybean seeds are shown, controlled and uncontrolled conditions during the storage time.

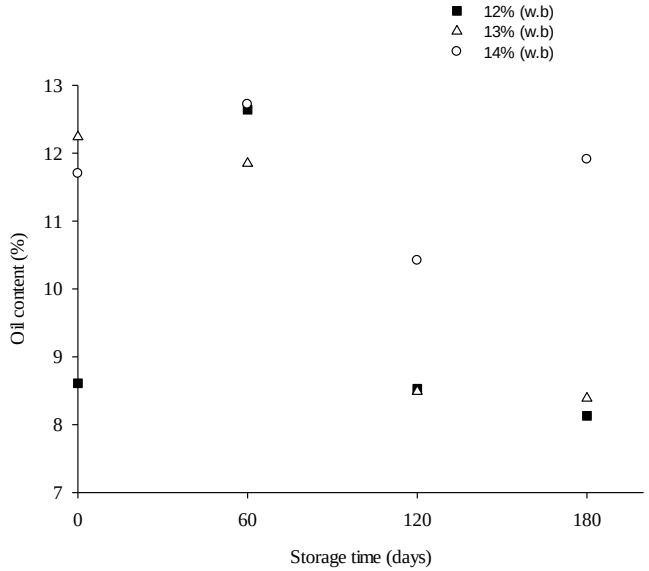

Figure 2 – Experimental values of the extracted oil content of soybean seeds for the different moisture contents during the storage time.

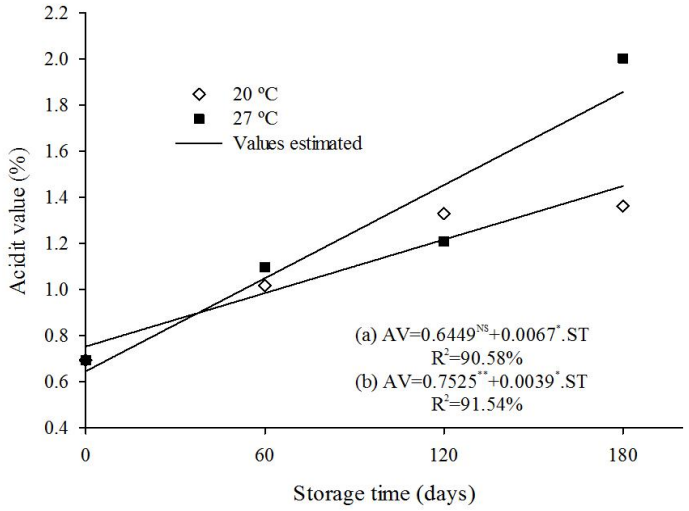

Figure 3 - Experimental and estimated values of oil acidity value extracted of soybean seeds, for 20 ºC and 27 °C temperatures, during the storage time.

It is noted in Figure 3 that there was an increase in acidity value during the storage for the two studied conditions, the stronger for higher temperature (27 °C) at the end of the storage (180 days), demonstrating further intensification in oxidation of lipids, thus contributing to the degradation of the quality of grains. The linear equations can be used to describe this increase, with an increased acidity value of 0.0067 and 0.0039% for each day of storage at temperatures of 27 ºC and 20 ºC, respectively. According to Ribeiro & Seravalli (2004), oil conservation status is closely related to the nature and quality of crude material and especially with the conditions of storage, the decomposition of glycerides is accelerated by heat and light, while rancidity is always almost accompanied by the formation of free fatty acids.

The high temperature is a generator factor of the acidity value, because the breathing reactions (oxidation) and hydroperoxide degradation is accelerated by increasing temperature. According to Araujo (2008), this lipid oxidation release acids compounds that can be measured by titration. According to the author, besides acids, free radicals are formed, which attack unsaturated fatty acids of long chain, forming new free radicals and hydrogen peroxide, that later will form volatile compounds of unpleasant smell by enzymatic action.

The pattern established by MAPA (2006) for the commercialization of soy crude oil in Brazil is of 2% acidity. In this context, it can be said that the crude oil extracted from the seeds stored at the two temperatures, 20 ºC and 27 ºC, showed acidity percentage below or even, within the limits of minimum conditions of quality.

Soares (2004) observed that the acidity values of the oil extracted from soybean increased during the storage, being more intense in the grains thermally damaged. Alencar et al. (2010), stored soybeans with different temperatures (20 ºC, 30 ºC and 40 °C) and moisture contents (11.2; 12.8 and 14.8% w.b.) and concluded that the storage with up to14.8% (w.b.) moisture content at 20 °C did not negatively affect the quality of crude oil when the temperature was increased to 30 °C, the quality remained satisfactory until 180 days of storage with moisture content up to 12.8 % (w.b.).

In Figure 4, the peroxide value of the extracted oil from soybeans stored during 180 days at temperatures of 20 º C and 27 º C is showed.

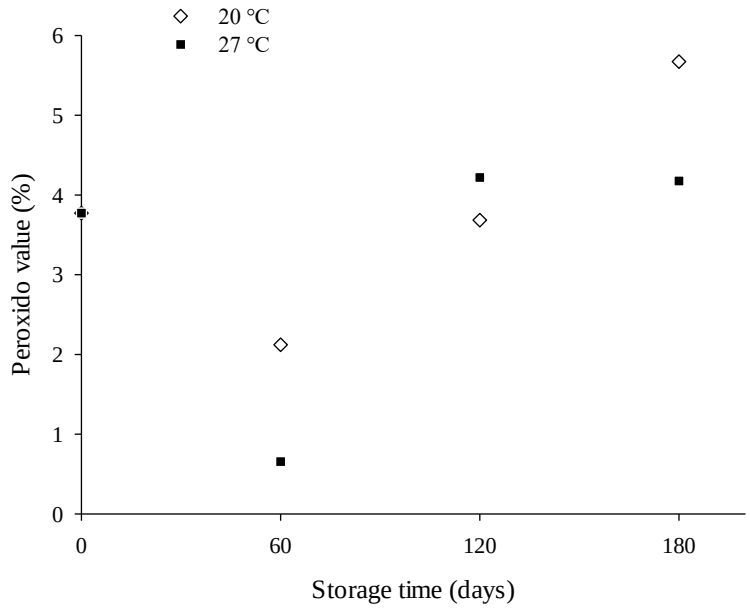

Figure 4 - Experimental values of peroxide value of the oil extracted from soybeans for the temperatures of 20 ºC and 27 ºC during storage time.

The peroxide value reduced after 60 days for both studied temperatures, being more evident under uncontrolled condition. During the 120 days of storage there was an increase in the index, this did not differ among the studied conditions. At the last 180 days the peroxide value increased only under the controlled condition, differing from uncontrolled condition, which remained fairly constant in the last days of storage (Figure 3). It was not possible to adjust equations to describe the phenomenon as a function of time.

According to Allen & Hamilton (1983), peroxides are inevitably decomposed even at room temperature, producing small molecules such as carbonyl compounds. At high temperatures, formation rate of peroxides is lower than the decomposition. Therefore, this measure is limited due to the transient nature of peroxide, its decomposition in secondary

products may underestimate the degree of oxidation, i.e., lower values may represent the early stage or advanced of oxidation. Bordignon (2009) reports that during the oxidation process, the formation and degradation of peroxides are constant, the peroxide values reach certain concentration and decrease.

Zeni (2010) observed increased peroxide value at the last 300 days of storage at all temperatures studied (20-25 °C, 35-40 °C, 55-60 °C, 75-80 °C) in oil extracted from canola grains. Alencar et al. (2010) analyzing soybean oil also observed increased peroxide value after 180 days of storage at temperatures of 20 °C, 30 °C and 40 ºC.

According to the Agência Nacional de Vigilância Sanitária – Anvisa (2005), the oil with the peroxide value of maximum 10 meq O_2 kg^{-1} may be used for human consumption.

By comparing the peroxide values obtained in oil extracted from soybean analysis with the standard of Anvisa, it demonstrates that all obtained results had values below 10 meq O_2 kg^{-1}, suggesting that are suitable for commercialization and consumption.

It was found in soybean seeds the incidence of the following fungi genera: *Aspergillus spp., Curvularia sp., Diplodia sp., Fusarium spp., Penicillium sp., Rhizopus sp. and Trichoderma sp*.. It was determined that both disinfected and not disinfected soybean seeds, there was a higher rate of the genera *Aspergilus spp.,* followed by *Fusarium* spp., and *Penicillium* sp. When working with cooled artificially soybean and stored in paper bags, Zuchi (2013) reported that the incidence of *Fusarium sp., Phomopsis sp.* decreased and *Aspergillus sp.* increased with the storage. The author relates this behavior with the variations in temperature and relative humidity occurred in the warehouse, since each genus has a great condition for its development.

In Figure 5 the experimental and estimated values of fungi incidence in soybean seeds disinfected, depending on the storage time, are showed. In the evaluation performed before seed storage, there was a result of 46.39%. At 60 and 120 days, there was an increasing rate in fungal incidence of 97.64% and 98.19%, respectively, demonstrating that there were favorable conditions for fungi development during the storage. At 180 days there was a decreased incidence with a value of 90.14%. Yaja (2005) noted an increase in the incidence of fungi in soybean seeds at 120 days of storage. Regarding the estimated values, after 80 days of storage was observed increased incidence of fungi.

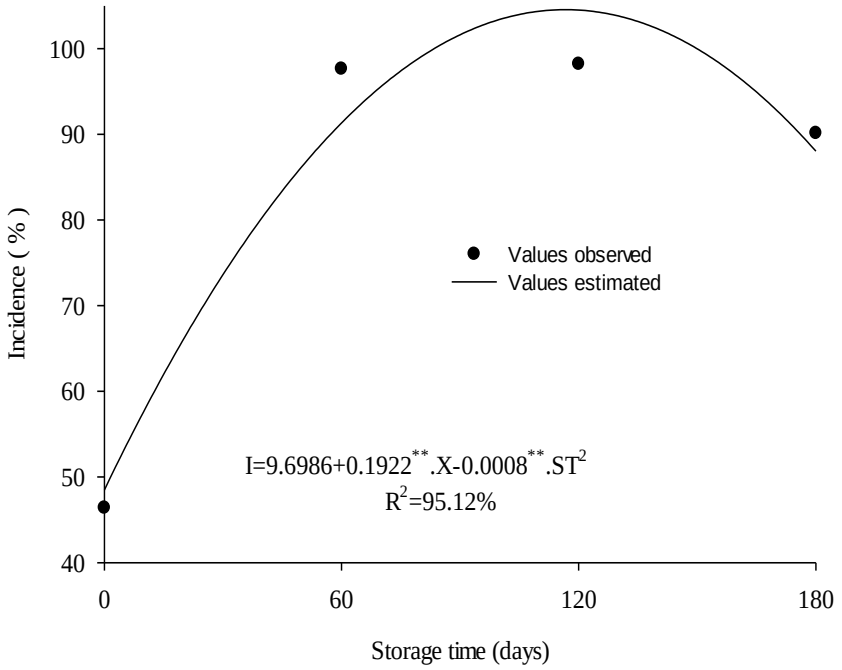

Figure 5 - Experimental and estimated values of fungi incidence in disinfected soybean seeds, depending on storage time at laboratory environments and air-conditioned.

In figure 6, the experimental and estimated values of fungi incidence in soybean seeds not disinfected, depending on function of storage time are presented. It is observed that there was an increase in the fungi incidence in soybean seeds not disinfected from the beginning to the 60 days, with 97.35%, showing small variations by the end of storage, 98.75% and 97.20% of incidence for the 120 and 180 days of storage, respectively.

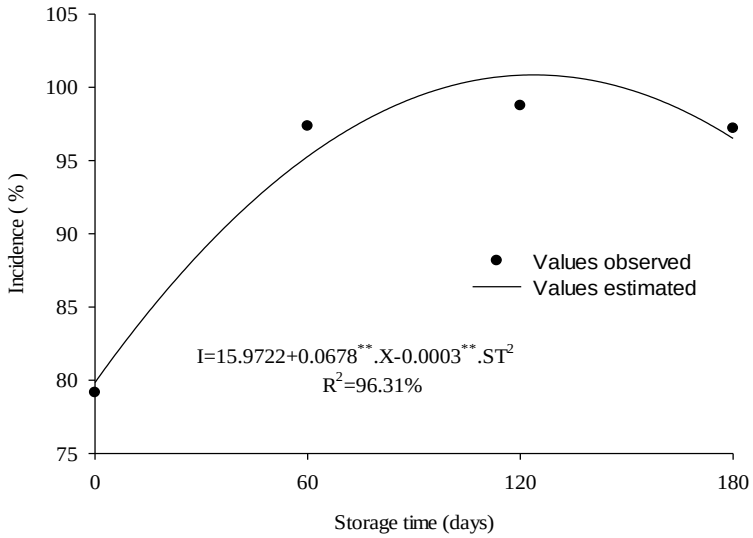

Figure 6 - Experimental and estimated values of fungi incidence in not disinfected soybean seeds, depending on storage time, at laboratory environments and air-conditioned.

Analyzing Figures 3 and 4, it is noted that regardless of the initial condition of the seed, the period of 60 days was sufficient for fungi incidence was above 97%, probably related to the increased fungi storage *(Aspergillus* and *Penicillium)*.

In Figure 7, the experimental and estimated values of fungi incidence in soybean seeds not disinfected are shown, for the interaction between initial moisture content and storage time, for the two studied sites. It was observed that there was an increased incidence of fungal at the first 60 days of storage practically maintaining constant until the end of storage for all studied moisture contents. The initial moisture content of 14% (w.b.) was at first the lower incidence, ending the 180 days without differ from others. This fact shows that the moisture content influences in the health of soybean seeds. According to Patel & Misrha (2010), colonization and survival of fungi in oilseeds during storage is primarily determined by the prevailing temperature and the moisture content of the seeds. Pereira et al. (2007) observed that there was an increased percentage of *Aspergillus, Penicillium* and *Fusarium,* at three months of storage, with subsequent reduction trend.

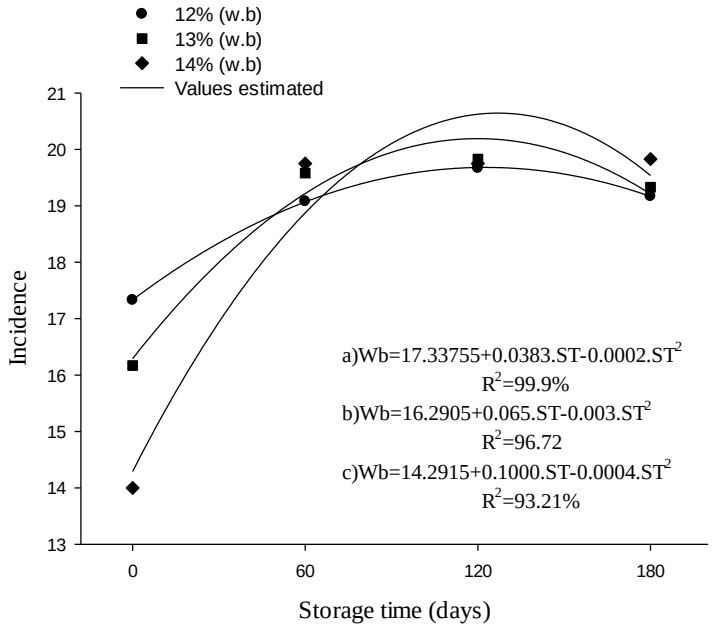

Figure 7 - Experimental and estimated values of fungi incidence in not disinfected soybean seeds, depending on moisture content and storage time, for the two studied sites.

Conclusions

The initial moisture content of 14% (w.b.) provides greater fungal incidence at the end of storage in soybean seeds not disinfected. The temperature of the environment during storage interferes in the quality of the crude oil extracted from soybean seeds. The temperature of 27 °C causes more increment in acidity value of soybean oil during storage.

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