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Effects of temperature and moisture during semi-hermetic storage on the quality evaluation parameters of soybean grain and oil

Efeitos da temperatura e umidade durante o armazenamento semi-hermético sobre parâmetros de avaliação da qualidade dos grãos e do óleo de soja

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Abstract

The soybean (Glycine max (L.) Merrill) is the most cultivated oilseed in the world, being of major importance in food, feed and biodiesel production. The quality of the product being marketed is directly influenced by storage techniques, and the grain moisture, temperature and storage time are primarily responsible for most of the amendments. The aim of this study was to evaluate the effects of temperature and humidity during the semi-hermetic storage on chemical and technological parameters for assessing the quality of soybean. The kernels were stored for 12 months in semi-hermetic system with two combinations temperature (15 to 25 °C) and humidity (12 and 16%) and then evaluated for the occurrence of plagues, variations in humidity, levels of lipids and proteins, protein solubility, oil quality, carotenoids, color, enthalpy and thousand grain weight. The results showed that the increase in storage time leads to changes in chemical and technological parameters. The preservation of the parameters of acid oil and thousand grain weight was dependent reductions in moisture, while the incidence of pests, protein solubility, enthalpy, carotenoid levels and parameters of color preservation were preserved by reducing temperature.
Key words: *Glycine max*, storage, refrigeration, quality parameters

Resumo

A soja (*Glycine max* (L.) Merrill) é a oleaginosa mais cultivada no mundo, sendo de grande importância na alimentação humana, animal e para produção de biodiesel. A qualidade do produto a ser comercializado é diretamente influenciada pelas técnicas de armazenamento, sendo que a umidade dos grãos, a temperatura e o tempo de armazenamento são os principais responsáveis pela maioria das alterações. O objetivo deste estudo foi avaliar os efeitos da temperatura e da umidade durante o armazenamento semi-hermético sobre parâmetros químicos e tecnológicos de avaliação da qualidade dos grãos de soja. Os grãos foram armazenados durante 12 meses, em sistema semi-hermético, com duas combinações de temperatura (15 a 25 °C) e humidade (12 e 16%) e, em seguida, avaliados quanto a ocorrência de

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pragas, as variações da umidade, os níveis de lipídios e proteínas, solubilidade proteica, qualidade do óleo, carotenoides, cor, entalpia e peso de mil grãos. Os resultados mostraram que o aumento do tempo de armazenamento conduz a alterações nos parâmetros químicos e tecnológicos. A preservação dos parâmetros de acidez do óleo e peso de mil grãos são mais dependentes da umidade, enquanto que a incidência de pragas, a solubilidade da proteína, a entalpia, os níveis de carotenoides e os parâmetros de preservação da cor foram preservados pela redução da temperatura.
Palavras-chave: *Glycine max,* armazenamento, refrigeração, parâmetros de qualidade

Introduction

The soybean (*Glycine max* (L.) Merrill), a dicotyledonous species in the Fabaceae family, was originally grown in Northeast Asia, including China, South Korea and the surrounding regions (KIM et al., 2005). The cultivation of the soybean in Brazil began in 1908 and was expanded in the 70's, when the international demand for this product and its derivatives increased (SOUZA et al., 2000).

In the post-harvest stage, soybeans pass through a series of operations, including drying, cleaning and storage, prior to industrialisation, where they are processed for foods, beverages, biofuels and other by-products. During storage, the beans, which are living tissues, suffer physical, chemical and biochemical changes that alter their technological properties, with direct consequences on their quality (KARUNAKARAN et al., 2001). The main factors that affect the quality of the grains are moisture of grains, humidity and ambient temperature, the presence of light, the duration of storage and the storage operations system (FREO et al., 2011; KONG; CHANG, 2013).

Soy proteins are ingredients in various foods, including embedded meat, soybean juice, noodles, soup, and tofu, among others. Therefore, the study of soybean storage for long periods is important to determine the negative effects of storage on the concentration and physico-chemical and technological properties of the proteins, such as their solubility, structural changes and denaturation (LIU et al., 2008; KONG; CHANG, 2013).

Soy is used to produce the biodegradable vegetable oil that dominates the world market for food and biofuels (LIU et al., 2001). Its chemical composition, which is rich in unsaturated fatty acids and carotenoids, makes it valuable for human consumption because it can aid in lowering cholesterol (PETER; HAKAN, 1998). Soybean oil is also valuable for the production of biofuels through the transesterification of triglycerides (MARCHETTI et al., 2007). Qualitative and quantitative losses of oil during soy storage occur predominantly due to reactions with atmospheric oxygen, i.e., through oxidative rancidity, or via hydrolytic reactions catalysed by the lipases in the grain, which can stem from the grain itself or from microorganisms (NAZ et al., 2004).

Although some studies have investigated the effects of storage on the quality parameters of soybeans (KONG; CHANG, 2013; LEE; CHO, 2012; LIU et al., 2008), additional quality changes, such as losses of dry mass of grain, qualitative and quantitative losses of proteins, and changes in solubility, thermal stability, and oil content, require further investigation. Therefore, this study aimed to evaluate the effects of the storage of soybeans in a semi-hermetic system for twelve months on the soybean protein content, lipids and carotenoids, protein solubility, oil quality (acidity, K_{232} and K_{270}), colouring, thermal properties and thousand grain weight. These are important for the quality control of soybeans as a raw material for the food and biofuel industries.

Materials and Methods

Material

The soybeans used (*Glycine max* L.) were grown in commercial tillage in the municipality of Primavera do Leste, latitude S: 15º 24' 58.37" and

longitude W: 54º, state of Mato Grosso do Sul, in the Central-West region of Brazil.

Methods

Preparation, conditioning and storage of samples

The grains were harvested at 18% moisture and dried using the conventional system in the region where they were grown. A part of the grain was dried in a continuous industrial dryer system to 16% moisture, and the other part was dried to 12% moisture. For each moisture level (16% and 12%), from each ton of grain leaving the dryer, samples of 50 kg were collected and separated into samples of 5 kg. After collected, samples were transferred to pilot chambers for storage in the *Laboratório de Pós-Colheita, Industrialização e Qualidade de Grãos* (*LABGRÃOS*) in the *Universidade Federal de Pelotas* (*UFPel*) in the city of Capão do Leão, state of Rio Grande do Sul, in the southern region of Brazil.

Two storerooms with temperature control of 15 and 25 °C each were used to store the grain samples at 12 and 16% moisture, and analysis was performed at the time of storage (initial time), 6 and 12 months thereafter.

The grains were packed in low density polyethylene bags that were 10 µm thick with a capacity of 5 kg with the samples kept in the dark. To simulate a semi-hermetic storage system, the experimental methodology consisted of opening the packages to aerate the grain every three months during storage, as is done in silos and grain warehouses that uses the aeration process. The aeration hold regular airings as a way to avoid an anaerobic environment and reduce non-uniformities in temperature, with the adverse consequences of internal convective currents according to the methodology described by Paraginski et al. (2014).

Analysis

Infestation and pest control

The detection of pests during storage was performed according to methods described in GRDC-BPG (AUSTRALIAN, 2011) and Papadopoulou and Buchelos (2002) with monthly evaluations. Pest control was performed according to the purge technique described by Weinzierl and Higgins (2008), with the purges carried out as soon as the presence of pests was detected so that the effects of purging would not compromise the evaluation of the other parameters in the search.

Thousand grain weight

To determine the grain weight, 10 samples of 100 grains were weighed according to the method described in BRASIL (2009).

Moisture

The water content was determined as proposed by the American Society of Agricultural Engineers – ASAE (2000) method, where samples of 10 g were dried at 105 °C for 24 hours.

Protein

The protein content was determined in a Microkjeldahl apparatus according to the method described by American Association of Cereal Chemists – AACC (2000). To convert the nitrogen content to % protein, a correction factor of 6.25 was used.

Protein solubility

The protein solubility was determined by the method described by Liu et al. (1992) with modifications. A 50 mL volume of distilled water was added to each 2 g of sample, and the samples and water were mixed with the aid of a magnetic

stirrer for 1 hour, after which they were centrifuged at 5300 x g for 20 minutes at 24 °C. An aliquot of 1 mL of the supernatant was collected, and the soluble protein content was determined by the method described by American Association of Cereal Chemists – AACC (2000), the same method used to evaluate the crude protein content.

Differential scanning calorimetry (DSC)

The protein denaturation was analysed by DSC (Differential Scanning Calorimetry) in a Shimadzu DSC 60 (Osaka, Japan) using a 2.5 mg sample of defatted bran in aluminium crucibles with 0.75 µL of PBS buffer at a concentration of 0.05 M (pH 7.0). The crucibles were sealed and subjected to a rest period of 24 hours for full hydration of the sample before heating. The heating rate was 5 °C per minute from 20 to 110 °C, and an empty crucible was used as a reference. All the tests were performed under a nitrogen atmosphere.

Lipid

The lipid content was determined by the method described by the Association of Official Analytical Chemists – AOAC (2006).

Oil acidity index

The oil acidity index was determined by the method described by the American Oil Chemist's Society – AOCS (1997).

Specific extinction coefficient (K_{232} e K_{270})

The specific extinction coefficients were determined according to the method proposed by the – American Oil Chemists' Society – AOCS (1997).

Carotenoids

The carotenoids index was determined according to the method described by RODRIGUES-AMAYA (2001). The results were expressed in mg of β-carotene.$100g^{-1}$ of oil.

Color

The soybean grain colour was evaluated using a colorimeter (Minolta, model CR-310, Osaka, Japan). The parameter of colour used was "*b,*" which evaluates the colour variation from blue to yellow (negative – blue and positive – yellow).

Statistical analysis

A comparison of the means was performed using Tukey's test at a 5 % level of significance using an analysis of variance (ANOVA). All evaluations were performed in triplicate and utilised the statistics software SAS (SAS, INSTITUTE, 2002).

Results and Discussion

Pest control and detection

The results of pest detection are presented in Table 1. In the first month of storage, pests were detected in all the samples as a result of the migration of pests from other storage environments; there were no pests present in the grain before storage. Whenever the presence of pests was detected, a purge was performed. It was observed that after the second month there were insects only in the grain stored at 25 °C for both grain moisture contents. In the grains stored at a temperature of 15 °C, the performance of a purge in the first month of storage was sufficient to control the initial infestation and there was no re-infestation, which highlights the importance of using cooling to control pests during soybean storage.

Table 1. Detection of insects in soybeans stored for twelve months in a semi-hermetic system under four combinations of temperature and moisture.

Treatment	Storage period (months)											
	1	2	3	4	5	6	7	8	9	10	11	12
12% / 15 °C*	d	u	u	u	u	u	u	u	u	u	u	u
12% / 25 °C	d	d	d	d	d	d	d	d	d	d	d	d
16% / 15 °C	d	u	u	u	u	u	u	u	u	u	u	u
16% / 25 °C	d	d	d	d	d	d	d	d	d	d	d	d

* Treatment (% of grain moisture / storage temperature – (°C)).
d = detected; u = undetected

The pests found in the grain were the beetles *Lasioderma serricorne* and *Cryptolestes spp.*, belonging to the families Anobiidae and Cucujidae, respectively. Although not specific, *Lasioderma* has become the most important pest affecting soybean storage in Brazil (LORINI, 2002).

In the 25 °C storage system, the grains were attacked by pests, whose action was accompanied by fungal contamination, which was more intense in the higher moisture condition. Baldassari et al. (2005) and Weinzierl and Higgs (2008) reported similar effects for the use of low temperatures for the control of reproduction in insects, with the major determinant being the metabolic activity of the insects present in the storage environment, and reported that moisture levels correlated with the presence of microorganisms.

Thousand grain weight

The thousand grain weight (Figure 1) was reduced after 6 months of storage, independent of the temperature and moisture during storage. The thousand grain weight was also reduced at 12 months in the four combinations of temperature and moisture tested, with the lowest observed reduction occurring in the grain with a 12 % moisture content stored at a temperature of 15 °C, from 172.89 g to 164.57 g. The largest decreases were observed in the grain with 16 % moisture, which decreased from 175.62 to 158.03 and 154.65 g, respectively, at 15 and 25 °C. The actions of insects (Table 1), microorganisms and the grain metabolism contributed to the reductions in grain weight (Figure 1) during storage, along with the loss of water observed by reducing the moisture content (Figure 2).

Alencar et al. (2009) working with soybeans with a 12.8% moisture content stored at 30 °C, observed a decrease in the thousand grain weight after six months of storage. The thousand grain weight is a measure widely used to evaluate mass loss during storage, but there are several factors that can change the values of the thousand grain weight, such as an intensification of metabolism during storage, which consumes energy reserves and results in the formation of carbon dioxide and water as the end products of respiration. The main utilised substrates for the metabolism of grains during storage are lipids and proteins, depending on the water content. Weight losses reflect decreases in the levels of proteins and lipids during storage (Figure 3a and 5a) and are also associated with reductions in the amounts of moisture (Figure 2).

The actions of insects (Table 1), microorganisms and grain metabolism itself, according to Maier et al. (1992) and Srzerdnicki et al. (2006) explain the reductions in grain weight (Figure 1). Such reductions also influence oil quality during storage.

Figure 1. Thousand soybean grain weight (g) after storage for twelve months in a semi-hermetic system under four combinations of temperature and moisture.

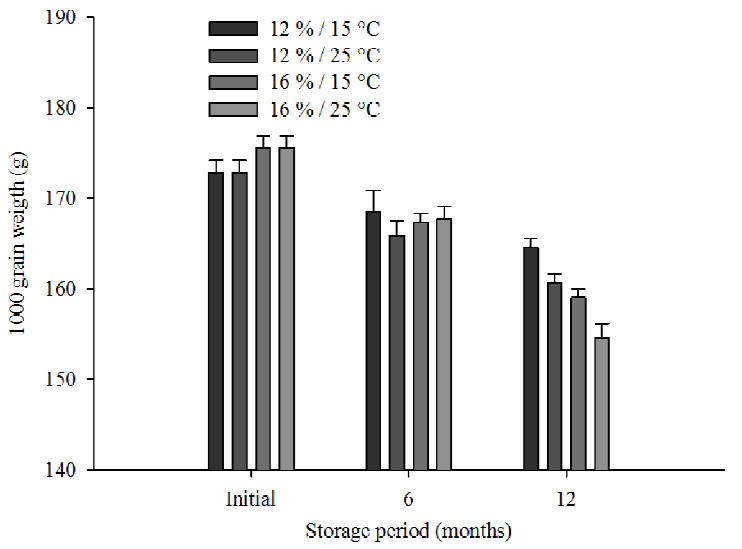
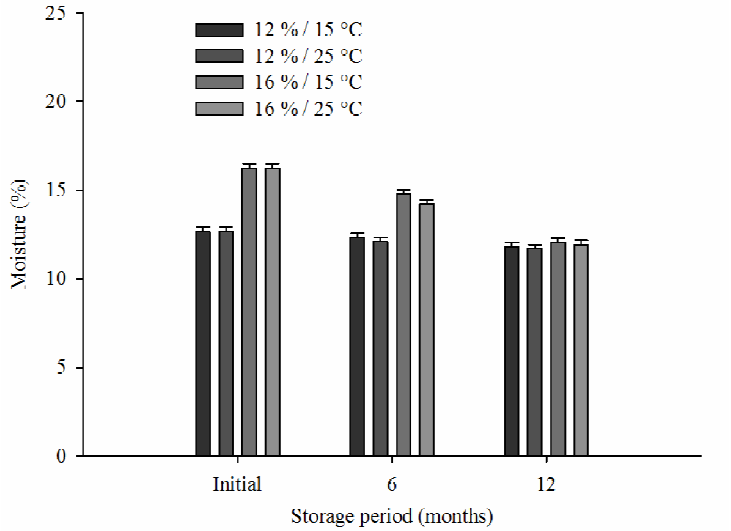


Figure 2. Moisture content (%) in soybeans after storage for twelve months in a semi-hermetic system under four combinations of temperature and moisture.



Moisture

It can be observed in Figure 2 that the grain moisture decreased over time in the stored grain for all four combinations of moisture and temperature tested. The greatest reductions was observed in the grains with higher initial moisture, in which the grain moisture decreased from 16.26 to 12.04% and 11.91%, respectively, at 15 °C and 25 °C at the end of 12 months. In the grain with 12.68% moisture, the moisture was reduced, respectively, to 11.81% and 11.71%. In the grains storage partially dry (16.26% moisture), gradual reductions were observed at six and twelve months, while the dry stored grain (12.68% moisture) exhibited smaller reductions.

The results showed that the equilibrium moisture content of the soybeans in the studied conditions was between 11 and 12%.

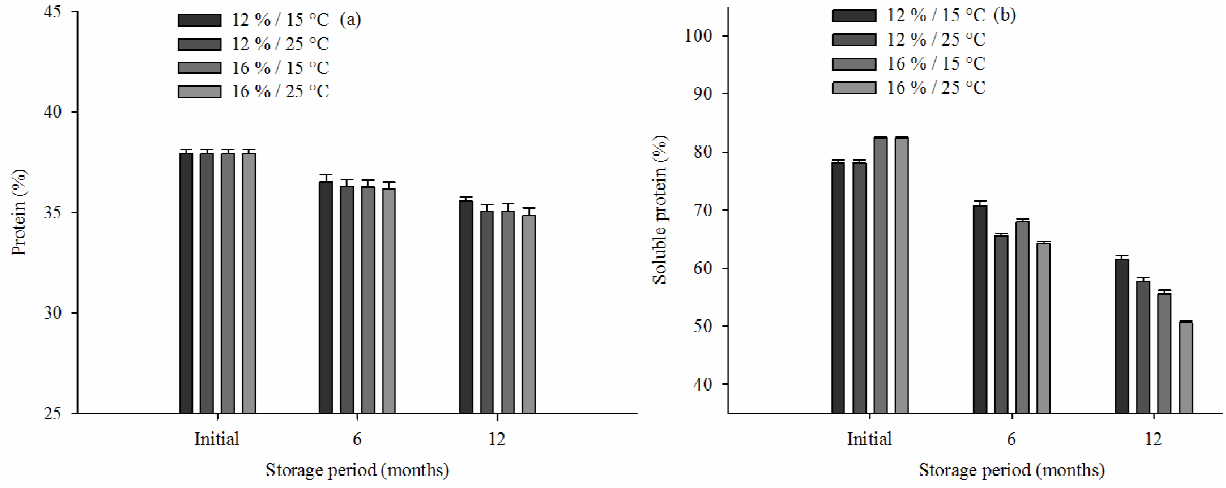
The behaviour observed for the moisture (Figure 2) was comparable to that reported by Kong et al. (2008) who stored soybeans at a relative humidity of 60% and a temperature of 40 °C and observed reductions in grain moisture from 13% to 8% after 10 months of storage. The decrease in moisture content with storage time is due to the semi-hermetic system, which allows air exchange with the external storage environment. When the vapour pressure of the grains is larger than that of the surroundings, desorption occurs (SUN; WOODS, 1997), transferring water vapour to the air, which reduces the grain moisture until the equilibrium moisture content is reached.

Protein content and protein solubility

The data in Figure 3a show that the grains initially contained 37.92% crude protein, in agreement with the literature for the species. The soybean has a protein profile consisting predominantly of globulins (90%), which have hydrophilic characteristics. This property is very important for yield and quality in the production of soy products, such as tofu and soymilk (HOU; CHANG, 2004).

The solubility at the beginning of storage was lower in the 12% grain than in the 16% grain (Figure 3b). This initial significant difference in solubility was due to the longer exposure to air grain drying used to reduce the moisture to 12%, which caused the complexation of protein structures. Prachayawarakorn et al. (2006) reported similar behaviour.

Figure 3. Protein content (a) and protein solubility (b) in soybeans stored for twelve months in a semi-hermetic system under four combinations of temperature and moisture.



At the end of storage, the content of crude protein (Figure 3a) decreased approximately 2 percentage points in the four combinations of temperature and humidity, with no significant difference between them. The protein solubility decreased slightly after twelve months of storage for the four combinations of moisture and temperature (Figure 3b), with the greatest reductions observed in the grain stored at 16% moisture, which showed 55.57 and 50.67% protein contents, respectively at 15 and 25 °C. In the grains stored at 15 °C with 16% moisture, the solubility was equivalent to that of the grains stored with 12% moisture at 25 °C (57.68%), which suggests that it may be possible to store grain at

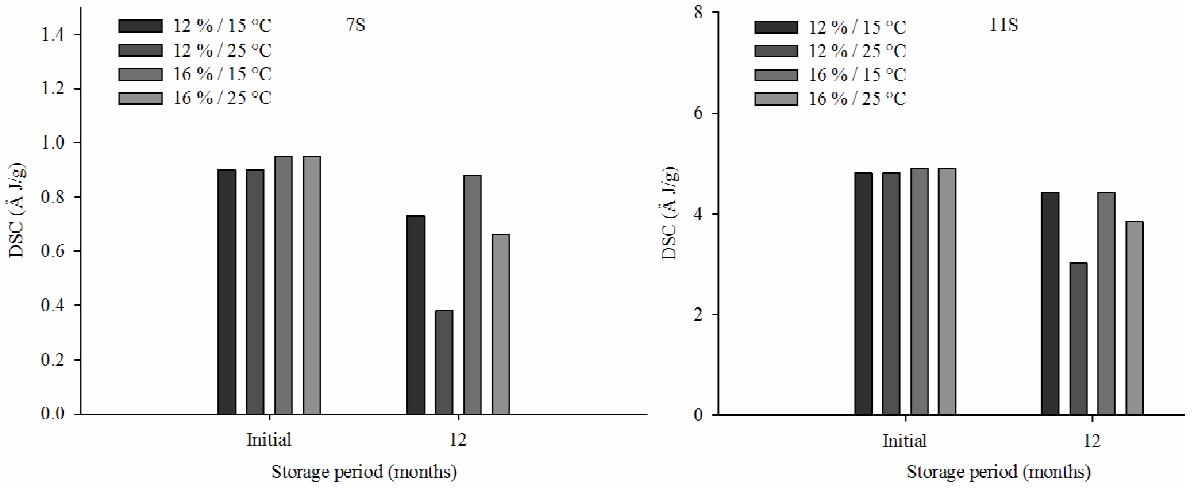
higher moisture levels under cooling (15 °C), which decreases the metabolism of the grains.

Reductions in the levels of proteins during storage have been reported in the literature by Lee and Cho (2012) who stored soybeans for two years at room temperature. They observed reductions in protein levels from 43 to 38.3% after one year and to 33.8% after two years of storage. Reduced protein contents were also observed by Rani et al. (2013) in beans under different storage conditions. Liu et al. (2008) found similar results, with significant reductions in protein content and protein solubility during soybean storage in adverse conditions (88% relative humidity and 30 °C), with the protein solubility similar to that in studies by Hou and Chang (2004).

The occurrence of protein denaturation and of molecular changes in the β-pleated structure and disulphide bonds during storage has been described in the literature (CHEN et al., 2011; STANOJEVIC et al., 2011). This phenomenon suggests that proteins can be degraded into small peptides and amino acids due to the metabolic needs of the grains, which explains the observed solubility (Figure 3b) and enthalpy changes (Figure 4a and 4b).

Glycinin, also known as 11S soy protein, is composed of six subunits of acidic and alkaline polypeptides each that are linked through disulphide bonds, while β-conglycinin, a 7S protein, is a glycoprotein that has three subunits (HOU; CHANG, 2004). Figure 4 shows that the enthalpy values decreased during storage for the four combinations of moisture and temperature in both fractions (7S and 11S), as evidenced by the two peaks formed in the calorimetric scan. The enthalpy values recorded by DSC indicate the energy needed for protein denaturation to occur.

Figure 4. Differential scanning calorimeter (DSC) (J/g) of defatted soybean bran after storage for twelve months in a semi-hermetic system under four combinations of moisture and temperature. (a) Peak enthalpy in the 7S fraction, (b) peak enthalpy in the 11S fraction.



DSC (Differential scanning calorimetry)

The grains stored at a temperature of 15 °C exhibited a higher enthalpy than did those stored at 25 °C for the same initial moisture content for both fractions 7S and 11S, which suggests that temperature is a more important factor than is moisture content. Other studies of thermal properties also revealed reductions in soybean enthalpy with increasing storage time, as was observed in this study (Figure 4). Liu et al. (2008) analysed

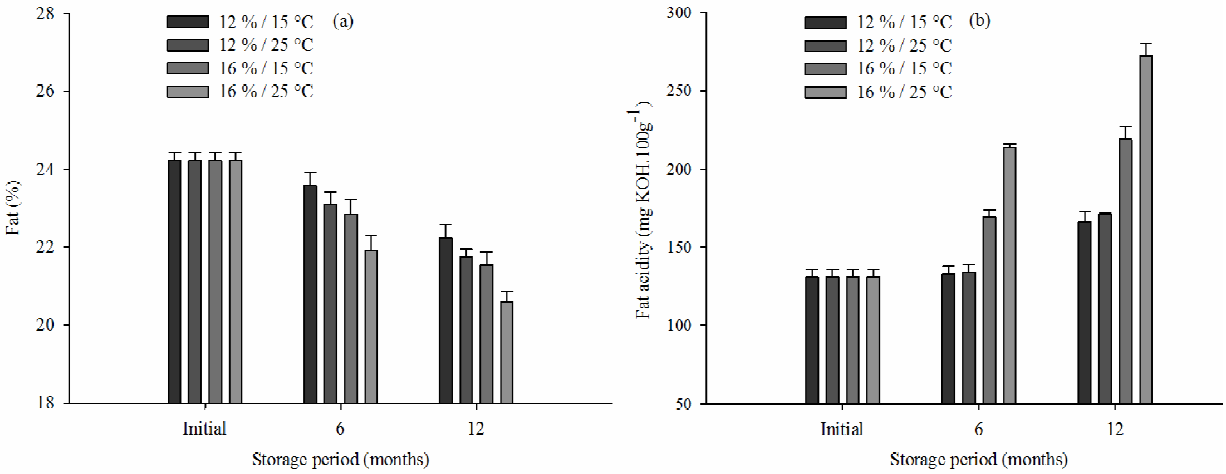
isolated soy protein stored under adverse conditions (88% relative humidity and 30 °C) and showed a significant reduction in the enthalpy of both 7S and 11S globulins during storage.

Regarding the enthalpy values (Figure 4), as related to protein solubility (Figure 3b), some distinct interference between the temperatures of 15 and 25 °C can be addressed: at 15 °C oxidizing reactions has probably occurred, accompanied by the formation of disulphide bonds and aggregates, which reduces the susceptibility of the grain to enzymatic hydrolysis. On the other hand, at 25 °C the protein was probably more able to enzymatic hydrolysis, which, in turn, provided lower formation of disulphide bonds and aggregates (GRUNE et al., 2004). The formation of disulphide bonds and aggregates at 15 °C may be contributed for the highest enthalpy values of stored soybean.

Lipid and fat acidity index

In the four combinations of temperature and moisture tested, decreases in the lipid content (Figure 5a) and increases in the fat acidity (Figure 5b) were observed with increasing storage time. In the grain with 12% initial moisture, there was no significant reduction in lipid content up to 6 months of storage, but there were reductions between 6 and 12 months at both temperatures. In the grain with a 16% moisture content, there was a significant reduction in lipid content at 6 months for both temperatures, and this reduction continued until 12 months of storage. Studying coffee storage at ambient 30 ºC, Toci et al. (2013) observed reductions in the levels of triglycerides after six months, which were attributed to the action of hydrolytic enzymes.

Figure 5. Lipid content (a) and acid value of the crude oil (b) of soybeans stored for twelve months in a semi-hermetic system under four combinations of temperature and moisture.



The lipid acid indices (Figure 5b) increased after 6 months of storage for the grains with 12% moisture. In the grain with 16% moisture, the acidity increased at 6 months of storage for both temperatures, and the largest increase in acid value was observed in the 25 °C environment at 12 months. The results are similar to those reported by Park et al. (2012) who studied the effects of rice storage at temperatures of 30 and 40 °C and found significant increases in acidity in the fourth month of storage, with further increases in the following months. Similar results for corn stored under

different temperature conditions were reported by Rehman et al. (2002).

Reductions in lipid levels (Figure 5a) and increases in acidity (Figure 5b) are due to the actions of lipases and phospholipases present in the grains themselves or produced by associated microflora (RAJARAMMANNA et al., 2010). These enzymes contribute to the breakdown of the ester bonds of triglycerides and to the oxidation of unsaturated carbon chains in the fatty acids (NAZ et al., 2004).

Proteins and lipids, the predominant components in soybean, are macromolecules that possess high reactivity, and interactions between proteins and lipids during storage can lead to the formation of protein radicals (REFSGAARD et al., 2000). The radicals formed during the oxidation of lipids (hydroperoxides) can react with oxygen and produce peroxide radicals of protein and subsequently protein hydroperoxides by abstracting hydrogen from other molecules.

Hydroperoxides of protein can then, in the presence of transition metals, be decomposed via cleavage to form carbonyl group proteins (CHOE; MIN, 2006) which explain the behaviour of the components in the present work.

Specific extinction coefficient of oil (K_{232} and K_{270}), carotenoids and "b" value of the colorimeter

Specific extinction coefficients are important in determining the quality parameters of oils. K_{232} is indicative of the presence of peroxides, hydroperoxides and conjugated dienes (primary oxidation products), while K_{270} is indicative of the presence of secondary oxidation products (alcohols, ketones, aldehydes) and conjugated trienes (CLODOVEO et al., 2007; RODRIGUES et al., 2012).

The results in Table 2 show that the specific extinction coefficients of the oil, i.e., K_{232} and K_{270}, increased with storage time, and for K_{232} the largest increase occurred in the treatment with 16% moisture at 25 °C, increasing from 1.83 at the beginning of storage to 2.46 at 12 months. The smallest increase occurred in the oil obtained from the grain stored with 12% moisture at 15 °C, remaining at 2.08 after 12 months. The same behaviour was observed for K_{270}, with larger increases in the oil of the grain with 16% moisture stored at 25 °C, from 0.15 at the beginning of storage to 0.46 at 12 months. The lowest observed reduction in oil was obtained from the grain with 12% moisture stored at 15 °C.

Carotenoids are bioactive compounds derived from secondary metabolism in plants, and the carotenoid composition can vary according to the cultivar, the growth location, and the weather and stress to which the plant is subjected (OOMAH et al., 2010; SLAVIN et al., 2009). Carotenoids have antioxidant capacity, are nonpolar, and are constituents of lipids. The data presented in Table 2 reveal differences in the levels of carotenoids at the beginning of storage, which is attributed to the longer drying time to which these grains were submitted to reach the desired moisture for this study. There was a reduction in the levels of carotenoids over the 12 months of storage. The largest decreases were observed in the grain with 16% moisture stored at 25 °C, which fell from 25.61 to 14.26 mg of β-carotene.100 g^{-1}. The smallest reductions occurred in the 12% moisture grains stored at 15 °C. The content of carotenoids was not different between treatments 12%/25 °C and 16%/15 °C (grain moisture/storage temperature) at 6 months. This highlights the importance of reducing the storage temperature on the maintenance of these compounds, as previously observed for other parameters, such as protein solubility (Figure 3b), enthalpy (Figure 4) and the "*b*" value of colour (Table 2).

Table 2. Specific extinction coefficients of oil (K_{232} *and* K_{270}), carotenoid levels (mg of β-carotene.100g^{-1}) and "*b*" value of colour.

Treatment	Storage period (months)		
	K_{232}		
	0	6	12
12% / 15°C**	A 1.83 ± 0.01 c*	D 1.94 ± 0.02 b	D 2.08 ± 0.02 a
12% / 25°C	A 1.83 ± 0.01 c	C 2.11 ± 0.01 b	C 2.18 ± 0.01 a
16% / 15°C	A 1.83 ± 0.01 c	B 2.21 ± 0.01 b	B 2.37 ± 0.05 a
16% / 25°C	A 1.83 ± 0.01 c	A 2.24 ± 0.05 b	A 2.46 ± 0.02 a
	K_{270}		
12% / 15°C	A 0.15 ± 0.01 c	C 0.21 ± 0.07 b	D 0.28 ± 0.07 a
12% / 25°C	A 0.15 ± 0.01 c	B 0.23 ± 0.06 b	C 0.31 ± 0.05 a
16% / 15°C	A 0.15 ± 0.01 c	B 0.23 ± 0.01 b	B 0.34 ± 0.04 a
16% / 25°C	A 0.15 ± 0.01 c	A 0.29 ± 0.03 b	A 0.46 ± 0.07 a
	Carotenoids (mg de β-caroteno.100 g^{-1})		
12% / 15°C	B 24.33 ± 0.40 a	A 22.02 ± 0.57 b	A 19.24 ± 0.36 c
12% / 25°C	B 24.33 ± 0.40 a	B 20.91 ± 0.30 b	B 17.74 ± 0.24 c
16% / 15°C	A 25.61 ± 0.16 a	B 20.48 ± 0.50 b	C 17.01 ± 0.10 c
16% / 25°C	A 25.61 ± 0.16 a	C 17.09 ± 0.11 b	D 14.23 ± 0.10 c
	Value "b"		
12% / 15°C	B 27.88 ± 0.46 a	A 26.14 ± 0.82 b	A 25.19 ± 0.27 b
12% / 25°C	B 27.88 ± 0.46 a	A 25.65 ± 0.95 b	B 24.02 ± 0.21 c
16% / 15°C	A 29.04 ± 0.43 a	A 26.08 ± 0.58 b	B 23.92 ± 0.64 c
16% / 25°C	A 29.04 ± 0.43 a	A 24.83 ± 0.85 b	C 21.21 ± 0.87 c

* Mean of three repetitions followed by different lowercase letters in the same row and uppercase letters in the same column, for the same parameter, are significantly different ($p<0.05$).
** Treatment (% of grain moisture / storage temperature – (°C)).

The yellowing of soybeans, verified by the "*b*" value for colour (Table 2), is influenced by storage conditions and follows the same trends as do carotenoids. It is understood that a decrease in yellow colour is directly related to a reduction of carotenoids in the grain. With increasing grain moisture and storage temperature, the metabolism accelerates, creating grains with higher oxidative enzyme and lipase activities as well as greater polyphenol contents. These increases are followed by increased respiration, which consequently increases the incidence of intermediate radicals derived from lipid peroxidation (NONIER et al., 2004), as well as chemical autoxidation reactions, these being accelerated or delayed according to the temperature at which the oil or the grains are exposed (HOUHOULA et al., 2003). These changes were reflected in the K_{232} and K_{270} values (Table 2) and reduced the amount of carotenoids, which is directly related to the observed changes in grain colour. The results presented in Table 2 are in accordance to the results presented by Yousif (2014), which observed a decrease in "b" value of soybean stored at 13% moisture content at 30 °C for 12 months, as compared to freshly harvested soybean, due to the degradation of carotenoids.

When the grains were stored for 12 months at a temperature of 25 °C, the rates of degradation of carotenoids varied from 33 to 44% for the grains with moisture levels of 12 and 16%, respectively. However, when stored in an environment at 15 ºC, the degradation rates ranged from 21 to 33%, showing that the degradation of this metabolite depends the temperature at which the grains are packed.

Conclusions

Increasing the storage time causes changes in the chemical composition of soybeans, which varies depending on the temperature and moisture at which they are stored. During storage, there are decreases in protein and lipid levels, protein solubility, enthalpy and the thousand grain weight along with increases in the acidity and K_{232} and K_{270} of the lipid fraction of the grains. These are important for the quality control of soybean as raw material for the food industries and agro-energy parameters because it directly affects the derived products.

The acidity parameters of the oil and the thousand grain weight were more dependent on reducing the moisture level, while the protein solubility, thermal properties, carotenoids and colour were more dependent on reducing the temperature. Similar behaviour was observed in relation to the grain insect infestation, wherein cooling to 15 °C controlled re-infestation, even in the grains with higher moisture contents.

These results show that grains cooling during storage can be an appropriate alternative for the storage of grain with higher water contents for short periods, because it helps to control contamination by insects and the preservation of nutritional and technological properties of grain and oil both for food and for biodiesel.

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