

Physicochemical and technological properties of soybean as a function of storage conditions

ABSTRACT

This study aims to evaluate the effect of moisture content (9, 12, 15 and 18%) and storage temperature (11, 18, 25 and 32 °C) on the centesimal composition, bulk density, protein solubility, fat acidity and fatty acid profiles of 12-months stored soybeans. The protein and fat contents decreased after 12 months of storage, with lower values observed in the grains stored with 18% moisture content at 32 °C. Significant decrease was observed in bulk density of grains stored with 15 and 18% moisture content. After 12 months of storage, significant changes in the protein solubility of 32 °C-stored soybeans were observed, regardless of the grain moisture content. The fat acidity was well controlled in grains stored with 9 and 12% moisture content, even at 32 °C. The free fatty acid profile revealed a decrease in linolenic (C18:3) acid and an increase in linoleic (C18:2) acid in grains stored at 15 and 18% moisture content. Grain stored for 12 months with 15 and 18% moisture content at 11 °C showed minimal changes in bulk density, protein solubility, lipid acid and fatty acids profile compared with those metrics determined in soybeans on the first day of storage.

KEYWORDS: *Glycine max.* Fat acidity. Protein solubility. Fatty acid profile.

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INTRODUCTION

Soybean (*Glycinemax*) is the main oilseed crop cultivated worldwide. In 2013, the USA and Brazil accounted for 32.3 and 29.6% of the world soybean production, respectively (FAO, 2016). The grains are considered an important source of proteins for populations from both developed and developing countries. China is the principal soybean importer, where the grains are used as raw material for several food products. According to Liu *et al.* (2008), approximately 60% of industrialized products worldwide possess some soybean constituent in their composition, such as industrialized meat, pasta, soups and canned foods.

Soybean is primarily composed of proteins, which account for approximately 40% of the grain's composition. Approximately 90% of soybean protein is constituted by hydrophilic globulins. The hydrophilic characteristic of soybean globulins determines the extraction yields and the quality of products prepared from soy protein, such as tofu and soymilk. The quality of these products is dependent on the soybean storage conditions (LEE; CHOO, 2012; SMANIOTTO *et al.*, 2014). In addition to the importance of soybean protein, the grains possess approximately 20% oil, which is fully extracted for use in food preparation worldwide. The soybean oil has a high content of poly-unsaturated fatty acids, which are more susceptible to oxidation and enzymatic digestion during storage of the grains than oils mainly composed of saturated fatty acids (NIKOLIC *et al.*, 2014).

In general, soybean production is cyclic because of the changing seasonal climate conditions, making storage a fundamental step for meeting soybean demand year-round. After harvesting, grains are susceptible to changes in their physicochemical, technological and nutritional properties. The main parameters that affect soybean quality during storage include the grain moisture content, storage temperature, relative humidity and storage time (KONG; CHANG, 2013). Recently, long-term storage studies were conducted in maize (PARAGINSKI *et al.*, 2014), lentil (SRAVANTHI *et al.*, 2013), beans (RANI *et al.*, 2013) and rice (PARK *et al.*, 2012) to evaluate the physicochemical and technological properties of the grains. Yousif (2014) studied the effects of soybean storage conditions on grain color, texture and cooking quality. The authors stored soybeans at 9, 11 and 13% moisture content at 10, 20 and 30 °C regimes during 9 months, and reported higher soybean test darkening and cotyledon hardening at the highest moisture content and storage temperature (13% and 30 °C).

Given the importance of soybean preservation in the food industry, it is also necessary to study the changes that occur in protein and lipid fractions as a function of storage conditions. Therefore, the present study aims to evaluate the effects of different grain moisture contents (9, 12, 15 and 18%) and storage temperatures (11, 18, 25 and 32°C) on centesimal composition, bulk density, protein solubility, fat acidity and fatty acid profiles of soybeans stored for 12 months.

MATERIALS AND METHODS

MATERIALS AND STORAGE CONDITIONS

Soybeans (*Glycinemax*) used in this study were harvested in 2013. The harvest site is located on Canguçu country-RS, Brazil, coordinates 31°23'44"S and 52°41'11"W; 408 meters. The grains were harvested mechanically when the moisture content was approximately 22%, placed into raffia bags, and immediately transported to the Postharvest, Industrialization and Quality of Grains Laboratory of DCTA-FAEM-UFPEL, where the study was carried out. The grains were subjected to artificial drying in an oven dryer (model 400-2ND, Nova Ética, Brazil) set at 38°C until they achieved 18, 15, 12 and 9% of moisture, and these were subsequently purged using aluminum phosphide to prevent the interference of insects in the experiment. The dried grains were stored in polyethylene bags composed of 0.2-mm-thick plastic film with a capacity of 0.9 kg at temperatures of 11, 18, 25 and 32°C for 12 months, in triplicate. The grains were covered with aluminum foil to block light. Every 60 days, the bags were replaced, thus avoiding the accumulation of carbon dioxide generated by the respiration and metabolic processes of grains and associated microorganisms. This procedure was similar to what occurs during soybean storage in silos (non-hermetic storage), where the carbon dioxide is removed by an aeration process. For analysis, the grains were ground in a laboratory mill (Perten 3100, Perten Instruments, Huddinge, Sweden) equipped with a 35 mesh sieve to obtain flour with a uniform particle size.

CENTESIMAL COMPOSITION

The moisture content of the soybeans was determined using a drying oven set at 105±3°C, with natural air circulation for 24 h, following the recommendations of the American Society of Agricultural Engineers (ASAE, 2000). The moisture content was expressed as a percentage (%). The fat content was determined following method 30-20 of the American Association of Cereal Chemists (AACC, 1995). The nitrogen content was determined according to AACC method 46-13 (AACC, 1995), and the protein content was obtained using a conversion factor of nitrogen to protein of 6.25. The ash content was determined according to the AACC method 08-01 (AACC, 1995).

BULK DENSITY

The bulk density of the paddy rice was determined using a DalleMolleHectolitre recipient (DalleMolleLtda, Caxias do Sul, Brazil) with a capacity of 0.25 liters. The grains retained in the recipient container were weighed using an analytical balance, and the weight was converted to g.100mL⁻¹.

PROTEIN SOLUBILITY

The protein solubility in water was determined according to the method described by Liu, Mcwatters e Phillips(1992), with some modifications. One gram of soybean flour was dispersed in 50 mL of distilled water with constant stirring for 1 h. The slurry was centrifuged at 5300 x *g* for 20 min, and 2.0 mL of supernatant was collected to determine protein content. The nitrogen content was determined by the Kjeldahl method, and the resultant nitrogen value was converted to protein using a factor of 6.25. The protein solubility, expressed as a percentage (%), was calculated by the ratio of soluble protein content to crude protein content.

FAT ACIDITY

Fat acidity was determined following the titrimetric procedure described in the AACC method 02-01A (AACC, 2000). The titratable acidity was expressed as the mass in milligrams of sodium hydroxide required to neutralize the acids in 100 g of sample using phenolphthalein solution as an indicator.

FATTY ACID PROFILE

A gas chromatograph (GC-14B, Shimadzu, Kyoto, Japan) with a flame ionization detector (FID) and a fused silica capillary column measuring 30 m x 0.25 mm x 0.25 μm DB-225 (50% cyanopropyl methyl and 50% methyl phenyl silicone, J&W Scientific, Folsom, CA, USA) was used. The injector and detector were both maintained at 250 °C. Nitrogen, at a rate of 1.0 mL.min⁻¹, was used as the carrier gas.

The oils obtained from the whole and milled rice grains by continuous extraction using the AACC method 30-20 (AACC, 2000) were used. The derivatization of the fatty acids was performed according to the method of (ZAMBIAZlet *al.*, 2007); briefly, samples of 45 mg of oil were weighed in test tubes with lids, and 1 mL of petroleum ether and 12 mL of 0.5 M HCl in methanol were added. The tubes were vortexed and heated at 65 °C for 1 h. Then, 5 mL of iso-octane and 6 mL of distilled water were added, and the tubes were shaken. The upper layer was partially transferred to a 1.5 mL flask, from which 1.5 μL was taken and injected into the gas chromatograph with a 1:50 split ratio. The initial column temperature of 100 °C was maintained for 0.5 min and then brought up to 150 °C at a rate of 8 °C.min⁻¹. After 0.5 min at 150 °C, the temperature was increased to 180 °C at a rate of 1.5 °C.min⁻¹. The column was held at 180 °C for 5 min and was increased to a final temperature of 220 °C at a rate of 2 °C.min⁻¹. The temperature was maintained for 6 more min, for a total analysis time of 58 min.

The identification of free fatty acids was performed in accordance to the retention time of the chromatographic patterns (myristic, palmitic, stearic, oleic, linoleic, arachidic and behenic acids, all obtained from Sigma-Aldrich Co., USA). The Class-GC10 software (Shimadzu, Kyoto, Japan) was used to acquire and process the GC data.

STATISTICAL ANALYSIS

Analytical determinations for the samples were performed in triplicate, and standard deviations were reported. A comparison of the means was ascertained with Tukey's test to a 5% level of significance using an analysis of the variance (ANOVA).

RESULTS AND DISCUSSION

CENTESIMAL COMPOSITION

There was a decrease in the moisture content of grains stored at 32 °C. The polyethylene bags used in the present study were not impermeable to water vapor. Moreover, the bags were opened every 60 days to remove the carbon dioxide accumulated inside the package due to grain metabolism processes, to simulate the semi-hermetic storage system used in practice. The 32 °C provided a greater water vapor pressure in the grains than in the ambient air, thus reducing the soybean moisture content (Figure 1). Similar behavior was observed by Paraginskiet *al.* (2014), who found a 54.5% reduction in the moisture content of maize grains stored in the same polyethylene bags at the temperature 35 °C after 12 months of storage.

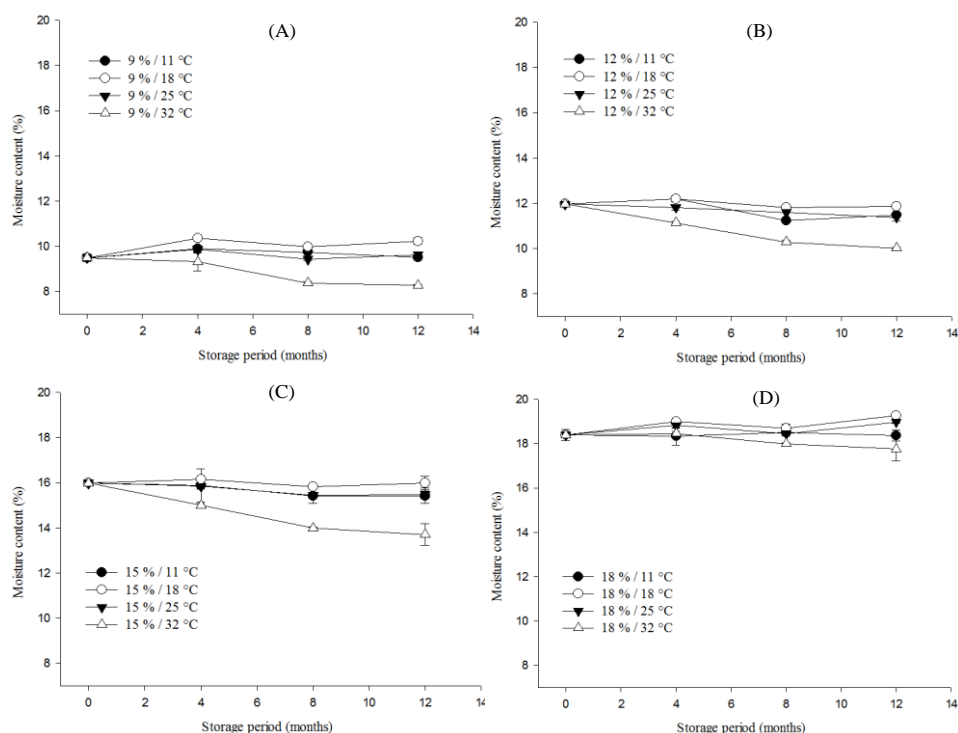


Figure 1 - Changes in moisture content (% wet basis, \pm standard deviation) of soybeans initially stored at 9% moisture content (A), 12% moisture content (B), 15% moisture content (C), and 18% moisture content (D), at different temperatures for 12 months of storage.

The protein content of soybeans as a function of storage conditions is presented in Figure 2. There was a decrease ($p < 0.05$) in the protein content of grains stored at 9, 12 and 15% moisture content under all storage temperatures after 8 months of storage compared with their respective counterparts on the first day of storage. For soybeans stored with 18% moisture content at 32 °C, the protein content was significantly ($p < 0.05$) reduced in the fourth month of storage compared with that of the grains on the first day of storage. Soybeans stored at 18% moisture content at 18 and 25 °C presented a decrease ($p < 0.05$) in the protein content after 8 months of storage. Initially, the protein content of soybeans stored at 9% moisture content was 39.0%. After 12 months of storage at 11°C, the protein content was 38.0%, whereas the grains stored at 18% moisture content at 32 °C, which was the most severe storage condition used in the present study, presented 35.4% of protein content.

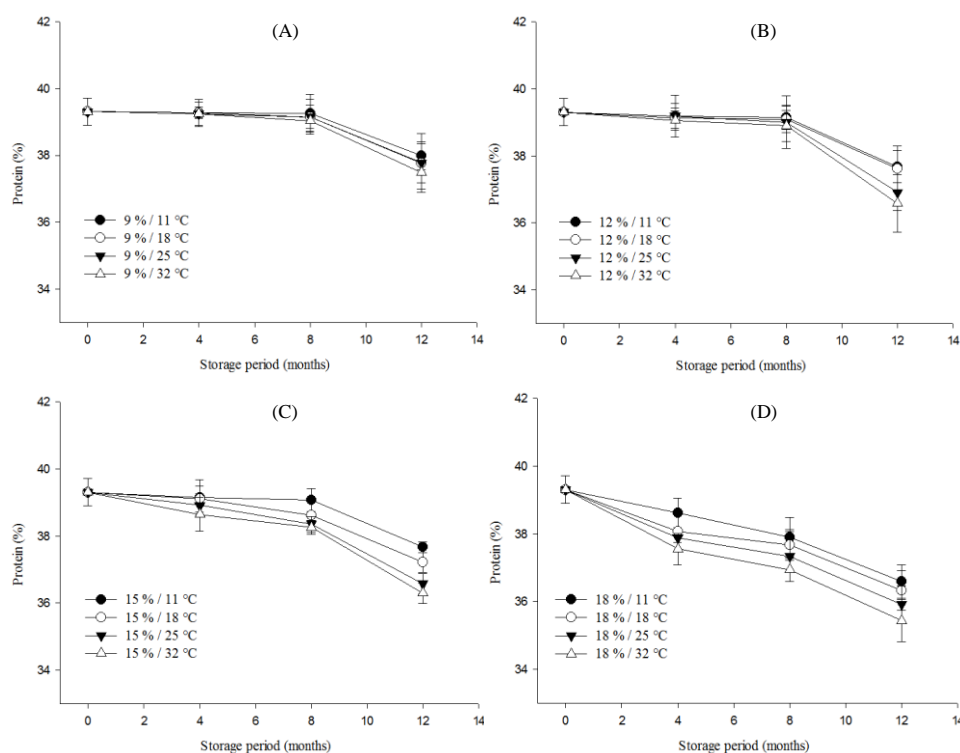


Figure 2 - Changes in protein content (% wet basis, \pm standard deviation) of soybeans stored at 9% moisture content (A), 12% moisture content (B), 15% moisture content (C), and 18% moisture content (D), at different temperatures for 12 months of storage.

A decrease in the protein content of stored soybeans was also observed by Lee and Cho (2012). The authors stored black soybeans for 2 years at ambient temperature, reporting a reduction in the protein content from 43.0% to 38.3% and 33.8% in the first and second years of storage, respectively. According to the same authors, the decrease in protein content as a function of storage may be attributed to the degradation of small peptides and amino acids due to grain metabolic processes.

The fat content decreased for all treatments after 12 months of storage (Figure 3). The most intense decrease in fat content was verified in grains stored at 18% moisture content at 32 °C, decreasing from 24.9 to 19.7% after 12 months

of storage (Figure 3D). Conversely, grains stored at 12% moisture content at 11 °C presented 22.4% fat content, which was the highest fat content found in soybeans stored for 12 months, when comparing all treatments (Figure 3B).

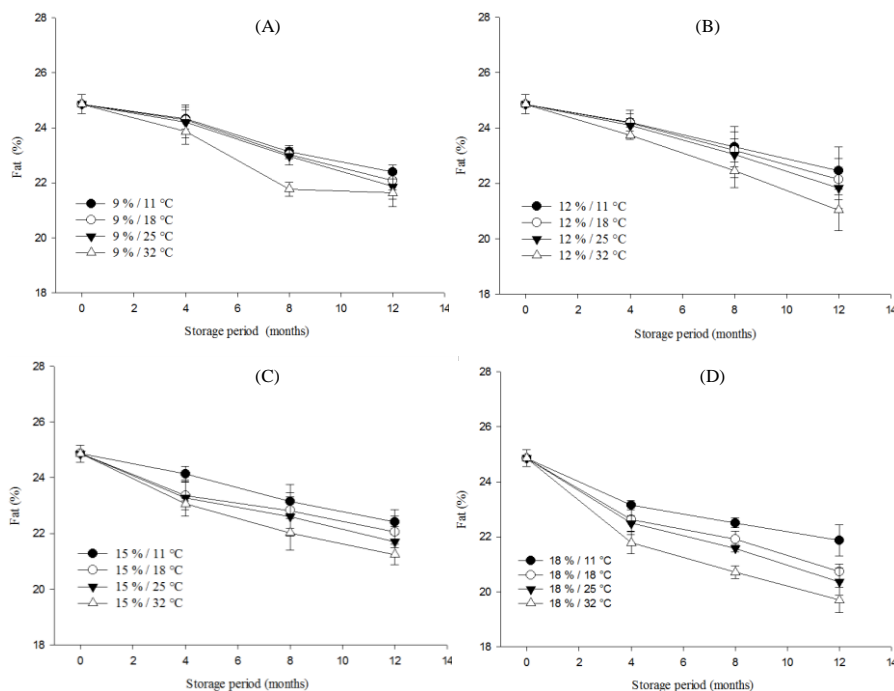


Figure 3 - Changes in fat content (% wet basis, \pm standard deviation) of soybeans stored at 9% moisture content (A), 12% moisture content (B), 15% moisture content (C), and 18% moisture content (D), at different temperatures for 12 months of storage.

These results are in agreement with those presented by Lee and Cho (2012), who reported a 10.8 and 16.7% decrease in fat content of soybean cultivar Milyang 147 stored for 12 and 24 months, respectively. The decrease in fat content during storage may be a result of enzymatic hydrolysis and/or oxidation of fat. As reported by Rani *et al.* (2013), the presence of microorganisms associated with the grains promotes an increase in free fatty acids and a decrease in fat content. Interestingly, the 9% moisture content (Figure 3A) was not advantageous for fat content preservation compared with a 12% moisture content (Figure 3B). This may be explained by the higher time of heat-exposure of grains dried up to 9% moisture content during the drying process, which most likely promoted physical damage to the grain, such as cell wall rupture and general changes in cell structures (Niamnuy *et al.*, 2011).

There was an increase in the ash content in soybeans after 12 months of storage (data not shown). The major increase was determined in grains stored with 18% moisture content at 32 °C, increasing from 5.1 to 6.1%. The increase in ash content may be associated with the decrease in the protein and fat contents, observed in Figures 2 and 3, respectively.

BULK DENSITY

Bulk density is an important tool for evaluating the mass loss of grains during storage. There were no changes ($p < 0.05$) in the bulk density of soybeans stored at 9 and 12% moisture content, even after 12 months of storage. The higher alterations in bulk density were observed in soybeans stored at 15 and 18% moisture content (Figure 4). The most severe storage condition used in the present study of 18% moisture content and 32 °C provided a significant ($p < 0.05$) 8.0% decrease in the bulk density of soybeans after 12 months of storage. The reduction in bulk density is associated with a decrease in the protein and fat contents, as verified in Figures 2 and 3. According to Adhikarinayakea, Palipanab and Muller (2006), the bulk density is dependent on the grain's specific mass. These authors reported a decrease in the caryopsis mass of rice stored at 14.0% moisture content for 6 months at room temperature. The metabolic processes of the grains and associated microorganisms consume cotyledon mass (protein, carbohydrates and primarily fat, which is the most energetic constituent), thus reducing the bulk density value. 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.

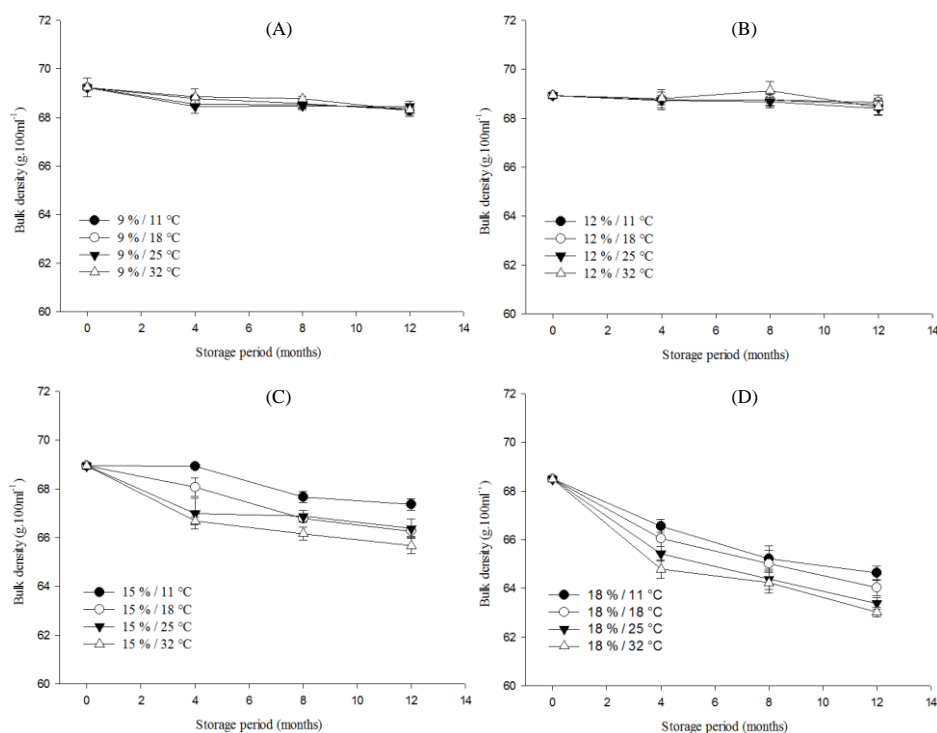


Figure 4 - Changes in bulk density (g/100 mL, \pm standard deviation) of soybeans stored at 9% moisture content (A), 12% moisture content (B), 15% moisture content (C), and 18% moisture content (D), at different temperatures for 12 months of storage.

PROTEIN SOLUBILITY

The protein solubility of soybeans as a function of storage conditions is presented in Figure 5. Initially, on the first day of storage, there was a difference in protein solubility as a function of soybean moisture content. Grains with 9, 12, 15 and 18% moisture contents showed protein contents of 43.8, 49.1, 59.0 and 60.7%, respectively. The lower protein solubility of grains with lower moisture contents reflect the impact of a greater exposure of grains to heat during drying. The air temperature used for drying process was set at 38 °C. Studies by Prachayawarakorn, Prachayawasin and Soponronnarit (2006) reported a 38.5% decrease in the protein solubility of soybeans dried for 10 min at 150 °C compared with non-dried grains. According to the data presented in Figure 5, larger decreases in protein solubility were found in soybeans that were stored at 15 and 18% moisture content at 32 °C, changing from 60.7 and 59.0 to 6.9 and 12.3%, respectively, after 12 months of storage. The grains that were stored for 12 months at 11 °C, even with the highest moisture content of 18% tested in the present study, had 54.6% protein solubility. This fact indicates the importance of decreasing storage temperature to enhance the preservation of protein solubility during a long storage period of soybeans. The highest protein solubility after 12 months of storage was presented by grains stored with 15% moisture content at 11 °C.

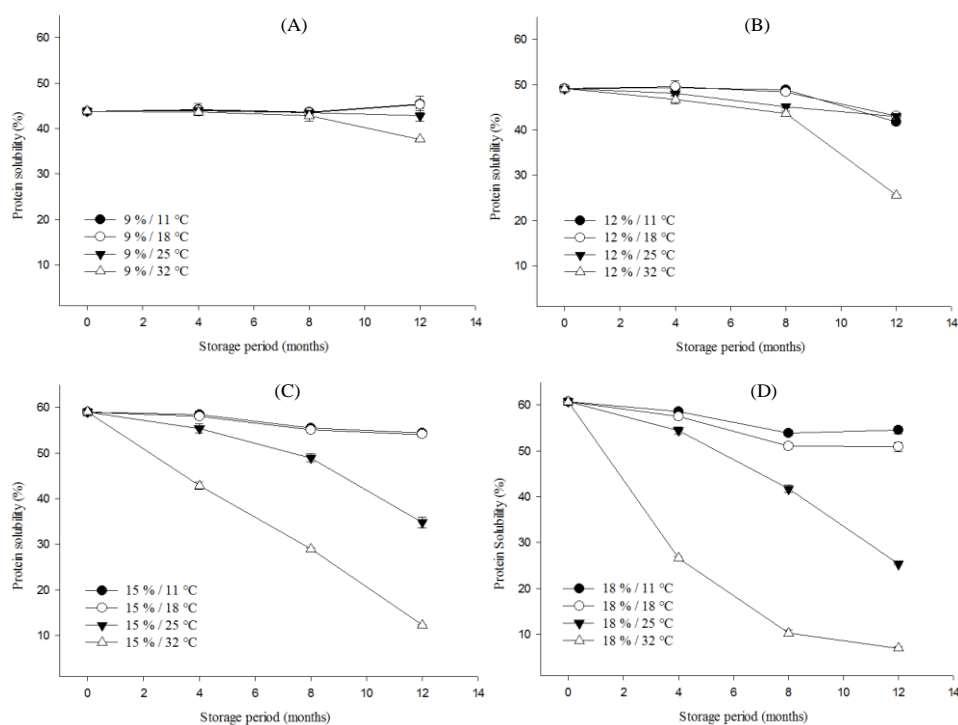


Figure 5 - Changes in protein solubility (% , \pm standard deviation) of soybeans stored at 9% moisture content (A), 12% moisture content (B), 15% moisture content (C), and 18% moisture content (D), at different temperatures for 12 months of storage

Similar results were reported by Liu *et al.* (2008), who observed a decrease in the protein solubility of soybeans stored at 30 °C and 88% relative humidity over 8 months compared with freshly harvested grains. According to Hou and Chang (2004), a decrease in protein solubility is a result of protein denaturation and molecular alterations of β -sheets and disulfide linkages.

FAT ACIDITY

The fat acidity of soybeans stored for 12 months at different moisture content-temperature regimes is presented in Figure 6. There was no difference ($p < 0.05$) in the fat acidity as a function of grain moisture content in the first day of storage. The storage condition of 9% moisture content at 11 °C provided an increase of 42.0% in fat acidity after 12 months of storage, whereas the storage condition of 18% moisture content at 32 °C provided an 1835.0% increase in the fat acidity of soybeans.

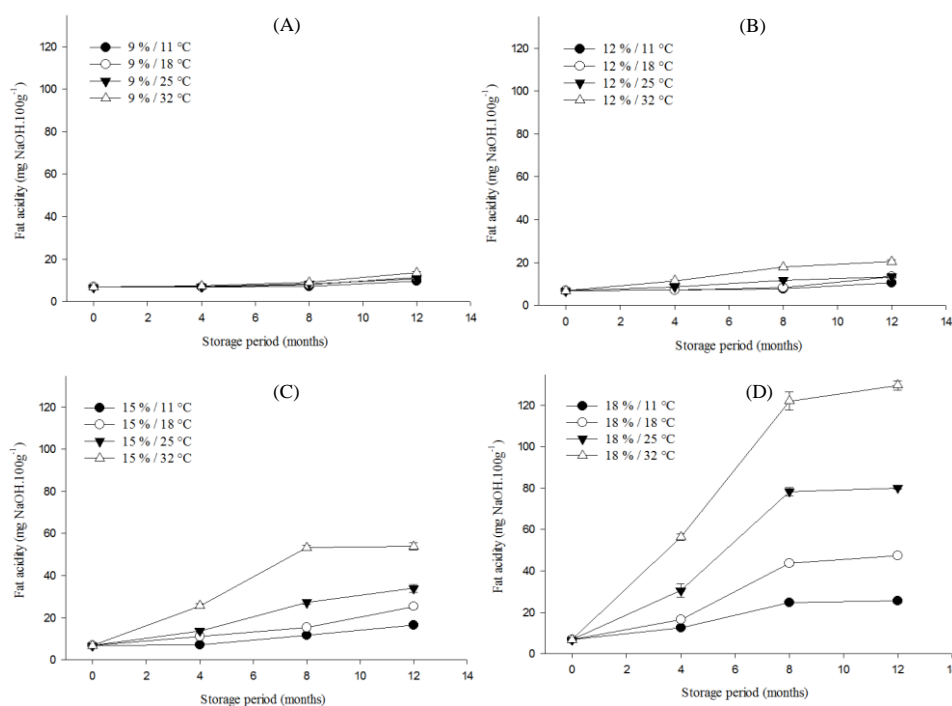


Figure 6 - Changes in fat acidity (mg NaOH/100 g, \pm standard deviation) of soybeans stored at 9% moisture content (A), 12% moisture content (B), 15% moisture content (C), and 18% moisture content (D), at different temperatures for 12 months of storage.

The increase in fat acidity is indicative of fatty acid hydrolysis via the enzymatic attack of the ester linkage between fatty acids and glycerol. The enzymes naturally occur in the grains but can also be produced by molds present in the grains (RAJARAMMANNA, JAYAS and WHITE 2010). Studies by Park *et al.* (2012) reported an increase in the fat acidity of polished rice stored at 16.3% moisture content at 4, 20, 30 and 40 °C over 4 months. Similarly, Rehman, Habib and Zafar (2002) observed an increase in the fat acidity of maize stored at 12.0% moisture content at 10, 25 and 45 °C over 6 months. The high preservation of the initial fat acidity in grains stored at low moisture contents and temperatures is related to the low activity of lipolytic enzymes, primarily lipases, which are well controlled when the grains are conditioned at low moisture content and temperatures.

FATTY ACID PROFILE

Table 1 shows the fatty acid profile of soybeans stored at different moisture contents and different temperatures. Seven fatty acids were identified: palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0) and behenic (C22:0). Only the data from the most representative fatty acids were included in Table 1. There was no difference in the fatty acid profiles of grains stored at 9 and 12% moisture content over 12 months compared with their respective initial treatments (Table 1). However, some changes were observed in the fatty acid profiles of soybeans stored at 15 and 18% moisture content. In general, for soybeans stored at 15 and 18% moisture content, the increase in storage temperature was accompanied by a reduction in linolenic (C18:3) acid and an increase in linoleic (C18:2) acid. For soybeans stored at 15% moisture content, some variations were also observed in palmitic (C16:0) and stearic (C18:0) acids as a function of storage temperature (Table 1). Similar behavior was reported by Lee and Cho (2012), who observed a decrease in linoleic acid (C18:2) content accompanied by an increase in oleic acid (C18:1) content in black soybeans stored for 24 months. Studies by Morelló *et al.* (2004) attributed the decrease in polyunsaturated fatty acids as a function of storage to fatty acids oxidation, which in turn increased the presence of less unsaturated fatty acids.

Table 1 - Effects of grain moisture content and storage temperature on fatty acid profile (%) of soybean stored for 12 months

Treatment	C16:0	C18:0	C18:1	C18:2	C18:3
9% moisture content					
Initial	10.6±0.0 ^{ab*}	4.7±0.1 ^a	18.2±0.0 ^a	55.8±0.1 ^a	9.1±0.1 ^a
11°C	10.5 ± 0.1 ^b	4.8±0.1 ^a	18.4±0.1 ^a	55.8±0.1 ^a	9.2±0.1 ^a
18°C	10.7 ± 0.1 ^{ab}	4.8±0.0 ^a	18.3±0.1 ^a	55.6±0.0 ^a	9.2±0.0 ^a
25°C	10.7 ± 0.1 ^{ab}	4.9±0.1 ^a	18.3±0.1 ^a	55.7±0.1 ^a	9.1±0.1 ^a
32°C	10.8 ± 0.1 ^a	4.9±0.1 ^a	18.2±0.1 ^a	55.7±0.1 ^a	9.2±0.1 ^a
12% moisture content					
Initial	10.7±0.3 ^a	4.7±0.1 ^a	18.5±0.4 ^a	54.6±0.9 ^a	9.1±0.2 ^a
11°C	10.8±0.0 ^a	4.9±0.1 ^a	18.4±0.1 ^a	55.7±0.1 ^a	9.1±0.1 ^a
18°C	10.8±0.2 ^a	5.0±0.0 ^a	18.3±0.2 ^a	55.3±0.1 ^a	9.2±0.2 ^a
25°C	10.8±0.1 ^a	5.1±0.3 ^a	18.4±0.2 ^a	55.3±0.4 ^a	9.1±0.4 ^a
32°C	10.9±0.2 ^a	4.9±0.2 ^a	18.6±0.1 ^a	55.5±0.4 ^a	8.7±0.1 ^a
15% moisture content					
Initial	10.7±0.1 ^{ab}	4.9±0.1 ^a	18.1±0.1 ^b	55.8±0.1 ^b	9.1±0.1 ^a
11°C	10.4±0.1 ^c	4.6±0.1 ^{bc}	18.3±0.1 ^{ab}	55.8±0.1 ^b	8.8±0.1 ^b
18°C	10.5±0.1 ^{bc}	4.5±0.1 ^c	18.0±0.1 ^b	56.2±0.1 ^a	9.1±0.2 ^a
25°C	10.7±0.0 ^{bc}	4.7±0.0 ^{ab}	18.2±0.0 ^{ab}	56.3±0.0 ^a	8.7±0.0 ^b
32°C	10.9±0.1 ^a	4.8±0.0 ^a	18.6±0.2 ^a	56.0±0.1 ^a	8.0±0.1 ^c
18% moisture content					
Initial	10.7±0.1 ^a	4.7±0.1 ^a	18.3±0.1 ^a	55.7±0.3 ^{ab}	9.1±0.0 ^a
11°C	10.8±0.2 ^a	4.8±0.1 ^a	18.4±0.4 ^a	55.6±0.5 ^{ab}	9.0±0.1 ^{ab}
18°C	10.6±0.5 ^a	4.8±0.1 ^a	20.3±2.0 ^a	54.5±1.1 ^b	8.6±0.2 ^{cd}
25°C	10.8±0.1 ^a	5.0±0.1 ^a	18.3±0.1 ^a	55.9±0.1 ^a	8.7±0.1 ^{bc}
32°C	10.8±0.1 ^a	5.0±0.2 ^a	18.3±0.2 ^a	56.3±0.1 ^a	8.3±0.1 ^d

* Means of three determinations ± standard deviations followed by different uppercase letters in the same column, for each moisture content, statistically differ by Tukey's test (p<0.05).

CONCLUSION

In general, grain moisture contents up to 12% and temperatures lower than 25 °C were sufficient for maintaining similar protein content, bulk density, protein solubility, fat acidity and free fatty acid profile in soybeans compared with those metrics determined in soybeans on the first day of storage.

Grain stored for 12 months with 15 and 18% moisture content at 11 °C showed minimal changes in bulk density, protein solubility, lipid acid and fatty acids profile compared with those metrics determined in soybeans on the first day of storage.

Propriedades físico-químicas e tecnológicas da soja em função das condições de armazenamento

RESUMO

Este estudo tem como objetivo avaliar o efeito do conteúdo de umidade (9, 12, 15 e 18%) e temperatura de armazenamento (11, 18, 25 e 32°C) na composição centesimal, peso volumétrico, proteína solúvel, acidez da gordura e o perfil de ácidos graxos de soja armazenada por 12 meses. O conteúdo de proteína e gordura diminuiu após 12 meses de armazenamento, com os menores valores observados nos grãos armazenados com 18% de umidade a 32°C. Redução significativa foi observada no peso volumétrico de grãos armazenados com 15 e 18% de umidade. Após 12 meses de armazenamento, foram observadas alterações significativas na proteína solúvel de soja armazenada a 32°C, independentemente do conteúdo de umidade do grão. A acidez da gordura foi bem controlada em grãos armazenados com 9 e 12% de umidade, mesmo a 32°C. O perfil de ácidos graxos revelou uma diminuição no ácido linolênico (C18: 3) e um aumento no ácido linoleico (C18: 2) em grãos armazenados com 15 e 18% de umidade. Grãos armazenados por 12 meses, com teor de umidade de 15 e 18% a 11 °C apresentaram mínimas alterações na densidade, solubilidade da proteína, acidez lipídica e perfil de ácidos graxos, comparando com esses parâmetros determinados no primeiro dia de armazenamento.

PALAVRAS-CHAVE: Glycinemax. Acidez da gordura. Proteína solúvel. Perfil de ácidos graxos.

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