



Physical alterations of soybean during accelerated and natural aging



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ABSTRACT

The aim of the present study was to investigate the effect of accelerated aging (AA) and natural aging (NA) on the physical characteristics of soybeans (*Glycine max*). Soybeans from two cultivars (Coodetec 214 and BRS 267) were stored under AA (30 °C and 84% RH up to nine months), NA (ambient temperature and RH, fluctuation in the period = 17.3 at 24.5 °C and 59 at 93% RH, up to 18 months), and control conditions (−20 °C and 47% RH). Scanning electron micrographs (SEM) of the hull and cotyledons, the water sorption rate (*S*) during hydration and equilibrium moisture content (X_{eq}), the color of the grains and ground soybeans, and the hardness of cooked soybeans were analyzed. After storage of AA and NA, the surface of the palisade cells of the cotyledons became wrinkled and wilted; the browning of the hull, the ground soybeans, and the hardness of the cooked soybeans increased for both cultivars. It was observed that the increase of *S* and decrease of X_{eq} were more pronounced in Coodetec 214 than BRS 267.

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1. Introduction

Among the legume species, soybean (*Glycine max*) is one of the more idely cultivated oleaginous plants worldwide, with a production of approximately 245 million tons per year (USDA, 2012), and the United States and Brazil are the two largest producers. A large fraction of soybeans are stored or transported from one continent to another by ship. The deterioration of the soybeans may begin during transport because the temperature and relative humidity conditions are difficult to control. The factors associated with the physical and chemical changes in soybean are the following: ambient temperature and relative humidity (RH), moisture content of the soybeans, time of storage, quality of the soybeans, and presence of foreign materials (Kong, Chang, Liu, & Wilson, 2008). Studies also showed that legume deterioration rate during storage may vary according species-and cultivar-related characteristics (Beckert, Miguel, & Filho, 2000; Kong & Chang, 2013; Kong et al., 2008; Santos, Menezes, & Villela, 2005; Stanley, Michaels, Plhak, & Caldwell, 1990; Trawatha, TeKrony, & Hildebrand, 1995).

The ambient relative humidity and water content of the soybeans are more critical than the temperature during storage (Hou & Chang, 1998; Murphy, Chen, Hauck, & Wilson, 1997; Thomas, deMAN, & deMan, 1989). Kong et al. (2008) found that storing soybeans for long

periods (more than one year), ideal storage conditions are temperature of less than 22 °C, RH of 55–60%, and an initial moisture content of the soybean 8–10%. Hou and Chang (1998) observed that soybeans with a high percentage of broken and cracked hulls exhibited larger areas that were more susceptible to chemical reactions and therefore deteriorated after shorter storage times. The presence of foreign materials (soil, dead insects, leaves, sticks, etc.) mixed with the soybeans also accelerated their aging through chemical reactions, fungal growth, among others.

The following factors are evidence of the physical aging of the leguminous grains: change in color, decreased density (Kong et al., 2008; Narayan, Chauhan, & Verma, 1988; Thomas et al., 1989), alterations in the structure of the hull and cotyledons (Cárabez-Trejo, Pares-López, & Reyes-Moreno, 1989), increased water sorption during hydration (Braccini, Reis, Sedyama, Scapin, & Braccini, 1999), and increased hardness of the cooked (Paredes-López, Cárabez-Trejo, Palma-Tirado, & Reyes-Moreno, 1991) and raw (Narayan et al., 1988) grains.

The browning of the hull and cotyledons of the soybean has been explained by several hypotheses, including enzymatic browning reactions that may result from the oxidation of phenolic substrates and/or nonenzymatic browning reactions involving the Maillard reaction (Hou & Chang, 1998; Narayan et al., 1988).

Reduction of the grain density after aging is most likely due to the physical, chemical, and physiological changes that result in water loss, lipid oxidation, protein denaturation, cell membrane rupture, reduced respiratory activity, and loss of vigor (Coutinho et al., 2010; Narayan et al., 1988; Siadat, Moosavi, & Zadeh, 2012).

The water sorption rate (*S*) during the hydration of the grains is the measure of the amount of water absorbed by the grain over a given time

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until reaching equilibrium. The equilibrium moisture content (X_{eq}) indicates the amount of water absorbed by the grains after reaching equilibrium in time tending towards infinity (Sun & Breene, 1991). Both parameters are important in the process of hydration because most leguminous plants must be hydrated to facilitate the processes of grinding, extraction, and/or cooking. The S during soybean hydration depends on time, temperature of the water used for hydration, and soybean cultivar seed size (Beckert et al., 2000; Sun & Breene, 1991). Hsu, Kim, and Wilson (1983) noticed that the rate of water absorption by soybean varied with cultivars and it was correlated with the size and density of the beans studied.

Aging of the soybeans may also influence the S and X_{eq} of the soybean during hydration. Parrish and Leopold (1978) reported that soybean cell membrane deterioration under adverse storage conditions was the main cause for the loss of vigor or germinability, decrease in the respiratory activity, and loss of electrolytes from the grains to the hydration water. Saio et al. (1980) also observed a loss of solids during the hydration of aged soybeans. However, there are no studies in the literature on the S and X_{eq} of soybeans as a function of aging.

The increased hardness of the soybean as a function of aging may be similar to the phenomenon that occurs in leguminous plants, such as the common bean (Garcia & Lajolo, 1994) and peas (Liu, Phillips, Hung, Shewfelt, & McWatters, 1992). During storage under adverse conditions, lipid oxidation and hydrolysis reactions are triggered, resulting in the formation of peroxides and free fatty acids that may promote the rupture of the cell membrane (Priestley, 1986; Trawatha et al., 1995) and a reduction in the pH and solubility of the cotyledon proteins (Thomas et al., 1989). The soybean protein solubility decrease may be related to the denaturation of the proteins 11S and 7S. It could be evidenced by the reduced surface hydrophobicity and total free sulfhydryl content, caused by the increased intramolecular disulfide interactions (Hou & Chang, 2004a, 2004b).

In the common bean, softening of the cotyledons and incomplete gelatinization of the starch during cooking are associated with a reduced separation of the cotyledon cells, as observed by Cárabez-Trejo et al. (1989) using scanning electron microscopy (SEM). Micrographs indicated that the ground raw common bean was composed of clumps of cotyledon cells; cooking soft beans generated cell separation but this did not occur in HTC samples (Garcia-Vela & Stanley, 1989). However, there are few data on the changes at the cellular level for aged soybeans. Silva, Vieira, and Santos (2008) observed anatomical changes in the hypodermal layer of the hull of the soybeans subjected to aging under high temperature and RH using SEM.

The phenomena and alterations associated with the aging of the soybean are complex and gave rise to several hypotheses and some controversies. Therefore, these phenomena and alterations require further studies. The present study investigated some physical changes in the soybean cultivars Coodetec 214 and BRS 267 as a function of accelerated (30 °C and 84% RH) and natural (ambient temperature and humidity) aging.

2. Materials and methods

Two different soybean (*Glycine max*) cultivars were studied: BRS 267 (mainly used to food production such as tofu and soymilk according Benassi, Benassi, & Prudencio, 2011) and Coodetec 214 (mainly used for oil edible extraction). The BRS 267 cultivar are classified by size as large (19.4 g/100 grains) and Coodetec 214 is small (11.7 g/100 grains), according to Yokomizo, Duarte, and Vello (2000). By color, the second one is lighter than the first. Soybean cultivar BRS 267 was grown at the Embrapa Soy experimental field (Embrapa Soja – Londrina PR, Brazil) during the 2008/2009 harvest. Cultivar Coodetec 214 was grown in the municipality of Nova Fátima in Northern Paraná (Brazil) during the 2009 harvest. All the reagents used in this study were of analytical grade.

2.1. Soybean storage

Based on the studies of Hou and Chang (2004a, 2004b, 2004c), three storage conditions were used: accelerated aging (AA) at 84% RH and 30 °C, natural aging (NA) at non-controlled environmental conditions, and control at –20 °C and 47% RH.

The control batch was placed in plastic packages containing 900 g of soybeans and stored at –20 °C and 47% RH.

For AA, a relative humidity of 84% was achieved using five covered plastic boxes with a saturated solution of potassium chloride loaded on the bottom of the receptacle. Soybean samples of 650 g were packed in cloth (tulle) bags (25 × 16 cm) and placed on a metallic rack with a perforated base that separated the sample from the solution. The plastic boxes were placed in ovens (FREEZETEC, model D1004, Brazil) at 30 °C. The temperature and relative humidity inside the plastic boxes were monitored using a thermo-hygrometer (INCOTERM, model 7663.02.0.00, Brazil). Samples of 650 g were removed from the oven at one-month intervals up to nine months and stored under the same conditions of the temperature and RH as the control batch until the time of analysis.

For NA, the soybeans were packed in cotton bags (90 × 40 cm) and stored in a ambient under non-controlled conditions (changes in the period = 17.3 at 24.5 °C and 59 at 93% RH) up to 18 months. At three-month intervals, 650-g samples were placed in plastic packages and stored under the same conditions of the temperature and RH as the control batch until the time of analysis.

2.2. Experimental design

Four experiments were performed, one for each cultivar under both storage conditions (Coodetec 214-AA, Coodetec 214-NA, BRS 267-AA and BRS 267-NA), whereas time zero was represented by the control batch. The experimental design was fully randomized with two repetitions. All analyses were performed in duplicate for each repetition.

3. Physical analysis

3.1. Color of grains and ground soybeans

The color parameters of the grains and ground soybeans were measured using a colorimeter (BYK GARDNER, model Color Guide 45/0, Germany) with CIE-D65 illuminant and 45/0 geometry. The samples were placed in transparent plastic bags, and twelve measurements were performed daily on their surface. The colorimeter directly provided the parameters L^* (lightness), a^* (red–green), and b^* (blue–yellow), and the hue was calculated ($h^\circ = \arctan [b^* / a^*]$).

3.2. Scanning electron microscopy (SEM) of hulls and cotyledons

The samples were prepared following Kao, Su, and Lee (2003) with some modifications. The soybeans were subjected to fixation with 3% glutaraldehyde, 2% paraformaldehyde, and 0.1 M phosphate buffer at pH 7.0 for 24 h at room temperature. Next, the soybeans were fractured in liquid nitrogen. A small fraction of the cotyledons and hulls was rinsed three times for 15 min with 0.1 M phosphate buffer at pH 7.0 and fixed with 1% OsO₄ for one hour at room temperature. Next, the samples were subjected to dehydration in 70% ethanol (twice for 15 min), 80% ethanol (twice for 15 min), 90% ethanol (three times for 15 minutes), and 100% ethanol (four times for 10 min). Finally, they were placed in a vacuum evaporator (CPD 010, Bal-Tec, Germany) to remove the ethanol by sublimation. The dry samples were fixed on the surface of an aluminum stub and then coated with gold (Sputter Coater model SCD 050, Bal-Tec, Germany). The structure of the samples was observed using SEM at 20 kV (Quanta 200 model, FEI, Netherlands).

3.3. Water sorption kinetics

The S and X_{eq} of the soybeans during hydration were measured using the method described by Sun and Breene (1991). Approximately 10 g of soybeans with the hulls was immersed in 50 mL of distilled water and kept at 25 °C for 24 h. At two-hour intervals over 10 h, and 24 h later, the soybeans were drained on a sieve for 30 s and then weighed. The amount of water absorbed (A) was calculated as the difference between the sample mass at each hydration interval and the initial mass. The values of S and X_{eq} were established using the model shown in Eq. (1) and the software Statistica 7.0 (Statsoft, 2004) under the non-linear estimation module:

$$A = \frac{S \times X_{eq} \times t}{1 + t \times S} \quad 1$$

where:

- A = amount of absorbed water (g absorbed water/100 g soybeans),
- S = water sorption rate (g absorbed water/100 g soybeans $\times$ h),
- X_{eq} = equilibrium moisture content (g absorbed water/100 g soybeans $\times$ h)
- t = hydration time (h)

3.4. Hardness of cooked soybeans

The hardness of the cooked soybeans was measured using a texturometer (TA.XT2i, Stable Micro Systems, England) according to the method described by Liu et al. (1992). A total of 15 g of soybeans was hydrated in distilled water at a ratio of 1:5 (m/v) for four hours at room temperature. The soybeans were drained, placed in cloth (sheer mesh) bags, and cooked in pre-heated water at 100 °C at a ratio of 1:20 (grain:water, m/v) for 90 min. Next, the soybeans were cooled in an ice bath until attaining room temperature. A total of 30 cooked soybeans randomly chosen from each treatment were compressed at 2.0 mm/s constant speed using a 25-mm diameter cylindrical aluminum sensor. The results for the hardness measures are expressed in Newtons (N).

3.5. Statistical analysis

The data were subjected to analysis of variance and Tukey's means comparison test using software Statistica 7.0 (Statsoft, 2004).

4. Results and discussions

4.1. Color of whole and ground soybeans

The color of the control cultivar Coodetec 214 is lighter than the corresponding BRS 267 ($p \leq 0.05$, not shown in Tables 1 and 2), but under both investigated storage conditions, the whole and ground soybeans of both cultivars exhibited a reduction ($p \leq 0.05$) in L^* (lightness) and h^* (hue). The decrease in h^* was more remarkable in the ground grains compared with the whole soybeans (Tables 1 and 2). The reduced L^* and h^* denote browning and a change of color from yellow to red as a function of the aging conditions, respectively. Kong et al. (2008) observed the same color change behavior in soybeans stored under several different conditions. Hou and Chang (2004c) suggested that for the production of tofu, the color parameters of the soybeans might be used as indicators of their quality during storage. The change in the color during soybean storage may be mainly attributed to nonenzymatic reactions, particularly to the Maillard reaction (Hou & Chang, 1998). Another factor that may promote the browning of soybeans during

Table 1

Color parameters of soybeans of cultivars Coodetec 214 and BRS 267 under different storage conditions.

Storage Conditions	Time (Month)	Coodetec 214		BRS 267	
		L^*	h^*	L^*	h^*
C AA	0	53.9 $\pm$ 1.1 ^{a, A}	71.7 $\pm$ 1.9 ^{a, A}	51.1 $\pm$ 0.6 ^{a, A}	70.6 $\pm$ 2.3 ^{a, A}
	1	53.3 $\pm$ 0.4 ^a	70.6 $\pm$ 1.3 ^{ab}	50.1 $\pm$ 0.5 ^b	71.3 $\pm$ 2.4 ^a
	2	51.7 $\pm$ 0.5 ^b	68.9 $\pm$ 2.4 ^{ab}	49.5 $\pm$ 0.7 ^{bc}	69.2 $\pm$ 2.0 ^a
	3	50.5 $\pm$ 0.6 ^c	69.5 $\pm$ 2.0 ^{ab}	49.3 $\pm$ 0.5 ^c	70.4 $\pm$ 2.0 ^a
	4	50.4 $\pm$ 0.5 ^{bc}	70.0 $\pm$ 2.3 ^{ab}	49.2 $\pm$ 0.6 ^d	69.7 $\pm$ 1.9 ^a
	5	49.6 $\pm$ 0.5 ^{de}	68.5 $\pm$ 1.4 ^b	48.1 $\pm$ 0.6 ^d	68.6 $\pm$ 2.3 ^a
	6	49.2 $\pm$ 0.5 ^e	69.2 $\pm$ 1.8 ^{ab}	47.9 $\pm$ 0.5 ^d	68.3 $\pm$ 2.4 ^a
	7	49.2 $\pm$ 0.6 ^e	69.3 $\pm$ 1.9 ^{ab}	46.6 $\pm$ 0.9 ^e	69.7 $\pm$ 1.9 ^a
	8	47.1 $\pm$ 0.5 ^f	69.9 $\pm$ 2.3 ^{ab}	45.5 $\pm$ 0.4 ^f	70.5 $\pm$ 3.3 ^a
NA	9	46.1 $\pm$ 0.7 ^g	68.8 $\pm$ 3.3 ^b	44.5 $\pm$ 0.4 ^g	69.9 $\pm$ 3.1 ^a
	3	53.2 $\pm$ 0.7 ^A	71.0 $\pm$ 2.1 ^{AB}	50.3 $\pm$ 0.4 ^B	70.4 $\pm$ 2.1 ^A
	6	52.0 $\pm$ 0.6 ^B	69.4 $\pm$ 2.3 ^{AB}	49.3 $\pm$ 0.2 ^C	68.9 $\pm$ 1.9 ^{AB}
	9	50.8 $\pm$ 0.8 ^C	68.6 $\pm$ 2.4 ^B	47.5 $\pm$ 0.5 ^D	68.4 $\pm$ 2.3 ^{AB}
	12	49.4 $\pm$ 0.5 ^D	69.1 $\pm$ 2.0 ^B	46.3 $\pm$ 0.5 ^E	68.5 $\pm$ 2.9 ^{AB}
	18	49.2 $\pm$ 0.4 ^D	68.6 $\pm$ 2.0 ^B	47.1 $\pm$ 0.8 ^F	67.2 $\pm$ 2.5 ^B

Means of four measurements for each condition $\pm$ standard deviation.

Means followed by different letters in the columns for each aging experiment (small letters for AA; capital letters for NA) exhibit significant differences ($p \leq 0.05$).

L^* (lightness): 0 = black, 100 = white.

h^* (hue): $\arctan(b^*/a^*)$, 0° = pure red, 90° = pure yellow, 180° = pure green, 270° = pure blue.

C = control (storage time zero): -20 °C and 47% RH.

AA = accelerated aging: 30 °C and 84% RH, up to 9 months.

NA = natural aging: ambient temperature and RH (changes in the period = 17.3 at 24.5 °C and 59 at 93% RH, up to 18 months).

storage is the accelerated polymerization of low-molecular-weight tannins (Nozzolillo & Bezada, 1984).

4.2. Scanning electron microscopy (SEM) of hulls and cotyledons

The SEM results did not show any changes in the soybeans hulls associated with the aging conditions (AA and NA) in either investigated cultivar (Fig. 1A, B, C, D, E, and F), which is in contrast with the results of Silva et al. (2008), who observed a reduced thickness of the palisade

Table 2

Color parameters of ground soybeans of cultivars Coodetec 214 and BRS 267 under different storage conditions.

Storage Conditions	Time (Month)	Coodetec 214		BRS 267	
		L^*	h^*	L^*	h^*
C AA	0	82.5 $\pm$ 0.8 ^{a, A}	83.8 $\pm$ 0.5 ^{a, A}	83.0 $\pm$ 0.4 ^{a, A}	83.5 $\pm$ 0.5 ^{a, A}
	1	82.5 $\pm$ 0.9 ^a	83.2 $\pm$ 0.6 ^a	83.2 $\pm$ 0.4 ^a	83.7 $\pm$ 0.5 ^a
	2	81.8 $\pm$ 0.5 ^{ab}	82.1 $\pm$ 0.7 ^{bc}	83.0 $\pm$ 0.6 ^a	83.5 $\pm$ 0.7 ^a
	3	81.7 $\pm$ 0.5 ^{ab}	82.2 $\pm$ 0.7 ^b	82.1 $\pm$ 0.6 ^b	82.6 $\pm$ 0.4 ^b
	4	81.5 $\pm$ 0.8 ^b	82.4 $\pm$ 0.5 ^b	82.0 $\pm$ 0.5 ^b	82.1 $\pm$ 0.5 ^{bc}
	5	80.6 $\pm$ 0.7 ^c	81.3 $\pm$ 0.6 ^{cd}	81.0 $\pm$ 0.4 ^c	81.6 $\pm$ 0.6 ^{cd}
	6	79.5 $\pm$ 0.6 ^d	80.6 $\pm$ 0.6 ^d	81.3 $\pm$ 0.5 ^c	81.2 $\pm$ 0.4 ^d
	7	79.9 $\pm$ 0.5 ^{cd}	80.6 $\pm$ 0.7 ^d	79.4 $\pm$ 0.8 ^d	79.7 $\pm$ 0.6 ^e
	8	76.4 $\pm$ 0.8 ^e	77.9 $\pm$ 0.5 ^e	76.7 $\pm$ 0.6 ^e	77.1 $\pm$ 0.5 ^f
NA	9	75.2 $\pm$ 0.3 ^f	75.0 $\pm$ 0.6 ^f	75.6 $\pm$ 0.5 ^f	75.9 $\pm$ 0.6 ^g
	3	82.3 $\pm$ 0.5 ^A	83.8 $\pm$ 0.5 ^A	82.9 $\pm$ 1.0 ^A	83.4 $\pm$ 0.8 ^A
	6	81.7 $\pm$ 0.5 ^{BC}	82.3 $\pm$ 0.5 ^{BC}	82.6 $\pm$ 0.4 ^{AB}	82.9 $\pm$ 0.5 ^{AB}
	9	81.8 $\pm$ 0.6 ^{BC}	82.5 $\pm$ 0.3 ^B	82.1 $\pm$ 0.8 ^{BC}	82.5 $\pm$ 0.8 ^{BC}
	12	81.3 $\pm$ 0.4 ^C	81.9 $\pm$ 0.5 ^{CD}	82.0 $\pm$ 0.5 ^C	82.2 $\pm$ 0.5 ^{BC}
	18	79.6 $\pm$ 0.6 ^C	81.7 $\pm$ 0.4 ^D	81.5 $\pm$ 0.4 ^C	82.0 $\pm$ 0.6 ^C

Means of four measurements for each condition $\pm$ standard deviation.

Means followed by different letters in the columns for each aging experiment (small letters for AA; capital letters for NA) exhibit significant differences ($p \leq 0.05$).

L^* (lightness): 0 = black, 100 = white.

h^* (hue): $\arctan(b^*/a^*)$, 0° = pure red, 90° = pure yellow, 180° = pure green, 270° = pure blue.

C = control (storage time zero): -20 °C and 47% RH.

AA=accelerated aging: 30 °C and 84% RH, up to 9 months.

NA = natural aging: ambient temperature and RH (changes in the period = 17.3 at 24.5 °C and 59 at 93% RH, up to 18 months).

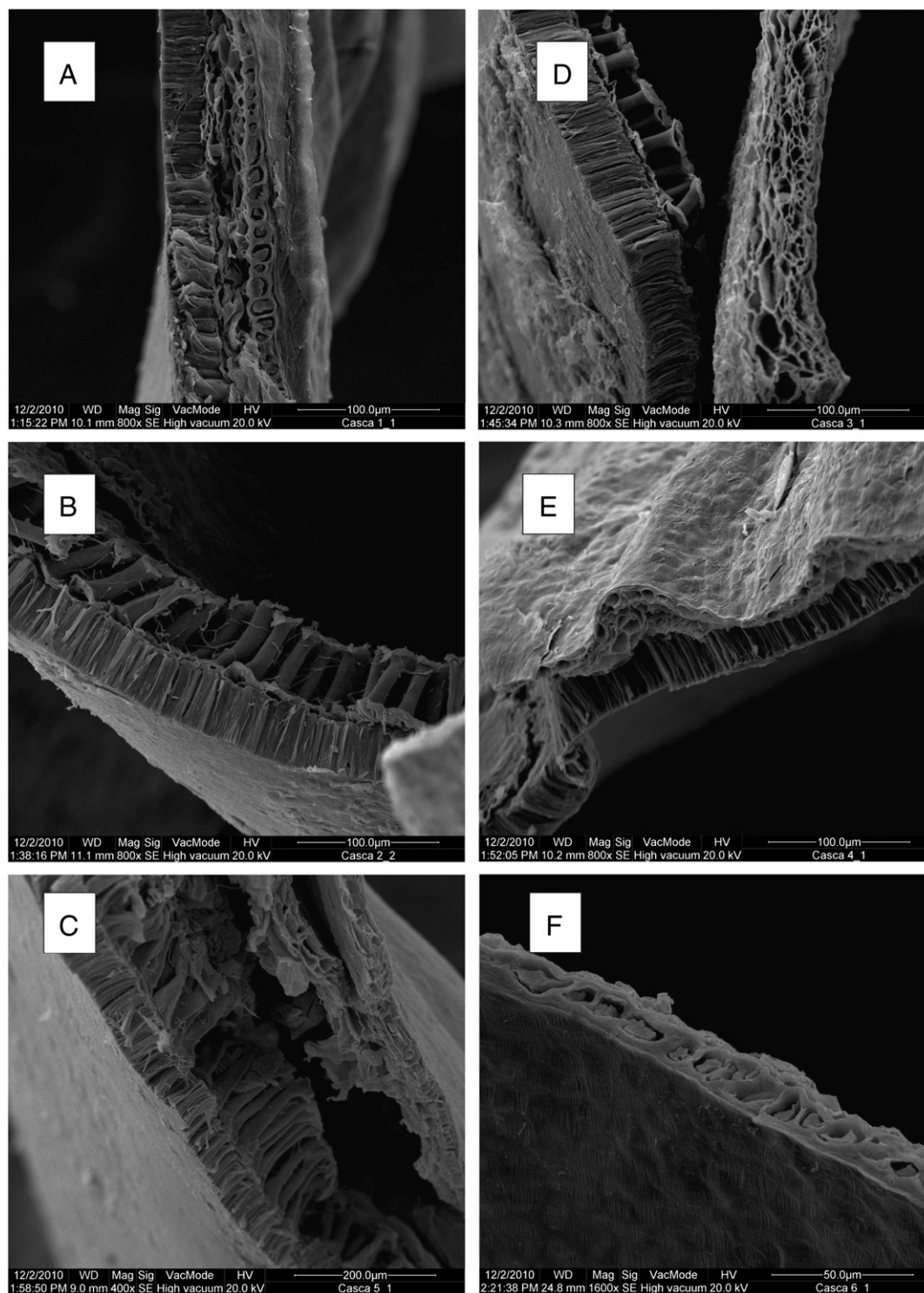


Fig. 1. Micrograph of palisade cells of soybean hulls. A = Coodetec 214 (C); B = Coodetec 214 (AA); C = Coodetec 214 (NA); D = BRS 267 (C); E = BRS 267 (AA); F = BRS 267 (NA). C = control storage: -20°C and 47% RH; AA = accelerated aging: 30°C and 84% RH for nine months; NA = natural aging: 21°C and 69% RH for 18 months.

cells and the hypodermal and parenchymal layers of the hulls of the soybeans subjected to aging at 42°C and 100% RH for 48, 72, and 96 h, suggesting collapse of the cells composing such layers. No other studies on the microstructural alterations of the soybean during storage could be located.

In the common bean, according to the review by [Reys-Moreno and Paredes-López \(1993\)](#), most studies on aging found more alterations in the cotyledons than in the hulls. However, [Cárabez-Trejo et al.](#)

(1989) observed the compaction of the palisade and mesophylic cells of the hulls in two varieties of common bean stored for 120 days at 35°C and 76% RH, which they correlated with alterations in the cell membranes or the loss of integrity of the cell membranes.

The present study demonstrated similar alterations in the surface of the palisade cells of both cultivars the cotyledons after the storage. The surface of the cells of the control sample was smooth ([Fig. 2A and D](#)), whereas the surface of the cells of the samples stored under the AA

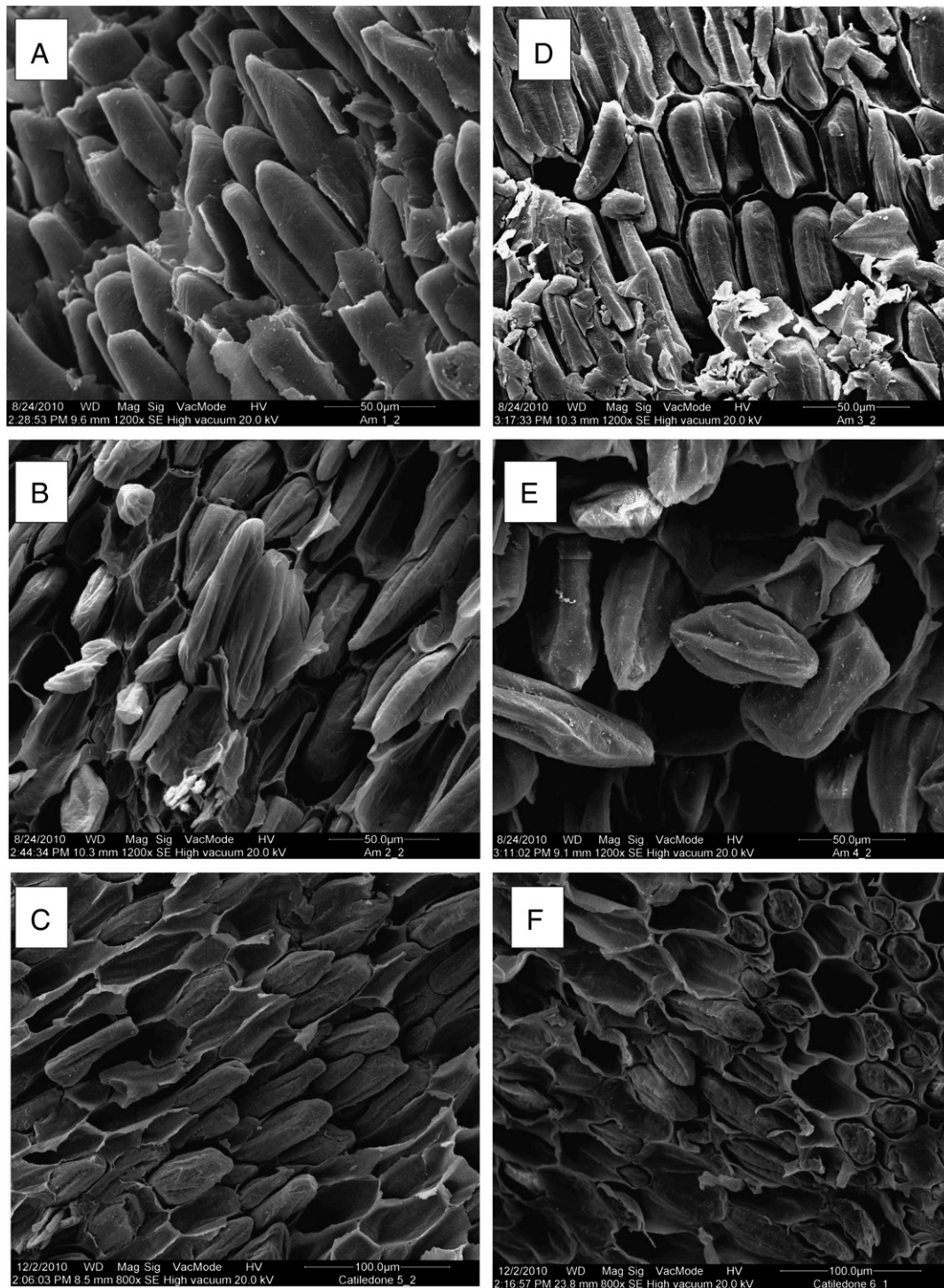


Fig. 2. Micrograph of palisade cells of soybean cotyledons. A = Coodetec 214 (C); B = Coodetec 214 (AA); C = Coodetec 214 (NA); D = BRS 267 (C); E = BRS 267 (AA); F = BRS 267 (NA). C = control storage: -20°C and 47% RH; AA = accelerated aging: 30°C and 84% RH for nine months; NA = natural aging: 21°C and 69% RH for 18 months.

conditions for nine months and NA conditions for 18 months was wrinkled and wilted (Fig. 2B, C, E, and F). These physical alterations on the surface of the palisade cells of the cotyledons may be due to water loss caused by chemical changes in the lipid and protein molecules of the soybeans stored at high temperature and RH, as observed by several authors (Hou & Chang, 2004a, 2004b, 2004c; Narayan et al., 1988; Saio et al., 1980). The mechanism for the wilting of the surface of the palisade cells of the cotyledons of aged soybeans may be related to lipid hydrolysis and oxidation reactions, increased acidity, and subsequent rupture of

the cell membranes, resulting in a surface more permeable to water and electrolyte loss (Siadat et al., 2012; Trawatha et al., 1995).

4.3. Water sorption kinetics

The model (Eq. (1)) used to calculate the S and X_{eq} adequately represented the water absorption kinetics of both cultivars the soybean (control and stored beans), whereas the value of the coefficient of determination (R^2) varied between 0.94 and 0.98.

The values of S and X_{eq} of cultivars Coodetec 214 and BRS 267 at the different times of AA and NA are described in Table 3. There was an increase in the S for both investigated cultivars under the AA conditions from the second month onwards; on the ninth month, the values of S in cultivars Coodetec 214 and BRS 267 were approximately four- and threefold higher compared with the control, increased from 5.4 to 19.3 and 5.0 to 15.3 g absorbed water/100 g soybeans $\times$ h, respectively. Under the NA conditions, S also increased approximately three- and twofold in cultivars Coodetec 214 and BRS 267 compared with the control, increased from 5.4 to 13.1 and 5.0 to 10.4 g absorbed water/100 g soybeans $\times$ h, respectively. This phenomenon is most likely due to physical alterations in the membrane of the palisade cells of the cotyledons, the surface of which became wrinkled and wilted after aging (Fig. 2B, C, E and F). Thus, we can infer that when a cell is wilted (aged soybean) it tends to absorb water more quickly than a cell hydrated (early harvested soybeans).

The value of X_{eq} decreased in the stored soybeans after three months under AA ranging from 152.1 to 137.8 g absorbed water/100 g soybeans $\times$ h to cultivar Coodetec 214 and 145.6 to 130.2 g absorbed water/100 g soybeans $\times$ h to BRS 267 (Table 3). These results may be due to the deterioration of the cell membranes and loss of water and electrolytes (Braccini et al., 1999; Santos et al., 2005; Siadat et al., 2012; Silva et al., 2008; Trawatha et al., 1995) as well as to alterations in the water retention ability of aged soybean proteins and carbohydrates (Narayan et al., 1988; Yoshino, Iwasaki, Okubo, & Okuyama, 1997). Hou and Chang (2004a, 2004b) found alterations in the structure of protein fractions 11S and 7S, such as reduced surface hydrophobicity (2.7 to 2.4 and 3.4 to 3.0 cps $\times 10^6$ for the 11S and 7S fractions, respectively) and increased intra-molecular disulfide bridges (15.2 to 18.6 and 1.2 to 2.0 mol/mol of protein for the 11S and 7S fractions, respectively), but there was no alteration in the molecular mass of the soybeans stored at 84% RH and 30 °C for control and seven months, respectively, thus indicating that the molecules were more aggregated or contracted/shrunk. When molecules are more aggregated, the availability of the water binding sites is decreased, and thus, water retention becomes more difficult. The reduction in the X_{eq} values might also be due to the loss of electrolytes or other components of the soybeans, such as proteins and free sugars, secondary to the loss of integrity of the cell membranes as a function of the adverse storage conditions (Saio et al., 1980; Santos et al., 2005; Siadat et al., 2012). Therefore, the time needed to hydrate the aged soybeans might be shorter than for new grains.

Table 3
Water sorption rate (S) and equilibrium moisture content (X_{eq}) of soybeans during hydration as a function of the storage conditions.

Storage Conditions	Time (Month)	Coodetec 214		BRS 267	
		S	X_{eq}	S	X_{eq}
C	0	5.4	150.4	5.0	155.4
AA	1	5.3	156.8	4.6	157.6
	2	6.6	157.4	6.4	150.3
	3	8.7	152.1	7.3	145.6
	4	9.9	149.0	7.4	145.4
	5	13.3	143.8	9.0	137.4
	6	13.6	141.0	10.7	136.2
	7	15.5	141.1	11.5	133.6
	8	18.4	138.6	13.0	133.3
	9	19.3	137.8	15.3	130.2
NA	3	6.1	148.8	5.7	153.6
	6	5.9	152.6	6.7	148.0
	9	7.9	157.1	9.4	140.2
	12	9.1	155.7	9.7	145.9
	18	13.1	145.7	10.4	146.9

S = water sorption rate (g water/100 g soybeans/h).

X_{eq} = equilibrium moisture content (g water/100 g soybeans/h).

C = control (storage time zero): –20 °C and 47% RH.

AA = accelerated aging: 30 °C and 84% RH up to 9 months.

NA = natural aging: ambient temperature and RH (changes in the period = 17.3 at 24.5 °C and 59 at 93% RH, up to 18 months).

The effect of soybean aging on the water absorption (increase in S and decrease X_{eq}) by grains resulted in similar behavior for both cultivars. Although, was more pronounced in Coodetec 214 probably because its grains are smaller and thus show a larger surface to contact with water during hydration. Beckert et al., 2000 reported that soybeans seed of small size showed a higher water absorption rate decreasing the seed germination potential. Stanley et al. (1990) verified that the amount of water absorbed following storage (six months at 30 °C/85% RH) of twenty cultivars of common bean was related to cultivar color, seed volume and helium area but not seed coat thickness.

4.4. Hardness of cooked soybeans

The values of hardness for the cooked soybeans of cultivars Coodetec 214 and BRS 267 as a function of the AA and NA conditions are described in Table 4. Comparing the grain controls, Coodetec 214 have a higher hardness than the BRS 267 ($p \leq 0.05$, not shown in Table 4). Both cultivars exhibited similar increased hardness ($p \leq 0.05$) as a function of storage time (2.8-fold for AA and 1.8-fold for NA). Narayan et al. (1988) found increased hardness of raw soybeans stored under room temperature and humidity conditions for one, two, three, and nine years.

Studies on the hardness of cooked soybeans as a function of the time and conditions of storage were not reported in the literature. The parameter of cooked grain hardness as a function of aging have been more often studied in other leguminous plants, such as the common bean (Garcia & Lajolo, 1994; Reys-Moreno & Paredes-López, 1993) and peas (Liu et al., 1992), which are more commonly consumed cooked. However, the hardening mechanism of grains from other leguminous plants is likely different from that of soybean because the latter has high protein and lipid contents.

The increased hardness of cooked soybeans as a function of the time and conditions of storage might be due to possible chemical alterations of its main components: lipids (20%) and proteins (40%). It is possible that the sequence of the events involved in the hardening of cooked soybeans might begin during storage with lipid oxidation and the subsequent formation of peroxides and free fatty acids, reduction in the pH, rupture of the cell membrane and water loss, and denaturation of the main protein fractions, 11S and 7S (Kong & Chang, 2013). Hardening might also be associated with the polymerization of phenolic compounds (phenolic acids

Table 4
Hardness of cooked soybeans of cultivars Coodetec 214 and BRS 267 under different storage conditions.

Storage Conditions	Time (Month)	Hardness of cooked grain (N)	
		Coodetec 214	BRS 267
C	0	14.5 $\pm$ 4.0 ^{g, D}	12.9 $\pm$ 3.3 ^{h, D}
AA	1	16.8 $\pm$ 5.4 ^{fg}	14.3 $\pm$ 4.0 ^{gh}
	2	19.2 $\pm$ 5.8 ^{ef}	16.6 $\pm$ 4.7 ^{fg}
	3	20.7 $\pm$ 6.6 ^e	18.2 $\pm$ 6.8 ^{ef}
	4	24.4 $\pm$ 8.4 ^d	18.9 $\pm$ 4.9 ^{ef}
	5	28.3 $\pm$ 8.9 ^c	21.7 $\pm$ 7.1 ^{cd}
	6	31.3 $\pm$ 11.6 ^{bc}	22.6 $\pm$ 7.1 ^{de}
	7	32.7 $\pm$ 10.1 ^b	25.7 $\pm$ 9.0 ^c
	8	36.9 $\pm$ 11.0 ^a	30.7 $\pm$ 14.0 ^b
	9	40.0 $\pm$ 9.9 ^a	36.8 $\pm$ 16.5 ^a
NA	3	18.0 $\pm$ 6.3 ^C	14.8 $\pm$ 3.3 ^C
	6	17.9 $\pm$ 6.2 ^C	15.6 $\pm$ 3.6 ^C
	9	19.7 $\pm$ 5.8 ^{BC}	17.4 $\pm$ 4.5 ^B
	12	21.7 $\pm$ 6.9 ^B	18.5 $\pm$ 5.2 ^B
	18	26.8 $\pm$ 5.3 ^A	22.4 $\pm$ 3.9 ^A

Means of four measurements for each condition $\pm$ standard deviation.

Means followed by different letters in the columns for each aging experiment (small letters for AA; capital letters for NA) exhibit significant differences ($p \leq 0.05$).

C = control (storage time zero): –20 °C and 47% RH.

AA = accelerated aging: 30 °C and 84% RH up to 9 months.

NA = natural aging: ambient temperature and RH (changes in the period = 17.3 at 24.5 °C and 59 at 93% RH, up to 18 months).

and flavonoids) into high-molecular-weight phenolic polymers (tannins and lignins) containing enough hydroxyl groups to form stable cross-links with proteins and carbohydrates, similar to the processes that occur in the common bean (Garcia-Vela & Stanley, 1989; Hincks & Stanley, 1987). Cooking makes the alterations that occur during aging become evident, and the grains fail to soften. The denatured proteins aggregate, i.e., coagulate and form a physical barrier that hinders the entrance of enough water to allow for the separation of cells and that limits the decomposition and solubilization of the cell wall pectin during cooking (Liu et al., 1992). Garcia-Vela, Del Valle, and Stanley (1991) showed that softening black beans by soaking them in an aqueous salt solution was affected by cations as well as anions. Liu, Phillips, and McWatters (1993) suggest that one part of HTC inducement occurs through loss of the cell membrane integrity, which allows cations to migrate inside cells to intracellular components, causing HTC defect.

The physical alterations of aged soybeans might be due to physiological (decreased respiratory activity and germinability) and chemical alterations that become irreversible when they begin during storage. Further studies are needed to elucidate the mechanism of these complex phenomena and to identify the means to avoid losses in the quality of the soybeans during storage, thus contributing to the industry of soy products.

5. Conclusion

The storage under the ambient and adverse conditions during long period of time of two different soybeans cultivars Coodetec 214 and BRS 267 causes darkening of the grain, wrinkling and wilting of the surface of palisade cells of the cotyledons, increasing of water sorption rate during hydration of the grain and hardness of the cooked grain. The increasing of water sorption rate during hydration of grains and decreasing of equilibrium moisture content were more pronounced in Coodetec 214 than in BRS 267. All of these physical changes of the grain are undesirable because they may affect the quality of the final products of soybeans, in particular non-fermented ones.

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