EFFECT OF STORAGE PERIOD ON ISOFLAVONE CONTENT AND PHYSIOLOGICAL QUALITY OF CONVENTIONAL AND TRANSGENIC SOYBEAN SEEDS[1]

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ABSTRACT - The objective in this research was to evaluate the isoflavone content and the physiological quality of seed from conventional and transgenic soybean cultivars before and after 180 days of storage. Twenty one soybean cultivars: CD 202, CD 206, CD 208, CD 213RR, CD 214RR, CD 215, CD 216, CD 217, CD 218, CD 221, BRS 184, BRS 185, BRS 214, BRS 244RR, BRS 245RR, BRS 246RR, BRS 255, BRS 257, BRS 258, BRS 261 and BRS 262, grown in the 2005/2006 crop season, were assayed. The seeds were packed in Kraft paper bags and stored at room temperature under laboratory conditions. Seeds were evaluated with respect to their germination and vigor (first germination count, accelerated aging and tetrazolium test) and their total isoflavone contents and respective aglycon forms (daidzein, genistein and glycitein),glycosides (daidzine, genistine and glycitine) and malonyl conjugates. A completely randomized block design with six replications with the treatments set out within a subplot scheme (21 cultivars x 2 storage periods) was used. The F-test was used to compare means between storage periods and the Scott-Knott test to compare cultivars for each storage period, both with a 95% probability. It was concluded that isoflavone contents differ between cultivars and show a distinct behavior throughout storage.

Index terms: aglycon, *Glycine max*, daidzein, genistein, vigour.

EFEITO DO PERIODO DE ARMAZENAMENTO NOS TEORES DE ISOFLAVONAS E NA QUALIDADE FISIOLÓGICA DE SEMENTES DE SOJA CONVENCIONAL E TRANSGÊNICA

RESUMO- Objetivou-se no presente trabalho avaliar o conteúdo de isoflavonas e a qualidade fisiológica de sementes de soja antes e após 180 dias de armazenamento. Para tal utilizou-se vinte e uma cultivares de soja CD 202, CD 206, CD 208, CD 213RR, CD 214RR, CD 215, CD 216, CD 217, CD 218, CD 221, BRS 184, BRS 185, BRS 214, BRS 244RR, BRS 245RR, BRS 246RR, BRS 255, BRS 257, BRS 258, BRS 261 e BRS 262 produzidas na safra 2005/2006 as quais foram acondicionadas em sacos de papel Kraft multifoliado e armazenadas em condições de ambiente natural de laboratório. As sementes foram avaliadas quanto a germinação, vigor (primeira contagem de germinação, envelhecimento acelerado e tetrazólio) e quanto ao teor de

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isoflavonas totais e suas respetivas formas agliconas (daidzeína, genisteína e gliciteína), glicosídeos (daidzina, genistina e glicitina) e seus conjugados malonil. O delineamento experimental utilizado foi o inteiramente casualizado com 6 repetições e tratamentos dispostos no esquema de parcelas subdivididas (21 cultivares x 2 tempos de armazenamento). Para a comparação de médias entre tempos de armazenamento, utilizou-se o teste F e para comparações de cultivares para cada tempo de armazenamento utilizou-se o teste de agrupamento de Scott-Knott, ambos com 95% de probabilidade. Pelos resultados foi possível concluir que os teores de isoflavonas diferenciam-se entre as cultivares e possuem comportamento distinto ao longo do armazenamento.

Termos para indexação**:** agliconas, *Glycine max*, daidzina, genistina, vigor.

INTRODUCTION

Soybean is an essential food, which provides proteins, saturated and unsaturated fatty acids and some vitamins, besides possessing polyphenolic compounds such as isoflavones.

Isoflavones comprise aglycons (daidizein, genistein and glycitein), the respective glycosides (daidzine, genistine and glycitine), as well as their malonyl- and acetyl-conjugates (Carrão-Panizzi, 1996). Genistin and daidzin comprise 50% to 90% of the flavonoids found in soyflour (Fukutake et al., 1996), while malonyl genistin and malonyl daidzin forms constitute around 66% of total isoflavones in mature soybean seeds (Kudou et al., 1991).

In seed tissue cells, the antioxidant effect is one of the main properties of isoflavones (Shahidi and Wanasundara, 1992) and could be an important warranty mechanism for seed quality, since the degradation of cell plasma membranes by free radicals is one of the most discussed and accepted theories of seed deterioration. Krzyzanowski et al. (2001) demonstrated that isoflavone content is related to high seed quality.

Several factors are linked to deterioration, including a decrease in enzyme activity; reduction in cell respiration and biosynthesis; and an increase in cellular and sub-cellular permeability followed by consequent mitochondrial degradation (França Neto and Henning, 1984).

Seed quality includes a group of characteristics which determines its value for sowing, and indicating that a seed's potential performance can only be consistently identified when an interaction of genetic, physical, physiological and health characteristics is considered (Hampton and Tekrony, 1995; Marcos Filho, 2005). Deterioration is a process which involves cytological, biochemical and physical changes, which eventually cause seed death. This deterioration process has been characterized by Delouche (1982) as inexorable and irreversible, dependent on the physiological maturation time and varying among seed lots of the same cultivar and species. This process is determined by genetic factors, stink bug attack, environmental conditions at the post-maturation/pre-harvest periods, harvest and processing procedures, as well as storage and transport conditions (Braccini et al., 2001).

Seeds' storage is accomplished right after seeds reach their physiological maturation stage even before harvesting (Vieira and Carvalho, 1994). Storage after harvest must be conducted in order to minimize as much as possible biochemical reactions, which cause physiological quality loss, provide unfavorable conditions or those ones that do not allow insects and fungi development which contributes to this reduction on quality (Villa et al., 1979). A lower storage potential leads to a higher seeds' deterioration, a decrease on germination percentage and an increase of abnormal seedlings' incidence (Delouche and Baskin, 1973).

The objective in this study was to evaluate isoflavone content as well as the physiological quality of soybean seeds before and after 180 days of storage.

MATERIAL AND METHODS

Laboratory evaluations were made at the Seed Technology Laboratory of the Applied Agricultural

Research Nucleus (NUPAGRI) in the Agricultural Science Center of the State University of Maringá, Paraná, and also at EMBRAPA SOYBEAN (National Soybean Research Center) in Londrina, Paraná.

The soybean cultivars evaluated were: CD 202, CD 206, CD 208, CD 213 RR, CD 214 RR, CD 215, CD 216, CD 217, CD 218, CD 221, BRS 184, BRS 185, BRS 214, BRS 244 RR, BRS 245 RR, BRS 246 RR, BRS 255, BRS 257, BRS 258, BRS 261 and BRS 262 from COODETEC and EMBRAPA SOYBEAN, produced in the 2005/2006 crop season.

Seeds were packed in Kraft paper bags, then labeled, sealed and stored at room temperature in the laboratory with no control of atmospheric relative humidity and without light, from March to August, 2006, totaling up to 180 days of storage (Figure 1).

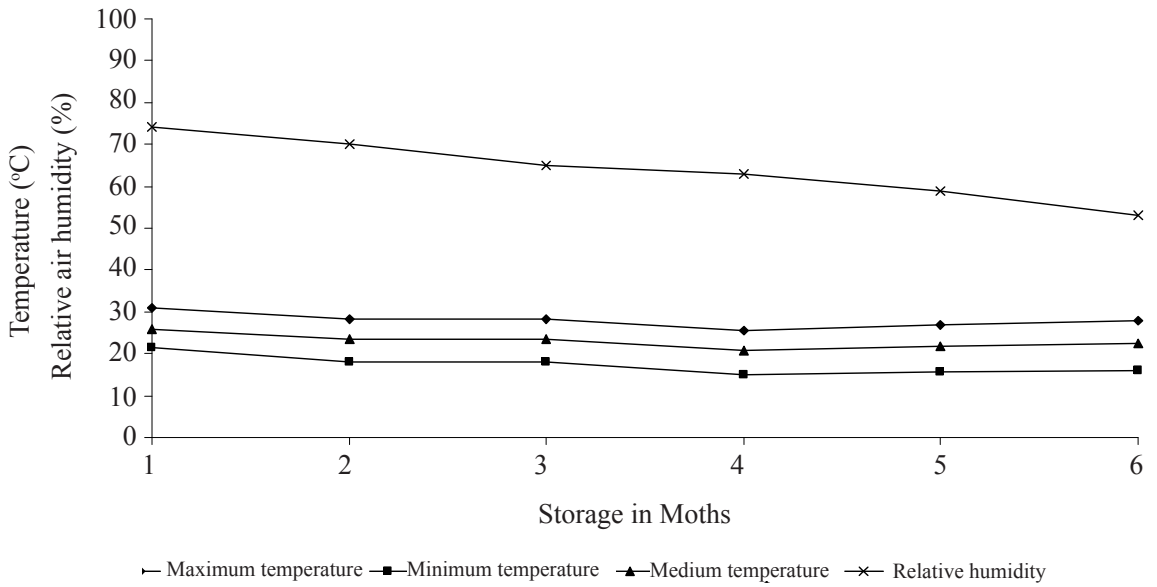

FIGURE 1. Temperature (ºC) and relative air humidity (%) during storage between March and August.

When stored, seeds had 12% water content, which is considered suitable for their conservation (Aguirre and Peske, 1992).

The experiment was set out in a completely randomized block design with six replications and the treatments in a subplots scheme (21 cultivars x 2 storage periods).

Evaluations were made before storage (time zero) and after 180 days of storage for each soybean cultivar using the following tests:

Germination test: six replications of 50 seeds were placed between a three-leaf paper-towel imbibed in distilled water (3 times the dry paper weight) to germinate. They were then rolled up and placed in a Mangelsdorf germinator kept at a constant temperature of 25 ºC. Evaluations of the percentage of normal seedlings were made, both on the fifth (first count) and eighth (final count) days, after the beginning of the test, according to the established criteria of the Seed Analysis Rules (Brasil, 1992).

First count of the germination test: done together with the previous procedure, using the same methodology to assess the percentage of normal seedlings obtained on the fifth day after the beginning of the test (Brasil, 1992).

Accelerated aging test: six replications of 50 seeds were placed on stainless steel screens inside plastic boxes (gerbox) containing 40 mL water. The relative humidity inside the boxes was approximately 100% according to the methodology described by Krzyzanowski et al. (1991). The boxes were then taken to a germination B.O.D-like chamber and kept at a constant temperature of 41 ºC for 48 hours. After the aging period, the seeds were submitted to the germination test, as described previously. Germination was assessed on the fifth day after sowing and the percentage of seedlings considered normal was calculated (Marcos Filho, 1999).

Tetrazolium test: six replications of 50 seeds, which were osmoconditioned in paper towels imbibed

in distilled water and placed in a germinator at a temperature of 25 °C for 16 hours. Seeds were then transferred to 50 mL plastic cups, and totally submerged in a 0.075% tetrazolium solution (2,3,5-triphenyl-2H-tetrazolium chloride) and kept at a temperature of 40 °C for 180 minutes inside a germination chamber in the absence of light. After the seed staining process, they were washed under running water and kept submerged until evaluation when they were individually sectioned longitudinally and symmetrically with a razor blade and classified according to the criteria proposed by França Neto et al. (1998). Viability was measured by the ratio of seeds belonging to classes 1 to 5; vigor level by classes 1 to 3 and viability loss by classes 6 to 8. Both vigor and viability potentials were expressed as a percentage (França Neto et al., 1999).

Isoflavone content: soybean seeds were milled by using a knife Mill for 60 seconds at 17,000 r.p.m. Afterwards, 15 g of this flour was mixed with 50 mL of hexane constantly shaken for 24 hours at room temperature before being filtered to obtain non-fat soybean flour (FDS).

For isoflavone extraction, 100 mg of this non-fat soybean flour was stored in assay tubes (10 mL) with 4 mL of ethanol solution (70%) plus 0.1% of acetic acid and then sealed with a lid. The assay tubes were kept at room temperature for one hour and vigorously shaken every 15 minutes. Later, 2 mL of this extract were centrifuged at 15,000 r.p.m. for 4 minutes. The overfloating fractions obtained from this process were then transferred to tubes with the auto-injection system of a chromatographer, 40 µL samples of the overfloating fractions were used for direct injections into the device. Each sample had six replications for analysis using high performance liquid chromatography (HPLC).

The separation and quantification of isoflavones were made according to the methodology described by Berhow (2002) using a Waters 2690 HPLC. A reverse-phase column ODS C18 (YMC Pack ODS-AM Columm) - 250 mm long x 0.4 mm internal width and 5 µm particles - was used. The binary linear gradient system was used to separate the isoflavones. The mobile phases were: methanol with 0.025% of Trifluoroacetic acid (TFA) as solvent **A** and ultrapure deionized distilled water with 0.025% of TFA as solvent **B**. The initial gradient was 20% for solvent **A**, which reached 100% concentration in 40 minutes and immediately after that, it returned to 20% at 41 minutes, remaining steady until the end of the running (60 minutes) for each sample. The mobile phase flow rate was 1 mL min^{-1} and the temperature during running was 25 ºC. Isoflavones were detected using a Waters 996 photodiode array detector, adjusted to a 260 nm wavelength. In addition, daidizin, daidzein, genistin and genistein standard operating protocols (Sigma Chemical Co.), solubilized in methanol (HPLC degree) were used at the following concentrations: 0.00625 $mg.mL^{-1}$; 0.0125 $mg.mL^{-1}$; 0.0250 $mg.mL^{-1}$; 0.05 $mg.mL^{-1}$ and 0.1000 $mg.mL^{-1}$. Extreme standards were used as a reference as well as a molar extinction coefficient from each malonyl form to quantify them. The isoflavone content was calculated in milligrams per 100 g of soybean flour.

Statistical analysis: variance analysis of the results was made and when there was a significant interaction, were found the necessary partitioning was performed. Cultivar effects for each storage period were compared by the Scott-Knott (1974) grouping method while a comparison between storage periods for each cultivar was made using the F-test. All tests were calculated at the 5% probability level.

RESULTS AND DISCUSSION

Data on temperature and relative humidity during the storage period are shown in Figure 1. Average temperature during storage was 22.4 ºC; average maximum temperature was 31.4 ºC and average minimum temperature was 13.5 ºC. Average relative humidity during storage was 64%. The result of variance analysis showed a significant interaction between cultivars x storage periods for all variables.

For the germination test (Table 1), the only cultivars which did not show any decrease in germination percentage during storage on first count (vigor) were the BRS 185 and BRS 244 RR cultivars. These cultivars maintained their performance at the final evaluation of the germination test, proving their high physiological potential.

The highest germination percentage at the beginning of storage was for the cultivars BRS 214, BRS 244 RR, BRS 246 RR and BRS 261 when initial germination was observed. These same genotypes continued to show the best performance even after storage, especially for cultivar BRS 244 RR, which had the best performance of all the materials tested. When seed quality was measured by the accelerated aging test (Table 1), all the cultivars were susceptible to storage and showed low germination percentages after storage. This highlights

the consequences of the non-artificial storage conditions, which accelerated the deterioration process inherent in seed physiology (Delouche, 1982; Hampton and Tekrony, 1995; Marcos Filho, 2005). Therefore, it was confirmed that the exposure of seeds to aging resulted in a reduction in vigor, mainly after storage.

TABLE 1. Normal seedlings obtained from first and count of Germination test germination, and the accelerated aging test, using Twenty one soybean cultivars, before and after a 180 day storage period.

Cultivar[1]	First count		Germination test		Accelerated aging	
	Storage[2] (days)		Storage[2] (days)		Storage[2] (days)	
	0	180	0	180	0	180
	---------- % ----------		--------- % ---------		---------- % ----------	
CD 202	76 Ba	40 Db	87 Aa	52 Cb	77 Ca	1 Db
CD 206	73 Ba	48 Cb	85 Aa	56 Cb	68 Da	12 Cb
CD 208	70 Ca	55 Bb	84 Aa	73 Bb	82 Ca	1 Db
CD 213 RR	62 Da	32 Db	80 Ba	50 Cb	43 Fa	1 Db
CD 214 RR	80 Ba	55 Bb	88 Aa	70 Bb	77 Ca	8 Db
CD 215	61 Da	27 Eb	76 Ba	35 Db	56 Ea	1 Db
CD 216	74 Ba	56 Bb	82 Aa	67 Bb	67 Da	0 Db
CD 217	66 Ca	13 Fb	77 Ba	21 Db	78 Ca	0 Db
CD 218	67 Ca	18 Fb	78 Ba	32 Db	54 Ea	0 Db
CD 221	69 Ca	25 Eb	84 Aa	36 Db	58 Ea	6 Db
BRS 184	50 Ea	23 Eb	62 Ca	31 Db	59 Ea	0 Db
BRS 185	59 Da	54 Ba	74 Ba	68 Ba	94 Aa	22 Bb
BRS 214	88 Aa	60 Bb	95 Aa	78 Bb	81 Ca	13 Cb
BRS 244 RR	90 Aa	89 Aa	94 Aa	92 Aa	90 Aa	30 Ab
BRS 245 RR	59 Da	32 Db	74 Ba	52 Cb	57 Ea	0 Db
BRS 246 RR	83 Aa	63 Bb	91 Aa	73 Bb	89 Ba	15 Cb
BRS 255	76 Ba	44 Cb	83 Aa	58 Cb	73 Ca	4 Db
BRS 257	78 Ba	37 Db	92 Aa	56 Cb	66 Da	2 Db
BRS 258	70 Ca	27 Eb	80 Ba	49 Cb	73 Ca	6 Db
BRS 261	90 Aa	76 Ab	93 Aa	86 Aa	87 Ba	20 Bb
BRS 262	67 Ca	21 Eb	76 Ba	29 Db	62 Da	0 Db
C.V. (%) Lot	11.62		10.03		12.89	
C.V. (%) Sub-Lot	10.89		9.23		9.63	

[1] Means followed by the same capital letter in each column belong to the same group according to the Scott-Knott grouping method (1974) at the 5% probability level.

[2] Means followed by the same non-capital letter in each line do not differ from each other based on the F-test at the 5% probability level.

Degenerative alterations in the internal seed structures promote a breakdown in metabolism as well as the exchange of water and solutes between cells and the external environment, resulting in reduced seed viability (Vieira and Carvalho, 1994). According to Delouche and Baskin (1973), the seed aging process leads to a destructuring of the cell membrane system mainly due to the action of high-reactivity chemical groups called free radicals.

Regarding the differences between cultivars, the result from the BRS 244 RR cultivar was significant

since it had the highest germination percentage even after the high temperature and moisture stresses of the aging test both before and after storage, thus demonstrating its high physiological quality compared to other cultivars.

Results from the tetrazolium test on 1 - 3 and 1 - 5 (Table 2) also confirmed the superiority of the BRS 244 RR cultivar compared to most cultivars, not only for vigor potential but also for viability. This test showed that that CD 215 and BRS 245 RR cultivars had the lowest performance. The tetrazolium test, as an indicator of viability (1 - 5) as well as of vigor, confirmed the results observed for the germination (initial and final) and accelerated aging tests, where the results obtained before storage were higher than those after storage. The only deviation from this tendency was for the tetrazolium test, where the seed physiological quality of the BRS 262 cultivar showed higher values after 180 days of storage. Since this was only observed for this cultivar, it may be due to sampling but should be more carefully investigated in the future

TABLE 2. Vigour potential (1 - 3) and viability (1 - 5), measured by the tetrazolium test and total isoflavones of twenty-one soybean cultivars, before and after a 180 day storage period.

Cultivar[1]	Tetrazolium (1 - 3)		Tetrazolium (1 - 5)		Total of Isoflavones	
	Storage[2] (days)		Storage[2] (days)		Storage[2] (days)	
	0	180	0	180	0	180
	---------- % ---------		--------- % --------		mg $100g^{-1}$ of flour	
CD 202	61 Da	47 Db	82 Ba	77 Ca	310 Ea	246 Gb
CD 206	66 Da	64 Ba	78 Ca	83 Ba	152 Jb	181 Ja
CD 208	75 Ca	55 Cb	85 Ba	81 Ba	247 Ha	239 Ha
CD 213 RR	56 Da	58 Ca	84 Ba	80 Ba	213 Ia	199 Ib
CD 214 RR	71 Ca	55 Cb	83 Ba	70 Db	356 Cb	420 Ba
CD 215	49 Ea	51 Ca	67 Db	76 Ca	302 Ea	305 Ea
CD 216	60 Da	56 Ca	78 Ca	76 Ca	277 Ga	199 Ib
CD 217	57 Da	58 Ca	73 Cb	82 Ba	301 Ea	278 Fb
CD 218	58 Da	63 Ba	75 Cb	83 Ba	258 Ha	233 Hb
CD 221	58 Da	44 Db	82 Ba	73 Cb	153 Ja	86 Mb
BRS 184	59 Da	44 Db	79 Ca	65 Db	276 Ga	251 Gb
BRS 185	85 Ba	64 Bb	94 Aa	85 Bb	330 Da	327 Da
BRS 214	80 Ba	80 Aa	88 Aa	90 Aa	286 Fb	327 Da
BRS 244 RR	95 Aa	84 Ab	95 Aa	91 Aa	507 Aa	327 Db
BRS 245 RR	45 Ea	39 Da	64 Da	68 Da	414 Ba	345 Cb
BRS 246 RR	84 Ba	58 Cb	93 Aa	77 Cb	260 Hb	290 Fa
BRS 255	70 Ca	57 Cb	88 Aa	67 Db	152 Ja	123 Lb
BRS 257	66 Da	56 Cb	82 Ba	73 Cb	304 Eb	326 Da
BRS 258	74 Ca	71 Ba	94 Aa	89 Aa	163 Ja	168 Ka
BRS 261	69 Da	60 Cb	89 Aa	75 Cb	259 Hb	307 Ea
BRS 262	59 Db	67 Ba	76 Cb	86 Ba	411 Bb	533 Aa
C.V. (%) Lot	8.69		6.96		3.23	
C.V. (%) Sub-Lot	7.96		8.34		4.23	

[1] Means followed by the same capital letter in each column belong to the same group, according to the Scott-Knott grouping method (1974) at the 5% probability level.

[2] Means followed by the same non-capital letter in each line do not differ from each other based on the F-test at the 5% probability level.

The results of our study agreed with those of other studies, in that total isoflavone content (Table 2) showed a significant difference ($P<0.05$) between genotypes (Park et al., 2001). Overall, all cultivars showed a higher total isoflavone content before storage. Nevertheless, exceptions were observed for cultivars CD 206, CD 214 RR, BRS 214, BRS 246 RR, BRS 257, BRS 261 and BRS 262 where greater isoflavone content was observed after storage. This may be a result of the action of active cell repair agents.

The highest total isoflavone content before storage was observed in cultivar BRS 244 RR, which stood out because of its higher total isoflavone content after storage. This may be related to the high physiological potential of the seeds (Ávila, 2004) due to the antioxidant activities of isoflavones (Shahidi and Wanasundara, 1992; Esaki et al., 1998; Aguiar, 2002) and a possible phytoalexin action of isoflavonoids as a defense against pathogens (Yoshikawa et al., 1978; Pelicice et al., 2000), which permitted a delay in the post-harvest deterioration process and also during storage.

The lowest isoflavone contents before and after storage were predominantly identified for the conventional cultivars CD 206, CD 221, BRS 255, before storage and for BRS 255, BRS 258, CD 221 after storage. There is no relationship between the transgeny for glyphosate resistance and the increase in isoflavone content. It is known that alterations in metabolism may occur (Jaworski, 1972; Duke et al., 2003) and isoflavonoids are derived from secondary metabolism in crops (Taiz and Zeiger, 2004).

Isoflavone contents may be specifically associated with the physiological quality of seeds considering the deterioration process measured from the accelerated aging test. The lowest isoflavone contents for cultivars, such as BRS 258 and CD 221 can be related to low physiological potential, especially after 180 days of storage (Table 2).

The isoflavones identified in seeds are given in Tables 3 (aglycons), 4 (malonyl) and 5 (glycosil), revealing differences among genotypes as recorded in the literature (Park et al., 2001 and Carrão-Panizzi et al., 2003).

The highest aglycons content before storage was shown by the following cultivars: CD 208 (daidzein, glicitein and genistein); CD 213 RR (glicitein); BRS 184 (genistein). The highest aglycons content after storage: CD 206 (daidzein and glicitein); CD 217 and CD 262 (genistein). Superior malonyl contents before storage: BRS 244 RR (daidzin and genistin); CD 217 (glicitin). The highest malonyl contents after storage: BRS 262 (daidzin and genistin); CD 214 RR (glicitin). Regarding glycosil contents before storage, the highest values were found in cultivars: BRS 245 RR (daidzin); CD 217 (glicitin); BRS 262 (genistin) and after storage: BRS 262 (daidzin, glicitin and genistin); CD 214 RR (glicitin).

Results like the ones above are useful considering the pharmacological interest in the search for the beneficial health care properties provided by isoflavonoids (Naim et al., 1974; Murphy, 1982; Coward et al., 1993; Miyazawa et al., 1999). Also, crop improvement and increased crop yield can supply a higher market demand.

Focusing on seed quality, malonyl content may be more related to increments in seed physiological potential (BRS 244 RR). This is logical when comparing low malonyl content with inferior physiological quality. This is observed for cultivars CD 215, CD 217, CD 218, CD 221, BRS 184 and BRS 255, when the storage time is compared.

Overall, the post-storage contents of malonyl and glycosil were higher than before storage, while aglycons tended to decrease during storage. This fact has demonstrated the possibility of a higher expression of malonyl and glycosil contents during storage and also their potential action as antioxidants and phytoalexins.

Importance should be given to the antioxidant activity since Park et al. (2001) demonstrated the possibility of flavonoid biosynthesis and interconversion from one form to another via enzymatic processes in an antioxidative action on free radicals.

The storage conditions, to which seeds were submitted, such as at non-climatized (natural) room temperatures, can lead to a deterioration process caused by high temperatures or temperature oscillations and moisture (Bewley and Black, 1985), as shown in Figure 1. Thus, alterations can occur to seeds during storage, including genetic injuries, loss of membrane system integrity, decrease of selective capacity, peroxidation of lipids, lixiviation of solutes, changes in seed respiratory activity, modification of enzyme activity and protein synthesis. The inability to maintain the electrochemical gradient, loss of cell compartmentalization, as well as the accumulation of toxic substances, consequently leads to physiological alterations such as: germination delay, decrease in tolerance to suboptimum environmental conditions

during germination, reduction in growth and/or seedling vigor, increase in abnormal seedlings, higher susceptibility to pathogenic microorganism attack, irregular emergence, yield reduction, complete loss of germination capacity and seed death (Wilson and Mcdonald, 1986; Basavarajappa et al., 1991).

TABLE 3. Aglycone isoflavone content of seeds from Twenty one soybean cultivars, before and after a 180 day storage period.

Cultivar[1]	Daidzein		Glicitein		Genistein	
	Storage[2] (days)		Storage[2] (days)		Storage[2] (days)	
	0	180	0	180	0	180
	mg.100 g^{-1} of flour		mg.100 g^{-1} of flour		mg.100 g^{-1} of flour	
CD 202	5 Ea	4 Da	0 Ca	0 Ba	5 Fa	4 Cb
CD 206	2 Fb	30 Aa	0 Cb	2 Aa	2 Ia	2 Da
CD 208	15 Aa	3 Db	9 Aa	1 Ab	10 Aa	4 Cb
CD 213 RR	5 Ea	3 Da	8 Aa	2 Ab	6 Ha	3 Da
CD 214 RR	5 Ea	5 Ca	0 Cb	1 Aa	5 Fa	5 Ba
CD 215	7 Da	5 Ca	0 Cb	1 Aa	4 Ga	5 Ba
CD 216	7 Da	3 Db	0 Ca	1 Aa	6 Ea	2 Db
CD 217	7 Da	5 Cb	0 Ca	0 Ba	8 Ca	6 Ab
CD 218	7 Da	4 Db	0 Ca	0 Ba	9 Ba	4 Bb
CD 221	5 Ea	1 Fb	0 Ca	0 Ba	4 Ga	1 Eb
BRS 184	12 Ba	5 Cb	1 Ba	0 Ba	10 Aa	5 Bb
BRS 185	9 Ca	3 Db	1 Ba	0 Ba	8 Ca	2 Db
BRS 214	7 Da	3 Db	0 Cb	1 Aa	6 Ea	2 Db
BRS 244 RR	7 Da	3 Db	0 Cb	1 Aa	6 Ea	2 Db
BRS 245 RR	12 Ba	5 Cb	0 Ca	1 Aa	9 Ba	5 Bb
BRS 246 RR	5 Ea	3 Db	0 Cb	0 Ba	4 Ga	2 Db
BRS 255	3 Fa	1 Fb	0 Ca	1 Aa	2 Ia	1 Eb
BRS 257	4 Ea	2 Eb	0 Ca	0 Ba	5 Ea	3 Db
BRS 258	3 Fa	2 Ea	0 Ca	0 Ba	3 Ha	2 Db
BRS 261	4 Ea	2 Eb	0 Ca	0 Ba	6 Ea	3 Db
BRS 262	8 Ca	7 Ba	0 Ca	0 Ba	7 Da	6 Ab
C.V. (%) Lot	12.37		23.40		4.20	
C.V. (%) Sub-Lot	13.45		30.00		5.63	

[1] Means followed by the same capital letter in each column belong to the same group, according to the Scott-Knott grouping method (1974) at the 5% probability level.

[2] Means followed by the same non-capital letter in each line do not differ from each other based on the F-test at the 5% probability level.

TABLE 4. Malonyl isoflavone content for Twenty one soybean cultivars, before and after a 180 day storage period.

Cultivar[1]	Daidzin		Glicitin		Genistin	
	Storage[2] (days)		Storage [2] (days)		Storage [2] (days)	
	0	180	0	180	0	180
	mg.100 g^{-1} of flour		mg.100 g^{-1} of flour		mg.100 g^{-1} of flour	
CD 202	70 Fa	56 Gb	18 Ba	7 Gb	101 Ea	84 Gb
CD 206	36 Ja	30 Kb	13 Da	11 Fa	43 Kb	55 Ia
CD 208	39 Ja	42 Ia	8 Eb	14 Ea	94 Fa	96 Ea
CD 213 RR	40 Ja	40 Ia	12 Da	12 Fa	70 Ia	67 Ha
CD 214 RR	87 Da	89 Ca	11 Db	30 Aa	144 Bb	158 Ba
CD 215	63 Ja	64 Fa	13 Da	6 Gb	94 Fb	106 Da
CD 216	71 Fa	53 Gb	8 Ea	0 Ib	78 Ha	67 Hb
CD 217	49 Ha	47 Ha	30 Aa	11 Fb	79 Hb	94 Ea
CD 218	52 Ha	46 Hb	17 Ba	8 Gb	85 Ga	88 Fa
CD 221	33 Ka	16 Lb	19 Ba	5 Hb	32 La	31 Ja
BRS 184	64 Ga	64 Fa	18 Ba	15 Eb	72 Ia	71 Ha
BRS 185	81 Eb	92 Ca	17 Ca	18 Da	91 Fb	104 Da
BRS 214	68 Fb	92 Ca	14 Db	18 Da	88 Gb	104 Da
BRS 244 RR	149 Aa	92 Cb	19 Ba	18 Da	168 Aa	104 Db
BRS 245 RR	107 Ba	97 Bb	19 Ba	12 Fb	112 Da	107 Da
BRS 246 RR	64 Gb	79 Da	7 Eb	14 Ea	93 Fb	101 Da
BRS 255	50 Ha	40 Ib	2 Fa	1 Ia	34 La	36 Ja
BRS 257	62 Gb	67 Ea	14 Cb	24 Ba	106 Eb	112 Ca
BRS 258	31 Ka	33 Ga	7 Eb	11 Fa	59 Ja	59 Ia
BRS 261	44 Ib	61 Fa	6 Eb	20 Ca	106 Eb	117 Ca
BRS 262	91 Cb	125 Aa	11 Db	25 Ba	134 Cb	164 Aa
C.V. (%) Lot	3.88		12.25		4.20	
C.V. (%) Sub-Lot	3.95		13.78		5.78	

[1] Means followed by the same capital letter in each column belong to the same group, according to the Scott-Knott grouping method (1974) at the 5% probability level.

[2] Means followed by the same non-capital letter in each line do not differ from each other based on the F-test at the 5% probability level.

TABLE 5. Glycosil isoflavone content for Twenty one soybean cultivars, before and after a 180 day storage period.

Cultivar[1]	Daidzin		Glicitin		Genistin	
	Storage[2] (days)		Storage[2] (days)		Storage[2] (days)	
	0	180	0	180	0	180
	mg 100 g^{-1} of flour		mg 100 g^{-1} of flour		mg 100 g^{-1} of flour	
CD 202	38 Fa	31 Fb	13 Ca	6 Fa	59 Ea	54 Db
CD 206	20 Ja	15 Ka	10 Ea	5 Fa	25 Jb	31 Ga
CD 208	16 Kb	20 Ia	6 Gb	10 Da	49 Ga	48 Ea
CD 213 RR	22 Ja	21 Ia	10 Ea	9 Ea	42 Ha	40 Fa
CD 214 RR	33 Hb	37 Ea	6 Gb	21 Aa	64 Db	72 Ba
CD 215	44 Ea	42 Db	12 Da	5 Fb	66 Da	70 Ba
CD 216	46 Ea	31 Fb	10 Ea	0 Hb	52 Fa	41 Fb
CD 217	39 Fa	32 Fb	28 Aa	10 Db	60 Eb	72 Ba
CD 218	28 Ia	23 Hb	12 Da	6 Fb	46 Gb	53 Da
CD 221	22 Ja	8 Lb	16 Ba	2 Gb	21 Ja	20 Ha
BRS 184	36 Ga	35 Ea	14 Ca	11 Db	46 Ga	43 Fa
BRS 185	51 Da	45 Cb	14 Ca	11 Db	54 Fa	49 Eb
BRS 214	40 Fb	45 Ca	9 Fb	11 Da	54 Fa	49 Eb
BRS 244 RR	67 Ba	45 Cb	13 Ca	11 Db	76 Ba	49 Eb
BRS 245 RR	69 Aa	53 Bb	16 Ba	9 Eb	70 Ca	57 Db
BRS 246 RR	32 Hb	35 Ea	6 Gb	8 Ea	47 Ga	46 Fa
BRS 255	38 Fa	25 Hb	1 Ha	1 Ha	22 Ja	18 Ha
BRS 257	36 Ga	38 Ea	8 Fb	17 Ba	61 Ea	62 Ca
BRS 258	17 Ka	18 Ja	6 Ga	7 Ea	35 Ia	33 Ga
BRS 261	21 Jb	27 Ga	6 Gb	13 Ca	64 Da	62 Ca
BRS 262	59 Cb	75 Aa	8 Fb	19 Aa	88 Ab	108 Aa
C.V. (%) Lot	4.52		11.20		5.67	
C.V. (%) Sub-Lot	5.79		14.84		6.78	

[1] Means followed by the same capital letter in each column belong to the same group, according to the Scott-Knott grouping method (1974) at the 5% probability level.

[2] Means followed by the same non-capital letter in each line do not differ from each other based on the F-test at the 5% probability level

It was stated previously that the loss of seed viability during storage is accompanied by several processes. Nevertheless, the increase of lipid peroxidation and the accumulation of free radicals, which can react with hydrogen peroxides, producing singlet oxygen and hydroxyl radical (OH^-), toxic to cells (Hendry, 1993) and that are able to injure cell constituents, such as proteins, DNAs and membranes (Hoekstra et al., 1996), are the most accepted theories.

At the same time as the process mentioned above, the ability to prevent, tolerate or repair the attack of free radicals occurs (Nkang et al., 2000). For Hoeskstra et al. (1996), the action of free radicals may be attenuated by a number of removers, *e.g.* antioxidant activity-like molecules (Rosa et al., 2005), already existent in cells or applied exogenally on seeds (Kikuti et al., 2002). Among these antioxidant molecules, we can name the tocopherol isomers (Vitamin E), beta-carotene, ascorbic acid (vitamin C), glutathione (Rosa et al., 2005; Kikuti et al., 2002) and isoflavonoids (Shahidi and Wanasundara, 1992; Esaki et

al., 1998; Esaki et al., 1999), some of which are present in soybean seeds.

CONCLUSIONS

Seed isoflavone contents possess a genotypical distinction, whether the cultivars are conventional or transgenic.

The behavior of isoflavonoid contents and forms is variable among cultivars during non-controlled storage conditions.

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