ELECTRICAL CONDUCTIVITY AND DETERIORATION OF SOYBEAN SEEDS EXPOSED TO DIFFERENT STORAGE CONDITIONS[1]

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ABSTRACT – Research with soybean seeds has revealed that the results of the electrical conductivity test may be influenced by storage temperature, particularly low temperature, such as 10ºC, suggesting that seed deterioration at low storage temperatures does not seem to be directly related to the loss of the cell membrane integrity. This study was conducted with seeds of two soybean cultivars with the objective of: a) studying the effect of different storage temperatures (10ºC; 20ºC; 25ºC; 20/10ºC and 25/10ºC) on the results of the electrical conductivity test; b) observing the behavior of fatty acids and carbohydrates during storage and studying its relation with the electrical conductivity results. Every three months, from a total of 18 months of storage, the physiological quality of seeds was evaluated using the germination, accelerated aging and electrical conductivity tests. Based on the obtained results, it can be concluded that the electrical conductivity test was not shown to be a good indicative of the deterioration process of seeds stored at low temperatures, and no direct relationship between changes in the fatty acids and carbohydrates and the behavior of the mentioned test for seeds stored at 10ºC was found.

Index terms: *Glycine max*, vigor, physiological quality.

CONDUTIVIDADE ELÉTRICA E DETERIORAÇÀO DE SEMENTES DE SOJA SUBMETIDAS A DIFERENTES CONDIÇÕES DE ARMAZENAMENTO

RESUMO – Pesquisas com sementes de soja têm revelado que os resultados do teste de condutividade elétrica podem ser influenciados pela temperatura de armazenamento, especialmente as mais baixas, como 10ºC, sugerindo que a deterioração da semente a baixas temperaturas de armazenamento parece não estar relacionada diretamente com a perda da integridade das membranas celulares. Este trabalho foi conduzido com sementes de dois cultivares de soja objetivando: a) estudar o efeito de diferentes temperaturas de armazenamento (10ºC; 20ºC; 25ºC; 20/10ºC e 25/10ºC) sobre os resultados do teste de condutividade elétrica; b) observar o comportamento dos ácidos graxos e carboidratos durante o armazenamento e estudar sua relação com os resultados do teste de condutividade elétrica. A cada três meses, num total de 18 meses de armazenamento, a qualidade fisiológica das sementes foi avaliada pelos testes de germinação, envelhecimento acelerado e condutividade elétrica. O teste de condutividade elétrica não mostrou ser um indicador eficiente do processo de deterioração de sementes armazenadas a baixas temperaturas, também não foi observada nenhuma relação direta entre as mudanças nos ácidos graxos, nos carboidratos e no comportamento do referido teste, para sementes armazenadas a 10ºC.

Termos para indexação: *Glycine max*, vigor, qualidade fisiológica.

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INTRODUCTION

The main causes for the seed ageing process are not yet fully elucidated, given the high number of cytological and metabolic alterations involved. Several researchers have considered cell membrane disintegration, with consequent increased permeability as one of the first detectable signs of deterioration, even if the mechanisms involved in such process are not yet fully clear.

Thus, reviewing the deterioration process of seeds, McDonald (1999) reported that lipid peroxidation has frequently been pointed to as the main cause of deterioration and considering that lipids are part of the cell membrane of seeds, there would be a direct relation between lipid peroxidation and the loss of the cell membrane integrity. On the other hand, alterations in carbohydrates during storage could affect the cell membrane permeability, thus contributing to the reduction in the physiological quality and germination of seeds (Crowe et al., 1984; Bernal-Lugo and Leopold, 1992).

The damage caused to membrane through deterioration that provides lower selectivity and hence increase in the leakage of solutes to the environment has been one of the main causes of the decline in the physiological quality of seeds. As result, the electrical conductivity test is considered as an important tool to evaluate the seed vigour, since it indirectly assesses the cell membrane degradation degree by determining the amount of electrolytes released in the seed soaking solution.

The electrical conductivity results may be affected by several factors. Tao (1978) evaluated the use of this test for soybean seeds and reported the influence of seed size, water quality, initial seed water content, volume of water used and the presence of physically damaged seeds. Loeffler et al. (1988) verified the effect of these factors; however the authors stressed that the variables indicated could be controlled by standardizing procedures in order to provide a non-subjective evaluation of the physiological quality of soybean seeds. Later researchers (Hampton et al., 1992; Vieira et al., 1996 and 2002) corroborated these results.

Although this test has been used for several species, especially pea and soybean, not many studies on electrical conductivity to estimate changes in the physiological quality of seeds during storage have been developed. Some research has revealed that the storage temperature may influence the electrical conductivity test, especially lower ones. There are results showing that soybean seeds stored at 10ºC, when evaluated by the germination and accelerated ageing tests, present reduction in physiological quality; however, this fact is not detected by the conductivity test (Vieira et al., 2001; Fessel, 2001). Thus, the deterioration of seeds at low storage temperatures does not seem to be directly related to the loss of membrane integrity.

The present study was conducted with soybean seeds with the following objectives: a) to study the effect of different storage temperatures on the results of the electrical conductivity test; b) to observe the behavior of fatty acids and carbohydrates during storage and to study their relation with the results of the electrical conductivity test.

MATERIAL AND METHODS

This study was carried out at the Seed Analysis Laboratory (Department of Crop Production) and at the Microorganism and Plant Biochemistry Laboratory (Department of Technology), UNESP, Jaboticabal Campus, SP from November 2003 to July 2005. Seeds from two soybean (*Glycine max* (L.) Merrill) cultivars were used (BRSMG-68 and BRS-133), each cultivar was represented by two lots with distinct physiological quality: superior and inferior (Table 1).

The seed water content was initially adjusted to 120g kg^{-1} by the following procedures: a) **drying** (when the initial water content was above the desired level): each sample was placed in a polystyrene tray and taken to a dryer with air forced circulation, where the temperature ranged from 30 to 33^{o}C; b) **moistening** (when the initial water content was below the desired level): this procedure was conducted in adapted plastic boxes (26.0 x 16.0 x 8.5cm) with seed samples placed on a wire mesh screen, distributed in a single layer; these boxes covered with lid and containing 500 mL water were incubated at 20^{o}C. During artificial drying and moistening, the seed water content was controlled through successive weighings until the desired values were obtained. As soon as the desired seed water content was reached, each sample was placed in an aluminum recipient sealed and properly identified.

The samples were submitted to different storage temperatures: a) 10ºC (constant); b) 20ºC (constant); c) 25ºC (constant); d) 20ºC for six months with transference to 10ºC until the end of the storage period; e) 25ºC for six months with transference to 10ºC until the end of the storage period with the objective of verifying whether the seed stored at higher temperature maintained its physiological quality or delayed its deterioration process when transferred to an environment with a lower temperature.

Every three months, in a total of 18 months of storage, the physiological quality of soybean seeds was evaluated for each treatment using the following tests: **Germination** – four 50-seed samples for each lot were used, sown in rolled paper towels moistened with water amount equivalent to 2.5 times the weight of the dry paper substrate and set to germinate at 25ºC. The evaluations were performed on the fifth and eight days after sowing (ISTA, 1999); **Accelerated ageing -** test conducted with the use of plastic boxes (11.0 x 11.0 x 3.5cm) with a wire mesh screen inside, where the seeds were distributed in such way that they formed a single layer on the screen surface; 40mL deionized water were added inside each individual compartment. The sealed boxes were kept in an ageing chamber (water jacketed chamber) at 42ºC for 48h (Vieira et al., 1999). Following the seed ageing period, four 50-seed samples per treatment were set to germinate. The evaluation was performed on the fifth day after sowing, counting the percentage of normal seedlings (Hampton and TeKrony, 1995). **Bulk electrical conductivity** – four 50-seed samples were weighed (precision 0.01g) and placed in plastic cups (200mL capacity) containing 75mL deionized water for 24h at 25ºC (Loeffler et al., 1988; Vieira and Krzyzanowski, 1999). After that, the electrical conductivity of the solution was determined by reading on a conductivimeter (DIGIMED DM-31) and the average values obtained for each lot were expressed as $\mu S\ cm^{-1}\ g^{-1}$.

Biochemical analyses were performed during storage with the objective of verifying the behavior of fatty acids and carbohydrates and studying their relationship with the results of the electrical conductivity test. The soybean cultivar BRSMG-68 was used, represented by two lots, where seed samples stored at the following temperatures were analyzed: (a) 10ºC (constant); (b) 20ºC (constant); (c) 20ºC for six months with transference to 10ºC until the end of the storage period in four evaluation times (0, 6, 12 and 18 months of storage) according to the following procedures: **fatty acids** – lipids were extracted from seeds by the method described by Bligh and Dyer (1959). Then, the methylation of fatty acids was performed according to methodology developed by Maia and Rodrigues-Amaya (1993). To determine the fatty acids chain through Gas Chromatography, a gas chromatograph label (Shimadzu model 14b) was employed, using a melted silica capillary column Omegawax 250 (30m x 0.25mm x 0.25µm), catalog nº 24136 – Supelco. For better separation, the following column temperature program was used: 100ºC for two minutes, heating of 4ºC/minute up to 220ºC, remaining at this temperature for a further 25 minutes. The injector and the detector temperatures were 250ºC and 280ºC, respectively; the drag gas velocity was 40cm $second^{-1}$. The sample divisor was split: 1/100 with injection of 1µL/sample with the use of the FID detector. The identification standard for fatty acids was Sigma, catalog nº 189-19; **Carbohydrates** – the carbohydrates were extracted according the analytical model proposed by Bernal-Lugo and Leopold (1992), with adaptations. Thus, soybean seed samples placed in stove with forced circulation of air at 65ºC for 48h were crushed and sieved. One mL of distilled water was added to 50mg of the crushed powder, remaining for three hours at 4ºC. The material was centrifuged at 12,000 rpm for five minutes at 4ºC. The supernatant was collected and the liofilization process was performed in Hetovac equipment and later solubilization in 1ml of acetonitrile: H_2O buffer in the proportion of 75 : 25. Later, the filtration process was performed at 0.45µm. For the standardization of the High Performance Liquid Chromatography (HPLC), the following standard sugars were used: fructose, glucose, sucrose, raffinose and stachiose, where 50µmoles of each standard sugar were employed. For the construction of the calibration curve, the following concentrations of the standard sugar mixture were used: 0.12; 0.25; 0.37 and 0.5µmol. The

TABLE 1. Initial seed water content (SWC), standard germination (SG), accelerated ageing (AA) and electrical conductivity (EC) of soybean seeds, BRSMG-68 and BRS-133 cultivars.

LOTS	SWC (%)	SG (%)	AA (%)	EC ($\mu S.cm^{-1}.g^{-1}$)
cv. BRSMG-68				
Lot 1	11.0	87 b	75 b	71 b
Lot 2	10.2	93 a	84 a	62 a
C,V.(%)	-	2.19	2.41	2.23
cv. BRS-133				
Lot 1	9.4	83 b	73 b	89 b
Lot 2	9.2	90 a	82 a	76 a
C.V.(%)	-	2.43	2.50	1.70

Comparison of averages in each column by the Tukey test at 5% probability analyses of samples were developed in a HPLC system equipped with a RID detector (Shimadzu, model RID - 10A). The separation of sugars was performed through a Supelcosil column LC-NH_2 (25cm x 4.6mm) with constant flow velocity of one mL/min using the acetonitrile : H_2O buffer (75 : 25). The amount of standard and samples injected was 10mL.

A completely randomized experimental design with four replications per treatment was used. The analysis of variance was performed applying the F test and the obtained averages were compared by the Tukey test at 5% of probability.

RESULTS AND DISCUSSION

Figure 1 (lots of the BRSMG-68 cultivar) and Figure 2 (lots of the BRS133 cultivar) show a significant decrease in germination percentage during the storage periods (3, 6, 9,

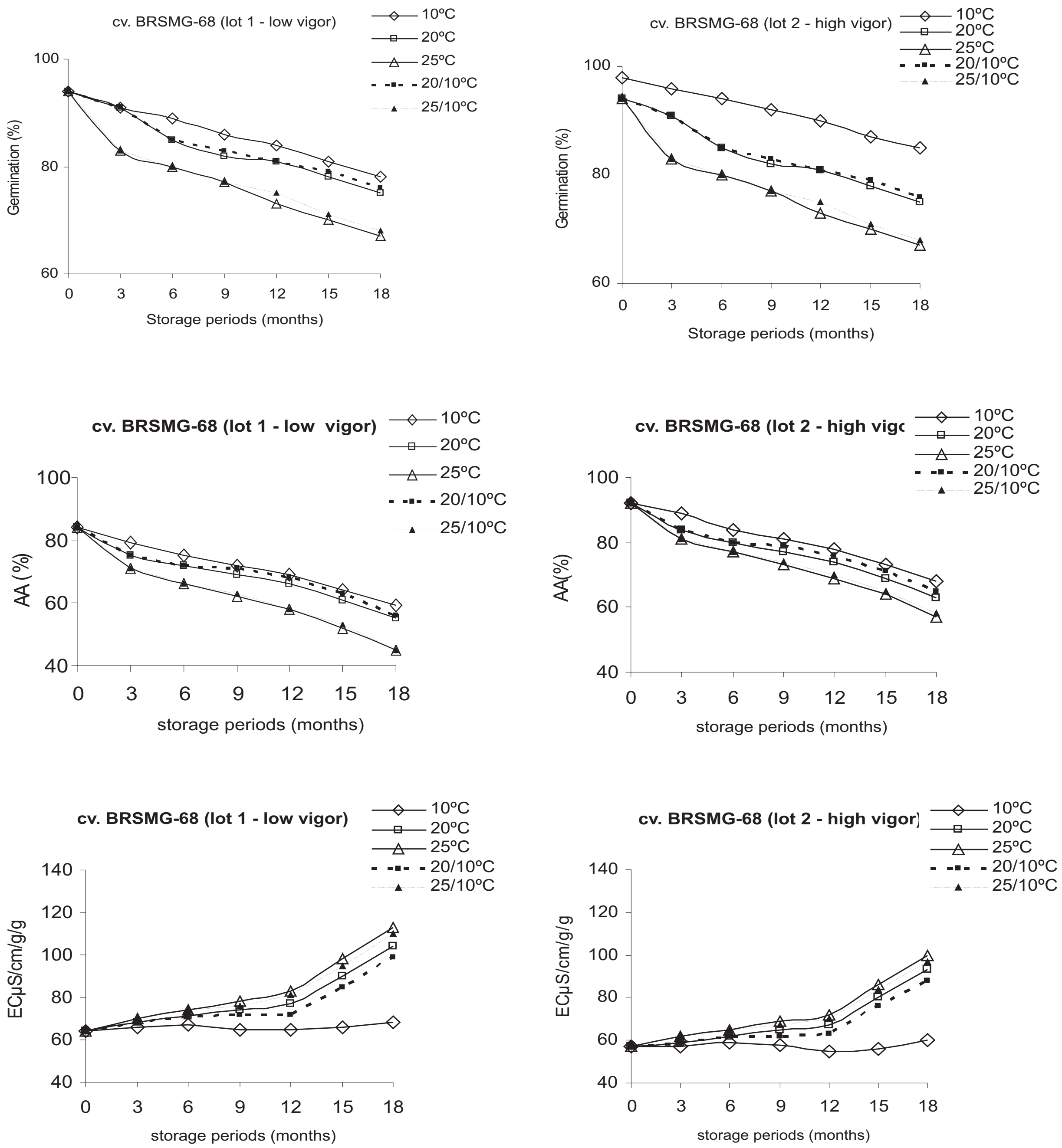

FIGURE 1. Data of standard germination, accelerated ageing (AA) and electrical conductivity (EC) tests of soybean seeds, BRSMG-68 cultivar, lots 1 (low vigor) and 2 (high vigor).

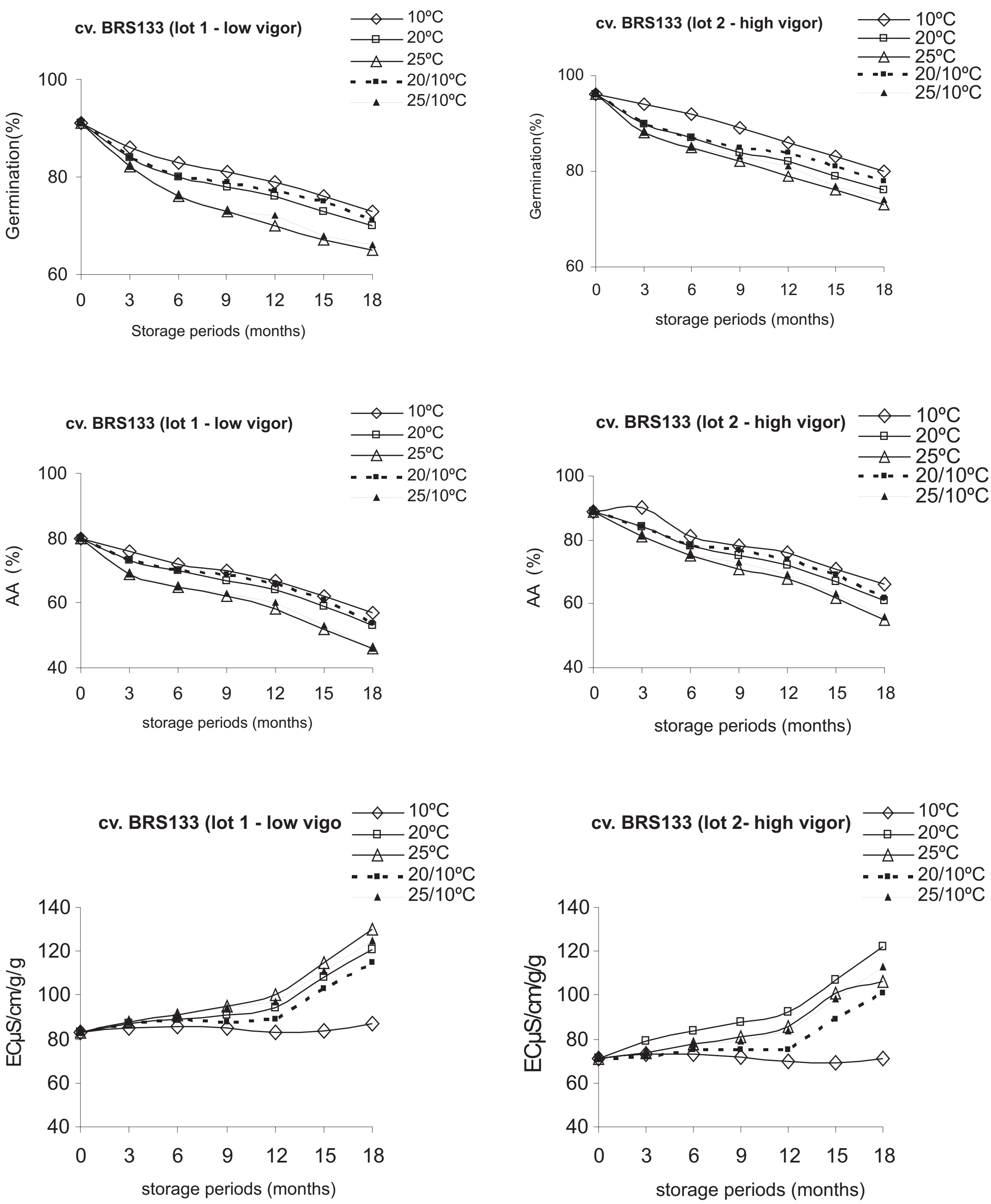

FIGURE 2. Data for standard germination, accelerated ageing (AA) and electrical conductivity (EC) tests of soybean seeds, BRS133 cultivar, lots 1 (low vigor) and 2 (high vigor).

12, 15 and 18 months) at all temperatures studied (10, 20, 25, 20/10 and 25/10ºC).

In this context, it is worth emphasizing that as the storage temperatures increased, reductions in the seed physiological quality were observed. The transference to a lower temperature after six months of storage (treatments 20/10ºC and 25/10ºC) also revealed significant decrease in the soybean seed germination. It is emphasized that the behavior described above was observed in both cultivars studied and in both superior and inferior physiological quality seed lots.

In relation to the results of the accelerated ageing test, a significant reduction in seed germination after accelerated ageing over evaluation times (3, 6, 9, 12, 15 and 18 months) was verified at all the temperatures studied. It was also observed that temperature increased promoted significant decreases in the values obtained during storage periods. In the case of treatments 20/10 and 25/10ºC, the transference to an environment with a lower temperature (10ºC) after six months did not result in delay in the seed deterioration process either. The facts reported above were verified for all the lots studied, for both the BRSMG-68 (Figure 1) and BRS133 (Figure 2) cultivars.

For the electrical conductivity test, when the seeds were stored at 10ºC, no statistical differences were observed among the evaluation times in general (3, 6, 9, 12, 15 and 18 months) and similar behavior was verified for soybean lots. Unlike results obtained in the germination and accelerated ageing tests, the electrical conductivity test indicated that the seed maintained its physiological quality when stored at lower temperature (10ºC).

When working with higher temperatures (20 and 25ºC), significant alterations were verified in the electrical conductivity values during the storage periods, revealing increase on the loss of lixiviated solutes with time. It is should be emphasized that in the mentioned test, the release of higher amounts of exudates in the soaking solution is related to seeds with lower physiological quality, revealing a higher disarrangement intensity of the cell membrane systems.

For treatments involving the transference of seeds to environments with temperature of 10ºC after six months (20/10ºC and 25/10ºC), lower electrical conductivity readings were observed from the ninth month of storage on, in comparison to treatments with the respective constant temperature (in other words, 20ºC and 25ºC). However, this transference to the lower temperature did not result in delay in the seed deterioration process, since a significant increase in the conductivity values among evaluation times was verified.

Data obtained at 10ºC for soybean seeds corroborated some studies in the literature, which indicated that the electrical conductivity results might be influenced by the storage temperature, questioning the use of the test as indicative of seed deterioration and vigour after storage at lower temperatures.

Also working with soybean seeds, Vieira et al. (2001) observed that the germination obtained after accelerated ageing was reduced for all lots stored at 10ºC, while the electrical conductivity data remained unchanged during the same period. In samples of seeds stored at 20ºC, a quick decrease in the values of the standard germination and accelerated ageing tests as well as an increase in the electrical conductivity values were verified. The authors concluded that the electrical conductivity test did not seem to be suitable to evaluate the vigour of soybean seeds stored at low temperature.

Seed hydration allows the cell membrane structure to reorganize becoming less permeable and consequently reducing it leakage (Bewley and Black, 1994). The results found suggest that soybean seed membranes also stabilize at low temperatures, since no significant increase in the electrical conductivity over time was observed.

Table 2 presents data from the fatty acid analysis in soybean seeds from the BRSMG-69 cultivar, lots 1 and 2. The saturated fatty acids (palmitic and estearic) and unsaturated fatty acids (oleic, linoleic and linolenic) were determined because these fatty acids are considered as the most common in plant tissue (Carvalho, 1994).

The cell membranes present a wide superficial area and a high proportion of unsaturated fatty acids that makes lipids particularly susceptible to damage caused by peroxidation (Ferguson et al., 1990). Peroxidative alterations in the composition of fatty acid constituents of the polar lipids would affect the viscosity, permeability and the functioning of cell membranes (Priestley, 1986).

There was an increase in the proportion of saturated fatty acids (palmitic and estearic) during the storage periods for the three water contents (8, 10 and 12%) and for all the temperatures tested. On the other hand, a decrease in the unsaturated fatty acid contents (oleic, linoleic and linolenic) was observed during storage for the three water contents under different temperatures.

Unsaturated fatty acids are considered less stable, in other words, more susceptible to oxidation during the seed deterioration process. In this context, the increase verified in the saturated fatty acids may be due to the transformation of the unsaturated (C18:1, C18:2 and C18:3) into saturated fatty acids (C16:0 and C18:0), as suggested by Priestley and Leopold (1983) in research involving soybean seeds.

The stored seeds revealed a decline on their physiological quality during the evaluation times (0 to 18 months) detected by the germination and accelerated ageing tests at all the temperatures studied. In the case of electrical conductivity, such a decline was only not verified at 10ºC, at which no significant increase in the amount of lixiviated ions over time was observed (Figures 1 and 2).

Comparing the results of the mentioned test after seed

TABLE 2. Data of fatty acids in soybean seeds, BRSMG-68 cultivar (lots 1 and 2) in function of the storage period and temperature.

Period	BRSMG-68 (Lot 1)			BRSMG-68 (Lot 2)		
(month)	10ºC	20ºC	20/10ºC	10ºC	20ºC	20/10ºC
			Palmitic acid (C16:0)			
0	11.59 d	11.59 c	11.59 c	11.61 d	11.61 b	11.61 b
6	11.83 c	11.97 b		11.66 c	11.72 a	
12	12.23 b	11.93 b	12.01 b	12.08 b	11.51 c	11.62 b
18	12.37 a	12.35 a	12.56 a	12.30 a	11.69 a	12.59 a
			Estearic acid (C18:0)			
0	4.30 b	4.30 b	4.30 a	3.57 d	3.57 c	5.57 a
6	4.76 a	5.75 a		4.13 a	5.08 a	
12	3.50 d	3.62 d	3.56 c	3.71 c	3.43 d	3.54 c
18	4.00 c	3.87 c	3.81 b	3.96 b	3.83 b	3.99 b
			Oleic acid (C18:1)			
0	21.80 a	21.80 a	21.80 a	23.85 a	23.85 a	23.85 a
6	21.25 b	20.96 b		20.43 b	20.20 b	
12	20.66 c	20.97 b	20.53 b	19.74 c	20.20 b	19.85 b
18	19.92 d	20.82 c	20.36 c	19.45 d	20.19 b	19.81 c
			Linoleic acid (C18:2)			
0	52.69 a	52.69 a	52.69 a	53.21 a	53.21 a	53.21 a
6	51.88 b	51.69 b		51.60 b	49.74 b	
12	51.75 c	51.49 c	52.55 b	51.53 c	49.79 b	53.01 b
18	51.47 d	51.12 d	52.29 c	51.33 d	49.44 c	52.82 c
			Linolenic acid (C18:3)			
0	7.79 a	7.79 a	7.79 a	9.07 a	9.07 a	9.07 a
6	7.66 b	7.54 b		8.43 b	7.21 b	
12	7.63 b	7.17 c	7.43 b	8.26 c	7.13 c	8.26 b
18	7.40 c	7.10 d	7.28 c	8.11 d	7.05 d	8.20 c

Averages followed by the same lower case letter in a column do not differ by the Tukey test at 5% probability.

storage at 10ºC with the results of the fatty acid analysis (Table 2), no evident relation was verified between them, one of the reasons for this study. The possible stabilization or reorganization of the membranes of seeds stored at low temperatures does not seem to be directly related with alterations in the fatty acid contents during the deterioration process.

It is worth emphasizing that the facts described above were verified for both inferior and superior physiological quality seed lots of soybean studied.

Table 3 shows the data corresponding to the analysis of carbohydrates in soybean seeds, BRSMG-68 cultivar, lots 1 and 2.

Reviewing the dynamic of seed mortality, Bernal-Lugo and Leopold (1998) reported that the transition from a period of relative membrane stability to quick seed ageing could occur through the loss of the glassy state. This loss could be affected by an increase in the temperature and in the seed water content or by a separation of phases of the involved sugars.

Alterations in the carbohydrate contents during storage may contribute to reduction in soybean seed vigour and germination. It is known that the soluble carbohydrates generally decrease with seed ageing (Petruzelli and Taranto, 1989) and that this decrease may reduce the protective effect of sugars on the structural integrity of cell membranes (Crowe et al., 1984).

Generally, a reduction in the monosaccharide (fructose and glucose), disaccharide (sucrose) and oligosaccharide (raffinose and stachiose) contents was observed in the present study for the three water contents and all the temperatures evaluated, corroborating reports by Yaklich (1985) and Bernal-Lugo and Leopold (1992).

In an endeavour to better understand the results obtained in the electrical conductivity test for seeds stored at 10ºC, a test intended to determine the proportion of carbohydrates in the seeds during the storage periods was performed and

TABLE 3. Data of carbohydrates in soybean seeds, cultivar BRSMG-68 (lots 1 and 2) in function of the period and temperature storage.

Period (month)	BRSMG-68 (Lot 1)			BRSMG-68 (Lot 2)		
	10ºC	20ºC	20/10ºC	10ºC	20ºC	20/10ºC
			Fructose			
0	0.55 a	0.55 a	0.55 b	0.58 a	0.58 a	0.58 a
6	0.35 b	0.29 b		0.42 b	0.36 b	
12	0.14 c	0.13 c	0.88 a	0.11 c	0.24 c	0.22 b
18	0.10 c	0.12 d	0.16 c	0.07 c	0.13 d	0.09 c
			Glucose			
0	0.32 a	0.32 a	0.32 a	0.36 a	0.36 a	0.36 a
6	0.16 b	0.10 b		0.16 b	0.14 b	
12	0.15 b	0.09 b	0.18 b	0.10 c	0.13 b	0.17 b
18	0.03 c	0.03 c	0.01 c	0.09 c	0.04 c	0.01 c
			Sucrose			
0	5.49 a	5.49 a	5.49 a	5.57 a	5.57 a	5.57 a
6	2.97 b	2.78 b		3.23 b	2.58 b	
12	1.05 c	0.78 c	1.04 b	0.66 c	1.25 c	1.22 b
18	0.60 d	0.17 d	0.58 c	0.13 d	0.29 d	0.52 c
			Raffinose			
0	0.40 a	0.40 a	0.40 a	0.70 a	0.70 a	0.70 a
6	0.18 b	0.16 b		0.33 b	0.20 b	
12	0.10 c	0.14 b	0.06 b	0.20 c	0.19 b	0.11 b
18	0.02 d	0.10 c	0.03 b	0.10 d	0.09 c	0.02 c
			Stachiose			
0	2.07 a	2.07 a	2.07 a	2.75 a	2.75 a	2.75 a
6	0.76 b	0.74 b	-	1.06 b	0.70 b	
12	0.58 c	0.48 c	0.58 b	0.94 c	0.36 c	0.36 b
18	0.30 d	0.08 d	0.11 c	0.71 d	0.16 d	0.21 c

Averages followed by same small letter in column are not different from each other through the Tukey test at 5% of probability.

verified that a relationship could not be established, since the carbohydrates presented similar behavior at all temperatures tested.

CONCLUSIONS

The bulk electrical conductivity test is not a good indicator of the deterioration process of seeds stored at low temperatures. No direct relationship between changes in the fatty acid and carbohydrate contents during storage and the performance of the electrical conductivity test was verified for seeds stored at 10ºC.

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REFERENCES

BERNAL-LUGO, I.; LEOPOLD, A.C. Changes in soluble carbohydrates during seed storage. **Plant Physiology**, Bethesda, v.98, n.3, p.1207-1210, 1992.

BERNAL-LUGO, I.; LEOPOLD, A.C. The dynamics of seed mortality. **Journal of Experimental Botany**, Eynsham, v.49, n.326, p.1455-1461, 1998.

BEWLEY, J.D.; BLACK, M. **Seeds**: physiology of development and germination. New York: Plenum Press, 1994. 444p.

BLIGH, E.G.; DYER, W.J. A rapid method of total lipid extraction and purification. **Canadian Journal of Biochemistry and Physiology**, Ottawa. v.37, n.8, p.911-917, 1959.

CARVALHO, N.M. O conceito de vigor em sementes. In: Vieira, R.D.; Carvalho, N.M.(Ed.) **Testes de vigor em sementes**. Jaboticabal: FUNEP, 1994. p.1-30.

CROWE, L.M.; MOURDIAN, R.; CROWE, J.H.; JACKSON, S.A.;

WOMERSLY, C. Effects of carbohydrates on membrane stability at lower water activities. **Biochimica et Biophysica Acta**, Amsterdam, v.769, p.141-150, 1984.

FERGUSON, J.M. **Metabolic and biochemical changes during the early stages of soybean seed deterioration.** 1988. 138p. Thesis (Ph.D. in Crop Science) - University of Kentucky, Lexington, Kentucky, EUA, 1985.

FERGUSON, J.M.; TEKRONY, D.M.; EGLI, D.B. Changes during early soybean seed and axes deterioration: II. Lipids. **Crop Science**, Madison, v.30, n.1, p.179-182, 1990.

FESSEL, S.A. **Condutividade elétrica em sementes de soja em função da temperatura e do período de armazenamento**. 2001. 100f. Dissertação (Mestrado em Agronomia/Produção e Tecnologia de Sementes) - Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, 2001.

HAMPTON, J.G.; JOHNSTONE, K.A.; EUA-UMPON, V. Bulk conductivity test variables for mungbean, soybean and frenchbean seed lots. **Seed Science and Technology**, Zürich, v.20, n.3, p.677-686, 1992.

HAMPTON, J.G.; TEKRONY, D.M. **Handbook of vigor test methods**. Zürich: ISTA, 1995. 103p.

INTERNATIONAL SEED TESTING ASSOCIATION. International Rules for Seed Testing. **Seed Science and Technology**, Zürich, v.27**,** p.1-333, 1999 (Supplement).

LOEFFLER, T.M.; TEKRONY, D.M.; EGLI, D.B. The bulk conductivity test as an indicator of soybean seed quality. **Journal of Seed Technology**, Springfield, v.12, n.1, p.37-53, 1988.

MAIA, E.L.; RODRIGUES-AMAYA, D. Avaliação de um método simples e econômico para metilação de ácidos graxos com lipídios de diversas espécies de peixes. **Revista do Instituto Adolfo Lutz**, São Paulo, v.53, n.1, p.27-35, 1993.

MCDONALD, M.B. Seed deterioration: physiology, repair and assessment. **Seed Science and Technology**, Zürich, v.27, n.1, p.177-237, 1999.

PETRUZELLI, L.; TARANTO, G. Wheat aging: the contribution of embryonic and non-embryonic lesions to loss seed viability. **Physiologia Plantarum**, Copenhagen, v.76, n.2, p.289-294, 1989.

PRIESTLEY, D.A. **Seed ageing**: implications for seed storage and persistence in the soil. Ithaca: Cornell University Press, 1986. 304p.

PRIESTLEY, D.A.; LEOPOLD, C. Lipid changes during natural ageing of soybean seeds. **Physiologia Plantarum**, Copenhagen, v.59, n.3, p.467-470, 1983.

TAO, J.K. Factors causing variations in the conductivity test for soybean seeds. **Journal of Seed Technology**, Springfield, v.3, n.1, p.10-18, 1978.

VIEIRA, R.D.; AGUERO, J.A.P.; PERECIN, D. Electrical conductivity and field performance of soybean seeds. **Seed Technology**, Lincoln, v.21, n.1, p.15-24, 1999.

VIEIRA, R.D.; KRZYZANOWSKI, F.C. Teste de condutividade elétrica In: KRZYZANOWSKI, F.C.; VIEIRA, R.D.; FRANÇA-NETO, J.B. (Ed.). **Vigor de sementes**: conceitos e testes. Londrina: ABRATES, 1999. p.4.1-4.26.

VIEIRA, R.D.; PANOBIANCO, M.; LEMOS, L.B.; FORNASIERI FILHO, D. Efeito de genótipos de feijão e de soja sobre os resultados da condutividade elétrica de sementes. **Revista Brasileira de Sementes**, Brasília, v.18, n.2, p.220-224, 1996.

VIEIRA, R.D.; PENARIOL, A.L.; PERECIN, D.; PANOBIANCO, M. Condutividade elétrica e teor de água inicial das sementes de soja. **Pesquisa Agropecuária Brasileira**, Brasília, v.37, n.9, p.1333-1338, 2002.

VIEIRA, R.D.; TEKRONY, D.M.; EGLI, D.B.; RUCKER, M.. Electrical conductivity of soybean seeds after storage in several environments. **Seed Science and Technology**, Zürich, v.29, n.3, p.599-608, 2001.

YAKLICH, R.W. Effect of aging on soluble oligosaccharide content in soybean seeds. **Crop Science**, Madison, v.25, n.4, p.701-704, 1985.

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