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Effect of Harvesting and Drying Conditions on Chlorophyll Levels of Soybean (*Glycine max* L. Merr)

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Chlorophyll in soybean represents a downgrading factor for the crops. Five Brazilian cultivars were harvested between R_6 and R_8 stage of development (Fehr & Caviness scale) and dried at 25° and 40 °C. The effect of maturity stages and two drying conditions after harvest were studied to achieve reduction of moisture and chlorophylls to acceptable levels. When seeds were dried at 25 °C, even harvesting at early stages of development such as R_6, the green pigments were almost degraded, and 16 ppm of chlorophyll were found at maximum, accompanied by loss of moisture. Moisture and chlorophyll declines as seed matures, but at intermediary stages (R_6–R_7), chlorophyll degrades first, so the rate of moisture loss should not be used to predict chlorophyll contents. At 40 °C, complete degradation of chlorophyll pigments is only achieved when seeds are swathed from R_7 stage up, otherwise the seed quality could be compromised. Slow drying allows almost complete removal of green pigments, even when seeds are swathed a few days before the physiological maturity stage.

KEYWORDS: Soybean seeds; maturation; drying conditions; chlorophyll contents

INTRODUCTION

The presence of chlorophyll in oilseeds such as canola and soybean is considered a downgrading factor since the presence of green seeds is one of the parameters used for quality control of these grains (*1*–*3*). Chlorophylls are extracted with the oil when green seeds are crushed producing dark-colored crude oils, which are commercially unacceptable and may require additional special treatment during refining. The oil is aesthetically unappealing to consumers for a few reasons. When present in oils, chlorophyll pigments act as photosensitizers, reduce its oxidative stability, lead to rancidity, and reduce its shelf life (*4*–*6*). Chlorophyll derivatives can also act as catalyst poisons, impairing the hydrogenation process. The pigments could be removed from the oil by adsorption on bleaching clays but it increases costs of refining and as larger amounts of clays are needed, oil loss increases as a result of retention (*7*–*9*). Besides that, it has been reported that high contents of chlorophyll impair germination of seeds (*10*). These problems are common in canola seeds produced in the United States and Canada but also are observed in soybean, mainly in Brazil and other tropical countries, with high pluviometric indexes and hot weather, which are environmental conditions commonly associated with the maintenance of green seeds (*11*, *12*).

Various classification systems have been used for international trade to control and standardize the production of soybean seeds for marketing. Each country has set up rules regarding trading procedures, grades, and standards. The various criteria used to evaluate soybean quality are based on several physical characteristics as damaged kernels, splits, other colors than yellow, and foreign matter. Different countries are more or less tolerant and grade standards differ in their maximum percent limits (*13*, *14*). In Brazil, the parameters for soybean are based on moisture contents, damaged kernels, splits, impurities, and the presence of green-colored seeds. The Brazilian grade standards, established in 1983 and not modified until now, are more tolerant than those in international trade concerning the allowed proportion of green seeds. While the threshold for off-colors of No. 1 and No. 2 U. S. graded soybeans is 1% and 2% respectively, the maximum permitted level of discolored seeds is 10% in Brazil and seeds are not subclassified into categories (*15*). There is a worldwide increasing interest in classifying soybeans according to their chlorophyll and pheophytin levels.

The degradation process of chlorophylls is not completely understood but previous studies performed with canola reported that chlorophyll contents can be strongly affected by both the stage of maturity and the postharvest drying conditions, although

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adverse growing conditions as hot weather associated to water stress or frost damage can also interfere on normal ripening (*11*, *12*). Retention of green color can be observed as well as on field, when desiccants are applied on the cultures to reduce seed moisture. Another situation where seeds stay green occurs with the common practice of harvesting immature crops to hasten the harvesting process and to reduce the shattering losses. These studies correspond to canola (*16*, *17*) but have also been observed with soybeans (*10*). Immature and high-moisture-containing seeds require fast drying processes to reduce moisture contents to acceptable levels for safe storage (below 13%). The temperatures commonly used for fast drying are over the range from 40° to 80 °C, depending on the destiny of grains; lower temperatures are used to produce soy protein isolates and higher temperatures prevail for the seed-crushing industry (*13*). The fast removal of moisture contents can result in the maintenance of considerable amount of green seeds. Although the green pigments could be eliminated during oil refining, a better solution would be the avoidance of green seeds on the basis of a deeper knowledge about the harvest and postharvest conditions to achieve the chlorophyll degradation.

Despite the fact that there are many refined techniques for direct quantification and structure elucidation of chlorophyll and its derivatives (*18*), the preferred routine procedures to classify seeds for oil processing are based on rapid and simple methods such as spectrophotometry besides colorimetric methods, which are indirectly related to chlorophyll contents.

In view of the requirements of moisture contents below 13% and chlorophyll contents lower than 30 ppm, which are set as the maximum levels for first-grade seeds according to the International Commercial Standards of Canadian Grain Commission for exporting canola and soybeans (*1*, *3*, *19*), the objective of the present study was to compare the effect of slow and fast drying conditions on chlorophyll contents of soybean seeds harvested at different maturity stages. We intended to get better knowledge about the conditions of swathing and drying which may be able to avoid retention of green-colored pigments to guarantee marketing quality of seeds.

MATERIALS AND METHODS

Plant Material and Sampling. Five Brazilian soybean (*Glycine max* L. Merr) cultivars, IAS-5, IAC-17, IAC-18, IAC-20, and IAC− Foscarin 31, were grown at the Agronomic Institute of Campinas, São Paulo, Brazil. The experiments were conducted between November of 1996 (planting) and March of 1997 (swathing). Each cultivar was planted in a field divided into 10 rows, each of them having 10 m of length for each variety. There was a distance of 60 cm between rows and 1.5 m between each variety. The soybeans were harvested at intervals of approximately 5 days from the beginning to the end of March, with five intervals of development, beginning at physiological maturation (R_6), which occurs nearly 125 days after planting, continuing until the commercial maturation (full maturity or R_8) (*20*), adding two more intermediate steps R_6-R_7 (I) and R_6-R_7 (II). For each stage of development, samples were harvested early in the morning from four randomly selected subplots to minimize located effects of crops in the planted area. After swathing, samples from the subplots were combined and submitted to threshing by hand.

Drying Conditions. Seeds were divided into three groups. One part was used for analysis of moisture of freshly harvested samples to monitor the moisture loss that occurs naturally during field maturation. The two other groups were submitted to two drying conditions until their moisture contents dropped down to 13%. Slow-dried seeds were obtained by allowing intact pods to air-dry at ambient temperature (25° ± 5 °C) for approximately 10 days and fast-dried seeds were obtained by dehydration in an air-circulating oven (40° ± 2 °C) for approximately 5 days. The period of drying depended on the initial moisture content of the seeds in each maturity stage. After drying, seeds were threshed and stored at 2 °C until analysis of moisture and chlorophyll.

Experimental Design. The factorial experimental design was 5 × 5 × 2 (five cultivars × five maturation stages × two drying conditions). Quantification of moisture and chlorophyll contents were made in three genuine replicates for each assay. Results are presented in terms of mean values with their respective standard deviations.

Moisture Determination. The moisture contents of fresh intact seeds recently harvested were determined by drying 10 g of samples in oven at 105 °C until achieving constant weight. Residual moisture of fast- or slow-dried seeds was determined on 2 g powdered soybean seeds at 105 °C until constant weight.

Chlorophyll Analysis. Total chlorophyll contents and its components a and b were determined spectrophotometrically, according to the method n° 942.04 for chlorophyll in plants described by the Association of Official Analytical Chemists (*21*). To extract the green pigments, 2 g of dried soybean seeds were ground in a laboratory mill (Polymix KCH-Analytical mill A10, Kinematica AG, Luzern, Switzerland) and extracted for 1 h, protected from light, in 30 mL of heptane/ethanol (3:1 v/v) in a shaker (Julabo SW20, Julabo Labortechnik GmbH, Seelbach, Germany) at constant rotation of 110 rpm/min. The homogenate was then centrifuged at 20 000 rpm for 15 min at 15 °C (Sorvall RC-5C, rotor SS-34); the pellet was re-extracted under the same conditions and the supernatants were combined. The pigment-containing extracts were dehydrated with anhydrous sodium sulfate, dried under vacuum in a rotary evaporator (Heidolph Elektro GmbH & Co., WB2000, Kelheim, Germany), and the residues were made up to 10−25 mL with diethyl ether. Pigments were quantified spectrophotometrically (DU-70, Beckman Instruments, Palo Alto, CA). Absorbance readings of the solutions were made at both 660 and 642.5 nm. The concentrations of chlorophyll a and b and total chlorophyll contents were estimated using the following equations and the extinction coefficients for diethyl ether, found in the literature (*21*):

$$\text{Chl t (total chlorophyll, mg/kg)} = 7.12\,[A_{660}] + 16.8\,[A_{642.5}]$$

$$\text{Chl a (chlorophyll a, mg/kg)} = 9.93\,[A_{660}] - 0.777\,[A_{642.5}]$$

$$\text{Chl b (chlorophyll b, mg/kg)} = 17.6\,[A_{642,5}] - 2.81\,[A_{660}]$$

where A_λ is the absorbance of the solution at the respective wavelengths. Samples were analyzed in triplicate. The values obtained are reported on a dry basis. Quantification of individual chlorophyll a and b contents were performed to investigate the changes in chlorophyll a/chlorophyll b ratios caused by drying seeds at different temperatures.

Data Analysis. The entire experiment was conducted through two consecutive years, and there were triplicate of each sample analysis. Results were similar in both experiments; however, only data from the second experiment are reported here. Analysis of variance was performed, using the SAS statistical software package. Significance was determined at the 0.05 level. Data presented in graphs show calculated means and standard deviations of the mean values.

RESULTS AND DISCUSSION

Moisture Contents of Soybeans. The moisture contents of freshly harvested seeds from standing plants sampled at regular time intervals of the five cultivars were analyzed and numerical results are presented in **Table 1**, showing the natural loss of moisture that occurs on field. Moisture contents were similar among the cultivars and of about 69.4 ± 2.48% at the first sampling time corresponding to the stage R_6. Over the next two weeks, the seed moisture contents declined very slowly up to 59.2 ± 3.83% until the seeds of all cultivars reached a stage

Table 1. Moisture Contents (%) of Soybean Seeds Harvested at Five Sampling Times and Dried at Two Conditions

cultivars	freshly harvested seeds[a]					dried seeds[a]	
	R_6	R_6–R_7 (I)	R_6–R_7 (II)	R_7	R_8	25 °C	40 °C
IAS-5	67.3 ± 0.6	65.3 ± 1.0	63.3 ± 1.1	15.0 ± 0.2	11.0 ± 0.1	10.7 ± 0.7	9.4 ± 0.6
IAC-17	72.1 ± 0.7	65.6 ± 0.4	60.7 ± 0.5	41.1 ± 2.1	11.4 ± 0.3	10.6 ± 0.3	9.4 ± 0.5
IAC-18	68.5 ± 0.5	64.5 ± 0.6	60.3 ± 0.3	36.5 ± 3.7	11.6 ± 2.8	11.0 ± 0.2	10.2 ± 0.1
IAC-20	67.1 ± 3.0	62.4 ± 2.6	58.9 ± 0.8	18.0 ± 0.6	11.6 ± 0.0	11.0 ± 0.2	9.7 ± 0.3
Foscarin 31	72.0 ± 1.2	63.6 ± 0.4	53.0 ± 0.4	14.3 ± 0.7	11.4 ± 0.1	11.3 ± 0.2	10.0 ± 0.2

[a] Mean values of triplicate analysis of moisture contents (%).

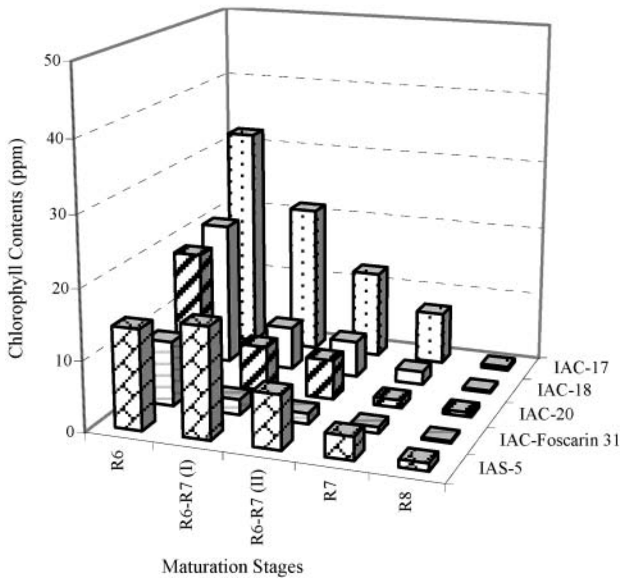

Figure 1. Total chlorophyll contents in five Brazilian soybean seeds harvested at five maturation stages and dried at 25 °C.

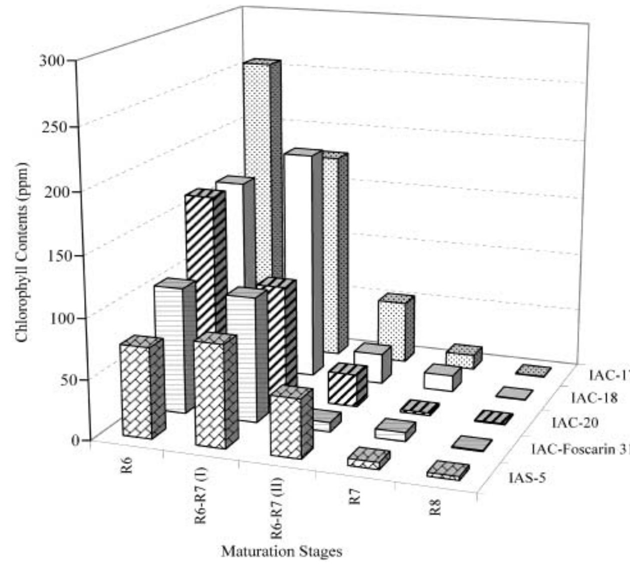

Figure 2. Total chlorophyll contents in five Brazilian soybean seeds harvested at five maturation stages and dried at 40 °C.

between R_6 and R_7. Afterward, the average moisture contents decreased faster but we observed significant differences between cultivars: IAS-5, IAC-20, and Foscarin 31 reached an average value of 15.8%, whereas cultivars IAC-17 and IAC-18 showed still an average moisture content of 38.8% at physiological maturity (R_7), pointing out that the length of growing differs from each other according to the variety. At stage R_8, 23 days after the first sampling, the moisture had decreased uniformly to 11.4%. However, the final moisture contents could have varied significantly with the air humidity and length of growing. Therefore, in wet years, even harvesting at R_8 stage is not a guarantee for adequate moisture contents. Frequent and long rainfall alternated with dry periods may affect moisture contents of maturing seeds when they are still on the field, accelerating deterioration processes or impairing the grain germination (*7*, *9*, *19*).

Samples from each harvest time were dried separately at 25 °C and 40 °C and their final moisture contents are also shown in **Table 1**. All seeds had their moisture contents decreased to values ranging between 9.4% and 11.3%, which are considered safe for storage, avoiding the growth of storage fungi producing hydrolytic lipases (*1*).

Chlorophyll Contents after Drying at Ambient Temperature (25 °C). The chlorophyll contents of seeds of the five cultivars swathed at regular time intervals over the seed development and maturation stages beginning at physiological maturity stage (R_6) are presented in **Figure 1**. Prior to chlorophyll quantification, the intact pods were dried at ambient temperature (25 ± 5 °C) similar to conditions that commonly occur on field. As a matter of course, drying these immature seeds at ambient temperature permitted an effective yellowing in all cultivars analyzed, once degradation of chlorophyll continued characteristically after harvesting, and breakdown was almost complete. We observed that even immature and quite green seeds swathed at R_6 stage could have their chlorophyll contents reduced to levels as low as 9 ppm as observed for IAC-Foscarin 31cultivar whereas the maximum level observed among the five cultivars was 32 ppm. As expected, seeds harvested at later stages of development had their chlorophyll contents degraded to almost undetectable levels. This behavior suggests a strong physiological activity associated with maturation at ambient temperature.

Chlorophyll Contents after Drying at 40 °C. The chlorophyll contents of seeds dried at 40 ° C are shown in **Figure 2**. The fast drying stopped the chlorophyll degradation and did not allow yellowing to proceed to the same extent as for slow-dried seeds. Seeds harvested at R_6 and immediately submitted to a fast drying showed variable but high chlorophyll contents, ranging from 75 to 257 ppm, depending on the cultivars. Fast-dried seeds coming from stage R_6−R_7(II) presented still chlorophyll contents varying between 8 and 53 ppm. On the other hand, fast drying did not play a significant role on the rate of chlorophyll breakdown when plants are harvested after stage R_6−R_7(II) while only residual levels of the pigment were found (**Figure 2**).

Therefore, we concluded that seeds harvested from stage R_6 until R_6−R_7(II), when dried at 40 °C, actually present a risk to stay green.

The reasons for the maintenance of green color are not well understood yet but seem to be related to the moisture content and rate of dehydration, as previously reported by Adams et al.

Table 2. Chlorophyll a (Chl a) and Chlorophyll b (Chl b) Contents and Chlorophyll a/b Ratio of Five Brazilian Soybean Seeds Harvested at Five Maturation Stages and Dried at 25° and 40 °C

		chlorophyll content in soybean seeds mg/kg (dry weight)[a]					
		drying at 25 °C			drying at 40 °C		
variety	maturation stage	chl a[b]	chl b[c]	a/b[d]	chl a	chl b	a/b
IAS-5	R_6	8,8 ± 0,8	5,5 ± 1,0	1,6	53,6 ± 4,0	21,8 ± 3,5	2,5
	R_6–R_7 (I)	10,2 ± 0,2	5,6 ± 0,5	1,8	65,5 ± 3,0	19,5 ± 1,6	3,4
	R_6–R_7 (II)	4,4 ± 0,4	3,3 ± 0,5	1,3	34,2 ± 1,7	14,5 ± 1,0	2,4
	R_7	2,0 ± 0,1	1,3 ± 0,2	1,5	3,9 ± 0,1	1,9 ± 0,1	2,0
	R_8	0,5 ± 0,1	0,4 ± 0,2	1,3	2,2 ± 0,2	0,9 ± 0,2	2,4
IAC-17	R_6	21,5 ± 0,7	10,8 ± 0,3	2,0	189,6 ± 11,9	67,4 ± 4,3	2,8
	R_6–R_7 (I)	12,1 ± 1,4	9,2 ± 1,4	1,3	131,3 ± 5,2	46,8 ± 5,7	2,8
	R_6–R_7 (II)	7,6 ± 0,5	5,0 ± 0,4	1,5	39,9 ± ,03	13,3 ± 0,4	3,0
	R_7	4,7 ± 0,4	2,8 ± 0,4	1,7	7,6 ± 1,0	4,8 ± 1,0	1,6
	R_8	0,3 ± 0,04	0,2 ± 0,1	1,1	0,26 ± 0,0	0,24 ± 0,1	1,1
IAC-18	R_6	13,2 ± 0,3	7,4 ± 0,5	1,8	121,6 ± 1,2	42,6 ± 0,8	2,9
	R_6–R_7 (I)	3,6 ± 0,2	2,5 ± 0,2	1,4	146,3 ± 4,0	47,0 ± 1,6	3,1
	R_6–R_7 (II)	3,4 ± 0,3	1,9 ± 0,5	1,8	20,0 ± 0,1	5,6 ± 0,4	3,5
	R_7	1,1 ± 0,1	0,8 ± 0,1	1,4	1,01 ± 0,4	3,7 ± 0,2	2,7
	R_8	0,16 ± 0,05	0,14 ± 0,1	1,1	0,24 ± 0,0	0,26 ± 0,1	0,9
IAC-20	R_6	12,5 ± 0,6	6,6 ± 1,6	1,9	122,7 ± 0,3	43,9 ± 0,6	2,8
	R_6–R_7 (I)	3,8 ± 0,1	2,7 ± 0,2	1,4	71,0 ± 3,7	24,7 ± 0,3	2,9
	R_6–R_7 (II)	3,4 ± 0,2	2,3 ± 0,2	1,4	20,4 ± 2,3	6,9 ± 0,5	2,9
	R_7	0,67 ± 0,1	0,43 ± 0,1	1,5	1,8 ± 0,0	0,5 ± 0,2	4,1
	R_8	0,32 ± 0,03	0,28 ± 0,0	1,1	0,5 ± 0,0	0,4 ± 0,0	1,2
IAC-Foscarin 31	R_6	6,2 ± 0,2	3,1 ± 0,4	2,0	81,8 ± 11,1	23,9 ± 0,1	3,4
	R_6–R_7 (I)	1,3 ± 0,1	0,9 ± 0,3	1,4	81,5 ± 1,7	22,9 ± 1,5	3,6
	R_6–R_7 (II)	0,9 ± 0,02	0,6 ± 0,02	1,5	5,6 ± 0,4	2,3 ± 0,5	2,5
	R_7	0,5 ± 0,05	0,4 ± 0,2	1,2	4,8 ± 0,3	2,8 ± 0,7	1,7
	R_8	0,15 ± 0,04	0,14 ± 0,5	1,1	0,46 ± 0,05	0,44 ± 0,2	1,0

[a] Mean values of three genuine replicates ± s.d. [b] Chl a: Chlorophyll a (mg/kg). [c] Chl b: Chlorophyll b (mg/kg). [d] a/b: ratio chl a/chl b.

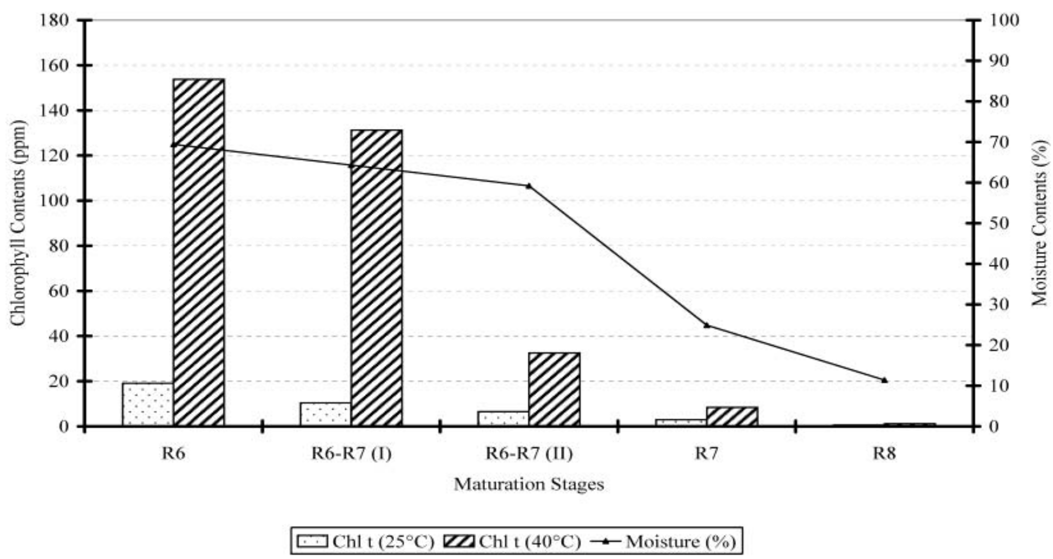

Figure 3. Chlorophyll contents in soybean seeds (mean values of all varieties) dried at 25 °C and 40 °C, over the whole maturation period, in comparison with moisture levels of recently harvested seeds.

(*10*) and Ward et al. (*11*). According to these authors, a rapid desiccation turns moisture unavailable for respiration, so metabolism within the seed stops and the seed fails to ripen. Similar conclusions were presented by Nwufo (*22*) pointing out that transpiration on hot weather causes water stress, which could be responsible for disintegration of membranes and leakage of cell contents. We observed in a previous assay that an attempt to rehydrate the fast-dried seeds and then dehydrate them slowly was unsuccessful to achieve chlorophyll breakdown, indicating that changes that occurred during the fast drying were irreversible (results not shown).

Changes on Chlorophyll a/b Ratios over the Whole Maturation Period after Drying. The contents of individual components (chlorophyll a and chlorophyll b) of slow-dried seeds (25 °C) and fast-dried seeds at 40 °C of the five varieties over the whole maturation period are shown in **Table 2**. In general, all varieties presented significant higher chlorophyll a levels compared to chlorophyll b but both contents declined during the period of degreening. As expected, the degradation of chlorophyll a was slightly faster than chlorophyll b. According to the newest reports, the enzymes involved in chlorophyll degradation seem to be specific for chlorophyll a and it has been suggested that the reduction of chlorophyll b to chlorophyll a plays a role in the process of chlorophyll degradation (*23*). Therefore, in our assumption, the rate of chlorophyll a degradation mediated by specific enzymes was higher than the rate of reduction of chlorophyll b to chlorophyll a, as observed by a decreasing of chlorophyll a/b ratio.

Seeds dried at 25 °C presented a decrease of chlorophyll a/b ratios related to the maturation stage with a concomitant alteration of the pigment patterns. At stage R_6, the mean ratio was 1.9 and at full maturity (R_8) it changed to 1.3. On the other hand, seeds dried at 40 °C showed, at stage R_6, a higher chlorophyll a/b ratio (2.9) than that observed for slow-dried seeds and with the progress of maturation till R_8, the ratio decreased to 1.1.

These results are similar to those reported by Johnson-Flanagan and Thiagarajah (*17*) who observed a slight decrease in the a/b ratios from 2.5 to 2.0 in canola seeds during maturation on field. The decrease of a/b ratio is related to the lower stability of chlorophyll a. Drying at 40 °C, the chlorophyll degradation was blocked favoring the maintenance of higher chlorophyll a levels than chlorophyll b.

We noticed that seeds from varieties IAS-5 and IAC-18 harvested at stage R_6 seemed to not have reached full physiological maturity and chlorophyll contents increased until R_6-R_7(I). The chlorophyll synthesis from R_6 to R_6-R_7(I) became evident during fast drying when the rate of chlorophyll synthesis was higher than chlorophyll degradation. We do suppose that drying at 25 °C, both processes, synthesis and degradation, take place, but in this case, degradation seems to be faster than synthesis not allowing visualization of the metabolism.

Interaction of Initial Moisture Contents of Seeds and Chlorophyll Contents after Drying. Figure 3 presents the average moisture contents of recently harvested seeds and chlorophyll levels after drying seeds at each harvesting time to verify if there was a relationship between these two parameters (*10*, *12*). A significant correlation would be useful for predicting chlorophyll contents at any maturation stage just on the basis of moisture contents.

Several studies have shown that a decrease in chlorophyll levels after harvest is only possible if seed contains sufficient moisture for metabolic reactions (*7*, *19*). We observed that harvesting at any time from stage R_6 on and drying at 25 °C permits chlorophyll breakdown reaching very low levels independent of initial moisture contents at harvest and did not show any abnormal maturation process. Although both moisture and chlorophyll decline as seed matures when dried at 25 °C, the rate of chlorophyll breakdown does not depend on the rate of moisture loss, or vice versa, resulting always in grains with acceptable levels of green pigments.

Moisture loss did not show a linear pattern during maturation and the degree of development played a significant role in retention of the green color. At first, it was observed a slow decrease from 70% to 60% corresponding to R_6 and R_6-R_7(I), respectively, followed by a fast decrease of moisture until reaching 11% at R_8. Drying the soybeans at 40 °C, the pigment contents declined in a similar pattern in comparison to moisture loss. Seeds harvested from R_6 until R_6-R_7(II) showed a parallel decline of both green pigments and moisture levels. Afterward, at intermediate maturation stages, chlorophyll breakdown seemed to occur first, with an exponential reduction. Besides that, at this period of degreening, the moisture loss occurs quickly and in our opinion, it seems to be too risky predicting chlorophyll levels on the basis of only moisture contents.

On the basis of our experiment, we conclude that at 40 °C, complete degradation of chlorophyll pigments is only achieved when seeds are swathed from R_7 up, otherwise the seed quality could be compromised. Slow drying allows almost complete removal of green pigments, even when seeds are swathed a few days before the physiological maturity stage.

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