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Relationship between Color (Instrumental and Visual) and Chlorophyll Contents in Soybean Seeds during Ripening

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The correlation between chlorophyll content and quantitative color parameters was investigated in order to find an indirect method for predicting green pigment in ripening soybean seeds. Five Brazilian soybean varieties harvested at different maturity stages (R_6 to R_8 according to the scale of Fehr & Caviness) and dried under two conditions (in oven at 40 °C with circulating air and at ambient temperature around 25 °C) were analyzed in two consecutive years. The slow-dried seeds at 25 °C lost chlorophyll faster, whereas drying at 40 °C did not result in yellowing of seeds. High and significant linear correlations between *a** value and total chlorophyll were obtained over the whole maturation period and on both conditions of drying. From an industrial point of view it appears that *a** value, obtained by the CIE-L*a*b* method, seems to be a good tool to be applied for quality control and classifying soybean seeds for different purposes.

KEYWORDS: Soybean; maturation stages; drying conditions; chlorophyll contents; color correlation

INTRODUCTION

The most important edible-oil-producing raw materials in terms of volume produced and exported in world oil trade are soybean, canola, sunflower, palm kernel, and coconut (*1*). In 1999, the global production of soy was 160 million metric tons with the major production countries being the United States, Brazil, and Argentina (74.6, 31.0, and 19.9 millions of tons, respectively). In Brazil 90% of total soybean is exported as kernels and meal (*2*).

A major portion of annual soybean production is crushed for oil and defatted meal (*3*), and the use of the latter as animal feed has been increasing after the outbreak of bovine spongyform encephalopathy (BSE), known as “mad cow disease”.

In face of the expanding market, various classification systems have been used for international trade to control and standardize the production of soybean seeds for marketing. To promote fair trading of soybean commodity and provide a medium of communication between buyers and sellers at national and international levels, each country has set up rules regarding trading procedures, grades, and standards. The various criteria used to evaluate soybean quality are based on a number of physical characteristics such as damaged kernels, splits, colors other than yellow, and foreign matter. Different countries are more or less tolerant, and grade standards differ in their maximum percent limits (*4*, *5*). In Brazil, the parameters for soybean are based on moisture contents, damaged kernels, splits, impurities, and the presence of green seeds. The grade standards adopted in Brazil are more tolerant than those in international trade concerning the allowed proportion of green seeds. Whereas 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.

The importance of the presence of chlorophyll in oilseeds has been recognized as it increases processing costs, and payments to farmers take into account the chlorophyll levels in seeds (*1*, *6*). Chlorophyll can be extracted with the oil, promoting its oxidation, reducing its shelf life, and producing dark-colored oil, which is aesthetically unappealing to consumers (*7*−*9*). These problems are common in canola seeds produced in U.S. and Canada, but also are observed in soybean, mainly in Brazil and other tropical countries with high pluviometric indexes and hot weather, which are conditions commonly associated to the appearance of green seeds. Besides that, high chlorophyll content requires additional bleaching steps in the hydrogenation process (*10*).

The rate of chlorophyll loss in oilseeds such as canola or soybean varies with both the stage of maturity and the temperature of drying. The green pigment content can be eliminated almost completely by ambient air-drying, whereas rapid drying with hot air results in retention of the green color (*11*−*14*).

The direct quantification of chlorophyll and its derivatives can be achieved by chemical techniques such as spectropho-

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tometry (UV/Vis or fluorescence), high-performance liquid chromatography (HPLC), or thin-layer chromatography (TLC); as well as by combining these methods with nuclear magnetic ressonance (NMR), mass spectrometry (MS), infrared (IR), and circular dichroism (CD) for structural elucidation of green pigments (*15*).

The content of green pigments has not been evaluated by chemical characterization, but several physical methods for color measurement, which are indirectly related to chlorophyll, have been used in industries (*16*). Among the techniques for color measurements, there are colorimetrics, the Munsell color notation system, and also methods based on reflectance spectrophotometry. These methods are the most commonly used for routine procedures to classify oilseeds.

The Munsell system is a rapid and portable system of color determination with widespread usage and low cost. The Munsell notation is decimal and can be refined to any degree. Also, the data can be easily converted to a continuous numeric scale for statistical analysis. Three aspects of perceived color are directly determined with the Munsell system. These are value (lightness from black to white on a scale of 0 to 10), chroma (degree of departure from gray toward pure chromatic color), and hue (red, orange, yellow, green, etc.). But this method depends on sensory evaluation by human panelists to measure the color, and many laboratories prefer, when it is possible, to replace human judgment and chemical analysis by instrumental techniques that are easier to handle (*17*).

High correlation between color parameters obtained by instrumental methods and pigments are reported in the literature for crops and crop-derived products (*18*, *19*). Thus, the use of the system of color measurement established by the Commission International d'Eclairage (CIE) (*20*) may provide a methodology to assess the green pigment contents in soybean seeds. This system has become widely used with the availability of reflectance spectrophotometric instrumentation. In this CIE system, colors may be regarded as existing in a three-dimensional space in which each particular color has a unique location. Accordingly, colors are measured in terms of their fundamental tristimulus values (X,Y, and Z) which are further used to calculate their derivatives, namely L*, a*, and b*(CIE-L*a*b*) color space values. Additionally, CIE-L*a*b* space has the advantage of providing means for measuring two important functions: the hue angle ($\tan^{-1}$ (b*/a*), which is recommended by the CIE as the psychometric correlate of the visually perceived attribute of hue; and chroma ($[(a^*)^2 + (b^*)^2]^{1/2}$) as the color saturation. Such measurements were found to be quantitative, repeatable, and reproducible.

The objective of this study was to evaluate the correlations between chlorophyll contents and quantitative CIE L*a*b* color measurements (visual and instrumental) in soya seeds in order to find an indirect method for predicting green pigment levels over the whole maturation period and after submitting seeds to two drying conditions.

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. The experiments were conducted in two consecutive years, 1996 and 1997, with just two of the varieties (IAS-5 and IAC-17) harvested in 1996, and all five cultivars swathed in 1997 in the same conditions. Each cultivar was planted in a field divided into 10 rows of 10-m 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 the stage of physiological maturation (R_6), which occurs nearly 125 days after planting, continuing until the commercial maturation (full maturity or R_8), based on the scale of Fehr & Caviness (*21*), 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 in order to minimize location effects of crops in the planted area. After swathing, samples were mixed and hand threshed.

Drying Conditions. Swathed threshed seeds were sorted into two groups which were submitted to two drying conditions until their moisture had dropped down to 13% (moisture needed for safe storage). Slow-dried seeds were obtained by allowing intact pods to air-dry at ambient temperature (25 ± 5 °C) for 10 days, and fast dried seeds were obtained by dehydration in an oven (40 ± 2 °C) with circulating air for 5 days. After drying, samples were stored at 2 °C until analysis of moisture, chlorophyll, and color.

Experimental Design. The factorial experimental design was 5 × 5 × 2 (five cultivars × five maturation stages × two drying conditions). Total chlorophyll content and color assessments were monitored through the experiments. Three genuine replicates were made for each assay. Results are presented in terms of mean values with their respective standard deviations.

Moisture Determination. Residual moisture of dried seeds was determined on 2 g of powdered samples in triplicate at 105 °C until constant weight (approximately 24 h) to confirm that all seeds reached moisture contents below 13%.

Spectrophotometric Chlorophyll Analysis. Total chlorophyll contents were determined spectrophotometrically, according to method 942.04 for chlorophyll in plants as described by the Association of Official Analytical Chemists (*22*). Two grams 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, with 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 47 807*g*, for 15 min at 15 °C in a centrifuge (Sorvall RC-5C). The supernatant was stored, and the precipitate was reextracted under the same conditions described before, and then the collected supernatants were combined. The extracts containing the pigments were dehydrated with anhydrous sodium sulfate; these solutions were concentrated 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 in a spectrophotometer (DU-70, Beckman Instruments, Palo Alto, CA). Absorbance readings of the solutions were made at both 660 and 642.5 nm. The concentration of total chlorophyll contents was estimated according to the following equation, using the extinction coefficients for diethyl ether found in the literature (*22*):

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

where A_λ corresponds to the absorbance of the solution at the respective wavelength. Samples were analyzed in triplicate. The values obtained are presented on a dry basis (DB).

Instrumental Color Assessments. Color was monitored by the CIE-L*a*b* system where L* indicates lightness, a* indicates hue on a green (−) to red (+) axis, b* indicates hue on a blue (−) to yellow (+) axis, hue angle is $\tan^{-1}$ (b*/a*), and chroma is $[(a^*)^2 + (b^*)^2]^{1/2}$ (*17*). Dried soybean seeds were milled and sieved until passing a 48-mesh screen. Instrumental color measures were made by three-stimulus colorimetry on a spectrophotometer (Hitachi Ltd. U-3410, Tokyo, Japan) connected to an integration spherical cell that calculates the tristimulus values (X,Y, and Z), luminosity index (L*), and chromaticness indexes (a*, b*). The reflectance readings of the samples were made over the 380 to 780 nm range, scan speed 120 nm/min, under illuminant C (day light). Anhydrous magnesium sulfate was used as standard. CIE-L*a*b*, a*/b*, hue angle, and chroma values were calculated from the spectral curve for each sample. Values for each sample were an average of three readings.

Visual Evaluation. Visual color evaluation was performed by the Munsell color notation system under illuminant C (day light) in a special

Table 1. Chlorophyll Contents and Colorimetric Values of Five Different Brazilian Varieties of Soybeans at Five Maturity Stages, Dried at 40 °C for 5 Days[a]

variety	maturity stage[b]	chlorophyll (ppm)	L*	a*	b*	a*/b*	chroma[c]	hue angle[d]	a_1*[e]
	R_6	121.3	76.0	−6.1	27.4	−0.22	28.0	−1.3	−3.9
IAS-5 (1996)	R_6–$R_{7(I)}$	172.6	83.5	−2.5	22.9	−0.11	23.0	−1.5	−1.1
	R_6–$R_{7(II)}$	138.9	85.4	−0.6	21.6	−0.03	21.6	−1.5	−0.4
	R_7	41.9	86.3	−0.1	20.3	0.00	20.3	−1.6	3.5
	R_8	7.9	88.2	0.6	19.5	0.03	19.5	1.5	4.6
	R_6	181.3	76.4	−6.2	26.6	−0.24	27.3	−1.3	−5.4
IAC-17 (1996)	R_6–$R_{7(I)}$	147.7	83.5	−4.2	23.5	−0.18	23.9	−1.4	−2.3
	R_6–$R_{7(II)}$	59.9	84.4	−2.5	22.9	−0.11	23.0	−1.5	−1.2
	R_7	37.1	85.8	−0.9	23.8	−0.04	23.9	−1.5	−0.6
	R_8	2.5	88.1	0.8	21.1	0.04	21.1	1.5	0.5
	R_6	75.4	80.1	−4.7	29.3	−0.16	29.7	−1.4	−4.4
IAS-5 (1997)	R_6–$R_{7(I)}$	85.0	84.2	−4.4	25.5	−0.17	25.5	−1.5	−1.1
	R_6–$R_{7(II)}$	48.7	81.1	−2.8	26.4	−0.11	26.7	−1.4	−0.8
	R_7	5.8	86.0	−0.1	20.9	0.00	20.9	−1.6	3.5
	R_8	3.1	87.5	1.2	21.0	0.06	21.0	1.5	3.9
	R_6	256.9	78.8	−8.1	27.5	−0.29	28.4	−1.3	−7.5
IAC-17 (1997)	R_6–$R_{7(I)}$	178.1	75.9	−7.0	29.2	−0.24	30.3	−1.3	−6.1
	R_6–$R_{7(II)}$	53.2	82.4	−3.0	28.1	−0.11	28.3	−1.5	−3.7
	R_7	12.5	87.6	−0.8	22.6	−0.03	22.6	−1.5	−1.1
	R_8	0.7	87.9	−0.2	21.5	−0.01	21.5	1.5	2.0
	R_6	162.2	79.4	−6.2	28.2	−0.22	28.9	−1.4	−4.8
IAC-18 (1997)	R_6–$R_{7(I)}$	193.3	75.9	−7.5	31.8	−0.23	32.6	−1.3	−6.2
	R_6–$R_{7(II)}$	25.6	84.5	−1.7	24.4	−0.07	24.4	−1.5	−0.5
	R_7	13.7	87.8	−0.2	22.6	−0.01	22.6	−1.6	2.4
	R_8	0.5	87.0	0.8	20.8	0.04	20.8	1.5	6.9
	R_6	166.6	74.7	−7.5	32.8	−0.23	33.6	−1.4	−4.8
IAC-20 (1997)	R_6–$R_{7(I)}$	95.7	82.9	−5.3	27.1	−0.19	27.6	−1.4	−4.6
	R_6–$R_{7(II)}$	27.3	82.3	−1.5	24.5	−0.06	24.5	−1.5	−1.3
	R_7	2.2	86.7	0.1	20.6	0.00	20.6	−1.6	4.2
	R_8	0.9	86.2	0.8	20.8	0.04	20.9	1.5	6.6
	R_6	105.7	80.1	−5.2	28.9	−0.18	28.4	−1.4	−4.5
IAC-Foscarin 31 (1997)	R_6–$R_{7(I)}$	104.4	78.3	−5.8	30.4	−0.19	31.0	−1.4	−4.1
	R_6–$R_{7(II)}$	7.9	88.4	−0.2	21.3	−0.01	21.3	1.6	2.7
	R_7	7.5	87.3	0.4	23.6	0.02	23.6	1.6	2.4
	R_8	0.9	87.7	0.7	22.0	0.03	22.0	1.5	4.8

[a] All the results are presented as an average of triplicate measurements. [b] Maturity stage according to Fehr & Caviness classification (*21*). [c] Chroma = $[(a^*)^2 + (b^*)^2]^{1/2}$. [d] Hue angle = $\tan^{-1}$ (b*/a*). [e] a_1*: parameter obtained by the visual color determination by Munsell System equivalent to the instrumental measure of a*.

booth. Evaluations were made by one trained observer. From each threshed sample, 50 g of seeds were weighed and 100 seeds were separated randomly. Their colors were evaluated by comparison with the Munsell color tiles. Each color tile was also analyzed instrumentally by the CIE-L*a*b* method in triplicate obtaining the a* values for each one, so that the visual evaluations could be converted to the same unit of CIE-L*a*b* system. After visual evaluation, the a* values obtained from the Munsell color tiles were used to obtain a parameter equivalent to average a* of the 100 seeds, that we called a_1*, by the weighted mean considering the percent frequency of the a* value from each seed. The a* values were obtained by visually matching each seed with a Munsell tile. Therefore, the a_1* value represents the average color of the sample obtained by subjective evaluation. The a_1* values were also correlated to the chlorophyll contents of samples. All analyses were made in triplicate and the arithmetic means were obtained. The parameter a* was considered because it showed the best correlation with chlorophyll contents.

Data Analysis. The data were statistically analyzed by the Statistica Software Package '98 Edition, and the correlations between the chlorophyll contents and the color parameters (L*, a*, b* values, hue angle, chroma, and a_1* values) were obtained.

Linear regression analysis was conducted between a* values and total chlorophyll contents. Significance was determined at the 0.05 level. Confidence levels (mean $\pm 2\sigma$), upper limit control (mean $+ 3\sigma$), and lower limit control (mean $- 3\sigma$) were also obtained. Data presented in graphs show calculated means and standards deviations of the means.

RESULTS AND DISCUSSION

Color changes in soybean seeds during maturation (from green to yellow) have been shown to reflect degradation of chlorophyll, and its degree depends on drying rates.

Tables 1 and **2** show data on chlorophyll contents and color parameters (L*, a*, b*, a*/b*, chroma, and hue angle) obtained by the CIE-L*a*b* system for each maturity stage, starting from early-harvested seeds until full maturity, when dried at 40 °C and at ambient temperature (25 °C), respectively. The values of a_1* coming from visual judgment and properly transformed into CIE-L*a*b* units are also presented in **Tables 1** and **2**.

Yellowing was characterized, as expected, by an increase in a* value (less green) and a decrease in b* value (more yellow), showing a reduction in chlorophyll concentration as the seeds lose their green color at advanced stages of maturity. Data show a more pronounced decrease in total chlorophyll contents and faster yellowing of seeds throughout the maturation period for seeds when dried at 25 °C, in comparison to those of seeds dried at 40 °C. Therefore, the chlorophyll retention varied with both the stage of maturity and the drying conditions of the seeds.

Visual color readings with the Munsell System (data not shown) were taken over the range from 5.0GY to 10.0YR, using 7 hue sheets of the Munsell Color chart (2.5GY, 5.0GY, 2.5Y, 5.0Y, 7.5Y, 10.0Y, and 10.0YR). A classification of seed color based only on hue or chroma seemed to be inadequate and we observed visual differences in seeds with the same value but different hue readings. The same is true with chroma. Even so, the variability of soybean seeds color could be described using just 36 different color tiles, covering the whole R_6−R_8 maturation stages and the two drying conditions of all cultivars analyzed.

The data were statistically analyzed and the correlation matrix is presented in **Table 3** for seeds dried at 40 °C and at ambient

Table 2. Chlorophyll Contents and Colorimetric Values of Five Different Brazilian Varieties of Soybeans at Five Maturity Stages, Dried at Ambient Temperature (around 25 °C) for 10 Days

variety	maturity stage[b]	chlorophyll (ppm)	L*	a*	b*	a*/b*	chroma[c]	hue angle[d]	a_1*[e]
	R_6	28.0	84.2	–1.2	19.2	–0.06	19.3	–1.5	–2.2
IAS-5 (1996)	R_6–$R_{7(I)}$	13.6	85.6	–0.4	19.2	–0.02	19.1	–1.6	2.8
	R_6–$R_{7(II)}$	13.4	85.8	–0.4	21.4	–0.02	21.4	–1.6	1.2
	R_7	8.6	87.9	–0.1	18.8	0.00	18.8	–1.6	4.6
	R_8	3.7	87.5	0.8	20.3	0.04	20.3	1.5	3.3
	R_6	21.2	87.8	–1.1	17.6	–0.16	17.6	–1.5	–0.2
IAC-17 (1996)	R_6–$R_{7(I)}$	19.9	87.6	–0.7	18.8	–0.04	18.8	–1.5	2.0
	R_6–$R_{7(II)}$	15.9	85.1	–0.5	21.7	–0.02	21.7	–1.6	0.2
	R_7	4.3	88.3	0.1	18.5	0.00	18.5	–1.6	4.6
	R_8	1.1	89.1	0.3	20.3	0.01	20.4	1.6	4.0
	R_6	14.3	84.0	–1.2	20.8	–0.06	20.9	–1.5	–1.2
IAS-5 (1997)	R_6–$R_{7(I)}$	15.8	86.0	–1.1	22.3	–0.05	22.3	–1.5	–1.4
	R_6–$R_{7(II)}$	7.7	86.8	–0.4	18.4	–0.02	18.5	–1.6	1.3
	R_7	3.3	88.0	0.5	22.4	0.02	22.4	1.6	4.1
	R_8	1.1	87.1	0.6	22.6	0.03	22.6	1.5	4.3
	R_6	32.2	86.6	–2.2	23.5	–0.19	23.6	–1.5	–2.7
IAC-17 (1997)	R_6–$R_{7(I)}$	21.3	80.9	–1.1	18.7	–0.06	18.7	–1.5	–3.3
	R_6–$R_{7(II)}$	12.6	88.4	–0.7	17.4	–0.04	17.4	–1.5	0.3
	R_7	7.5	85.2	–0.1	23.2	0.00	23.3	–1.6	2.9
	R_8	0.5	87.4	0.4	22.6	0.02	22.6	1.6	5.1
	R_6	20.6	85.1	–2.2	24.2	–0.09	24.3	–1.5	–2.1
IAC-18 (1997)	R_6–$R_{7(I)}$	6.2	86.1	–0.6	18.6	–0.03	18.6	–1.5	–1.5
	R_6–$R_{7(II)}$	5.2	85.8	–0.4	24.3	–0.02	24.4	–1.6	2.6
	R_7	1.9	87.6	0.1	21.4	0.00	21.4	1.5	4.3
	R_8	0.3	86.3	0.8	21.7	0.04	22.7	1.5	6.4
	R_6	19.0	82.9	–1.4	23.4	–0.06	23.5	–1.5	–5.0
IAC-20 (1997)	R_6–$R_{7(I)}$	6.5	87.1	–0.6	20.1	–0.03	20.2	–1.5	–1.0
	R_6–$R_{7(II)}$	5.7	85.2	–0.2	22.1	–0.01	22.1	–1.6	1.7
	R_7	1.1	85.4	0.5	21.6	0.02	21.6	1.5	5.7
	R_8	0.6	86.0	0.8	21.9	0.04	21.9	1.5	6.5
	R_6	9.3	86.5	–1.0	23.8	–0.04	23.8	–1.5	–1.8
IAC-Foscarin 31 (1997)	R_6–$R_{7(I)}$	2.2	88.2	–0.2	18.1	–0.01	18.1	–1.6	0.3
	R_6–$R_{7(II)}$	1.5	85.5	0.6	24.7	0.02	24.7	1.5	4.0
	R_7	0.9	85.3	0.7	22.8	0.03	22.8	1.5	3.6
	R_8	0.3	86.0	0.9	23.1	0.04	23.1	1.5	4.8

[a] All the results are presented as an average of triplicate measurements. [b] Maturity stage according to Fehr & Caviness classification (*21*). [c] Chroma = $[(a^*)^2 + (b^*)^2]^{1/2}$. [d] Hue angle = $\tan^{-1}$ (b*/a*). [e] a_1*: parameter obtained by the visual color determination by Munsell System equivalent to the instrumental measure of a*.

Table 3. Correlation Coefficients between Chlorophyll Contents (chl) and Colorimetric Values of Five Different Brazilian Varieties of Soybeans at Five Maturity Stages, Dried under Two Conditions (40 °C for 5 days, and 25 °C for 10 days)[a]

parameters	L*	a*	b*	a*/b*	chroma[b]	hue angle[c]	a_1*[d]
chl (ppm), seeds dried at 40 °C	–0.385	**–0.882**	–0.156	**–0.870**	–0.175	–0.664	–0.748
chl (ppm), seeds dried at 25 °C	**–0.815**	**–0.878**	0.687	**–0.885**	0.709	–0.524	**–0.824**

[a] Data in bold are significant ($p < 0.05$). $n = 35$. [b] Chroma = $[(a^*)^2 + (b^*)^2]^{1/2}$. [c] Hue angle = $\tan^{-1}$ (b*/a*). [d] a_1*: parameter obtained by the visual color determination by Munsell System equivalent to the instrumental measure of a*.

temperature (25 °C). Significant correlations ($p < 0.05$) were observed between chlorophyll contents and values of a* and a/b* (−0.882 and −0.870 respectively) for seeds dried at 40 °C, and values of a/b*, a*, a_1*, and L* (−0.885, −0.878, −0.824, and −0.815, respectively) for seeds dried at ambient temperature. The a_1* values presented significant and high correlation with chlorophyll contents for the seeds dried at ambient temperature; the correlation was lower for seeds dried in an oven. Therefore, the transformation of the Munsell System readings into instrumental a* values generating the parameter a_1* could not be applied in all cases. The a* value and a*/b* present the highest significant correlation coefficients with total chlorophyll in both cases, suggesting that these parameters may serve as suitable indexes of chlorophyll contents during soybean maturation in both drying conditions tested in those experiments. Although there was observed a marked visual difference in color hue between samples, the calculated hue angle was not sensitive enough to significantly detect this. Depending on the region of the color space, direct measure of a* or b* is more easily related to color changes than the hue angle. This is especially true for red or green samples.

Regression analyses were performed to develop equations in order to predict chlorophyll contents using instrumental a* color values. The regression curves (**Figures 1** and **2**), plotted by Statistica program package with confidence level of $\pm 3\,\sigma$, were linear in the R_6 to R_8 range of ripening. On the basis of these correlations, two formulas were derived for routine evaluation of chlorophyll contents in ripening soybean:

$$\text{TC (total chlorophyll, ppm)} = 17.305 - 21.58\ a^*(\text{instr.}),\ \text{for drying in oven at 40 °C}$$

$$\text{TC (total chlorophyll, ppm)} = 6.5296 - 9.230\ a^*(\text{instr.}),\ \text{for drying at ambient air conditions (25 °C)}$$

We were able to prove that a* values are well-correlated with chlorophyll contents over the whole maturation period, and it has been proven also that the same is true when seeds are

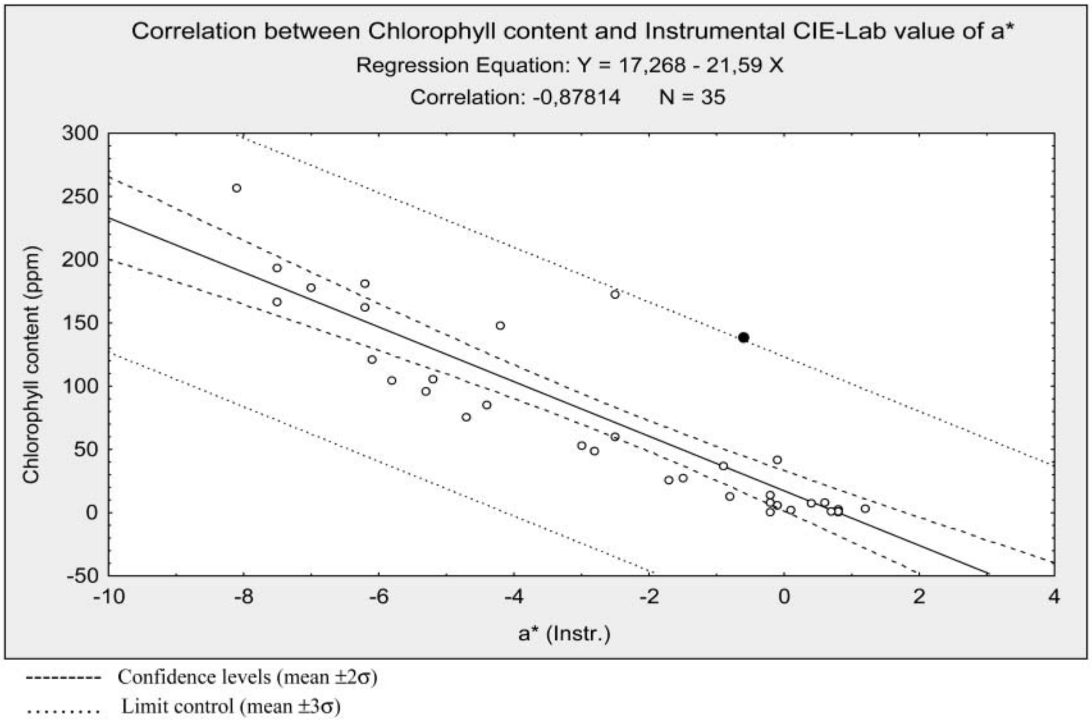

Figure 1. Linear regression between chlorophyll content (ppm) and value of a* (instrumental) of soybean seeds, during five maturation stages, dried at 40 °C for 5 days.

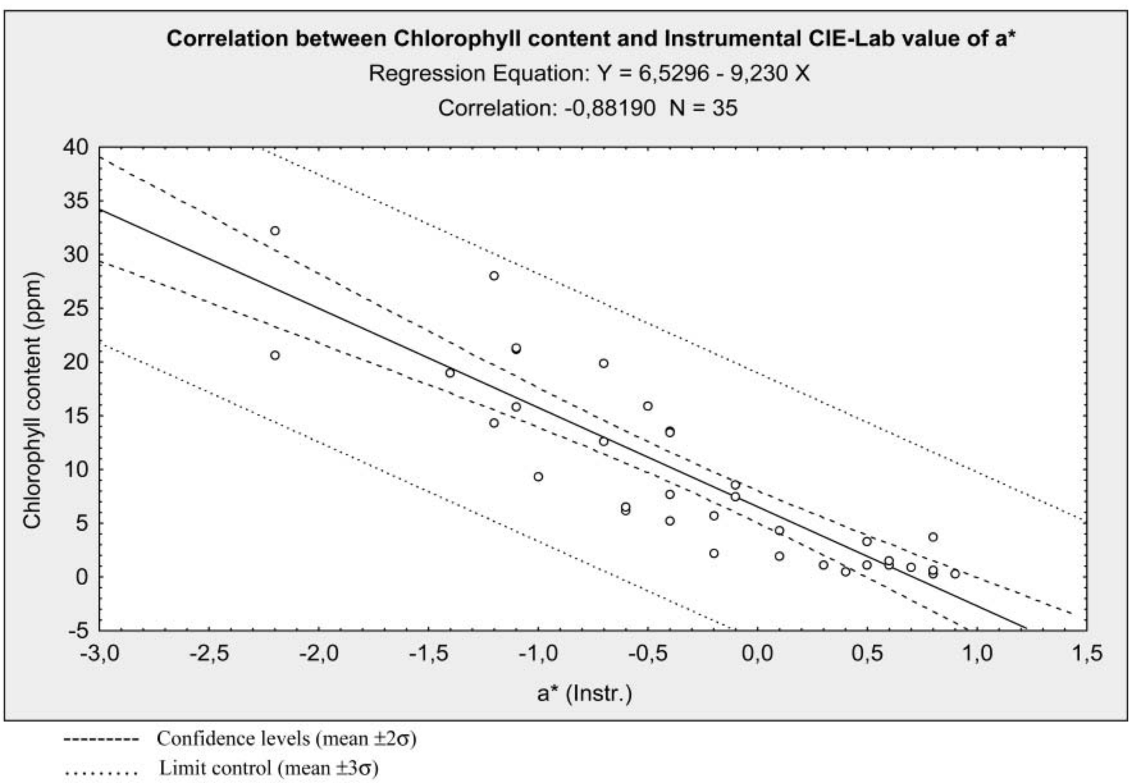

Figure 2. Linear regression between chlorophyll content (ppm) and value of a* (instrumental) of soybean seeds, during five maturation stages, dried at ambient temperature (mean 25 °C) for 10 days.

submitted to drying processes under two conditions: slow air-drying at 25 °C and fast oven-drying at 40 °C. From an industrial point of view, the parameter a* is easily measured and could be a useful tool for quality control in classifying soybean seeds for different purposes.

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