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Color measurements in annatto (*Bixa orellana* L.) seeds

Análise de cor em sementes de urucum (Bixa orellana L.)

Paulo Roberto Nogueira Carvalho[1]*, **Mariana Aparecida Almeida da Silva[2]**, **João Victor Silva de Oliveira[2]**, **Eliane Gomes Fabri[3]**, **Marta Gomes da Silva[1]**

[1]Instituto de Tecnologia de Alimentos (ITAL), Centro de Ciências e Qualidade de Alimentos, Campinas/SP - Brasil
[2]Faculdade de Tecnologia de Campinas (FATEC), Campinas/SP - Brasil
[3]Instituto Agronômico de Campinas (IAC), Centro de Horticultura, Campinas/SP - Brasil

***Corresponding Author:** Paulo Roberto Nogueira Carvalho, Instituto de Tecnologia de Alimentos (ITAL), Centro de Ciências e Qualidade de Alimentos, Av. Brasil, 2880, CEP: 13070-178, Campinas/SP - Brasil, e-mail: carvalho@ital.sp.gov.br

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Abstract

Brazil has emerged as the world's largest producer of annatto seeds (*Bixa orellana* L.). Fortunately, the increase in national production has been accompanied by an improvement in the quality of this raw material. Annatto is a typical crop of small producers who usually use their empirical knowledge to evaluate the quality of the crop. Thefore, the visual observation of the color of annatto seeds has been considered a quality standard by many producers. To evaluate the scientific evidence, the present study evaluated annatto seeds from 51 accessions of the germplasm bank of the Agronomic Institute (Instituto Agronômico de Campinas - IAC) to determine a possible correlation between the pigment's contents and the color of annatto seeds as well as the influence of humidity on this correlation. The correlations between the concentrations of pigments, humidity, lipids, and color (L*, a*, b*) were also evaluated. The color was evaluated using a colorimeter with measurements of the CIE L*, a*, b* scale. Only the correlation between the variables moisture and lipids was considered strong through the simple correlation, while the variables bixin content and coordinate a* presented a significant correlation while eliminating the effect of moisture (partial correlation). Thus, a correlation between the red color and the bixin concentration of annatto seeds was observed even though it was a weak correlation. In turn, the color difference (ΔE_{ab}) between the samples with higher and lower bixin contents was only 1.44. This difference indicates that large variations in the bixin concentrations may lead to small color changes in the annatto seeds, impairing the use of this criterion for quality control of these seeds.

Keywords: *Bixa orellana* L.; annatto; colorimetry; bixin; moisture; lipids.

Resumo

O Brasil tem se firmado como o maior produtor mundial de sementes de urucum (*Bixa orellana* L.). Felizmente, o aumento da produção nacional tem sido acompanhado por uma melhoria da qualidade dessa matéria-prima. O urucum é uma cultura típica de pequenos produtores, que em geral usa seu conhecimento empírico para avaliar a qualidade do material que está produzindo. A observação visual da cor das sementes de urucum tem sido considerada padrão de qualidade por muitos produtores. Para avaliar as evidências científicas, o presente estudo avaliou amostras de sementes de urucum de 51 acessos do banco de germoplasma do Instituto Agronômico de Campinas (IAC), para determinar se existe correlação entre as concentrações de pigmentos com a cor das sementes e a influência da umidade nessa correlação. Foram avaliadas também as correlações entre as concentrações de pigmentos, umidade, lipídios e a cor (L*, a*, b*). Para a avaliação da cor, utilizamos um colorímetro com a medida da cor pelo sistema CIE L*, a*, b*. Apenas a correlação simples entre as variáveis Umidade e Lipídios foi considerada forte, pela classificação usada neste estudo. Quando se remove o efeito da Umidade (correlação parcial), apenas as variáveis bixina e a* apresentaram correlação significativa, considerada fraca pela classificação utilizada. Portanto, mesmo considerada fraca, há uma correspondência entre a cor vermelha das sementes de urucum com a concentração de bixina das amostras estudadas. Contudo, a diferença da cor (ΔE_{ab}) entre as amostras com maior e menor teor de bixina foi de apenas 1,44. Essa diferença indica que grandes variações nas concentrações de bixina nas sementes de urucum podem levar a pequenas variações de cor, dificultando o uso desse critério para o controle de qualidade desses grãos.

Palavras-chave: *Bixa orellana* L.; urucum; colorimetria; bixina; umidade; lipídios.

Highlights

- A correlation between the red color and the bixin concentration of annatto seeds was observed
- Large variations in the bixin concentrations may lead to small color changes in the annatto seeds
- The color difference (ΔEab) between the samples with higher and lower bixin contents was only 1.44

1 Introduction

Annatto is an orange-red condiment extracted from *Bixa orellana* L. seeds with great importance in the Brazilian culture, known as colorífico or colorau. However, annatto seeds have gained more space as a raw material for the food coloring industry and the pharmaceutical industry as a source of herbal medicines. The market potential of these seeds in the coloring and seasoning industry is due to their concentration of pigments, including bixin, which is the majority carotenoid of annatto seeds.

Besides the bixin concentration, the lipids and moisture contents are relevant to the quality of annatto seeds. The lipids are correlated with important phytochemicals such as geranylgeraniol and tocotrienols (Carvalho, 2020), while moisture is an important criterion for the conservation of the grains. Seeds with moisture contents higher than 14% are susceptible to fungal infections, while low-moisture seeds with moisture contents below 8%, are susceptible to the loss of pigments due to friction between grains (Bezerra et al., 2019).

The lipid content of annatto seeds has been widely studied. Matos et al. (1992) reported a lipid content of 2.35%, and Carvalho et al. (2010) found a lipid content from 2.0 to 4.5% (w/w, on a dry basis) in 25 accessions from the germplasm bank of the Agronomic Institute (Instituto Agronômico de Campinas - IAC), located in the municipality of Pindorama, in the State of São Paulo - Brazil, from the 2007 crop. The analyses were repeated for 63 accessions from the 2011 crop of the same germplasm bank, and the lipid contents ranged from 2.14 to 7.11%, w/w, on a dry basis (Dequigiovani et al., 2017).

The drying of annatto seeds is usually performed by exposing the fruit and seeds to the sun. However, sun drying is empirical and leads to high variation in the moisture contents of the seeds (Bezerra et al., 2019). The market has set 12% as the maximum moisture content in annatto seeds. Oliveira et al. (2020a) studied seven seed samples from commercial annatto plantations and observed a variation in moisture contents from 9.28 to 14.12% (w/w).

The coloring of annatto seeds is given by a series of carotenoids with a predominance of 9-cis-bixin (methyl, hydrogen, 9'-cis-6,6'-diapocarotene-6'-methanoate-6-oic), and its structural formula is shown in Figure 1. It is the first cis-configuration carotenoid isolated from a natural source (Jondiko & Pattenden, 1989). The determination of the pigment concentration of annatto seeds is usually done using spectrophotometric methods to assess the total carotenoid concentration. Since bixin is the predominant pigment, accounting for approximately 80% of the carotenoids present in the seeds (Preston & Rickard, 1980), the determination is usually performed at the maximum absorption wavelength of bixin. An alternative to the direct determination of bixin is the quantification of the norbixin salt from the pigment extraction using an alkaline solution. This methodology has become a standard in the annatto coloring industries to determine the quality control of these grains and has the advantage of the absence of organic solvents in the analytical process. In the present study, the concentration of total carotenoids was expressed in bixin form.

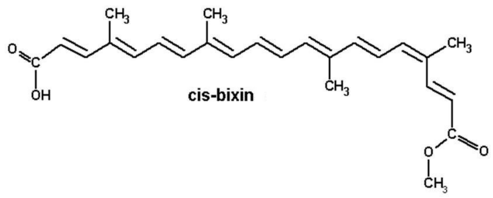

Figure 1. Structural formula of 9-cis-bixin (methyl, hydrogen, 9'-cis-6,6'-diapocarotene-6'-methanoate-6-oic).

Visual color classification has been used as a tool to indicate many factors, including the degree of fruit ripeness, the harvesting stage, and the quality of grain. While in some cases these empirical methods are proven by quality control processes, in other situations these procedures have no scientific basis.

Color perception is a sensory experience with a certain subjective quality. It is not unusual to find references to the color of annatto seeds as red, bright red, or brick brown, among others. The subjective analysis of color makes it very difficult to describe the seeds, which can be overcome with techniques that convert colors into numbers. One of these techniques uses a colorimeter to divide the three-dimensional color space into three axes. One of the axes represents the luminosity (L*) and varies from -L* (black or dark) to +L* (white or light); the other axis is represented by the symbol a* that presents the color coordinate ranging from -a* (green) to +a* (red), while the b* axis represents the color changes from -b* (blue) to +b* (yellow).

A study performed by our research team (Silva & Ferreira, 1997) with 53 samples of annatto seeds from the germplasm bank of the IAC, 1996 crop, evaluated the colors of the annatto seeds in the CIE L*a*b* color space. The authors correlated the color measured with a visual color scale that comprised the following descriptors: Brown; Reddish Brown; Dark Brown; Brick Brown; Dark Brick Brown; Red; Orange Red; Purple Brown Red; Brick Red; Light Brick Red; Dark Brick Red. The largest number of samples were classified as Brick Red (18 samples), Light Brick Red (9 samples), and Dark Brick Red (6 samples).

The CIE L*a*b* system allowed assessing the correlation between the color of annatto seeds and bixin contents, as well as the influence of humidity on this correlation.

2 Material and methods

2.1 Raw material

Annatto seeds from 60 accessions at the Germplasm Bank of the Agronomic Institute (IAC), located in the municipality of Pindorama - SP - Brazil - Latitude 21°11'0.09 "N; Longitude 48°54'0.26" E - of the 2019 crop, were collected, processed, and sent in plastic jars to the Natural Pigments Laboratory of the Institute of Food Technology (Instituto de Tecnologia de Alimentos – ITAL). The samples were identified by sequential numbering from A01 to A60. The samples with fungi incidence were discarded, resulting in 51 samples for the study. Each sample was then vacuum-packed in metallic packaging and stored under refrigeration (-15 ± 5 °C) until analysis. This type of storage keeps the concentration of bixin stable for up to one year (Oliveira et al., 2020b). At the time of the analyses, impurities from the seed samples (leaves, branches, and soil covers) were removed and discarded.

2.2 Methodologies

2.2.1 Total carotenoids expressed as bixin

The determinations of total carotenoids, expressed as bixin contents, were performed according to Carvalho et al. (2010). The analysis was based on the extraction and saponification of bixin in norbixin salt with an alkaline solution of castor oil and subsequent dilution of the pigments with an aqueous alkaline solution (0.5% NaOH, w/v). The norbixin salt was quantified by spectrophotometry at 453 nm using an absorption coefficient of 2,850 (Reith & Gielen, 1971).

2.2.2 Moisture content

The moisture content of the annatto seeds was determined by the gravimetric method by heating the samples in an oven at 110 ± 5 °C until constant weight, as described by Bezerra et al. (2019). The moisture content was calculated by the difference between the initial and final weight of the samples.

2.2.3 Lipid content

The lipid contents of annatto seeds were determined as described by Horwitz (2005). The extraction in the Soxhlet apparatus was performed with hexane at 70ºC for 8 hours, and the resulting residue after solvent evaporation was quantified as lipids.

2.2.4 Color measurements

Color analyses were performed in a Minolta colorimeter, model CR410, with a 53 mm sensor, using D65 illuminant (corresponding to daylight, including ultraviolet radiation) and a standard observer angle of 2º. The readings were taken in tristimulus values denoted by X, Y, and Z, and converted to the L*a*b* color space using Equations 1 to 3[1]:

$$L^* = 116\left(Y/_{Y_0}\right)^{\frac{1}{3}} - 16 \tag{1}$$

$$a^* = 500\left[\left(X/_{X_0}\right)^{\frac{1}{3}} - \left(Y/_{Y_0}\right)^{\frac{1}{3}}\right] \tag{2}$$

$$b^* = 200\left[\left(Y/_{Y_0}\right)^{\frac{1}{3}} - \left(Z/_{Z_0}\right)^{\frac{1}{3}}\right] \tag{3}$$

where: L* represents the luminosity scale, a* represents green (-a*) to red (+a*), and b* represents blue (-b*) to yellow (+b*).

The Chroma value (C*) was calculated using the Equation 4:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \tag{4}$$

The color difference (ΔE_{ab}) was calculated according to the Equation 5:

$$\Delta E_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \tag{5}$$

For the color measurements, annatto seeds without impurities were transferred to an opaque container with an internal diameter of 50 mm and a height of 15 mm, filling the entire volume. The readings were performed without the external light´s interference, in 6 replicates for each sample.

[1]For X/X0, Y/Y0, and Z/Z0 > 0.008856.

2.2.5 Statistical analysis

Statistical analyses were performed using the software Genes (version 2009.7.0) and Microsoft Excel (version 2010). Analysis of variance, comparison of means (Scott & Knott, 1974), regression analysis, and Pearson correlation coefficients were calculated. The partial correlation coefficients were calculated using the Equation 6:

$$r_{ij.m} = \frac{-c_{ij}}{\sqrt{c_{ii}c_{jj}}} \tag{6}$$

where *m* represents the set of characters without the influence of the correlation between *i* and *j*, and *cij* represents the element *ij* of the inverse of the simple correlation matrix (Cruz, 2006).

The correlation coefficient *r* was determined by estimated *t* (student) with *n*-2 degrees of freedom according to the Equation 7:

$$t_{n-2} = r\sqrt{\frac{n-2}{1-r^2}} \tag{7}$$

where *n* is the number of pairs of observations, and r^2 is the determination coefficient.

The strength of the correlation was classified according to Biaggi et al. (2017) as follows: for r = 1, the correlation is considered perfect; for 1 > r ≥ 0.75, the correlation is considered strong; for 0.75 > r ≥ 0.50, the correlation is considered moderate; and for r < 0.50, the correlation is considered weak.

3 Results and discussion

Table 1 presents the results on a wet basis. The moisture content of the samples ranged from 3.65 ± 0 to 18.77 ± 0.28% for samples 7 and 35, respectively, and only one sample showed moisture higher than 14%, which is considered the threshold for storage of annatto seeds at room temperature (Bezerra et al., 2019).

The average lipid content of the samples on a wet basis was 3.26 ± 0.94% and ranged from 1.69 ± 0.15 to 5.87 ± 0.04% for samples 22 and 37, respectively. Gaydou & Romanoelina (1983) reported 3.9% of lipids in annatto seeds. Matos et al. (1992) analyzed plants from the Prisco Bezerra Herbarium of the Federal University of Ceara and reported a lipid content of 2.35%, while Rao et al. (2015) found an average lipid content of 6.3% ± 0.1% in annatto seeds from three harvests (2009 to 2011). The lipid values of the present study are close to the results of two previous studies performed by our research team for samples from the same germplasm bank, from the 2007 and 2011 crops (Carvalho et al., 2010; Dequigiovani et al., 2017).

The average total carotenoid content, on a wet basis, expressed as bixin was 3.19 ± 0.86% and ranged from 1.78 ± 0.17 to 4.82 ± 0.10% for samples 47 and 46, respectively. Previous studies on samples from the IAC germplasm bank of the 2007 crop production indicated a variation of bixin concentration from 3.12 to 6.26% on a dry basis (Carvalho et al., 2010). This same collection was analyzed again in the 2011 harvest, with seeds from 62 accessions that showed bixin[3] contents ranging from 2.0 to 7.3% on a dry basis (Dequigiovani et al., 2017).

The color measurements of annatto seeds showed L* values ranging from 27.60 ± 0.21 to 31.08 ± 0.07 for samples 42 and 01, respectively, with an average value of 29.55 ± 0.63. The a* values ranged from 17.93 ± 0.19 to 25.06 ± 0.15 for the samples 42 and 51, respectively, with an average value of 21.83 ± 1.58. The b* values ranged from 9.95 ± 0.28 to 15.89 ± 0.04 for samples 42 and 50, respectively, with an average value of 13.66 ± 1.10. Consequently, the Chroma values ranged from 20.51 ± 0.30 to 29.65 ± 0.17 for samples 42 and 51, respectively, with an average value of 25.76 ± 1.88. Silva & Ferreira (1997) investigated 53 annatto seeds from the IAC germplasm bank from the 1996 crop and reported L* values from 22.47 to 26.97 with an average value of 24.09 ± 0.99, a* values from 13.65 to 25.83 with an average of 18.23 ± 1.69, and b* values from 12.15 to 19.21 with an average value of 14.74 ± 1.30. The results found by those authors are very close to the findings of this study.

Table 1. Moisture content (g/100g), total carotenoid expressed as bixin concentration (g/100g), lipid content (g/100g), and color coordinates L*, a*, b*, and Chroma of annatto seeds from the germplasm bank of the IAC.

S[1]	M[2]	s[3]	SK[4]	B[5]	s[3]	SK[4]	H[6]	s[3]	SK[4]	L*	s[3]	SK[4]	a*	s[3]	SK[4]	b*	s[3]	SK[4]	C*[7]	s[3]	SK[4]
1	11.51	± 0.38	d	3.50	± 0.11	d	2.18	± 0.06	i	31.08	± 0.07	a	21.62	± 0.03	m	13.76	± 0.06	h	25.63	± 0.05	m
2	10.22	± 0.51	e	3.04	± 0.03	f	3.12	± 0.04	f	30.54	± 0.07	c	21.36	± 0.04	n	13.77	± 0.05	h	25.41	± 0.05	n
3	7.30	± 0.23	g	2.18	± 0.08	h	2.23	± 0.02	i	30.43	± 0.17	c	19.78	± 0.23	t	13.40	± 0.13	j	23.89	± 0.26	t
4	7.58	± 2.53	g	2.18	± 0.13	h	3.24	± 0.24	f	29.66	± 0.10	g	19.47	± 0.03	u	13.01	± 0.04	l	23.42	± 0.03	u
5	7.85	± 0.13	g	2.17	± 0.06	h	2.13	± 0.06	i	29.51	± 0.08	h	18.72	± 0.11	x	12.08	± 0.09	p	22.28	± 0.14	x
6	8.91	± 0.98	f	2.17	± 0.06	h	2.58	± 0.01	h	29.59	± 0.06	g	20.84	± 0.05	p	13.03	± 0.05	l	24.58	± 0.06	q
7	3.65	± 0.00	h	1.97	± 0.00	i	2.23	± 0.17	i	29.35	± 0.13	h	20.76	± 0.08	q	12.41	± 0.07	o	24.18	± 0.10	s
8	8.21	± 0.18	f	1.95	± 0.05	i	2.73	± 0.03	h	28.82	± 0.14	k	21.09	± 0.07	o	12.71	± 0.19	n	24.62	± 0.15	q
9	7.12	± 0.77	g	2.34	± 0.09	h	2.95	± 0.03	g	29.09	± 0.03	i	19.84	± 0.18	t	12.46	± 0.12	o	23.43	± 0.21	u
10	7.72	± 0.28	g	2.39	± 0.01	h	2.78	± 0.03	h	29.34	± 0.14	h	20.44	± 0.13	r	12.88	± 0.09	m	24.16	± 0.15	s
11	6.43	± 0.14	g	3.54	± 0.07	e	3.35	± 0.06	f	29.40	± 0.05	h	19.28	± 0.17	v	12.39	± 0.13	o	22.92	± 0.21	v
12	8.33	± 0.15	f	3.14	± 0.11	f	2.94	± 0.19	g	29.64	± 0.16	g	21.15	± 0.16	o	13.18	± 0.13	k	24.92	± 0.20	p
13	9.31	± 0.97	e	1.86	± 0.03	i	2.84	± 0.06	g	28.67	± 0.15	l	21.36	± 0.11	n	12.84	± 0.05	m	24.92	± 0.12	p
14	9.76	± 0.25	e	2.21	± 0.01	h	2.86	± 0.17	g	28.97	± 0.11	j	21.61	± 0.16	m	13.49	± 0.15	j	25.48	± 0.21	m
15	8.93	± 0.34	f	2.21	± 0.04	h	3.93	± 0.81	d	28.64	± 0.14	l	21.07	± 0.19	o	13.17	± 0.19	k	24.85	± 0.26	p
16	8.34	± 0.03	f	3.23	± 0.09	e	2.59	± 0.05	h	29.87	± 0.08	f	22.66	± 0.04	i	13.85	± 0.07	h	26.55	± 0.06	j
17	7.99	± 0.09	g	2.93	± 0.09	f	2.73	± 0.02	h	29.15	± 0.11	i	21.90	± 0.07	l	13.35	± 0.16	j	25.65	± 0.03	m
18	8.16	± 0.28	f	2.60	± 0.10	g	2.85	± 0.02	g	29.65	± 0.01	g	22.41	± 0.03	j	13.73	± 0.11	h	26.28	± 0.08	k
19	8.86	± 0.11	f	2.58	± 0.00	g	2.47	± 0.01	h	29.27	± 0.25	h	22.03	± 0.11	l	13.40	± 0.23	j	25.78	± 0.19	l
20	7.02	± 0.26	g	3.38	± 0.01	e	2.38	± 0.06	i	28.91	± 0.18	j	20.50	± 0.04	r	12.68	± 0.12	n	24.11	± 0.07	s
21	7.93	± 0.03	g	4.12	± 0.10	c	2.77	± 0.09	h	29.18	± 0.10	i	22.79	± 0.21	h	13.33	± 0.12	j	26.41	± 0.21	j
22	7.16	± 0.39	g	1.99	± 0.01	i	1.69	± 0.15	j	30.06	± 0.20	e	21.22	± 0.03	o	14.29	± 0.12	f	25.58	± 0.08	m
23	7.79	± 0.08	g	3.18	± 0.11	g	3.18	± 0.33	f	29.13	± 0.21	i	20.65	± 0.25	q	13.01	± 0.27	l	24.41	± 0.35	r
24	6.62	± 0.03	g	4.59	± 0.05	a	2.91	± 0.09	g	29.57	± 0.22	g	21.64	± 0.15	m	13.59	± 0.25	i	25.56	± 0.27	m
25	7.49	± 0.25	g	3.15	± 0.14	f	2.49	± 0.04	h	29.30	± 0.04	h	21.64	± 0.12	m	13.65	± 0.04	i	25.59	± 0.12	m
26	9.82	± 0.30	e	2.98	± 0.09	f	3.45	± 0.06	e	29.20	± 0.21	i	21.31	± 0.19	n	13.36	± 0.12	j	25.15	± 0.22	o
27	7.74	± 0.32	g	3.73	± 0.04	d	2.68	± 0.21	h	30.68	± 0.16	b	22.18	± 0.06	k	14.08	± 0.02	g	26.27	± 0.04	k
28	8.57	± 0.13	f	4.16	± 0.04	b	2.46	± 0.14	h	30.36	± 0.18	d	21.94	± 0.05	l	13.73	± 0.02	h	25.89	± 0.05	l
29	8.76	± 0.16	f	4.55	± 0.15	a	2.99	± 0.15	g	30.35	± 0.05	d	22.88	± 0.05	h	14.38	± 0.12	f	27.02	± 0.10	h
30	7.45	± 0.35	g	2.74	± 0.21	g	2.97	± 0.02	g	29.37	± 0.04	h	21.78	± 0.08	m	12.73	± 0.06	n	25.23	± 0.09	n
31	9.64	± 0.36	e	2.10	± 0.01	h	3.28	± 0.12	f	29.72	± 0.21	g	21.96	± 0.03	l	14.36	± 0.06	f	26.24	± 0.02	k
32	8.44	± 0.12	f	3.01	± 0.01	f	2.53	± 0.02	h	29.70	± 0.12	g	22.21	± 0.22	k	14.06	± 0.03	g	26.28	± 0.20	k
33	8.97	± 0.26	f	3.50	± 0.04	g	3.19	± 0.02	f	29.70	± 0.11	g	23.22	± 0.13	g	14.61	± 0.01	e	27.44	± 0.11	f
34	8.97	± 0.07	f	3.45	± 0.06	e	2.93	± 0.15	g	28.60	± 0.07	l	19.97	± 0.08	t	12.29	± 0.08	o	23.45	± 0.11	u
35	18.77	± 0.28	a	3.97	± 0.08	b	5.50	± 0.08	a	30.01	± 0.07	e	22.58	± 0.03	i	15.00	± 0.04	c	27.11	± 0.03	h
36	14.01	± 0.49	b	4.11	± 0.13	b	5.64	± 0.22	a	30.09	± 0.09	e	23.95	± 0.02	e	15.89	± 0.04	a	28.74	± 0.02	c
37	14.01	± 0.46	b	4.03	± 0.01	b	5.87	± 0.04	a	29.40	± 0.13	h	24.63	± 0.09	b	15.00	± 0.06	c	28.84	± 0.10	c
38	10.14	± 0.64	e	4.56	± 0.05	a	3.21	± 0.03	f	29.28	± 0.15	h	22.26	± 0.06	k	13.37	± 0.04	j	25.97	± 0.03	l
39	15.45	± 0.83	a	3.67	± 0.18	c	3.74	± 0.14	d	29.48	± 0.34	h	22.74	± 0.36	h	14.28	± 0.12	f	26.85	± 0.36	i
40	13.01	± 0.47	c	3.13	± 0.02	e	4.81	± 0.17	c	29.73	± 0.17	g	24.27	± 0.19	d	15.45	± 0.15	b	28.77	± 0.24	c
41	11.19	± 0.39	d	3.72	± 0.02	d	3.39	± 0.11	e	28.96	± 0.16	j	20.29	± 0.18	s	12.63	± 0.04	n	23.89	± 0.17	t
42	11.65	± 0.11	d	3.79	± 0.05	c	3.93	± 0.11	d	27.60	± 0.21	m	17.93	± 0.19	z	9.95	± 0.28	q	20.51	± 0.30	z
43	13.28	± 0.02	c	3.96	± 0.11	b	3.91	± 0.00	d	30.29	± 0.11	d	23.16	± 0.15	g	15.05	± 0.03	c	27.62	± 0.14	f
44	10.76	± 0.09	d	4.14	± 0.15	b	3.59	± 0.02	e	28.62	± 0.18	l	20.98	± 0.18	p	12.93	± 0.07	m	24.65	± 0.18	q
45	8.64	± 0.33	f	4.72	± 0.27	a	3.17	± 0.15	f	30.22	± 0.13	d	23.54	± 0.05	f	14.17	± 0.06	g	27.47	± 0.01	f
46	8.90	± 0.16	f	4.82	± 0.10	a	3.43	± 0.01	e	30.09	± 0.09	e	24.48	± 0.18	c	14.51	± 0.10	e	28.46	± 0.20	d
47	10.09	± 0.22	e	1.78	± 0.17	i	3.10	± 0.01	f	29.75	± 0.10	g	23.08	± 0.06	g	14.51	± 0.01	e	27.26	± 0.06	g
48	9.78	± 0.18	e	3.75	± 0.16	d	4.86	± 0.08	c	29.39	± 0.09	h	22.42	± 0.30	j	14.22	± 0.17	f	26.55	± 0.34	j
49	12.33	± 0.30	c	3.38	± 0.04	e	3.73	± 0.05	d	29.68	± 0.18	g	24.09	± 0.18	e	14.76	± 0.22	d	28.26	± 0.27	e
50	13.10	± 0.00	c	3.28	± 0.07	e	4.88	± 0.03	c	30.07	± 0.09	e	24.77	± 0.10	b	15.89	± 0.04	a	29.42	± 0.09	b
51	12.34	± 0.41	c	4.09	± 0.09	b	5.17	± 0.03	b	30.09	± 0.11	e	25.06	± 0.15	a	15.84	± 0.11	a	29.65	± 0.17	a

Averages of at least two simultaneous and independent replicates (moisture, bixin, and lipids on a wet basis); [1]S = samples; [2]M = Moisture; [3]Estimated standard deviation; [4]Scott and Knott: means followed by the same letter in the same column are not significantly different ($p < 0.05$); [5]B = total carotenoid expressed as bixin; [6]H = lipids (hexane extract); [7]C* = Chroma.

The analysis of variance for the factors moisture, lipids, bixin concentration, L*, a*, b*, and Chroma indicated significant differences ($p < 0.05$) among the samples. Table 2 presents a summary of the analysis of variance of these factors.

Table 2. Analysis of variance of the factors studied.

	Moisture	Lipids	Bixin	L*	a*	b*	Chroma
F	50.22	57.95	150.73	113.30	755.36	508.21	755.17
RMS	0.2581	0.0261	0.0100	0.0208	0.0198	0.0142	0.0279
CV (%)	5.46	5.11	3.13	0.49	0.64	0.87	0.65

F = F Test (Snedecor); RMS = Residual Mean Square; CV (%) = Coefficient of variation among the samples.

Table 3 presents the simple and partial correlations when eliminating the effect of moisture content. Significant simple correlations ($p < 0.05$) were observed between most variables, except for the variable L* and the factors moisture, lipids, and bixin concentration, which presented no significant correlations between them. This result indicates that lighter (+L*) or darker (-L*) seeds are not indicators of changes in moisture, lipids, and bixin concentration of the grains within the range studied. Only the simple correlation between the variables moisture and lipids was considered strong by the classification used in this study, while the other simple correlations were considered moderate (Moisture × b*; Moisture × Chroma; Lipids × a*; Lipids × b* and Lipids × Chroma) or weak (Moisture × Bixin; Moisture × a*; Lipids × Bixin; Bixin × a*; Bixin × b*, and Bixin × Chroma).

When eliminating the effect of moisture (partial correlation), only the variables bixin concentration and a* showed significant correlation, considered weak by the classification used. Despite the weak relationship, there was a correlation between the red color and the bixin concentration of the annatto seeds. The differences between the simple and partial correlations highlight the effect of moisture on the color assessment of annatto seeds. Moreover, the higher the bixin concentration is, the redder the seeds (positive correlation) will be. The correlations between the color space (L*, a*, b*, and Chroma) were not considered.

When analyzing the two samples with the higher bixin concentrations, corresponding to Sample 46 and 47, with values of 4.82 and 1.78%, respectively, the color difference (ΔEab) was only 1.44. This result indicates that large variations in bixin concentrations of annatto seeds may lead to color changes, thus this evaluation criterion is not recommended for the quality control of these grains.

Table 3. Correlation matrices between the variables studied. The values in the left quadrant of the matrix represent the simple correlations, and the values in the right quadrant represent the partial correlations when eliminating the effect of moisture.

	Simple correlations			Partial correlations	
	Moisture	Lipids	Bixin	Lipids	Bixin
Lipids	0.7652^{s}				
Bixin	0.3568^{s}	0.4078^{s}		0.2242^{ns}	
L*	0.1362^{ns}	-0.0085^{ns}	0.2093^{ns}	0.1767^{ns}	0.1737^{ns}
a*	0.4831^{s}	0.5214^{s}	0.4220^{s}	0.2692^{ns}	0.3053^{s}
b*	0.5271^{s}	0.5274^{s}	0.2936^{s}	0.2268^{ns}	0.1330^{ns}
Chroma	0.5086^{s}	0.5359^{s}	0.3923^{s}	0.2648^{ns}	0.2621^{ns}

^{s}Significant ($p < 0.05$); ^{ns}Not significant ($p < 0.05$).

4 Conclusion

Only the variables moisture and lipids content exhibited a strong correlation with each other for the classification used in this study. When eliminating the effect of moisture (partial correlation) only the variables bixin and coordinate a* showed significant correlation, considered weak for the classification used.

Therefore, a correlation was observed between the red color and the bixin concentration of the annatto seeds, even though it was weak. However, the color difference (ΔEab) between the samples with higher and lower bixin concentrations was only 1.44. This result indicated that large variations in the bixin concentrations of annatto seeds can lead to small changes in color, thus the use of this evaluation criterion is not recommended for the quality control of these grains.

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