

# Optical Characterization in Annatto and Commercial Colorific

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**Abstract** Nowadays, the synthetic dyes (as erythrosine, ponceau and tartrazina) and natural colourants (as annatto, paprika, curcuma and anthocyanin) are indispensable in the food, pharmaceutical and cosmetics applications. However, the use of natural colourant has been considered safer for human consumption than synthetic dye. For practical applications of the coloring, optical properties are important for the understanding of the characteristics of them. In this work, we presented the absorption and fluorescence spectroscopic characterizations of annatto extracts obtained from the seeds of the tropical shrub *Bixa orellana* L. solutions and commercial colourant. The measurements were performed in annatto extracts with acetone and chloroform in different concentrations range (3.5–52.5)  $\mu\text{g/mL}$ . The main carotenoids detected in annatto seeds is bixin. The numerical calculus of the absorbance spectra for cis- and trans-bixin conformation is presented. In addition, for commercial colourant, the measurements were performed for six different brands and five lots each one. Modifications in the shape of the colorific fluorescence spectra were observed and it can be an indicative of differences in the industrial methods applied

for obtaining annatto pigments powders and/or the possibility of the presence of other impurities added in the commercial powders.

**Keywords** Annatto · Colorific · Spectroscopy · Fluorescence

## Introduction

New alimentary habits is growing recently due to the increased conscientization by the world population of the importance of the ingestion of natural and functional foods. On the other hand, for industrialized food products, such properties as long time of durability, reduced loss of the both functionality and sensorial quality are fundamental characteristics. With the intention of prolonging the durability of the foods, many additives are added, for example, to enrich the aroma, the color or to impede the proliferation of microorganisms [1–3]. The dyes are amongst the mostly used additives in the food industry, with the purpose of intensifying, to compensate or to join color to the product, maintaining a pleasant and attractive visual aspect close to the natural product. Despite the controversies that arise from their possible noxious effects, such as toxicity and mutagenic action, the synthetic colorants are widely used [1, 4–9]. The world tendency seeks an easy way of life for the population, but also worries about maintenance of the health and the reduction of risks of diseases. Then, there has been a growing demand for natural dyes as curcuma, paprika, carmin, annatto, among others.

The seeds of the annatto tree (*Bixa orellana* L.) is an important source of natural colorant. In Portuguese it is called urucum and, historically, the name comes from the Tupi-Guarani indigenous language, in which “uru-ku” means “red”. It was used by the natives in the feeding,

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tribal make-up for rituals and war, as repellent against insects and suntan lotion [7, 10]. Nowadays, annatto is an important sources of natural colorant used by the food industries, finding also applications in textiles, cosmetic and pharmaceutical products [1, 11–13]. The annatto tree (*Bixa orellana* L.) is a tropical bush of the family Bixaceae that possesses fruits denominated cachopas, the external part possesses inoffensive thorns and, inside the fruits, 10 to 50 seeds covered by a slightly red resin can be found. It is the main substance which is responsible for the dye characteristics from the yellow-orange-red range [4, 14].

Several carotenoids were detected in annatto seeds, in which 80%–90% of the total carotenoid content is bixin [4, 12, 15, 16]. In the indirect extraction of the annatto, the pigments are extracted from the seeds with solvent, producing concentrated extracts which contain mainly *cis*-bixin ( $C_{25}H_{30}O_4$ ) and much lesser quantities of *trans*-bixin and *cis*-norbixin ( $C_{24}H_{28}O_4$ ) [17, 18]. The intense red coloration indicates the presence of concentrated bixin, which is liposoluble. When the color is close to yellow, there is predominance of the norbixin structure. The bixin is a carotenoids with high antioxidant action, their conjugated double-bond system act as an excellent captor of free radicals. It presents potential importance for the human health for being absorbed easily by the organism and for being an effective biological singlet molecular-oxygen quenchers, what may contribute to the protection of cells and tissues [8, 19, 20].

On the other hand, the product constituted of mixed maize flour with powdered annatto or oily extract of annatto containing or not salt and eatable oils is labeled as colorific. The colorific is the final form of the commercial product obtained from annatto. Latin America produces ~60% of the annatto and both Africa and Asia together are responsible for ~39% of the world's annatto production [2]. In this work we present the optical characterization of the annatto pure solutions and the results are compared with those obtained for industrialized colorific.

## Experimental and Numerical Calculations

### Sample Preparation

It was used seeds of the fruit of the urucum (*Bixa orellana*) tree from Jacareí city, state of São Paulo/Brazil and six different brands of commercial colorific. After opening the fruits for the assessment of the seeds and removal of the pericarp, the larger seeds were collected and cleansed for the experiment. The chosen seeds were macerated and a powder mass of 3.5 mg was added to 0.5 mL of acetone and 4.5 mL of chloroform at the room temperature. The resulting solution was filtered and the final volume was obtained by adding 5 mL of chloroform. Samples with

different concentrations were obtained from aliquotes of the concentrated solution in chloroform.

The six commercials colorific were obtained from Korima (S1), Hikari (S2), KiSabor (S3), Kitano (S4), Chinezinho (S5) and Masterfoods (S6) comercialized at São José dos Campos and Jacareí cities, state of São Paulo/Brazil. For each commercial brand, five different batches were analyzed to validate this study. The powder mass of the commercial samples were measured (~14.3 mg) and the annatto extraction were performed with acetone (0.5 mL) and chloroform (4.5 mL), at room temperature. A paper filter was used to separate the filtrate and the solution, which was completed to the final volume with 5 mL of chloroform.

The absorption and fluorescence spectra were obtained in a quartz cuvette of 1 cm, using a Carry 50 Bio Varian, a Jobin-Yvon Spex Fluoro Max-2 spectrofluorimeter, and an Ocean Optics USB 4000 spectrometer configured for fluorescence.

### Computational

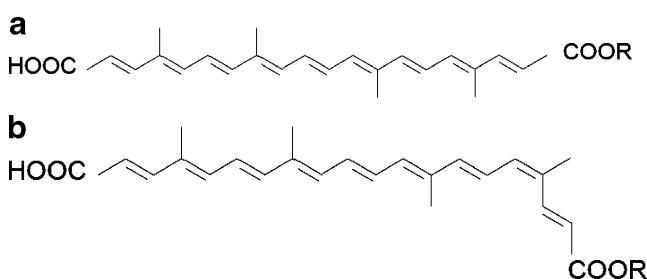
The chemical and electronic structures were calculated by Density Functional Theory (DFT). DFT calculations were carried out with the functional B3LYP (the exchange functional of Becke and Lee) starting from previous AM1 optimized geometry, and following subsequent harmonic frequency calculations. All calculations (DFT and semi-empirical) were performed with the molecular package Gaussian 03, in a PC computer.

The size of the molecule imposes some restrictions on the level of the quantum mechanical method used for an additional description of the electronic spectra. In practical terms, the option used in this work was limited to use a semi-empirical method approach to perform the TD-DFT (time-dependent DFT) calculations.

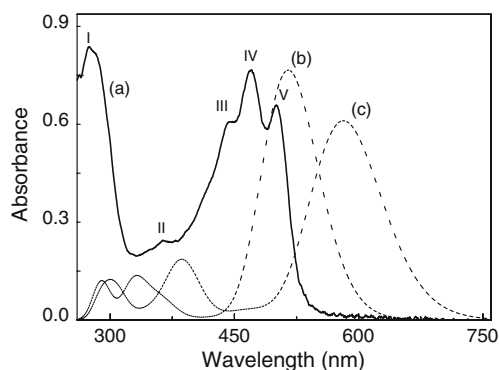
## Results and Discussions

### Molecular Geometry

The chemical structures are shown in the Fig. 1. The optimized molecular geometry of the compounds was



**Fig. 1** Chemical Structure of (a) *trans*- and (b) *cis*- bixin (for R=CH<sub>3</sub>) or norbixin (for R=H).



**Fig. 2** Absorbance [-log(I/I<sub>0</sub>)] of the (a) annatto extracted from seeds of the tropical shrub *Bixa Orellana* (A3, Table 1 and 1-cm quartz cuvette). Absorbance numerical calculus for (b) cis and (c) trans bixin suspended in chloroform solvent

calculated without symmetry constraints with exception of the molecule in the trans configuration. The harmonic frequency calculations (only positive frequencies) showed that the obtained molecular geometry corresponds to a minimum-energy structure. Geometry optimization using DFT method B3LYP/6-31G (d) predicts an almost planar structure with negligible deviations of the structural common parameters in both dyes.

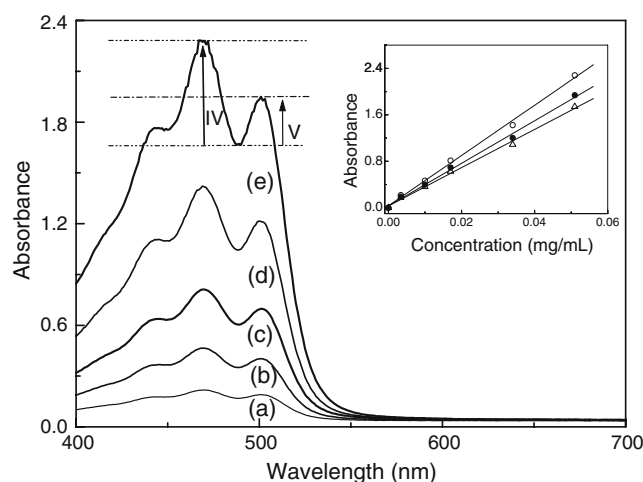
#### Frontier Orbital, Electronic Energy Levels

The major contributors for the color of the annatto are the carotenoids bixin (C<sub>25</sub>H<sub>30</sub>O<sub>4</sub>) and norbixin (C<sub>24</sub>H<sub>28</sub>O<sub>4</sub>). These carotenoids can be obtained at *-cis* and *-trans* conformations, as presented in the Fig. 1. The difference between HOMO and LUMO energy for *cis*-bixin (Fig. 1a) and *trans*-bixin (Fig. 1b) are  $-2.58$  and  $-2.37$  eV, respectively. Therefore, the most important transition, the HOMO→LUMO, is located approximately at the 480,56 and 523,14 nm wavelengths for *cis* and *trans* configurations, respectively, showing that the *trans* configuration promotes a significant change of the electronic energy levels of the compound.

**Table 1** Concentration and optical properties of the annatto solutions extracted from *Bixa Orellana*

Sample	C (μg/mL)	α* (cm <sup>-1</sup> )	V/IV (%)	<λ <sub>em</sub> > (nm)
A1	3.4	0.37	44.4	591.1
A2	10	0.83	45.7	590.8
A3	17	1.32	45.3	608.8
A4	34	2.50	45.2	602.8
A5	51	3.81	46.1	598.0

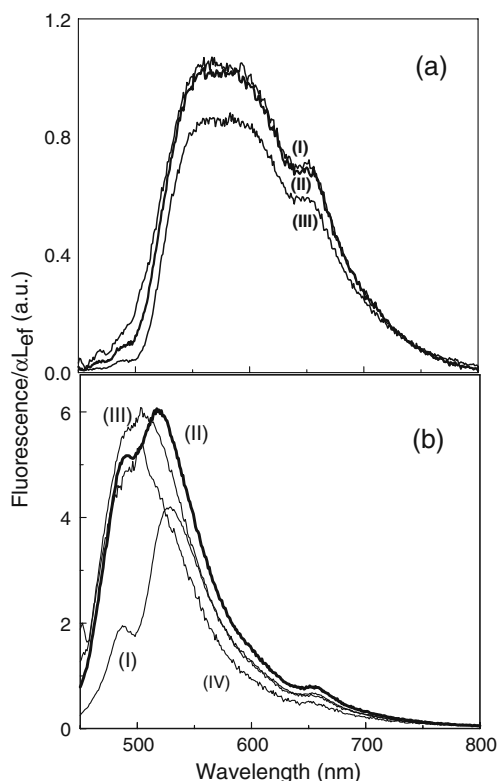
\*λ<sub>e</sub>=440 nm



**Fig. 3** Absorbance spectra for several concentrations of the annatto samples: (a) A1, (b) A2, (c) A3, (d) A4 e (e) A5 (Table 1). The inset shows the absorbance values in function of concentrations for λ= 440 nm (open triangle), 468 nm (open circle) e 503 nm (closed circle)

#### Electronics Spectra and Other Experimentals Photophysical Properties

Figure 2 shows the UV-Vis absorption spectra of the annatto solution extracted from the fruit of the *Bixa orellana* (A3, Table 1). The peak positions in the UV-Vis



**Fig. 4** Fluorescence spectra for (a) annatto solution: (I) A2, (II) A3 and (III) A4 (Table 1); b colorific sample S1 (Table 2) for the concentrations (I) 3.01, (II) 1.52, (III) 0.91 and (IV) 0.35 mg/mL

**Table 2** Composition of colorific samples for different lots L1-L5

	Industrial Brand	Ingredients						Date of the Product	
		maize flour	mffa <sup>a</sup>	Annatto	oil	osa <sup>b</sup>	salt	Lot	expiration
Korima (S1)									
L1	X	–	X	X	–	–	–	8/15/2008	
L2	X	–	X	X	–	–	–	5/20/2009	
L3	X	–	X	X	–	–	–	1/10/2009	
L4	X	–	X	X	–	–	–	6/20/2009	
L5	X	–	X	X	–	–	–	7/20/2009	
Hikari (S2)									
L1	X	–	X	X	–	–	L2322K	8/13/2008	
L2	–	X	X	X	–	–	1049K	11/6/2008	
L3	–	X	X	X	–	–	1619-1	12/28/2008	
L4	–	X	X	X	–	–	1836-2	1/29/2009	
L5	–	X	X	X	–	–	2013-2	2/13/2009	
KiSabor (S3)									
L1	X	–	X	X	–	–	70	9/25/2008	
L2	X	–	X	X	–	–	104	7/5/2009	
L3	X	–	X	X	–	–	108	9/5/2009	
L4	–	X	X	X	–	–	1A1	6/30/2009	
L5	–	X	X	X	–	–	110	9/5/2009	
Kitano (S4)									
L1	–	X	–	–	X	–	25IO7B	9/25/2008	
L2	–	X	–	–	X	–	F6L-B8BD	2/13/2009	
L3	–	X	–	–	X	X	F1L-B8EE	5/14/2009	
L4	–	X	–	–	X	–	F4L-B8EL	5/21/2009	
L5	–	X	–	–	X	–	F4L-B8FJ	6/19/2009	
Chinezinho (S5)									
L1	X	–	X	–	–	–	–	7/2/2008	
L2	X	–	X	–	–	–	–	3/10/2009	
L3	X	–	X	–	–	–	–	4/7/2009	
L4	X	–	X	X	–	X	–	7/21/2009	
L5	X	–	X	–	–	–	–	8/13/2009	
MasterFoods(S6)									
L1	X	–	X	X	–	–	713CM07	3/28/2008	
L2	X	–	X	X	–	–	725EM09	6/22/2008	
L3	X	–	X	X	–	–	L743CM07	10/24/2008	
L4	X	–	X	X	–	–	L826CM09	6/25/2009	
L5	X	–	X	X	–	–	L831BM09	7/29/2009	

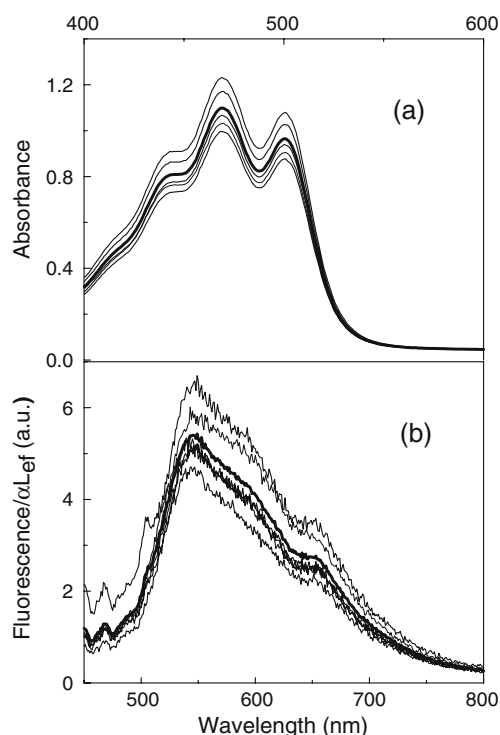
<sup>a</sup> Maize flour enriched with folic acid

<sup>b</sup> Oily suspension of annatto

spectrum of the carotenoids are characteristics and it gives information about the chromophores groups of the molecule [3, 21]. The peaks I, II, III, IV and V are observed at ~276, 363, 440, 468 and 503 nm, respectively. The position of the peaks I, III–V are the typical spectra for carotenoid bixin and the position and/or intensity of the absorption peaks can be influenced by change in the molecular environment of the carotenoid as, for example, the solvent [3, 16, 18, 22–24]. For carotenoids, the relevant transition is the  $\pi \rightarrow \pi^*$  transition, where the  $\pi$ -electrons are highly delocalized and the excited state is of comparatively low energy (energy corresponds to visible wavelength region)

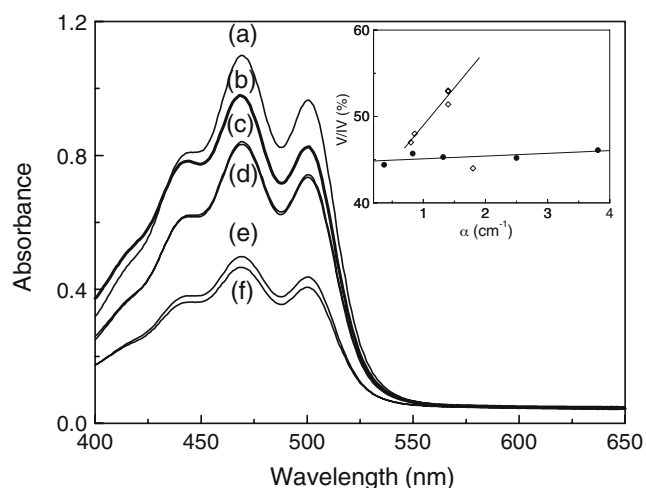
[3]. The absorption peak at ~360 nm is characteristic of the cis-bixin conformation [3]. Maximums wavelengths absorbance at 501 and 470 nm are typical from cis-Bixin conformation in chloroform; while 507 and 476 nm bands are characteristics for trans-bixin [25]. A complete study for the separation of cis- and trans- bixin conformation (prepared in chloroform) can be performed applying the reverse-phase HPLC method [25].

Figure 2(b-c) presents the numerical calculus for cis- and trans- bixin conformation, respectively, inserted in chloroform solvent. Spectral behavior of organic molecules can also be theoretically simulated from the energy gap between

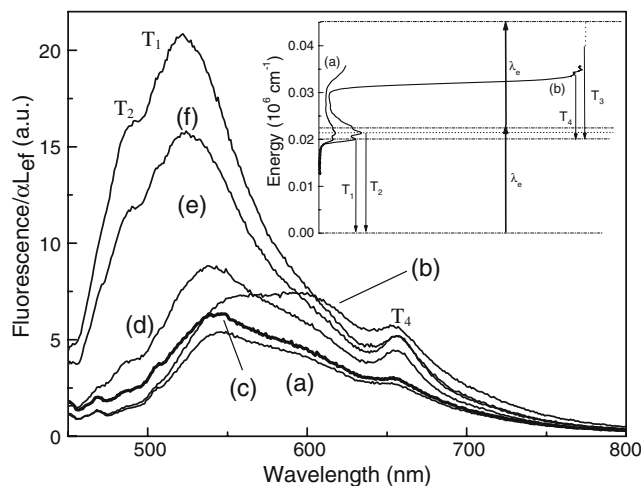


**Fig. 5** Absorbance (a) and Fluorescence (b) spectra for five different Kitano commercial colorific lots (Sample S4, Table 2). In bold the average results for S4 sample

the higher energy occupied orbital (HOMO) and the lower energy unoccupied orbital (LUMO). For this simulation, the calculated oscillator strength was employed and, taking the peak position, the band profile can be represented by a Gaussian function. Note that the solvent affects the absorption and the emission spectra of the compounds,



**Fig. 6** Average values of the absorbance spectra for five different lots of the commercial colorific: (a) S4, (b) S5, (c) S2, (d) S6, (e) S3 and (f) S1 (Table 2). The inset shows the spectral band structures results for bixin (close circle, Table 1) and colorific solutions (open diamond, Table 3)



**Fig. 7** Average values of the Fluorescence spectra for five different lots of the commercial colorific: (a) S4, (b) S5, (c) S2, (d) S6, (e) S3 and (f) S1 (Table 2). The inset shows an energy bands diagram to solutions: (a) A3 and (b) S1

promoting a Stokes shift. The maximum values of the absorption spectrum in the selected solvent correspond to energies of 2.95 and 2.14 eV, respectively. The predicted electronic transitions are little different from the observed ones, probably due to the solvent stabilization effect. The fluorescence emission spectra of the isomers are similar. In addition, it is observed that the main absorption band in the visible region for cis-bixin is blue shifted with relation to the trans-bixin visible absorption band. These results are in agreement with the differences that are reported between the spectra of trans- and cis- isomers of the carotenoids which are hypsochromic shift in the visible absorbance band for the cis-isomers [3].

Figure 3 presents the absorption spectra at the visible region for different concentration of annatto carotenoids (3.5–52.5)  $\mu\text{g/mL}$ . In the inset, the observed linear behaviors for the absorption coefficient as a function of the annatto concentration at 440, 468 and 503 nm are presented. The spectral band structures [3] results, expressed as the ratio of the peak heights V/IV, are showed in Table 1. The band IV increased with respect to the band V, when the concentration of the samples increased.

**Table 3** Commercial colorific properties at  $\sim 1.43$  mg/mL

Industrial Brand	Sample	$\alpha^*$ (cm <sup>-1</sup> )	$\lambda_{eMax}$ (nm)	IV/V (%)
Korina	S1	0.8±0.2	530±10	47±4
Hikari	S2	1.4±0.2	546±3	53±1
Kisabor	S3	0.86±0.08	540±10	48±2
Kitano	S4	1.4±0.4	547±2	51.4±0.6
Chinezinho	S5	1.8±0.3	590±20	42±2
Masterfoods	S6	1.4±0.2	541±3	52.9±0.9

\* $\lambda_e=440$  nm

For purified *cis*- and *trans*- bixin in chloroform, the average values of extinction coefficients  $A_{1\text{cm}}^{1\%}$  obtained are ~3161 (at 470 nm) and 3187 (at 475 nm), respectively [25, 26]. Applying the organic solvent extraction method to extract the pigment from the annatto seeds, the values for product with higher pigment concentrations, from 3.5% to 5.2% of bixin, are reported using acetone and methanol [27]; and the yield of the dye is 3.5% and its bixin content is 22% for chloroform [28].

The absorption coefficient  $\alpha$  was obtained from Fig. 3 using the expression of Beer-Lambert [29],  $I=I_0\exp\{-\alpha L\}$ , that it is applicable for samples in the condition of low concentration. Here,  $I_0$  is the light intensity incident into the material,  $I$  the transmitted intensity and  $L$  the thickness of the cuvette sample. The  $\alpha$  values at 440 nm are presented in the Table 1.

In Fig. 4 are presented, the fluorescence spectra for solutions with different bixin concentrations (Table 1), normalized by  $\alpha L_{\text{ef}} = (1 - e^{-\alpha L})$ . Where, the absorbed excitation beam  $P_{\text{abs}} = P_e(1 - e^{-\alpha L})$  and  $P_e$  is the excitation beam power at  $\lambda_e=440$  nm [30]. The values of the average emission wavelength  $\langle\lambda_{\text{em}}\rangle$  obtained for samples of Fig. 4 are presented in Table 1. A small decrease was observed in the intensity of the fluorescence normalized by  $P_{\text{abs}}$  with the increase of the concentration of the solutions of bixin studied [31, 32]. In the best of the authors' knowledge this is the first time that fluorescence spectra (at visible wavelength) from bixin is presented. For comparison, Fig. 4b shows the fluorescence spectra of the commercial colorific (S1, Table 2). The shape of the fluorescence spectrum is dependent of colorific concentration and the maximum intensity of the fluorescence ( $\lambda_{\text{eMax}}$ ) is at 520 nm for sample S1.

A significant difference was obtained in the shape of the band emission for annatto and commercial colorific. In this form, systematic investigations for six different brands of commercial colorific were performed. For each commercial brand, five different lots were available. For all commercial colorific brands the concentration used was maintained at the same value of the S1 sample that presented the more intense fluorescence (Fig. 4 b II). The compositions of colorific samples studied in this work are presented at Table 2.

Figure 5 presents the results of absorbance and fluorescence spectra for Kitano colorific (S4, Table 2) for different commercial lots. The same procedure was accomplished for the other five colorific brands. The average results for six different colorific are presented at Figs. 6 and 7, for absorbance and fluorescence, respectively. The spectral band structures results for the colorific and the pure annatto solutions are presented in the inset of the Fig. 6. The results show that for the colorific samples, the band V increases more than for the annatto samples, relative the band IV

(Table 3). The fluorescence spectra for colorific S5 (Tables 2 and 3) is closer to the format of the annatto fluorescence spectrum than for the others colorific. In fact, for S5 sample the results for relation between the bands V/VI is in agreement with the values obtained for annatto solution extracted from fruit of the *Bixa orellana* tree (see inset of Fig. 6). However all the others colorific samples presented similar shape of fluorescence. The values of  $\lambda_{\text{eMax}}$  are presented at Table 3. For colorific samples (S1–S4, and S6) the preferential transitions for emission, after absorption of  $\lambda_e=440$  nm, is T1 and T2 (see the diagram of energy inset of the Fig. 7). The small fluorescence at ~660 nm can be explained by two-photon absorbance process and corresponding transfer of energy T4. For annatto solution, the preference of the emission for the transfers of energy T4 or T3 is not understood. Modifications in the fluorescence spectra among of the commercial colorific were observed. Possibly, those modifications could be explained by differences in the procedure of production of the colorific powders and/or the presence of additive in the commercial colourant.

## Conclusions

In this work the spectroscopic characterization of annatto extracted from the fruit of the *Bixa orellana* and commercial colorific samples is presented. The absorption band peak at ~360 nm identified the annatto solution at *cis*-bixin conformation. The results have shown that fluorescence spectroscopy is a very sensitive method, which is suitable for identifying the processes of transference of energy in pure and mixture of annatto solutions. Differences in the fluorescence spectra of the samples were observed and it can be an indicative of differences in the form of powder production and/or additive presence in commercial colourant.

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