Spectroscopic and photothermal characterization of annatto: Applications in functional foods

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Dyes are among the most common additives used primarily to intensify, compensate or add color to manufactured products. The major color detected in annatto seeds comes from carotenoids bixin (C25H30O4) and norbixin (C24H28O4), depending on the extraction method. This article presents absorption and fluorescence spectroscopic characterizations of annatto extracted in aqueous solutions from seeds of the tropical shrub Bixa orellana L. Extractions from seeds were performed using aqueous solution (at 98 °C) with different potential of hydrogen values (pH 6.5–11.2), and the results were compared to those obtained with chemical extraction methods using other solvents. Thermo-optical parameters, such as refractive index temperature coefficient (dn/dT), thermal diffusivity (D), fraction thermal load (ϕ) and quantum yield (η) were determined for annatto solutions. Finally, the effectiveness of using of different concentrations of annatto dye in bread preparation is investigated as a functional food possibility.

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1. Introduction

Foods provide the necessary nutrients for human development and maintenance and are, therefore, of vital importance to human existence. The nutritional and functional properties of foods depend on active compounds that generate beneficial health effects [1,2]. One of the most important aspects of food marketing is its visual appearance, as depicted via the color and appearance of the product. Additives are applied to foods for numerous reasons, including prolonging durability, enriching aroma, imparting an attractive color or impeding the proliferation of microorganisms [3–5]. Among the most common additives used in the food industry, synthetic and natural dyes are used to intensify, compensate or add color to a manufactured product, thereby maintaining a pleasant and attractive appearance that resembles the natural product. Synthetic dyes, such as eritrosine, ponceau and tartrazine, remain in wide use, despite ongoing controversies arising from their possible noxious effects, including toxic and mutagenic actions [6–11]. However, the demand for natural dyes, such as curcumin, paprika, carmine and annatto, has increased due to the global trend of maintaining good health and reducing the risk of disease.

Annatto pigment is an important source of natural colorant used in food industries, textiles, and cosmetic and pharmaceutical products [1,3,12–16]. Commonly used in foods, the annatto dye is extensively applied in form of a colorific that is composed of maize flour mixed with powdered annatto or an oily extract of annatto that may or may not contain salt and edible oils [12]. The red resin that can be found in the pericarp of annatto seeds derived from fruit of the tropical tree Bixa orellana L. is the main substance responsible for the yellow-orange-red range of the dye [6,12,16–18]. Several carotenoids, including bixin and norbixin, can be obtained from annatto seeds, depending on the extraction method used [1,5,6,17,19]. The following three commercial processes have been applied to extract carotenoid pigment from dehydrated annatto seeds: indirect extraction with solvents, direct extraction using oil [1,5,6]. The indirect extraction method produces concentrated extracts that contain mainly cis-bixin (C25H30O4) and much lower quantities of

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trans-bixin and cis-norbixin (C₂₅H₂₈O₄) [20,21]. The oil extraction produces a dye that is primarily in the form of bixin [1,5]. Aqueous alkali extraction saponifies the methyl group of the bixin, producing norbixin as the principal natural dye [4,5,22,23]. An intense red coloration indicates the presence of concentrated bixin, which is liposoluble, while yellow coloration indicates predominance of norbixin. Bixin is a carotenoid with high antioxidant properties because its conjugated double-bond system constitutes an excellent captor of free radicals [24–26]. Bixin may have great potential for improving human health because it is easily absorbed and is an effective biological singlet molecular-oxygen quencher, which may provide protection for cells and tissues [10,27,28]. Annatto has also been reported to exhibit antimicrobial activity [29]. Furthermore, bixin and norbixin produce opposite effects on glycemia and lipidemia in diabetic rats [30].

The chemical environment involved in the extraction of dye pigments can have important effects on their absorption and emission spectra, their stabilization and thermal parameters, and other properties [5,31]. Therefore, it is important to obtain extraction-specific spectroscopic and thermo-optical characterizations for natural dyes. The present work reports the absorption and fluorescence spectroscopic and thermo-optical properties of annatto extracted from seeds of the tropical shrub Bixa orellana L. Spectroscopic measurements were obtained for different concentrations of annatto extracts in aqueous solutions with different pH values. The annatto samples were obtained using norbixin as the principal natural dye [4,5,22,23]. An intense red coloration indicates the presence of concentrated bixin, which is liposoluble, while yellow coloration indicates predominance of norbixin. Bixin is a carotenoid with high antioxidant properties because its conjugated double-bond system constitutes an excellent captor of free radicals [24–26]. Bixin may have great potential for improving human health because it is easily absorbed and is an effective biological singlet molecular-oxygen quencher, which may provide protection for cells and tissues [10,27,28]. Annatto has also been reported to exhibit antimicrobial activity [29]. Furthermore, bixin and norbixin produce opposite effects on glycemia and lipidemia in diabetic rats [30].

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\[ I(t) = I(0) \left[ 1 - (θ/2)\tan^{-1}\left(2mV\left[\left(1 + 2m + V^2\right)\tau_c/(2+1 + 2m + V^2)\right]^{-1}\right)\right]^2, \]

where \( I(0) \) is the on-axis intensity when \( t = 0 \); \( m = (w_1/w_2)^2 \); \( V = z_1/z_{op} \); \( z_1 \) is the distance between the sample and probe beam waist, \( z_{op} = πw_{op}^2/λ_p \) is the probe beam Rayleigh range, \( z_{op} = z_2 \) (cm) is the distance between the sample and TL detector and \( w_{op} \) is the probe beam radius at the focus with wavelength \( λ_p \).

The thermal lens (TL) effect is created when the excitation laser beam passes through a sample of thickness \( L \) and the absorbed energy is converted into heat. In TL experiments [32,33] employing a two-beam (pump and probe) configuration, the heat source profile, \( Q(r) \), is proportional to the Gaussian intensity profile of the excitation beam, which is expressed as \( L_e(r) = 2P_e/πw_e^2 \exp(-2r^2/w_e^2) \), where \( P_e \) is the power of the excitation beam with radius \( w_e \) at the sample. The temporal evolution of the temperature profile, \( ΔT(r, t) \), of the sample can be obtained by the heat conduction equation. In experiments that use short excitation pulses, heat diffusion can be neglected, and \( ΔT(r, t) \) is proportional to the Gaussian intensity profile of the excitation beam, \( L_e(r) \). For long-pulse or continuous-wave (cw) experiments, however, the effect of heat diffusion is important, and consequently, \( ΔT(r, t) \) is wider than \( L_e(r) \). For \( t = τ_e \) (where \( τ_e \) is the characteristic heat diffusion time), the on-axis temperature rise is proportional to the absorbed excitation power \( (P_{abs}) \) and inversely proportional to the thermal conductivity \( K/ΔT(0) = P_{abs}/K) \) but is independent of \( w_e \). Heating changes the refractive index of the material and causes a thermally induced phase change, \( Δφ_{TH} \), expressed as follows [32):

\[ Δφ_{TH} = (θ/τ_e) \int_0^t \left(1 + 2τ/(τ_c)\right)^{-1} \left[1 - \exp\left(-\left(2τ^2/w_e^2\right)/(1 + 2τ/(τ_c))\right)\right] dt, \]

and

\[ θ = -\varphi P_{abs}(Kλ_p)^{-1}(dn/dT), \]

where \( P_{abs} = P_{el}L_{eff} \), \( α (cm^{-1}) \) is the optical absorption coefficient at the excitation wavelength \( (λ_e) \), \( L_{eff} = (1 - e^{-αL})/α \) is the effective length, \( λ_p \) is the wavelength of the probe beam, \( dn/dT \) is the refractive index temperature coefficient, and \( φ \) is the absolute nonradiative quantum efficiency, which represents the fraction of the absorbed energy converted into heat. The characteristic thermal time constant \( τ_e \) is expressed as follows [32,33]:

\[ τ_c = w_e^2/4D, \]

where \( w_e \) is the excitation beam radius at the sample, \( D = K/ρC \) is the thermal diffusivity (cm²/s), \( K \) is the thermal conductivity (W/cm K), \( ρ \) is the density (g/cm³), and \( C \) is the specific heat (J/g K).

The electric field of the probe beam as it leaves the sample can be expressed as \( ζ_{el}(ρ_1) = ζ(ρ_1) × \exp(-Δφ_{NL}) \), where \( Δφ_{NL} \) is the phase change caused by the nonlinearity of the sample (which may include Kerr and thermal components), \( ζ(ρ_1) \) is the field of the probe beam at the entrance face of the sample, \( ρ_1 = [(x_1^2 + y_1^2)/w_1^2]^{1/2} \), and \( w_1 \) is the beam radius. In this case, \( Δφ_{NL} \) is approximately equal to \( Δφ_{TH} \) and is expressed by Eq. (1a) and (1b).

The field \( i(r_2) \) at a point \((x_2, y_2, z + d)\) in the observation plane \( P \) located at a distance \( d \) away from the sample, is given by the sum of the optical fields caused by all points in the \( P \) plane [34]. The variation in the probe beam on-axis intensity, \( I(t) = |i(r_2 = 0)|^2 \), can be calculated at \( r_2 = 0 \) (central part of the probe laser beam) in the cw excitation regime, as follows [32,33]:

\[ I(t) = I(0) \left[ 1 - (θ/2)\tan^{-1}\left(2mV\left[\left(1 + 2m + V^2\right)\tau_c/(2+1 + 2m + V^2)\right]^{-1}\right)\right]^2, \]
\[ \Theta = \varphi (K_{\varphi})^{-1} \frac{dn}{dT}, \quad (4a) \]

and \[ \varphi = 1 - \frac{\eta \lambda_e}{(\lambda_{em})}. \quad (4b) \]

where \( \lambda_{em} \) is the average emission wavelength and \( \eta \) is the fluorescence quantum efficiency or quantum yield.

Under conditions utilizing relatively high excitation beam power, analysis of the ring patterns generated in a laser beam (at observation plane P) due to thermally induced self-phase-modulation (TSPM) effects, denominated as the conical diffraction (CD) technique [31,37,38], was employed as a simple alternative method for nonradiative quantum efficiency measurements. SPM effects can be understood from the ability of the excitation beam to induce spatial variations in the refractive index, which leads to a phase shift that depends on the transverse distance from the beam axis. This transverse self-phase modulation [34,37,39,40] is also implicated in the emergence of rings in the pattern of transmitted light upon the change of phase that occurs when the nonlinearity \( \Delta \phi_{NL} = \pi p \). When the nonlinearity is a thermally induced phase change \( \Delta \phi_{TH} \), i.e., \( \Delta \phi_{NL} = \Delta \phi_{TH} \), the number of rings \( N \approx \Delta \phi_{NL}/2\pi \) [39,41] can be determined as a function of \( P_e \) using the following expression [42,43]:

\[ N = \left( \frac{\varphi \alpha_{diff}}{2\pi K_{\varphi}} \right) \left( \frac{dn}{dT} \right) P_e. \quad (5) \]

After traversing the nonlinear medium, interference occurs such that rays within the laser beam that have the same wave vector come out parallel with different phases. The interference will be constructive or destructive in the plane of the observation if \( \Delta \phi_{NL}(r_1) - \Delta \phi_{NL}(r_2) = \pi p \), where \( p \) is an even or odd integer; this is the origin of the diffraction rings [39,40].

3. Experimental

3.1. Annatto extracted from seeds of the tropical shrub Bixa orellana L. and commercial colorant

The experiments herein used seeds from the fruit of urucum (Bixa orellana) trees from Jacareí city (state of São Paulo/Brazil) and Uberlândia city (state of Minas Gerais/Brazil) and commercial colorific. After opening the fruits to assess the seeds and remove the pericarp, the seeds were collected and cleansed, and those seeds selected were macerated for the experiment. For indirect extraction, 3.5 mg of the powder mass was added to 0.5 mL of acetone and 4.5 mL of chloroform at room temperature. The resulting solution was filtered, and the final volume was obtained by adding 5 mL of chloroform. Samples of different concentrations were obtained from aliquots of the concentrated solution in chloroform. The same extraction processes were applied to extract annatto using toluene or acetone in the place of chloroform. For direct extraction in aqueous solutions, 3 mg of the urucum powder was added to 10 mL of distilled water (\(-98^\circ C\)), and the solution was filtered. Samples of different concentrations were obtained using 0.4, 0.8, 1.5, 3, 6, 9, 12, 15, 18 and 21 mg of urucum, each extracted separately in 10 mL of aqueous solution, and the solution was filtered. Another set of samples was extracted in aqueous solution to which the hydroxide of ammonium was added (NH4OH, pH 12.81 and molarity 4), and the solution was filtered. These samples were prepared using different amounts of NH4OH (0.001, 0.005, 0.025, 0.05, 0.5, 0.1, 0.3, 0.5, 0.7, 0.9 and 1 mL) while maintaining a fixed mass of annatto (15 mg) as well as using different annatto masses (0.4, 0.8, 1.5, 3, 6, 9, 12, 15, 18 and 21 mg) with a fixed value of NH4OH (0.5 mL).

The commercial colorifics were obtained in São José dos Campos and Jacareí cities in São Paulo/Brazil. Five different batches of the commercial brand were analyzed and the results presented. The powder mass of each commercial sample was measured (\(-14.3 \text{ mg}\)), and each annatto extraction was 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 brought to its final volume by adding 5 mL of chloroform.

3.2. Spectroscopic characterization

Absorption and fluorescence spectra were obtained using a 1-cm quartz cuvette, a Carry 50 Bio Varian UV-VIS-NIR from Shimadzu UV-3600, a Jobin-Yvon Spex Fluoro Max-2 spectrofluorimeter and an Eclipse from Varian. The commercial refractometer was from Atago RX-5000z. For pH measurements, a pH-meter HI 2221 from Hanna Instruments was used. A Leica microscope (DMLB2, Heidelberg, Germany) was used for seed imaging. For image acquisition, a digital camera (LEICA DFC280, Heidelberg, Germany) controlled by a software (LEICA IM50, version 4.0, Heidelberg, Germany) was used.

3.3. Thermo-optical measurements

The thermo-optical properties of natural dye solutions were investigated using TL [36-38] and CD [31,37,38] methods. TL transient measurements were performed in the mode-mismatched dual-beam (excitation and probe) configuration. A He–Ne laser (\( \lambda_p \) = 632.8 nm) was used as the probe beam, and an Argon ion laser (\( \lambda_e \) = 514.5 nm) was used as the excitation beam. The excitation and probe beam radii at the sample were measured as \( w_p = (24.5 ± 0.5) \mu m \) at 514.5 nm and \( w_e = (123 ± 3) \mu m \) respectively. Absorption of the excitation beam generated a TL heat profile and induced a phase shift proportional to \( \theta \). Modulation of the pump beam with a mechanical chopper allowed for time-resolved measurements. The transient curve was obtained from the weak probe beam, which counter-propagated in a direction that was nearly collinear with the excitation beam. On the other hand, the conical diffraction technique employed a single laser beam as the excitation at \( \lambda_e \) = 514.5 nm. Typical ring patterns were observed when the sample was positioned at the focus of the pump beam due to thermal self-phase-modulation (TSPM) effects [31,37,38].

3.4. Bread preparation

Bread production was developed using four formulations. The first was a standard or reference (F1) bread without colorific, and the other three formulations were modified by adding colorific at concentrations of 0.5% (F2), 1.0% (F3) and 3.0% (F4) (Table 1). The breads were processed using the G. Paniz method for mass homogenizing (mass AR15 series 291198cd90024), fermented in a fermentation cabinet and baked in an oven of the Venancio cilone digital brand.

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>60.0</td>
<td>59.5</td>
<td>59.0</td>
<td>57.0</td>
</tr>
<tr>
<td>Sugar</td>
<td>3.6</td>
<td>3.6</td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Fat</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Yeast biological</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Salt</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Water</td>
<td>33.0</td>
<td>33.0</td>
<td>33.0</td>
<td>33.0</td>
</tr>
<tr>
<td>Colorific [12]</td>
<td>0.5</td>
<td>1.0</td>
<td>3.0</td>
<td>5.8</td>
</tr>
<tr>
<td>Water lost</td>
<td>8.4</td>
<td>7.5</td>
<td>6.4</td>
<td>5.8</td>
</tr>
</tbody>
</table>
Production consisted of mixing the ingredients (Table 1) in the machine and adding small portions of water until the total mass of the mixture and the gluten network were obtained. The mass was weighed and fractionated into four samples, the first of which was called F1. Each of the other three masses was weighed, and colorific was added (Table 1), after which the masses, in sequence, were submitted to the rolling process for homogenization. Each mass was then divided to obtain a weight ranging between 400 and 420 g, modeled and placed into greased shapes and taken to the fermentation chamber. The time period for mass growth was 1.5 h, and the oven temperature was 160 °C for a period of 0.5 h. After completion, the breads were stored and refrigerated at room temperature for a period of 21 h, after which each bread was sliced and packed.

4. Results and discussion

The major contributors to the color imparted by annatto are the carotenoids bixin (C25H30O4) and norbixin (C24H28O4) [1,5,6,17,19]. These carotenoids can be obtained in cis and trans conformations, as presented in Fig. 1a and b [12]. The annatto tree Bixa orellana L. (Fig. 1c) is a tropical bush of the family Bixaceae that possesses fruits arranged in clusters (Fig. 1d), the external part possesses inoffensive thorns and, inside the fruits, seeds (Fig. 1e). The microscopic image of a dried seed of the fruit of the urucum tree is presented in Fig. 1f. The two inner regions of the seed shell depicting distinct colors are due to an artifact produced by the technique used for cleaving the dehydrated seed. First, the blade cuts the seed smoothly, after which the seed breaks off, producing a rough surface. The resin layer on the surface of the seed is responsible for the extraction of carotenoids.

Fig. 2 shows the UV–Vis absorption spectra of the annatto solution extracted from the fruit of the Bixa orellana using (a) acetone and chloroform, (b) heated water and (c) heated water with NH4OH. The characteristic peak positions in the UV–Vis spectrum of the carotenoids are observed, and information about the groups of chromophores within the molecule can be deduced [5,44]. Peaks I, II, III, IV and V (Fig. 2a) are observed at ~276, 363, 440, 468 and 503 nm, respectively. The position of peaks I and III e V are typical of spectra obtained for bixin, and the absorption peak at ~360 nm is characteristic of the cis-bixin conformation [5,12]. The position and/or intensity of each absorption peak can be influenced by changes in the molecular environment of the carotenoid, such as the solvent used for extraction of the natural dye [5,21,45–47].

Fig. 1. Chemical structures of annatto seed carotenoids: (a) trans- and (b) cis-bixin (for R = CH3) or norbixin (for R = H). The annatto tree (c) possesses fruits (d) and inside the fruits (e), seeds covered by a slightly red resin can be found. The optical microscopy image of the cross-section of a dehydrated annatto seed is presented in (f). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
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 (indicated by the visible wavelength region) [5]. For the dye solution of urucum powder extracted in heated water (Fig. 2b, pH ~ 7.9) the Vis absorption bands typical of annatto are not observed. In Fig. 2c, the maximum absorbance wavelengths obtained for IVN and VN peaks are ~452 and 481 nm, respectively, for annatto extracted in heated aqueous solutions with NH$_4$OH (pH ~ 11.4), which is typical for the carotenoid norbixin [24,48].

Fig. 3 presents the fluorescence spectra (normalized by the fluorescence peak) for annatto extracted as carotenoid (a) bixin [12] and (b) norbixin ($\lambda_e = 381$ nm) structures. Table 2 presents the values of the average emission wavelengths $<\lambda_{em}>$ obtained. The bixin fluorescence spectrum presents a red shift of ~140 nm in relation to the norbixin structure. Fluorescence spectra as a function of norbixin concentration are presented in Fig. 4. The preferential transitions for norbixin emission, after absorption at $\lambda_e = 381$ nm, are $T_2$ and $T_3$ (see the energy diagram inset in Fig. 4). An exponential decrease was observed in the fluorescence intensity normalized by $d_{L_{eff}}$ (transition $T_2$) with increasing concentrations (0.04–2 g/L) of the norbixin solutions studied. For the dye samples prepared (as a function of concentration), the pH values remained approximately constant (11 ± 1). Fig. 5 presents the fluorescence spectra of norbixin extracted in heated 1.5 g/L aqueous solutions using different amounts of NH$_4$OH (0–1 mL). The norbixin fluorescence process observed with increasing concentrations of norbixin (mass) and/or NH$_4$OH highlight the $T_2$ transitions, and the $T_3$ transition is quenched. This is most likely due to the increase in the IVN band with respect to the VN band that occurred with increasing sample concentrations [12]. For comparison, Fig. 6 shows the
The fluorescence spectra of the commercial colorific. The shape of the fluorescence spectrum is dependent on colorific concentration and commercial brand [12].

Fig. 7 shows typical TL transient signals for seeds of the tropical shrub Bixa orellana L. extracted in aqueous solution. The behavior of the curve in Fig. 7 indicates that dndT is negative, i.e., the created TL effect causes defocusing of the probe beam in the far-field. Fitting the experimental data of Fig. 7 using Eq. (3), θ and τc were obtained. Using Eq. (2) and the measured value of wa thermal diffusivity values were determined for the natural-dye carotenoids; the D values are presented in Table 2. The average values of D obtained for the norbixin carotenoids agree well with the published values for water solvents: D = (1.42 ± 0.02) × 10⁻⁷ m²/s [49,50]. The dyes do not significantly influence thermal diffusivity parameters at the concentrations analyzed in this work. In addition, dndT parameters were determined for all norbixin solutions using a commercial refractometer. As a function of the annatto concentration used, the dn/dT values obtained for aqueous norbixin samples are approximately constant (dn/dT = (−0.9 ± 0.1) × 10⁻⁴ K⁻¹) and agree well with values reported in the literature [49,50]. Fig. 8 presents the dn/dT and pH values of norbixin solutions containing different amounts of NH₄OH. On average, dndT = (−0.95 ± 0.05) × 10⁻⁴ K⁻¹ for solutions with average pH = (10 ± 1) values. From θ, the normalized thermal parameters of the carotenoids, θc, was determined. Using Eq. (1b) and the values of K for water [49,50] and dndT obtained, an average value of ϕ = 0.54 was determined from the TL results for the norbixin solution (Table 2).

For comparison, the ϕ and D values obtained for bixin (C₂₅H₃₀O₄) extracted in chloroform solvent are presented in Table 2. The average ϕ values were determined via TL and CD techniques. The numbers of rings (N) as a function of beam power (Pc) are shown in the inset of Fig. 7 for both samples extracted from seeds of the annatto tree and commercial colorific. The experimental results of N versus Pc were fitted with a linear equation, and the values of the angular coefficients A of the linear function fitted to the experimental data were determined. Using Eq. (5) and the K value characteristic for pure solvent [51,52], the value of [φdndT] was determined for extracted bixin and commercial colorific. Using dndT values from the literature for toluene, acetone and chloroform [51,52], Table 2 presents the average values of ϕ for bixin and for colorific extracted in chloroform for samples with different concentrations of each. The radiative quantum efficiency η and ϕ are related by Eq. (4b). Using λe = 514.5 nm and λem = 600 nm [12], a value of η < 0.20 is estimated for the bixin solution extracted in acetone, chloroform and toluene. On the other hand, using 514.5 nm and λem = 474 nm, a value of η = 0.42 was obtained for norbixin.

With regard to functional food application, Fig. 9 presents results obtained for conditions in which three different concentrations of commercial colorific were added to the bread preparation. Visual information regarding the macroscopic structure can be confirmed by the water-loss results reported in Table 1. For bread products, this loss of water should range between 10 and 12% [53–55]; thus, one may ascertain that the unit processing operations in the laboratory were not as efficient as those that occur in a processing factory where there is more effective process control. The decrease of water loss values (~10–30% compared to the reference F1) can be explained by the increased mass (2–12 g) of the corn meal or colorific added (Table 1). The colorific used is composed mainly of mixed maize flour with powdered annatto [12].

Fig. 7. Typical transient-thermal-lens signals for annatto extracted in heated aqueous solution containing NH₄OH at λe = 514.5 nm (pH 9.3, concentration 1.5 g/L, and Pe = 52 mW). The values derived from Eq. (3) are θ = (0.0980 ± 0.0002) rad and τc = (1.090 ± 0.005) ms. The inset presents the characteristic conical diffraction results [31] obtained for (a) annatto in chloroform, (b) colorific in chloroform and (c) annatto in acetone.

Fig. 8. dn/dT (at 20°C) and pH trends for annatto solutions (1.5 g/L) extracted using different amounts of NH₄OH.
5. Conclusions

This study reports the spectroscopic characterization of annatto extracted from the fruit of the Bixa orellana and within commercial colorant samples. The absorption band peak at ~452 and 481 nm identified the carotenoid as a norbixin solution. The results underscore the sensitivity of fluorescence spectroscopy as well as its suitability for identifying the processes of energy transference in bixin, norbixin and commercial colorant solutions. Annatto fluorescence spectra are dependent on the process as well as the concentration and pH of extraction of carotenoids. Norbixin fluorescence presents a blue shift in relation to bixin carotenoid. Thermo-optical characterizations involved applying conical diffraction (CD) and thermal lens (TL) techniques to pure as well as mixtures of annatto solutions. The primary parameter determined was $\frac{dn}{dT}$, allowing the fractions of thermal load ($\phi$) for pure and annatto solutions to be calculated. The thermal diffusivity parameters obtained were characteristic of the solvents in which annatto solutions were extracted. Finally, the coloric was applied to bread preparation to demonstrate its possible application in functional food production.

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