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Spectroscopic and photothermal characterization of annatto: Applications in functional foods

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ABSTRACT

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 ($C_{25}H_{30}O_4$) and norbixin ($C_{24}H_{28}O_4$), 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.

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 aqueous alkali solutions and direct extraction using oil [1,5,6]. The indirect extraction method produces concentrated extracts that contain mainly cis-bixin ($C_{25}H_{30}O_4$) and much lesser quantities of

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trans-bixin and cis-norbixin ($C_{24}H_{28}O_4$) [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 potential of hydrogen (pH) values. The annatto samples' thermo-optical properties, such as the refractive index temperature coefficients (dn/dT) and the thermal diffusivity (D), fraction thermal load (φ) and radiative quantum efficiency (η) values, were determined using photothermal techniques. The results obtained for annatto extracted in aqueous solutions are compared with those obtained for cis-bixin extracted from annatto seeds and commercial colorifics using other solvents (acetone, toluene and chloroform). In addition, we investigated the functional food potential in applying different colorant concentrations to bread preparation.

2. Photothermal technique

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 $I_e(r) = (2P_e/\pi w_e^2)\exp(-2r^2/w_e^2)$, where P_e is the power of the excitation beam with radius w_e at the sample. The temporal evolution of the temperature profile, $\Delta T(r, t)$, of the sample can be obtained by the heat conduction equation. In experiments that use short excitation pulses, heat diffusion can be neglected, and $\Delta T(r, t)$ is proportional to the Gaussian intensity profile of the excitation beam, $I_e(r)$. For long-pulse or continuous-wave (cw) experiments, however, the effect of heat diffusion is important, and, consequently, $\Delta T(r, t)$ is wider than $I_e(r)$. For $t \gg \tau_c$ (where τ_c is the characteristic heat diffusion time), the on-axis temperature rise is proportional to the absorbed excitation power ($P_{e,abs}$) and inversely proportional to the thermal conductivity $K(\Delta T(0, t) \propto P_{e,abs}/K)$ but is independent of w_e. Heating changes the refractive index of the material and causes a thermally induced phase change, $\Delta\phi_{TH}$, expressed as follows [32]:

$$\Delta\phi_{TH} = (\theta/\tau_c)\int_0^t (1 + 2t'/\tau_c)^{-1} \left[1 - \exp\left(-\left(2r^2/w_e^2\right)/(1 + 2t'/\tau_c)\right)\right]dt', \quad (1a)$$

$$\text{and} \quad \theta = -\varphi P_{e,abs}(K\lambda_p)^{-1}(dn/dT), \quad (1b)$$

where $P_{e,abs} = P_e\alpha L_{eff}$, α (cm^{-1}) is the optical absorption coefficient at the excitation wavelength (λ_e), $L_{eff} = (1 - e^{-\alpha L})/\alpha$ 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_c is expressed as follows [32,33]:

$$\tau_c = w_e^2/4D, \quad (2)$$

where w_e is the excitation beam radius at the sample, $D = K/\rho C$ is the thermal diffusivity (cm^2/s), K is the thermal conductivity (W/cm K), ρ is the density (g/cm^3), 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 $\varepsilon_S(\rho_1) = \varepsilon(\rho_1) \times \exp(-i\Delta\phi_{NL})$, where $\Delta\phi_{NL}$ is the phase change caused by the nonlinearity of the sample (which may include Kerr and thermal components), $\varepsilon(\rho_1)$ is the field of the probe beam at the entrance face of the sample, $\rho_1 = [(x_1^2 + y_1^2)/w_1^2]^{1/2}$, and w_1 is the beam radius. In this case, $\Delta\phi_{NL}$ is approximately equal to $\Delta\phi_{TH}$ and is expressed by Eq. (1a) and (1b). The field $\varepsilon(r_2)$ 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, $I(t) \approx |\varepsilon(r_2 = 0)|^2$, can be calculated at $r_2 = 0$ (central part of the probe laser beam) in the *cw* excitation regime, as follows [32,33]:

$$I(t) = I(0)\left[1 - (\theta/2)\tan^{-1}\left(2mV\left[\left((1 + 2m)^2 + V^2\right)\tau_c/2t + 1 + 2m + V^2\right]^{-1}\right)\right]^2, \quad (3)$$

where $I(0)$ is the on-axis intensity when t is zero; $m = (w_1/w_e)^2$, $V = z_1/z_{op}$, z_1 is the distance between the sample and probe beam waist, $z_{op} = \pi w_{op}^2/\lambda_p$ is the probe beam Rayleigh range, $z_{op} \ll z_2$ (where 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 thermally induced distortion of the laser beam as it passes through the sample is described by the optical path-length (S) change ($dS/dT = L^{-1}\, dS/dT$), which results in lensing at the sample. The propagation of a probe laser beam through the TL will result in either spreading ($dS/dT < 0$) or focusing ($dS/dT > 0$) of the beam, depending mainly on the temperature coefficients of the electronic polarizability of the sample, stress and thermal expansion (in the case of liquid samples, $dS/dT \approx dn/dT$).

In the dual beam mode-mismatched configuration with excitation and probe beams, the normalized transient signal amplitude is approximately the phase difference (θ) of the probe beam between $r = 0$ and $r = \sqrt{2}\, w_e$ that is induced by the pump beam, given by Eq. (1b). The normalized parameter, $\Theta = -\theta/P_e\alpha L_{eff}$, for liquid samples can be expressed using Eq. (1b) as follows [35,36]: