

## THERMOGRAVIMETRIC INVESTIGATIONS ON THE THERMAL DEGRADATION OF BIXIN, DERIVED FROM THE SEEDS OF ANNATTO (*BIXA ORELLANA L.*)

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The kinetic of the thermal decomposition of *cis*-bixin isomer isolated by solvent extraction from the seeds of Annatto have been investigated. The thermal decomposition data of the  $\alpha$ -bixin samples were analyzed by thermogravimetric analysis (TG/DTG) at different heating rates in the 25–900°C temperature range. Superimposed TG/DSC curves showed that thermal decomposition reactions for  $\alpha$ -bixin occurred in the liquid phase. The kinetic parameters  $E_a$  (activation energy) and  $A$  (pre-exponential factor) were determined using integral and approximate methods: Coats–Redfern (CR), Madhusudanan (MD), Horowitz–Metzger (HM) and Van Krevelen (VK). The results obtained with these methods are in good agreement. F2 mechanism describes well the first stage of the thermal decomposition while F1 model seems to be appropriate for the other stages.

**Keywords:** bixin, kinetic parameters, thermal degradation, thermogravimetric analysis

### Introduction

The tropical shrub *Urucum* internationally known as annatto (*Bixa orellana L.*) is a small tree with predominant occurrence in the South American regions (particularly in Brazil). Bixin is the major component of the coloring matter present on the external surface of its seeds (more than 80%) along with Norbixin. These diapocarotenoid pigments are widely used in the food industry (mainly in the butter, cheese and margarine production), condiments, cosmetics, pharmaceuticals, textiles and inks. Due to the higher toxicity of the synthetic colorants, the use of natural colorants increased in the last years. The currently available techniques are not good enough to extract these pigments at a low cost and with good quality.

Since 1990 the doubts concerning the toxicity of many coloring agents have been increased in several countries. The problem involves the regulation of the application of the same coloring compounds in nutritious products, medicinal cosmetics and others. Consequently, the interest concerning the natural colorings having generally lower toxicity is increasing in Europe. There are intensive researches to substitute the artificial colorings with natural ones, e.g. with annatto, curcuma, chlorophyll. Bixin ( $C_{25}H_{30}O_4$ ) is the diapocarotenoid formula present in the largest concentration in the annatto seeds, however this is not stable, and can be de-

graded when it is exposed to elevated temperature, light and time during the process of drying [1, 2].

Distinguished attention has been paid to the characterization of the bixin extracted from the annatto seeds and its thermal degradation products in recent years. Particularly, the stability of bixin under certain conditions such as light, oxygen, temperature and type of solvent was investigated. Bixin displayed a high stability in the absence of light and oxygen, but in the presence of oxygen and direct light it was degraded [3–5].

Annatto extract submitted to heat, exposed to light or stored for long time results considerable amounts of degradation products, including several products from the isomerization process such as all-*trans*-bixin,  $C_{17}$  *trans*-monomethyl ester of 4,8-dimethyl-tetradecahexane-dioic acid derived from 9'-*cis*-bixin [6], as well as volatile compounds like xylene, toluene, toluic acid and toluic acid methyl ester.

Recently, thermoanalytical methods as differential scanning calorimetry (DSC) and thermogravimetry/differential thermogravimetry (TG/DTG) have been used as powerful techniques to investigate the thermal properties of these samples [7, 8].

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## Experimental

### Extraction of bixin

The annatto seeds from the 'Piave' specie, were heated in hexane in a soxhlet extractor for 72 h. After the evaporation of solvent, the 'oily fraction' was obtained. The extracted seeds were later extracted with chloroform for 80 h using the same extracting instrument. After the evaporation of chloroform a red color solid residue was obtained. This solid residue was dissolved in an acetone:chloroform (1:2 v/v) solution under reflux for 8 h. After refluxing, the solid fraction was separated by filtration, yielding the pure form of  $\alpha$ -bixin micro crystals with red-purple hue.

### Physical measurements

Two techniques have been applied to measure the melting temperatures: A – Microquímica/MQAPF-301 with a controlled heating rate, B – by means of DSC using a Shimadzu/DSC-50 equipment.

The infrared absorption spectra have been recorded in KBr pellets, using a Bomem/MB-102 spectrophotometer operating in the 4000–400  $\text{cm}^{-1}$  range.

The elementary composition of  $\alpha$ -bixin was determined in a Perkin-Elmer PE 2400 elementary analyzer. The mass spectra were recorded in a Varian/MAT-311 spectrometer. The  $^1\text{H}$  and the  $^{13}\text{C}$  NMR spectra were obtained using a Varian 200 MHz spectrometer.

### Thermal analysis

The dynamic TG/DTG curves were obtained using a Shimadzu TGA-50 thermobalance in a nitrogen atmosphere (flow rate: 50  $\text{mL min}^{-1}$ ), alumina crucibles and at heating rates of 5, 10 and 15  $^{\circ}\text{C min}^{-1}$ . The initial sample masses were 7.0 $\pm$ 0.5 mg, and the TG curves were recorded in the 25–900  $^{\circ}\text{C}$  temperature range.

The DSC curves were obtained using a Shimadzu DSC-50 equipment in dynamic nitrogen flow (50  $\text{mL min}^{-1}$  flow rate), aluminium crucibles, mass of 3.0 $\pm$ 0.5 mg, at a heating rate of 10  $^{\circ}\text{C min}^{-1}$  in the 25 to 500  $^{\circ}\text{C}$  temperature range.

### Kinetic study

The theoretical basis for the kinetic calculations by dynamic thermogravimetry is summarized in the following equation:

$$g(\alpha) = \frac{A}{\theta} \int_0^T \exp\left(\frac{E_a}{RT}\right) dT \quad (1)$$

where  $A$  is the frequency factor,  $T$  is temperature,  $R$  is the universal gas constant,  $\theta$  is the heating rate,  $E_a$  is

the activation energy and  $\alpha$  represents the decomposed fraction. Equation (1) represents the reaction mechanism at a fixed heating rate. However, the right term of Eq. (1) cannot be solved analytically. Thus, several numerical methods to calculate this integral have been proposed giving rise to the different methods for the determination of the kinetic parameters from the TG curves [9].

In this study, Coats–Redfern (CR) [10] and Madhusudanan (MD) [11] integral methods and the Horowitz–Metzger (HM) [12] and Van Krevelen (VK) [13] approximation methods have been used.

## Results and discussion

### Characterization of bixin

The measure of the melting temperatures of  $\alpha$ -bixin obtained by the conventional and DSC methods were: 199 and 198  $^{\circ}\text{C}$ , respectively. The results are in a good agreement with the literature values [14]. The results of elementary analysis confirm the stoichiometric calculation based on the  $\text{C}_{25}\text{H}_{30}\text{O}_4$  formula, yielding less deviations than 0.4%. In the IR spectrum, the observed band at 1720  $\text{cm}^{-1}$  indicates the presence of C=O carbonyl band and a wide one between 3400–2400  $\text{cm}^{-1}$  indicating the presence of the OH group of the carboxylic acid. The mass spectrum of bixin showed the molecular ion as parent peak ( $m/z=394$ ), and there has not been detected other ions with higher masses with significant abundance originating from, e.g. the elimination of toluene as reported previously for seven other carotenoids [15] and methyl tropylium ion ( $m/z=105$ ).

The similarity between the  $^1\text{H}$  and carbon  $^{13}\text{C}$  spectra indicate that the doublet signals at 7.85 and 7.23 ppm as well as the signals at 148.2 and 145.5 ppm, are belonging to the protons linked to the C-3/C-18, respectively, and chemical displacement is belonging to these carbons atoms. The same analogy can be observed for the  $^1\text{H}$  doublet signals at 5.94 and 5.82 ppm, and for the 117.0 and 119.29 ppm signals in the  $^{13}\text{C}$  spectrum.

According to [16] in  $\alpha$ -bixin the 4 carbons of the methyl groups (C-21, C-22, C-23 and C-24) have the same chemical displacements. In our measurements signals 12.52 and 12.70 ppm and a signal at 19.96 ppm, indicating the presence of *cis*-bixin isomer.

### Thermal studies

The overlaid TG and DSC curves indicate that the decomposition of *cis*-bixin isomer starts in the liquid-phase (Fig. 1). The melting of  $\alpha$ -bixin is hardly visible around 200  $^{\circ}\text{C}$  at this sensitivity. The TG profile of  $\alpha$ -bixin was similar for all heating rates, as it

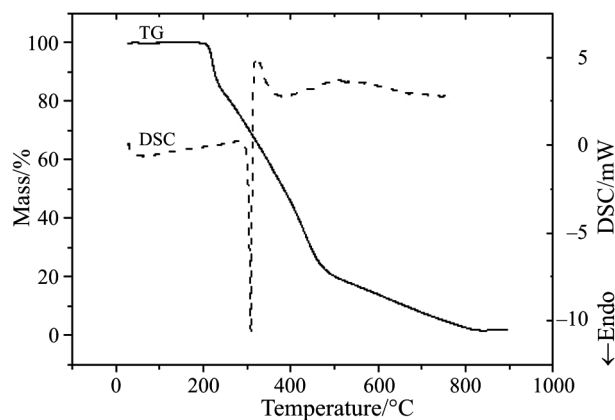


Fig. 1 TG/DSC curves of  $\alpha$ -bixin

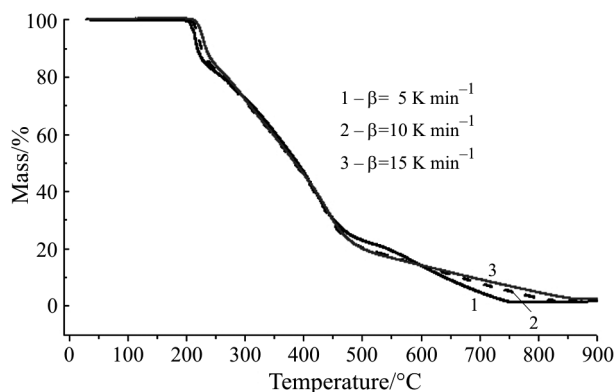


Fig. 2 TG curves of  $\alpha$ -bixin at different heating rates

can be observed in Fig. 2. All the TG curves present four decomposition stages in the 205–545°C temperature range.

Literature data [4] reveal that upon heating the *cis*-bixin isomer is converted to *trans* isomer. According to the present study, the isomerization takes place between 200–240°C. It indicates that the degree of degradation depends on the temperature. Therefore, in agreement with the observed thermal degradation, the authors believe the first mass loss stage is the most important.

#### Kinetic study

For the kinetic studies 5, 10 and 15 K min<sup>-1</sup> heating rates have been applied. The results of kinetic analysis are summarized in Tables 1–3, respectively.

The results of the different methods are in good agreement. The agreement between the two integral methods was much better, compared to the two approximation methods.

#### Determination of the mechanism

The selection of the kinetic parameters for the degradation stages was made from the mechanism chosen for each degradation stage which has been done using a software by Thermochemistry and Materials Laboratory (LTM) of the UFPB. This program employs the  $g(\alpha)$  function considering the maximum values of linear correlation coefficient and minor the standard deviation.

The nucleation order of Mampel's mechanisms, the F2 model (chaotic second order nucleation) and F1 model (chaotic first order nucleation) have been chosen in order to determine the reaction mechanism. Based on the above mentioned mechanisms for the different stages, the kinetic parameters were calculated by means of the referred software resulting the values presented in Table 4.

Table 1 Kinetic parameters of the thermal degradation of  $\alpha$ -bixin;  $\beta=5$  K min<sup>-1</sup>

Stages	Kinetic parameters	CR	MD	HM	VK
1 <sup>st</sup>	$n$	2.90	2.87	2.98	2.88
	$E/\text{kJ mol}^{-1}$	359.60	356.65	361.06	342.71
	$A/\text{s}^{-1}$	1.86E+36	9.11E+35	7.19E+38	8.12E+36
	$r$	0.9903	0.9903	0.9915	0.9972
2 <sup>nd</sup>	$n$	0.83	0.89	0.95	0.90
	$E/\text{kJ mol}^{-1}$	77.64	80.13	84.23	81.88
	$A/\text{s}^{-1}$	1.22E+11	2.37E+11	1.83E+05	1.01E+08
	$r$	0.9978	0.9979	0.9975	1.0000
3 <sup>rd</sup>	$n$	1.98	1.91	2.15	2.03
	$E/\text{kJ mol}^{-1}$	162.11	156.99	177.19	166.82
	$A/\text{s}^{-1}$	4.37E+07	1.81E+07	7.67E+08	4.92E+12
	$r$	0.9978	0.9999	0.9996	0.9986
4 <sup>th</sup>	$n$	1.36	1.36	1.52	1.48
	$E/\text{kJ mol}^{-1}$	107.31	107.79	122.33	120.12
	$A/\text{s}^{-1}$	1.69E+12	2.01E+09	2.64E+10	8.55E+08
	$r$	0.9967	0.9967	0.9967	1.0000

CR – Coats–Redfern, MD – Madhusudan, HM – Horowitz–Metzger, VK – Van Krevelen approximations

**Table 2** Kinetic parameters of the thermal degradation of  $\alpha$ -bixin;  $\beta=10$  K min<sup>-1</sup>

Stages	Kinetic parameters	CR	MD	HM	VK
1 <sup>st</sup>	$n$	2.52	2.63	2.85	2.64
	$E/\text{kJ mol}^{-1}$	296.64	310.17	331.35	307.41
	$A/\text{s}^{-1}$	3.04E+29	9.05E+30	2.41E+33	1.08E+36
	$r$	0.9961	0.9961	0.9966	0.9945
2 <sup>nd</sup>	$n$	0.92	0.97	1.09	1.0
	$E/\text{kJ mol}^{-1}$	71.09	73.10	85.04	77.77
	$A/\text{s}^{-1}$	5.18E+12	9.09E+10	1.84E+08	7.80E+07
	$r$	0.9965	0.9965	0.9965	0.9958
3 <sup>rd</sup>	$n$	2.05	2.18	2.61	2.31
	$E/\text{kJ mol}^{-1}$	165.01	175.75	212.53	187.99
	$A/\text{s}^{-1}$	1.04E+08	7.87E+08	4.36E+11	3.29E+17
	$r$	0.9999	0.9999	0.9999	1.000
4 <sup>th</sup>	$n$	1.11	1.06	1.39	1.24
	$E/\text{kJ mol}^{-1}$	76.27	74.76	98.13	87.39
	$A/\text{s}^{-1}$	2.04E+14	1.84E+13	7.43E+13	8.75E+05
	$r$	0.9969	0.9970	0.9970	0.9990

CR – Coats–Redfern, MD – Madhusudanan, HM – Horowitz–Metzger, VK – Van Krevelen approximations

**Table 3** Kinetic parameters of the thermal degradation of  $\alpha$ -bixin;  $\beta=15$  K min<sup>-1</sup>

Stages	Kinetic parameters	CR	MD	HM	VK
1 <sup>st</sup>	$n$	1.96	1.98	2.13	2.06
	$E/\text{kJ mol}^{-1}$	206.83	208.83	221.83	214.81
	$A/\text{s}^{-1}$	4.81E+19	8.23E+19	3.08E+21	8.74E+25
	$r$	0.9977	0.9978	0.9975	0.9953
2 <sup>nd</sup>	$n$	1.12	1.17	1.22	1.25
	$E/\text{kJ mol}^{-1}$	131.23	134.44	145.49	143.50
	$A/\text{s}^{-1}$	6.61E+07	1.44E+08	2.09E+09	3.24E+16
	$r$	0.9977	0.9976	0.9973	0.9984
3 <sup>rd</sup>	$n$	1.32	1.30	1.61	1.45
	$E/\text{kJ mol}^{-1}$	84.22	83.66	112.42	97.63
	$A/\text{s}^{-1}$	8.98E+10	8.90E+11	1.08E+07	5.45E+08
	$r$	0.9995	0.9995	0.9995	1.0000
4 <sup>th</sup>	$n$	0.90	1.02	1.21	1.02
	$E/\text{kJ mol}^{-1}$	71.02	76.26	96.53	83.08
	$A/\text{s}^{-1}$	9.47E+13	2.34E+13	5.12E+13	4.76E+05
	$r$	0.9980	0.9982	0.9978	1.0000

CR – Coats–Redfern, MD – Madhusudanan, HM – Horowitz–Metzger, VK – Van Krevelen approximations

**Table 4** Kinetic parameters of  $\alpha$ -bixin decomposition using the chosen mechanisms for each stage

Heating rate	Kinetic parameters	1 <sup>st</sup> stage	2 <sup>nd</sup> stage	3 <sup>rd</sup> stage	4 <sup>th</sup> stage
5 K min <sup>-1</sup>	$E/\text{kJ mol}^{-1}$	108.28	83.94	96.44	88.88
	$A/\text{s}^{-1}$	3.71E+09	5.20E+04	2.03E+04	1.02E+02
10 K min <sup>-1</sup>	$E/\text{kJ mol}^{-1}$	146.83	73.86	93.69	71.80
	$A/\text{s}^{-1}$	5.87E+13	9.82E+03	1.97E+04	1.04E+01
15 K min <sup>-1</sup>	$E/\text{kJ mol}^{-1}$	127.97	124.15	70.27	74.89
	$A/\text{s}^{-1}$	1.45E+11	1.36E+09	5.73E+02	1.69E+01
Chosen mechanism	–	F2	F1	F1	F1

## Conclusions

The physical and chemical characterization methods, developed in the present work, allow us to conclude that extraction method developed achieved the production of the *cis*-bixin isomer from the seeds of annatto (*Bixa orellana* L.). According to the thermal degradation results, the first stage is the most important. The results of the present work reveal that the dynamic method was good enough to analyze the decomposition reactions. F2 model was representative for the first stage of degradation while F1 was valid for the other stages.

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