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Effects of Light, Air, Anti-oxidants and Pro-oxidants on Annatto Extracts (*Bixa orellana*)

S. V. Najar, F. O. Bobbio & P. A. Bobbio*

Departamento de Ciência de Alimentos, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, 13081-Campinas-SP, Brazil

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ABSTRACT

Chloroform solutions of annatto extracts containing from 0·250 to 0·260 mg ml^{-1} of bixin were used as model systems to determine the effects on their stability of light (1380 or 430 lux), air, nitrogen, benzoyl peroxide and ascorbyl palmitate at 24 ± 1°C. Experiments were run for 12 days and the loss of bixin measured at 470 nm. Light was the most destructive agent, followed by benzoyl peroxide. Air was much less effective in promoting loss of colour. Ascorbyl palmitate effectively retarded the destructive effect of light.

The apparent first order reaction rate constants and $t_{1/2}$ *are presented.*

INTRODUCTION

Annatto powder or oily extracts from the seeds of *Bixa orellana* are widely used for colouring both industrial and home made foods. The red–orange colour of the extracts from the seed coats is due to a mixture of carotenoids and their degradation products. The major carotenoids of annatto are *cis*- and *trans*-bixin and *cis*- and *trans*-norbixin, whose structures have been studied by Karrer *et al.* (1929), Kuhn & L'Orsa (1932) and Barber *et al.* (1961). Decomposition products or other minor pigments present in annatto extracts were separated by paper chromatography by MacKeown (1961), but have not been clearly identified.

The content of bixin in several commercial annatto preparations was

* To whom correspondence should be addressed.

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determined by MacKeown & Mark (1962) and by Reith & Gielen (1971) who reported briefly on the poor stability of bixin toward oxygen.

A FAO/WHO report (1976) gives specifications and analytical methods for the assay of bixin and norbixin. A short review on the extraction and chemistry of annatto has been published by Preston & Richard (1980).

The objective of this work is to report on the stability of annatto extracts toward light and air with or without addition of pro-and anti-oxidants.

MATERIAL AND METHODS

Chloroform solutions of powdered annatto extracts (30% bixin) were distributed in tightly sealed screw-cap tubes of 10·5 ml capacity. Each tube contained 2·0 ml of the solutions which were made to contain 0·250–0·260 mg ml^{-1} of bixin ($1{\cdot}32 \times 10^{-6}$ moles). The experimental conditions used are summarized in Table 1.

The loss of bixin was determined according to the FAO/WHO (1976) report. The absorbance of the solutions was measured at 420 nm and $E_{1\,cm}^{1\%}$ was 2826.

The effect of light was determined by placing the tubes in a circle around a tungsten filament lamp with a nominal luminosity of 430 and 1380 lux,

TABLE 1
Experimental Conditions used in Determining the Specific Reaction Rate Constant, Period of Half-Life and Percentage of Bixin in each Model System. Reaction Time: 12 Days at 24° ± 1°C, using $CHCl_3$ as a Solvent

Model system	*Experimental conditions*	$k \times 10^{-2}$ (*day*$^{-1}$)	$t_{1/2}$ (*days*)	*Bixin after 12 days* (%)
1	Air, 100 W light,[a] 1380 lux	17·4	4	73·1
2	Air, dark	0·9	75	98·2
3	N_2, 100 W, light, 1380 lux	12·0	6	76·2
4	N_2, dark	—	—	100·0
5	Air, 40 W light, 430 lux	2·4	28	93·9
6	Air, 100 W light, 1380 lux, 10% ascorbyl palmitate[b]	5·7	12	85·9
7	Air, 40 W light, 430 lux, 10% ascorbyl palmitate	1·4	49	94·7
8	Air, 40 W light, 430 lux, 20% ascorbyl palmitate	1·2	58	96·0
9	Air, dark, 10% ascorbyl palmitate	0·9	77	98·5
10	Air, dark, 2·5% benzoyl peroxide	8·0	9	81·0

[a] Tungsten lamp (Osram Cat., 1985). Tubes were surrounding the light source, at a distance of 17·5 cm.
[b] % is w/v.

respectively. Distance from the geometrical centre of the lamp and tubes was 17·5 cm. Temperature was maintained at 24 ± 1°C. The amount of oxygen in the head space of the tubes was determined by gas chromatography as described by Saguy *et al.* (1985).

A nitrogen atmosphere was made by flushing the tubes' head spaces with nitrogen.

Spectra and absorbance of bixin were taken and measured with a Pye-Unicam spectrophotometer model 2000 using chloroform as solvent.

RESULTS AND DISCUSSION

Results are summarized in Table 1 and Figs 1, 2 and 3. A 12-day period was the maximum time of reaction which still permitted reliable absorbance readings for some model systems and therefore this was the standard reaction time used in all the experiments.

Effect of oxygen

The amount of oxygen in the tubes' head spaces was 5–6 × 10^{-5} moles of oxygen, a quantity considered sufficient to oxidize the bixin in each tube.

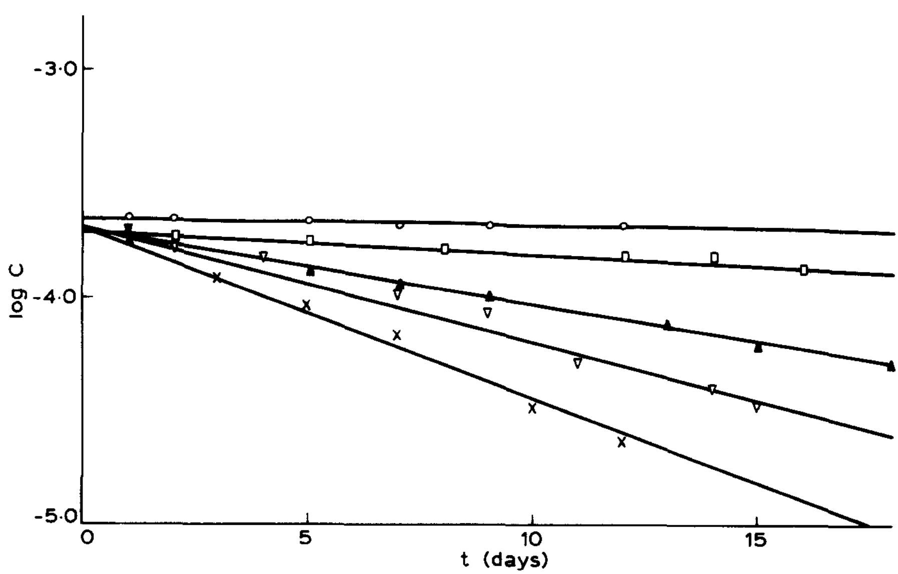

Fig. 1. The effect of reaction conditions and reaction time on the bixin concentration (log C): ○, oxygen; □, oxygen + light, 430 lux; ▲, oxygen + 2·5% benzoyl peroxide; ▽, nitrogen + light, 1380 lux; ×, oxygen + light, 1380 lux.

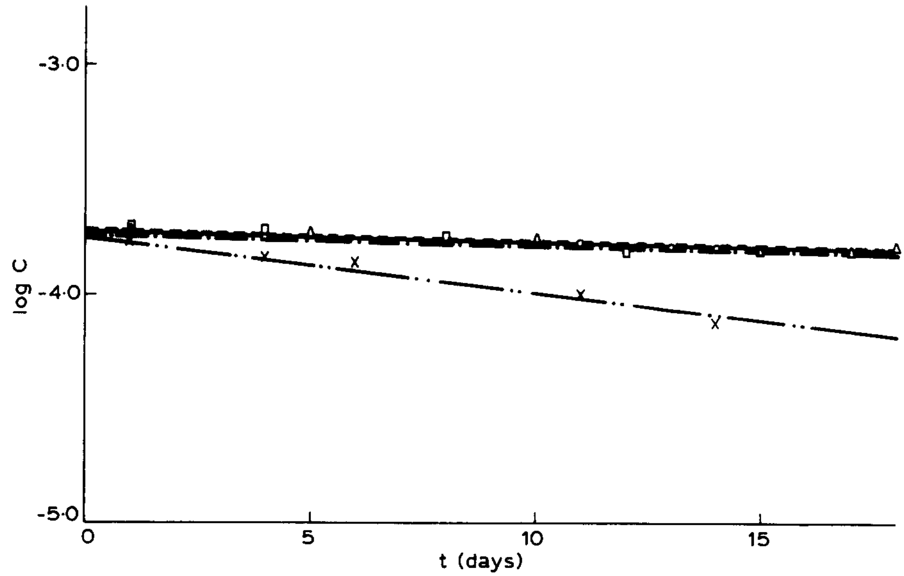

Fig. 2. The effect of reaction conditions and reaction time on the bixin concentration (log C): □, oxygen + light, 430 lux + 10% ascorbyl palmitate; △, oxygen + light, 430 lux + 20% ascorbyl palmitate; ○, oxygen + 10% ascorbyl palmitate; ×, oxygen + light, 1380 lux + 10% ascorbyl palmitate.

According to Teixeira Neto *et al.* (1981) for each mole of β-carotene discoloured, 6–7 moles of oxygen were consumed.

Carotenoids are highly unsaturated systems generally easily destroyed by oxygen (Ramakrishnan & Francis, 1979) depending on the nature of the solvent surrounding the carotenoid molecule and in a non-aqueous system losses of β-carotene were much higher than when aqueous systems were used; also, the degree of oxidation or the increase in the molecular polarity of the carotenoid had a depressing effect on the ease of the oxidation of such carotenoids (Ramakrishnan & Francis, 1980).

Bixin, $C_{25}H_{30}O_4$, is a dicarboxylic structure, and results in Table 1 and Fig. 2 indicate a weak response to oxygen even under the anhydrous conditions used. After 12 days, only 1·8% of the bixin was lost. The reaction half-life was estimated to be 75 days (Table 1, Fig. 1). Reith & Gielen (1971) found that 'α-bixin' losses by oxidation were concentration dependent and solutions of bixin stored in the absence of light suffered considerable losses in pigment which were attributed to destruction by atmospheric oxygen.

Effect of light

Results in Table 1 and Fig. 2 indicate a strong destructive effect of light on the colour of bixin. The fast reaction has a $k = 17{\cdot}4$ and $t_{1/2} = 4$ days.

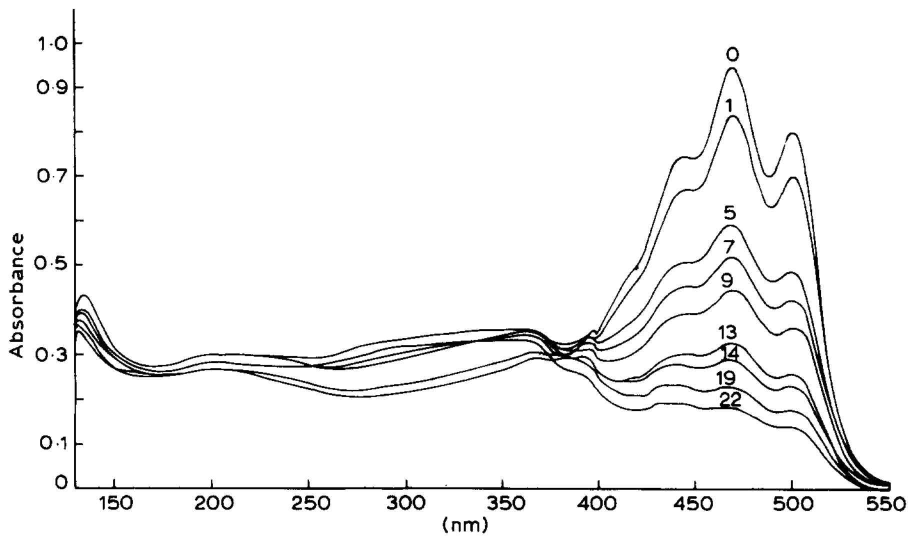

Fig. 3. Spectra of bixin in $CHCl_3$ with 2·5% benzoyl peroxide. (Numbers in each spectrum indicate days of reaction.)

Effect of light and oxygen

From Table 1 and Fig. 1 it can be seen that the combined effects of light and oxygen are similar to the effect of light without oxygen. The loss of bixin is strongly affected by increasing the luminosity of the lamp. The presence of oxygen does not affect the loss of bixin as much as the light does and results from systems 1 and 3 demonstrate this point: only 3·1% more bixin was lost due to the effect of oxygen combined with light, while oxygen in the absence of light accounted for only a 1·8% loss. The light-induced reaction predominates over the direct destruction by oxygen. Small differences can be attributed in part to a possible combination of effects.

The intensity of the irradiation is important as shown by a 7·5-fold increase in the values of k for a 3-fold increase in lamp luminosity (Fig. 1).

Effect of benzoyl peroxide and ascorbyl palmitate

The effect of adding benzoyl peroxide (Fig. 1), a well-known free radical promoter, is comparable to the effect of irradiating the system with a 1380 lux light in the absence of oxygen. Figure 3 shows the effect of benzoyl peroxide addition on the colour loss of bixin up to the 22nd day of reaction. After 12 days the absorbance was less than 50% of the original values.

Ascorbyl palmitate, an efficient anti-oxidant for fats and oils, was used because of the possible similarity of oxidative mechanisms for lipids and for carotenoids (Bourgeois, 1981). Results for systems 6, 7 and 8 (Table 1 and Fig. 2) demonstrate the efficiency of the anti-oxidant in retarding photo-induced transformation, affecting the extended π orbital system of the carotenoid with the participation of oxygen, since in the dark the values for the $t_{1/2}$, k and % of bixin in Table 1 are practically the same for systems 9 and 2.

The values of k for system 10 is approximately nine times greater than for system 2 (Table 1).

CONCLUSIONS

All results point to a considerable stability of annatto to oxidation by air in anhydrous media but to a lower resistance to the effects of light which are proportional to the light intensity. The addition of ascorbyl palmitate seems to be a reasonable way to increase colour persistence under strong illumination. The presence of free radicals or any promoter of such species in annatto extracts is to be avoided as results in systems 9 and 10 indicate that a rapid loss of colour might occur whenever free radical formation is promoted.

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