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Effect of water activity on the stability of bixin in an annatto extract–microcrystalline cellulose model system

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The influence of water activity on the stability of bixin in an annatto extract–microcrystalline cellulose model system during storage at 21 ± 1°C in the presence or absence of light and/or air was investigated. The water adsorption characteristic of the model system was determined. The samples were stored in desiccators containing saturated salt solutions for water activities of 0·33 to 0·97 and after equilibrium was reached, the samples were stored in the presence or absence of air and/or light. The degradation of bixin in every condition tested followed a first-order reaction. By comparing half-lives, bixin was observed to be more stable at intermediate/higher water activities.

INTRODUCTION

The annatto color originates from the annatto tree (*Bixa orellana* L.) which grows in the tropical areas of South America, India and East Africa (Prentice-Hernandez & Rusig, 1992). Having a hue varying from light yellow through orange yellow to orange red, annatto has been used since antiquity as a colorant for food, cosmetics and textiles (Engelhardt *et al.*, 1988). At present, the annatto color is widely used in the entire food industry. It has been added to margarine, mayonnaise, salad dressing, oil, bakery products, cheese (Ramamurthy & Krishna, 1982) and extruded products (Berset & Marty, 1986). It has also been used as a substitute for FD & C - yellow 5 - tartrazine (Andres, 1980).

Chemically, the annatto coloring principle bixin belongs to the carotenoid group. The presence of norbixin and methylbixin in annatto extract are due to degradation and isomerization reactions that take place during the extraction of annatto pigments (Mckeown & Mark, 1962) and can affect its coloring characteristics (Carvalho & Hein, 1989).

Berset and Marty (1986) investigated the stability of annatto extract to extrusion temperatures. The stability of annatto extract to the effects of light, air, anti- and pro-oxidants in chloroform has been described by Najar *et al.* (1988). They observed that light was the most destructive agent, followed by the pro-oxidant, benzoyl peroxide. However, the influence of water activity on the stability of annatto extract has not yet been evaluated.

The objective of this work was to investigate the effect of water activity on the stability of bixin in an annatto extract–microcrystalline cellulose model system simulating dehydrated foods.

MATERIALS AND METHODS

Powdered annatto extract containing 57% bixin was used in all experiments. Microcrystalline cellulose MC 201 (Microcel, SP, Brazil) was used as a solid support. It was vacuum dried at 60°C for 48 h and kept in a desiccator over phosphorus pentoxide.

A methylene chloride solution of bixin was thoroughly mixed with microcrystalline cellulose (1% w/w) and the residual methylene chloride was removed under vacuum at 40°C (Goldman *et al.*, 1983). All steps were carried out under subdued light.

The adsorption isotherm of the annatto extract model system was built at 21 ± 1°C by using saturated salt solutions for water activities from 0·33 to 0·97 (Rockland, 1960).

Table 1. First-order rate constant (and correlation coefficient for linear regression) for the degradation of bixin in an annatto extract–microcrystallinecellulose model system during storage at different water activities, in the presence or absence of air and/or light at 21 ± 1°C

Treatment[a]	First-order rate constants in 10^{-2} days^{-1} (correlation coefficient) at different water activities[b]			
	0·33	0·57	0·75	0·97
Nitrogen/darkness	1·19	1·20	0·90	0·60
	(0·98)	(0·99)	(0·95)	(0·97)
Nitrogen/light	3·23	2·53	2·01	3·09
	(0·94)	(0·98)	(0·99)	(0·99)
Air/darkness	1·65	2·35	1·20	2·79
	(0·96)	(0·90)	(0·90)	(0·95)
Air/light	12·79	11·80	11·87	9·56
	(0·91)	(0·90)	(0·90)	(0·96)

[a]Water adsorption characteristics (A_w = % moisture): 0·33 = 4·101 ± 0·137; 0·57 = 5·740 ± 0·219; 0·75 = 7·458 ± 0·286; 0·97 = 18·347 ± 0·736. All statistically different (Student's test, $P \leq 0{\cdot}05$).
[b]Light: test-tubes 10 cm away from a 40 W, 2500 lux day-light lamp at 21 ± 1°C. Nitrogen: test-tubes' headspace flushed with nitrogen. Air: air in the tubes' headspace should provide sufficient oxygen (Najar *et al.*, 1988).

Samples of the annatto extract–microcrystalline cellulose model system were distributed in petri dishes forming thin and uniform layers. The petri dishes were placed in desiccators equilibrated at water activities of 0·33, 0·57, 0·75 and 0·97, under vacuum at 21 ± 1°C in the dark. After equilibrium was reached, the samples were transferred to 15-ml capped test-tubes and submitted to the treatments indicated in Table 1. Periodically, samples were analyzed for bixin content according to FAO/WHO (1976).

RESULTS AND DISCUSSION

The degradation of bixin in the model system followed a first-order reaction as the correlation coefficient for linear regression of the logarithm of bixin concentration with storage time was very close to one (Table 1). Similar kinetic behavior was observed for degradation of bixin in chloroform solutions of annatto extract (Najar *et al.*, 1988) and of beta-carotene in model systems (Ramakrishnan & Francis, 1979; Goldman *et al.*, 1983) and in dehydrated carrots (Glória, 1987).

The half-lives for the degradation of bixin under the different treatments are summarized in Table 2. These values are higher than those observed by Najar *et al.* (1988) for bixin in chloroform solutions of annatto extract. According to Rusig and Martins (1992), the stability of a pigment is increased when it is impregnated in cellulose.

Loss of bixin was observed in the control sample (nitrogen atmosphere/darkness) possibly due to the presence of oxygen adsorbed or entrapped by the system, which remains available for oxidation (Teixeira Neto *et al.*, 1981). It was observed that bixin was very sensitive to light; however, the combined effects of air and light were the most detrimental at every water activity investigated. This is possibly due to light-induced free radical oxidation reaction (Bradley & Min, 1992). The slow oxidation of bixin by air/darkness could be an indication of a reduced but effective quenching of singlet oxygen by bixin (Di Mascio *et al.*, 1989).

The stability of bixin stored under air/darkness and nitrogen/light was higher at intermediate water activity (0·75). However, under nitrogen/darkness and air/light, greater stability was observed at higher water activities. According to Ramakrishnan and Francis (1979) and Goldman *et al.* (1983), increasing the water activity of a model system impregnated with beta-carotene, increased its stability. They suggested several mechanisms by which water reduced beta-carotene decolorization: water attaches to sites on the surface, excluding oxygen from lipid materials; hydrogen bonding of hydroperoxides with water; metal catalyst inactivation by water; and reduction of free radical content by interaction with water. Furthermore, light-induced free radical oxidation is slowed down by increasing water activity due to lower oxygen solubility and shorter half-life of singlet oxygen in water (Bradley & Min, 1992).

The results of the present study are also relevant in connection with best storage conditions for the annatto seeds prior to bixin extraction (Rodrigues-Amaya, 1993).

Table 2. Half-life of bixin in an annatto extract–microcrystalline cellulose model system during storage at different water activities, in the presence or absence of air/or light at 21 ± 1°C

Treatment[a]	Half-life (days) at different water activities[b]			
	0·33	0·57	0·75	0·97
Nitrogen/darkness	58·3	57·7	77·0	115·5
Nitrogen/light	21·5	27·4	34·5	22·4
Air/darkness	42·0	29·5	80·6	24·8
Air/light	5·4	5·9	5·9	8·3

[a]Water adsorption characteristics (A_w = % moisture): 0·33 = 4·101 ± 0·137; 0·57 = 5·740 ± 0·219; 0·75 = 7·458 ± 0·286; 0·97 = 18·347 ± 0·736. All statistically different (Student's test, $P \leq 0{\cdot}05$).
[b]Light: test-tubes 10 cm away form a 40 W, 2500 lux day-light lamp at 21 ± 1°C. Nitrogen: test-tubes' headspace flushed with nitrogen. Air: air in the tubes' headspace should provide sufficient oxygen (Najar *et al.*, 1988).

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