HPLC INVESTIGATION OF THE DEGRADATION OF SOME ARTIFICIAL FOOD COLORANTS IN THE PRESENCE OF ASCORBIC ACID

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Abstract: The influence of the concentration of ascorbic acid on some food colorant degradation in the solutions was studied. For this purposes some food colorants with naphtalenic structure, as: Ponceau 4R, Amaranth, Allura red, Carmoisine and Sunset yellow were investigated. Rate constant (k), time of colorant half-life ($t_{1/2}$), as well as the type of the kinetic reaction have been determined. At the same time, the ascorbic acid degradation in the solutions, and the degradation of the investigated colorants in the solutions without the presence of ascorbic acid under the similar conditions was followed. The colorants and the ascorbic acid have been determined in the solutions by a high performance liquid chromatography (HPLC) with a diode array detector (DAD) and UV-VIS spectrophotometry. It was found that when the concentration of ascorbic acid in the solutions of the colorants increases, the value of the rate constant also increases and according to that the values of the colorants half-life decreases. The values of the rate constants are 2.00-2.57 times higher when the colorant degradation was held in the presence of 500 mg/L of ascorbic acid. According to the values of correlation coefficient in all the cases it happened to be firs order kinetics.

Keywords: food colorants, ascorbic acid, degradation, rate constant, HPLC, UV-VIS spectrometry

1. INTRODUCTION

Artificial food colorants are widely used in food industry, for coloring foodstuff matter, but in fact they have no any nutritional value. The use of particular food colorant in food industry depends primarily of its toxicity. However, at the same time, of major importance are also some other factors such as stability of the colorant used for coloring certain food product and whether there is a possible interaction between some component of the foodstuff matter and the colorant. As it is known [1], some reducing agents present in the food may react especially with azo dyes forming degradation products, which may react further with other food components forming undesirable compounds. One of the agents, which can react with these dyes causing their degradation, is ascorbic acid [2, 3]. Ascorbic acid is often used in soft drinks as a nutritional supplement or as an antioxidant and provides a reducing environment in which azo dyes can easily be degraded.

Continuing our investigation on artificial food coloring [4], in the present study, the influence of the concentration of ascorbic acid on food colorant degradation in the solutions was studied. For this purpose some food colorants with naphtalenic structure (Ponceau 4R, Amaranth, Allura Red, Carmoisine and Sunset Yellow), were studied. Rate constant (k), time of colorant half-life ($t_{1/2}$), as well as the type of the kinetic reaction have been determined. At the same time, degradation of ascorbic acid in the solutions, and of the investigated colorants without the presence of ascorbic acid was also followed.

2. EXPERIMENTAL

2.1. Instrumental

HPLC system Perkin-Elmer equipped with Diode Array Detector 235 C, and binary pump series 200 and UV-VIS Spectrophotometer, model Milton Roy, were used. Liquid chromatography column was used. Stainless steel 10 cm x 4.6 inside diameter, packed with 5 mm Pecosphere C18, was used (Merck).

2.2. Reagents

Mobile phase was composed of solvent A (0.01 mol L^{-1} KH_2PO_4 adjusted to pH 4.5 with *o*-phosphoric acid) and solvent B (Methanol HPLC grade, Merck). Acetate buffer (pH = 5.5) was prepared by dissolving 6.8 g of sodium acetate, adjusted with acetic acid in 1 L. Reference food colorant standards were obtained from Etol (Slovenia) and Wurth (Austria).

2.3. Methods

Model solutions containing investigated dyes were made in acetate buffer (pH = 5.5). Concentration of added dyes in model solutions was 40 mg L^{-1}, and concentration of ascorbic acid was 100, 250 and 500 mg L^{-1}. All the solutions were preserved with potassium sorbate. Dissolved oxygen was removed from solutions by purging with ultrasonic treatment. Solutions were kept in dark at room temperature. Degree of degradation of ascorbic acid and food colorants was monitored within 4 weeks. The rate of ascorbic acid degradation was monitored by HPLC, and the rate of food colorant degradation was monitored simultaneously by HPLC and by visible spectrometry.

A gradient profile used was as follows: 3 minutes 100% A, 0 - 100% B in 4 minutes, held at 100% B for 8 minutes, returned to 100% A and held 10 minutes before next injection. HPLC determination of ascorbic acid was performed at 255 nm. Wavelengths for HPLC and spectrometric determination of artificial food colorants are given in Table I.

Table I. Wavelengths for determinations of some synthetic food colorants

Synthetic coloring	EEC serial number	Wavelength, nm	
		HPLC	Vis spectrophotometry
Amaranth	E 123	225	516
Allura Red	E 129	235	500
Carmoisine	E 122	225	505
Ponceau 4R	E 124	225	506
Sunset Yellow	E 110	245	370

3. RESULTS AND DISCUSSION

3.1. Degradation of ascorbic acid in model solution

Ascorbic acid is very rapidly oxidized by dissolved molecular oxygen in the model solutions. In order to decrease the degradation of ascorbic acid oxygen was removed from the solution by ultrasonic purging. Model solutions of ascorbic acid are made in acetate buffer pH the 5.5 and are preserved with potassium sorbate (48 mg L^{-1}). Results of the determination of degree of degradation at various concentration of ascorbic acid in the dark at the room temperature within 4 weeks show that hundred mg L^{-1} of ascorbic acid is rapidly degraded within an interval of 7 days in the dark and at room temperature, where as in the case of a higher concentration (250 and 500 mg L^{-1}) ascorbic acid is much more stable. After 28 days of storage, the remnants of ascorbic acid were found to be 38 % from the original concentration of 250 mg L^{-1} and 74.1 % from the initial concentration of 500 mg L^{-1}.

3.2. Degradation of food colorants in the presence of ascorbic acid

The rate of degradation of food colorants was established in the presence of ascorbic acid (100, 250 and 500 mg L^{-1}). They were also preserved by potassium sorbate (48 mg L^{-1}). The concentration of all added dyes was 40 mg L^{-1}, because this concentration happens to be most commonly used in the preparation of soft drinks.

The results obtained for the degradation of food colorants show that the remnants of the undegraded colorants in the presence of 100 mg L^{-1} ascorbic acid after 3 weeks of storage are between 83.4 % for Allura red and 88.0 % for Ponceau 4R. On the contrary, a higher concentration of ascorbic acid in the solution causes faster degradation of the artificial food colorants. Even after 4 weeks of storage in the presence of 500 mg L^{-1} ascorbic acid, the remnants of undegraded colorants in the solutions are between 59.0 % for Sunset Yellow and 69.4 % for Ponceau 4R. No significant changes in food colorant concentration were found within 4 weeks in the absence of ascorbic acid.

According to the results for the degradation of investigated food colorants in the presence of various concentrations of ascorbic acid vs. time, rate constant (k), time of the colorants half life ($t_{1/2}$) and correlation coefficient (r) were determined. The values of the rate constants were calculated from the slopes of the straight lines obtained by plotting $\ln\gamma$ vs. time. This dependence has the form of the linear equation ($\ln\gamma = -kt + \ln\gamma_0$) which shows that the plot of $\ln\gamma$ versus t yields a straight line, so the reactions are first order [5]. The values of k, $t_{1/2}$ and r obtained for the degradation of ascorbic acid and of all investigated colorants in the presence of 100, 250 and 500 mg L^{-1} of ascorbic acid are given in Table II and III. As it can be seen from the results for ascorbic acid given in Table II, the rate constant (k) and half-life ($t_{1/2}$) increases by increasing the initial concentration of ascorbic acid in the solution.

Table II. Kinetics of degradation of ascorbic acid

γ_0 (mg L^{-1})	k (d^{-1})	$t_{1/2}$ (d)	r
100	0.450	1.5	-0.976
250	0.09 5	7.3	-0.999
500	0.065	10.6	-0.997

Correlation coefficients (Table II) for degradation in the presence of 100 mg L^{-1} of ascorbic acid, were calculated for a 3 days period and the other parameters (k, $t_{1/2}$) refer to period of 3 weeks. All the parameters for the colorant degradation in the presence of 250 and 500 mg L^{-1} of ascorbic acid were obtained for the period of 4 weeks.

The higher concentration of ascorbic acid leads to the higher rate constant and lower colorant half-life. The values of the rate constants are 2.00-2.57 times higher when the colorant degradation was run in the presence of 500 mg L^{-1} of ascorbic acid than in the presence of 100 mg L^{-1} ascorbic acid.

Table III. Kinetics of degradation of the synthetic food colorants in the presence of ascorbic acid

Food colorant	Ascorbic acid (mg L^{-1})	k (d^{-1})	$t_{1/2}$ (d)	r
Amaranth	100	0.006	105.1	-0.995
	250	0.009	76.7	-0.999
	500	0.013	52.6	-0.999

Carmoisine	100	0.007	103.9	-0.995
	250	0.010	66.3	-0.997
	500	0.014	50.5	-0.999
Ponceau 4R	100	0.006	115.5	-0.993
	250	0.008	86.6	-0.997
	500	0.012	57.7	-0.998
Sunset Yellow	100	0.007	100.8	-0.992
	250	0.011	62.0	-0.997
	500	0.018	38.5	-0.998
Allura Red	100	0.008	85.0	-0.991
	250	0.014	50.2	-0.995
	500	0.018	38.0	-0.999

ИСПИТУВАЊЕ НА ДЕГРАДАЦИЈАТА НА НЕКОИ СИНТЕТИЧКИ ПРЕХРАНБЕНИ БОИ ВО ПРИСУСТВО НА АСКОРБИНСКА КИСЕЛИНА СО ПРИМЕНА НА HPLC

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Испитувано е влијанието на присуството на аскорбинската киселина на кинетиката на распаѓањето на некои прехранбени бои со нафталенска структура (понко 4R, амарант, алура црвено, кармоизин и сончево жолто). При тоа, определувана е константата на брзината на реакцијата на деградација (k), полувреме на разградување на боите ($t_{1/2}$), како и типот на кинетиката на реакцијата. Во исто време, испитувано е и распаѓањето на аскорбинската киселина како и на боите без присуство на аскорбинската киселина. Боите и аскорбинската киселина се определувани со примена на високоефикасна течна хроматографија (HPLC) со детектор со низа од диоди (DAD) и на UV-VIS спектрофотометрија. Утврдено е дека со зголемување на концентрацијата на аскорбинската киселина во растворите на боите, вредноста на константата на брзината на деградација се зголемува, додека полувремето на разградување на боите се намалува. Вредноста на константата на брзината на реакцијата на деградација е повисока за 2,00-2,57 пати во присуство на аскорбинската киселина со концентрација од 500 mg/L. Според вредноста на коефициентот на корелација најдено е дека овие процеси ја следат кинетиката на реакција од прв ред.