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Influence of water activity on the stability of betanin in various water/alcohol model systems

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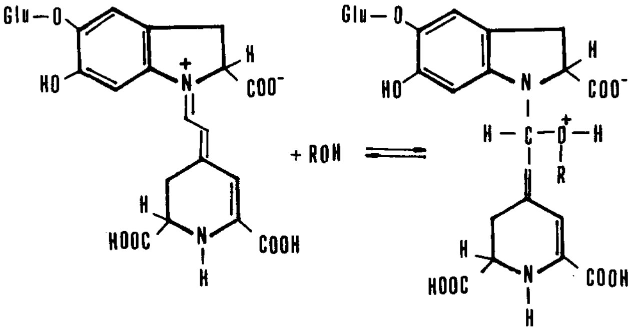
The influence of water activity on the degradation rate of betanin has been studied in the systems water/ethanol, water/ethylene glycol and water/glycerol. In order to eliminate the systematic error caused by the absorbance of degradation products and impurities, the rate constants have been obtained by the Guggenheim method. In all cases the dependence of the rate constant on the water activity exhibits a maximum. This finding is accounted for by nucleophilic attack on the carbon atom in the vicinity of N^+.

INTRODUCTION

Naturally occurring pigments are raw materials of increasing interest. Suitable sources of red pigments have been encountered in red beetroot (*Beta vulgaris* var. *rubra*). Such pigments are designated betalain which is an aggregative term and includes a red component of pigments (betacyanins) and a yellow component (vulgaxanthins). The dominant pigment in betacyanins is betanin (Scheme 1) which forms 75–95% of the natural pigment (von Elbe *et al.*, 1972).

The use of the natural pigments is limited by their stability. The main factors affecting the stability of betanin are temperature (Saguy, 1979; Havlíková & Míková, 1985; Drdák & Vallová, 1990), pH (von Elbe *et al.*, 1974; Saguy, 1979; Kearsley & Katsaboxakis, 1980; Havlíková & Míková, 1985), water activity (a_w) (Pasch & von Elbe, 1975; Drdák *et al.*, 1990), presence of oxygen (Saguy *et al.*, 1984), metal ions and light radiation (Aurstad & Dahle, 1973). Considering the water activity, its influence has been studied in water/glycerol model systems with a_w from 0·37 to 1·00. A non-linear, assumingly exponential, dependence has been found between the rate constant of betanin decay and a_w. This paper deals with the study of betanin decay in various water/alcohol model systems with the aim of investigating the influence of the nature of the alcohol on the rate of decay. The alcohols chosen are ethanol, ethylene glycol and glycerol.



Scheme 1. Nucleophilic attack on betanin.

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MATERIALS AND METHODS

Isolation of betanin

Red beet juice was fermented with the strain of *Saccharomyces cerevisiaie* sp. Badacsony 1. Fermentation took place at 26°C for 64 h. The fermented juice was separated in a column packed with Sephadex, eluted with 1% acetic acid solution. Purity of betanin determined by HPLC was >95%. Main impurities were isobetanin, betanidin and isobetanidin.

Preparation of samples

The water/alcohol model systems were prepared in the ratios (v/v): 100/0, 80/20, 70/30, 60/40, 40/60, 20/80, 15/85, 0/100 (except ethylene glycol) and additionally for ethanol 10/90. Alcohols for preparing the solvent mixtures were used without drying. The water activities were determined by Rotronic Hygroskop DT, Switzerland. Betanin in solution was added to the model systems and pH was adjusted to 5·0 with NaOH. The resulting concentration of betanin was approximately 8·25 mg/dm^3.

Betanin degradation

The samples were degraded in a thermostat at 75°C under aerobic conditions without exposure to light. At constant time intervals, the samples were chilled in mixture with ice-water and the absorbance was measured at 537 nm (Spekol 11, Carl Zeiss Jena, Germany).

RESULTS AND DISCUSSION

The rate constants were obtained from kinetic runs by the usual procedure as the negative slope of the dependence ln A = f(t), where A is absorbance and t is time. The dependence of the rate constants obtained by this classical method on a_w is shown in Fig. 1. It can be seen that, for the water/glycerol system, the rate constant increases with increasing a_w. This is in agreement with the published results (Pasch & von Elbe, 1975; Drdák *et al.*, 1990). For the water/ethanol and water/ethylene glycol systems the dependence exhibits a maximum. The betanin degradation also occurs in pure alcohols as is indicated by the related rate constants which are non-zero.

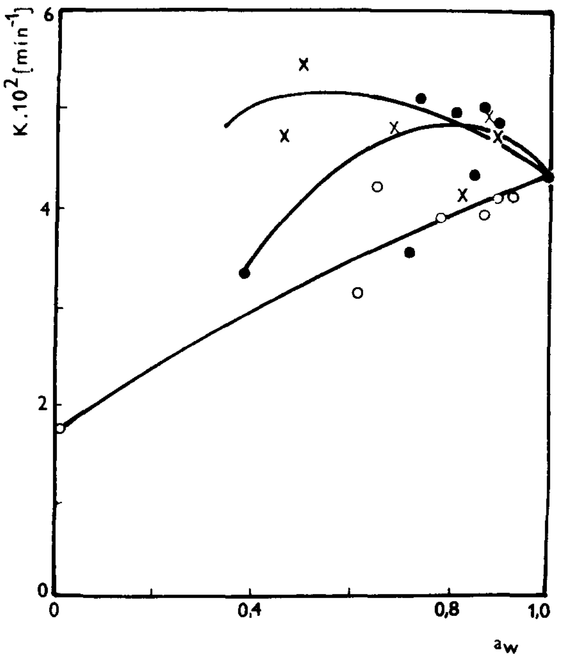

Fig. 1. Dependence of the rate constants obtained by the classical method on the water activity: (○) water/glycerol system; (×) water/ethylene glycol system; (●) water/ethanol system.

However, the transformed kinetic curves, ln A = f(t), are not straight lines; they are convex curves as when second-order kinetic curves are used for this transformation. Also other authors have observed that, initially, betanin degradation obeys first-order kinetics and then, as the reaction proceeds, the reaction order increases (Havlíková & Míková, 1985). The concentrations of water and alcohol in model solutions are much higher than the betanin concentration. If the betanin decay is caused by water or by alcohol, the reaction should consequently be quasi-first-order; the second-order seems to be highly improbable. The seeming change of the reaction order can reside in the presence of stable absorbing impurities or also in the fact that the reaction products exhibit a small absorbance at the wave-length used for the determination of betanin concentration. This idea is supported by the fact that, at the end of the reaction, the absorbance reaches a steady value of about 0·05. Then, the total absorbance, A, can be expressed as a sum of the absorbances of betanin, impurities and degradation products:

$$A = A_b + A_i + A_p \qquad (1)$$

where A_b, A_i and A_p stand for the absorbance of betanin, impurities and products, respectively. Equation (1) can be rewritten in the form

$$A = \epsilon_b c_b l + A_i + \epsilon_p c_p l \qquad (2)$$

where ϵ_b and ϵ_p are the extinction coefficients of betanin and products, c_b and c_p are the related concentrations and l is the width of the absorption cell. Assuming that the betanin decay can be expressed by the equation

$$\text{betanin} \rightarrow \text{product} \qquad (3)$$

it is obvious that c_b and c_p are connected by the relationship

$$c_b + c_p = c_o \qquad (4)$$

where c_o is the initial betanin concentration. Combination of eqns (2) and (4) gives

$$A = (\epsilon_b - \epsilon_p) c_b l + A_i + \epsilon_p c_o l \qquad (5)$$

Under the assumption that the betanin decay is a first-order reaction, eqn (5) has the form

$$A = (\epsilon_b - \epsilon_p) l c_o \exp(-kt) + A_i + \epsilon_p c_o l \qquad (6)$$

where k is the rate constant of betanin degradation. The conditions for betanin determination are chosen so that $\epsilon_b \gg \epsilon_p$. Hence, in the initial stage of the decay, when $t \rightarrow 0$, the second term is negligible and the decay is a first-order reaction. At the end of the reaction, for $t \rightarrow \infty$, the first term in eqn (6) disappears and the absorbance has a constant value. The simplest way to obtain the rate constant from eqn (6) is by the difference

$$A_1 - A_2 = (\epsilon_b - \epsilon_p) l c_o (1 - \exp(-k\Delta t)) \exp(-kt) \qquad (7)$$

where A_1 is the absorbance at time t and A_2 is the one at time $t + \Delta t$. The dependence $\ln (A_1 - A_2) = f(t)$ should be linear where the slope is the negative value of the rate constant and the intercept is $\ln [(\epsilon_b - \epsilon_p)lc_o (1 - \exp(-k\Delta t))]$. Of course, the time interval Δt for the measurement of absorbance must be constant. This method of treatment of first-order kinetic runs is known as the Guggenheim method (Laidler, 1965). Originally, the Guggenheim method was developed for the treatment of first-order kinetic runs with unknown initial concentration. In our case, the Guggenheim approach enables elimination of the systematic error caused by the absorbance of the degradation products and inert materials. A disadvantage of the Guggenheim method is that the relative error of the difference $(A_1 - A_2)$ is much greater than that of A. The experimental data have been treated up to about a 6% decrease of the original absorbance; below 6% the residual absorbance of impurities and products is so high in comparison with the absorbance of betanin that the difference $(A_1 - A_2)$ carries an unacceptably great relative error.

The rate constants obtained by the Guggenheim method are shown in Fig. 2. It can be seen that the values of rate constants are approximately twice those obtained by the classical method. The coefficients of correlation for the Guggenheim method are generally higher than for the classical method. Comparison of Figs 1 and 2 shows that the maxima in rate constants have become more pronounced. In contradiction with the results obtained by the classical method (Pasch & von Elbe, 1975; Drdák *et al.*, 1990; this work), the water/glycerol system also exhibits a maximum. Obviously, the systematic error caused by the absorbance of degradation products obscures the flat maximum for this system.

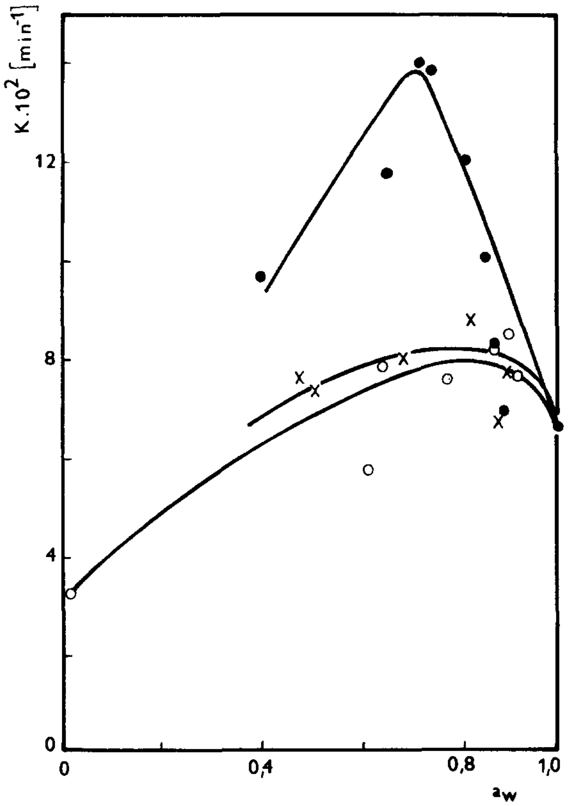

Fig. 2. Dependence of the rate constants obtained by the Guggenheim method on the water activity. Curves denoted as in Fig. 1.

The appearance of the maxima in the dependences of the rate constants on a_w is undoubtedly connected with the mechanism of the betanin degradation. A possible explanation for this behaviour is offered by the finding that, in aqueous solution, hydrolytic splitting of betanin occurs yielding betalamic acid and cyclodopa (von Elbe *et al.*, 1981; Schwartz & von Elbe, 1983). The first step of this splitting should be a nucleophilic attack on the carbon atom in the structure $>N^+ = C<$. Due to higher electron density on the oxygen atom, alcohols are stronger nucleophilic agents than water. Consequently, the presence of alcohol in water for high values of a_w brings about an increase of the rate constant owing to the higher rate of the intermediate formation (Scheme 1). A small amount of alcohol in water promotes the betanin decay.

If betanin reacts with pure alcohol, the intermediate formation is rapid. However, the intermediate is unstable since the addition of the alcohol is connected with the breakdown of the conjugated double bond system. It can be stabilized by the splitting-off of the proton. The stabilization of the intermediate is essential from the point of view of betanin degradation because the ether so formed should absorb light at shorter wave-lengths than betanin. Hence, in the ether formation the colour of betanin solution changes so that subsequent hydrolysis of the ether is not of interest from the point of view of using betanin as a pigment. Since water is better able to transfer protons than alcohols, a small amount of water in alcohol causes the increase of the rate constant. For a certain value of a_w, the rate constant reaches a maximum value in which the contribution of the increasing rate of proton transfer is maximum. Further increase of the water activity leads to a decrease of the rate constant owing to the decreasing concentration of the stronger nucleophilic agent (alcohol). Ethanol, as the strongest nucleophilic agent used here, exhibits a higher maximum than ethylene glycol and glycerol.

The mechanism of betanin degradation suggested is also supported by the fact that the highest betanin stability has been found for pH 4–5 (von Elbe *et al.*, 1974). In these slightly acid solutions the splitting-off of the proton from the intermediate is not so rapid as in neutral or basic solutions. For pH below 4 the acceleration of the degradation rate has been observed (Havlíková & Míková, 1985), connected obviously with a change of the reaction mechanism.

This paper has neglected the role of other degradation mechanisms which can compete with the one suggested here. Nevertheless, the results presented here lead to the conclusion that, under the above-mentioned experimental conditions, the hydrolysis (or expressed more generally, the solvolysis) plays a dominant role in betanin degradation.

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