Thermal Kinetic Degradation of Betanin and Betalamic Acid

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An investigation was undertaken with the aim of suggesting the kinetic model concerning the degradation of the red beet major pigment betanin to betalamic acid and the degradation of the latter to cleavage products. By using nonlinear least-squares technique, the formulated model was verified against the experimental data. The degradation of both pigments followed a first-order reaction (with energy of activation of 20.4 and 20.7 kcal/mol for betanin and betalamic acid, respectively). In addition, betalamic acid was one order of magnitude more thermally stable than betanin.

Large attention has been paid during the recent years to the usage of natural food pigments as permitted colorants. This interest was manifested by the growing restrictions in the usage of synthetic coloring compounds as food additives.

Red beet is one of the most logical sources for water-soluble food colorants and has been used as such quite extensively (Food Processing, 1973; von Elbe and Maing, 1973; von Elbe et al., 1974a; Pasch et al., 1975; von Elbe, 1975).

Many of the red and yellow pigments of the red beet were well characterized both chemically and structurally (Mabry, 1966; Mabry and Dreiding, 1968; Nilsson, 1970). However, stability and kinetic data of a particular interest to the food industry have been limited only to the major pigment betanin (Aurstad and Dahle, 1973; von Elbe et al., 1974b; von Elbe, 1975; Pasch and von Elbe, 1975). Data concerning the other beet pigments of importance are very limited, mainly due to the laborious and time-consuming techniques presently engaged in the pigments separation. Recently, using mathematical models Saguy et al. (1977a,b) outlined a procedure whereby all major beet pigments, namely: betanin, vulgaxanthin I, betalamic acid, and browning substances can be simultaneously determined in a mixture, dispensing completely with the separation step.

Betalamic acid seems to be a probable key intermediate in the degradation (pH 10.5) of betanin (Kimler et al., 1971). Nevertheless, only limited thermal kinetic data are available as to the cleavage of betanine to betalamic acid and the further degradation of the latter to various compounds yielding light brown pigments (von Elbe et al., 1974b; Aronoff and Aronoff, 1948a,b).

This investigation was undertaken with the aim of suggesting the kinetic model concerning the thermal degradation of betanin to betalamic acid and the degradation of the latter to cleavage products.

Mathematical Formulation. Let the reaction sequence describing the simultaneous formation and degradation of betalamic acid be represented by the following reaction sets:

$$\text{betanin} \xrightarrow{k_1} \text{betalamic acid} \xrightarrow{k_2} \text{betalamic acid cleavage products} \rightarrow \text{browning substances} \quad (1)$$

Let the letters A, B, and C represent the concentration of betanin, betalamic acid, and betalamic acid cleavage products, respectively, i.e.

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \quad (2)$$

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The following assumptions have been used in developing the analytical solution for the kinetic rate equation represented in eq 2: (1) as a first approximation, both reactions are assumed to be of a first-order nature; (2) the reactions are irreversible.

Assumption 1 is based upon the fact that first-order reaction is most successful in describing deterioration of nutritive components (Labuza, 1972) or pigment (von Elbe et al., 1974b) in food system. Nevertheless, this assumption will be justified later by the favorable agreement between the predicted values of the analytical solution with that of the experimental data.

Let the concentration of A and B be represented by a and b, respectively. Thus, the rate equations as function of time, t, can be formulated as follows:

$$da/dt = -k_1 a;\ a(o) = a_0 \quad (3)$$

$$db/dt = k_1 a - k_2 b;\ b(o) = b_0 \quad (4)$$

where k_1 and k_2 are the rate constants. The solution for the differential equation represented in eq 3 is:

$$a/a_0 = \exp(-k_1 t) \quad (5)$$

Dividing eq 4 by eq 3 yields:

$$db/da = -1 + k_2 b/k_1 a \quad (6)$$

By applying separation of variables technique and taking $b_0 = 0$ as a reference point, eq 6 can be integrated (Smith, 1970) resulting:

$$b = a_0 \frac{k_1}{k_1 - k_2}\left[\left(\frac{a}{a_0}\right)^{k_2/k_1} - \frac{a}{a_0}\right] \quad (7)$$

Substituting eq 5 into eq 7 one gets:

$$b = a_0 \frac{k_1}{k_1 - k_2}\left[\exp(-k_2 t) - \exp(-k_1 t)\right] \quad (8)$$

Equation 8 represents the dynamic concentration of betalamic acid, b as a function of time, initial betanin concentration, a_0, and the respective degradation rate constants k_1 and k_2.

EXPERIMENTAL SECTION

The experimental verification of the kinetic model presented above was carried out using betanin solutions of known concentration (= 4.29 mg/L) buffered with McIlvaine's citric-phosphate 0.1 M (CRC Handbook of Biochemistry, 1968) to simulate the normal pH (5.5) of beet-root. Betanin was separated according to the procedure outlined by Saguy et al. (1977b).

Ten milliliters of the freshly made betanin solution was filled up into vials, leaving no headspace. The vials were sealed and placed in a thermostatically controlled (±0.05

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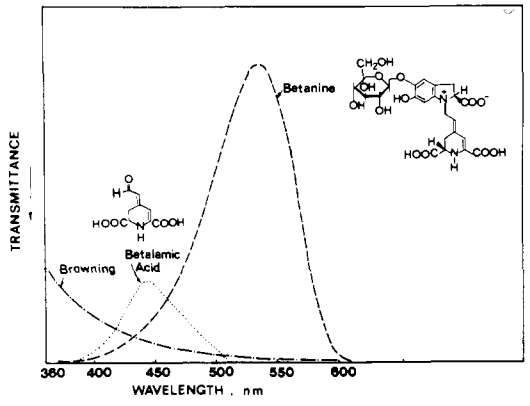

Figure 1. Characteristic visible spectra of betanin, betalamic acid, and browning substances.

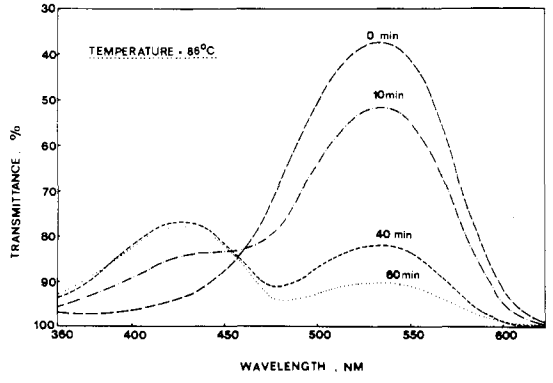

Figure 2. Typical visible spectra of the degradation process of betanin to betalamic acid and the latter to its cleavage products.

Table I. Degradation Rate Constants for Betanin Solution

Temperature, °C	$k_1 \times 10^3$, min^{-1}	$T_{1/2}$, min
60	4.9	141.5
75	17.5	39.6
81	29.6	23.4
86	46.1	15.0

°C) shaking bath for the heat treatment. Temperature within less than 1 °C was reached in less than 90 s. The vials (in triplicate) were drawn periodically and cooled down immediately in an ice bath. Subsequently, the visible spectrum (360–620 nm) of the solution was recorded using a D.B. Beckman Spectrophotometer.

Based upon the visible spectrum of the solution, the concentration of betanin, betalamic acid, and browning substances were determined simultaneously in accordance with the method described by Saguy et al. (1977a,b).

RESULTS AND DISCUSSION

Typical spectra (and their molecular formula) of betanin, betalamic acid, and browning substances are illustrated in Figure 1. Betanin shows a peak at approximately 535 nm, betalamic acid at 430 nm, while browning substances are characterized by the monotonous decrease of the transmittance toward the UV region.

When solutions of betanin pigment were heated for different lengths of time, the color gradually diminished, yielding betalamic acid and browning substances (Figure 2).

For each temperature tested, the concentration of the retained betanin vs. time generated a straight line when plotted on a semilogarithmic scale (Figure 3), thus, verifying the first-order kinetics assumed for betanin degradation. The slopes of the lines indicate that the degradation rate can be expressed in terms of the rate constant k_1 or alternatively in terms of half-life time, $T_{1/2}$ (Table I).

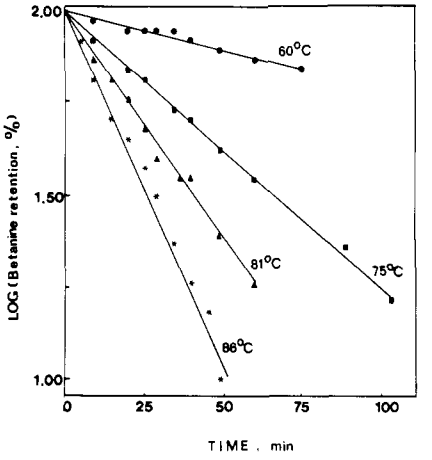

Figure 3. Degradation rates of betanin as a function of process temperature.

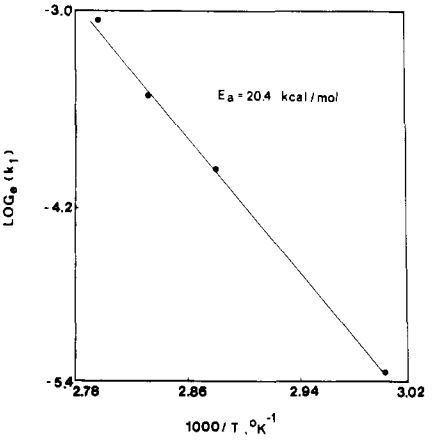

Figure 4. Rate constant (k_1) for betanin as a function of temperature.

Table II. Degradation Rate Constants for Betalamic Acid Solution

Temperature, °C	$k_2 \times 10^3$, min^{-1}	Error mean squares (EMS)
60	0.39	0.043
75	0.81	0.124
81	2.60	0.079
86	3.41	0.085

The rate constant (Table I) can be fitted into Arrhenius temperature coefficient pattern (Figure 4), yielding an energy of activation of 20.4 kcal/mol.

Obviously, for a given system both the betanin initial concentration (a_0) and its degradation rate constant (k_1) are known. Thus, by measuring the concentrations of betalamic acid (b) as a function of time, one might calculate, in an implicit manner, the value of the unknown betalamic acid degradation rate constant (k_2).

Using an IBM 370/168 computer and by applying nonlinear least-squares technique (BMDX85; Dixon, 1971), the best fitted betalamic degradation rate constant was derived (Table II). The high accuracy of the derived best fitted betalamic acid degradation constant (k_2) is illustrated in Figure 5, whereby the predicted betalamic acid (based upon eq 8) is compared with the experimental data. In addition, the small error mean squares (EMS, Table II), clearly indicate that the assumption of a first-order betalamic degradation kinetic is well justified.

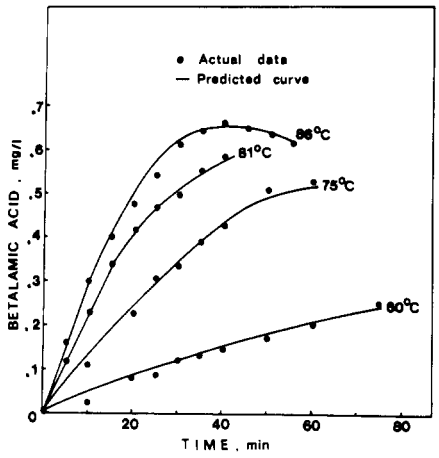

Figure 5. Predicted and actual concentration of betalamic acid.

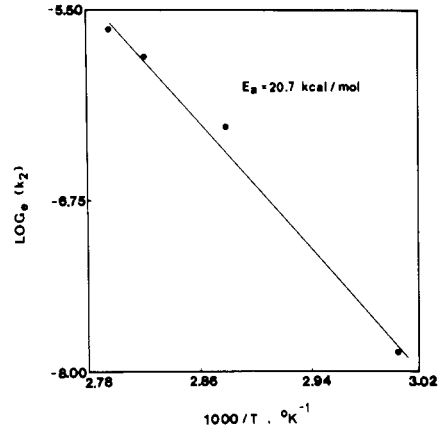

Figure 6. Rate constant (k_2) for betalamic acid as a function of temperature.

Similarly to betanin, the rate constant of betalamic acid can be fitted into Arrhenius temperature coefficient pattern (Figure 6), yielding an energy of activation of 20.7 kcal/mol.

While both betanin and betalamic acid have close value of energy of activation, betalamic acid seems to be approximate one order of magnitude more thermal stable than betanin; thus, explaining the shift toward the lower region of the visible spectrum of a thermal treated beet root juice or puree (Aurstad and Dahle, 1973; von Elbe et al., 1974b).

In conclusion, the proposed kinetic model describing the degradation of betanin to betalamic acid and the degradation of the latter to cleavage products was verified. The model enables one to predict the retention of these pigments under variable conditions of process temperature and time.

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Root, Hill, and Field Variance in Protein Content of North Carolina Sweet Potatoes

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Variation in protein content of Centennial and Jewel sweet potatoes grown in North Carolina was studied. Standard deviations of percent protein dry basis between roots of single hills were 0.79 for Centennial and 0.69 for Jewel and between hills within fields, 0.81 for Centennial and 0.73 for Jewel. The range of protein content from a number of hills was 5.27–7.24% for Centennial and 3.99–8.81% for Jewel.

Sweet potatoes have nutritional value that would recommend them for increased consumption. They are an excellent source of vitamin A value (Miller et al., 1949), and they provide a significant quantity of high quality protein (Nagase, 1957; Purcell et al., 1972). If the nutritional value of sweet potatoes is to be exploited in an effort to increase consumption, it will be necessary to provide nutritional labeling (Federal Regulation 21CFR101.9). Differences in protein content among cultivars has been reported to range from about 2–10% dry basis (Cooley, 1948; Purcell et al., 1972). Variation of protein content within cultivars has not been as well documented (Constantin et al., 1974; Li, 1976a,b).

During the conduct of other work some samples of sweet

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