Effect of pH on neohesperidin dihydrochalcone thermostability in aqueous solutions

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Abstract. The objective of this work was the evaluation of neohesperidin dihydrochalcone thermostability. For this accelerated stability study, we have used an isothermal method at three temperatures (50, 70 and 90 °C). The thermodegradation of aqueous diluted solutions of neohesperidin dihydrochalcone at various pH was studied. This degradation appeared to follow first-order kinetics and was found to be pH-dependent. In the best stability conditions (pH = 4.50), the experiments revealed an activation energy (E_a) of 45.8 kJ mol^{-1} and a $t_{90\%}$ of 164 days, at 20 °C.

Key words. Neohesperidin dihydrochalcone – thermodegradation – aqueous solutions – pH.

Introduction

Neohesperidin dihydrochalcone, an intense sweetener, is obtained by a simple chemical modification of some naturally occuring flavonoids (hydrogenation of naringin or neohesperidin). It was first prepared by Horowitz and Gentili [1].

The flavonoids are natural compounds that are widely distributed in fruits and vegetables, specially in citrus fruits like Seville orange (*Citrus aurantiacum*), grapefruit (*Citrus paradisi*), soft orange (*Citrus sinensis*), and lemon (*Citrus lemon*) [2-5].

Dihydrochalcones seem to be an attractive class of sweeteners from a safety point of view because neohesperidin dihydrochalcone has not been reported to show any ill effects [6].

Neohesperidin is about 1500 times sweeter than sucrose. These sweet taste appears and disappears slowly and there is an aftertaste of mint [7,8].

As far as we are concerned, we have studied neohesperidin dihydrochalcone to the warmth with isothermal method at three temperatures.

Material and methods

Chemicals

Neohesperidin dihydrochalcone (N.H.D.C.) was obtained from Interchemical, France (batch N° 29.044). It is a white, odourless, crystalline powder, freely soluble in hot water, sparingly soluble in ethanol and practically insoluble in ether and benzene. LC quality methanol and 85% phosphoric acid were purchased from Merck. All other chemicals used were of analytical reagent grade. Water, applied throughout the study, was purified by an Autostill 4000X (Jencons) apparatus. Demineralized, deionised water was obtained from MilliQ system (Millipore).

Experimental procedure

Aqueous diluted solutions (3.26×10^{-5} M) of neohesperidin dihydrochalcone at various pH were packaged in small glass bottles (Duran, Schott, 250 mL) hermetically closed by a polypropylen cork. The pH of these solutions was adjusted to the desired value with HCl 0.01 and 0.32 M and H_3BO_3 0.32 M. All pH measurements were performed on a Metrohm Herisau pH-meter, model E300B, equiped with a Refill 9811 Ingold I 3556 (pH = 0 – 14, $T = 0 – 80$ °C) electrode and standardized with Panreac solutions respectively at pH = 4 and pH = 10. These measures were carried out at 20 °C.

The various solutions were stored in thermostatically controlled ovens (Memmert, type UE-200), at 50 °C, 70 °C and 90 °C. Samples were withdrawn at specific time intervals, throughout 65 days, cooled to room temperature and neohesperidin dihydrochalcone concentration was then determined.

Preliminary study

The value of the absorption peak of neohesperidin dihydrochalcone was determined by a spectrophotometric method (Hitachi UV-visible, double beam spectrophotometer, model U-2000). Slit width was fixed at 2 nm. Solutions were recorded in 1 cm quartz cells over the 200 to 400 nm range (= 2.3 nm). The scan speed was 400 nm min^{-1}.

Assay

The neohesperidin dihydrochalcone concentrations initially and at various times were determined using High Performance Liquid Chromatography. HPLC was carried out with a system consisting of a Waters Model 6000 A pump, a Waters Lambda Max model 481 LC variable-wavelength

detector and a Merck D-2500 model integrator (Hitachi). Each solution was analysed under the following conditions: the best resolution of peaks was obtained on a 5-μm LiChrosorb C18 analytical column (125 × 4.1 mm I.D.) (Interchim); the most suitable mobile phase was found to be a gradient of methanol and water (Tab. I) which was pumped at a flow-rate of 2 mL min^{-1}; the pH of the buffer was adjusted to 3.2 with H_3PO_4; volume injected 10 μL. All analyses were performed in triplicates at room temperature.

Data analysis

The order of the thermodegradation reaction was determined by the least squares method of linear adjustment and by calculating the correlation coefficients, in order to choose between zero-order kinetics and first-order kinetics. The degradation rate constants (k) were determined from the slope of the line of peak area versus storage times at 50, 70 and 90 °C and have been calculated in accordance with the determined order of the reaction. These thermal treatments or accelerated stability studies are based on Arrhenius relationship where the degradation rate constant of the substance is a function of the temperature, according to the equation:

$$\log k = \log A - (E_a/2.303.RT) \qquad (1)$$

where k is the degradation rate constant (d^{-1}), A a constant for a given reaction, E_a the heat of activation (J mol^{-1}), R universal gas constant and T the absolute temperature (K) [9-13]. The statistical analysis of the results were conducted using the Student's t-test (.95 confidence level).

Results and discussion

Results concerning the preliminary study

Optimum sensitivity of neohesperidin dihydrochalcone was obtained at 281 nm (Fig. 1). Then, the detector was set to this wavelength. Under experimental conditions, a linear response was obtained over neohesperidin dihydrochalcone concentrations ranging from 5 to 35 mg L^{-1}. The regression equation is the following:

$$y = 0.0284x + 0.00824 \quad (r = 0.9997) \qquad (2)$$

where x is the concentration (mg L^{-1}) and y is the area of the peak of neohesperidin dihydrochalcone. r is the correlation coefficient.

Results concerning the pH of the various solutions

The values of pH of the various aqueous diluted solutions of neohesperidin dihydrochalcone can be found in table II.

Neohesperidin dihydrochalcone thermodegradation kinetics study and effect of pH

The thermodegradation of neohesperidin dihydrochalcone in aqueous diluted solution whatever pH is expressed as the rate of change of peak area of this molecule. The HPLC analysis demonstrates the gradual decrease at 281 nm during the thermal treatment. The degradation rate constant is calculated from the slope of the line of peak area versus time. The percentage of substance remaining is calculated and the ratio

Table I. Gradient composition during the HPLC analysis.

Solvent^a	Time (min)				
	0	*5*	*10*	*15*	*20*
A (%)	50	50	70	70	50
B (%)	50	50	30	30	50

^{a} A = Methanol; B = Water (adjusted at pH 3.2 with H_3PO_4)
At 20 min, 50% A and 50% B were run for 5 min, and then starting conditions were maintained for 5 min before the next injection.
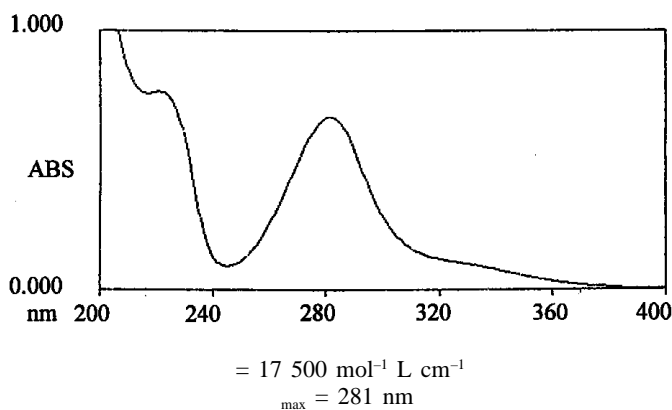

= 17 500 mol^{-1} L cm^{-1}
$_{max}$ = 281 nm

Fig. 1. Spectra obtained with the 3.26×10^{-5} M neohesperidin dihydrochalcone aqueous solution between 200 and 400 nm.

Table II. pH of various solutions studied.

Reagent employed	Concentration (M)	pH
None		6.00
HCl	0.32	0.50
HCl	0.01	2.50
H_3BO_3	0.32	4.50

C/C_0 at three temperatures for neohesperidin dihydrochalcone at pH 4.50 is shown in table III.

The degradation rate constants and the $t_{90\%}$ (time necessary to obtain a decrease of 10% of initial concentration) concerning neohesperidin dihydrochalcone in aqueous diluted solution (3.26×10^{-5} M) at pH 4.50 can be found in table IV.

The thermodegradation of neohesperidin dihydrochalcone in aqueous diluted solution followed apparent first-order kinetics and is described by the following equation:

$$C/C_0 = e^{-kt} \qquad (3)$$

where C and C_0 are the neohesperidin dihydrochalcone concentrations at time t and initially and k is the apparent first-order degradation rate constant (Fig. 2). The values of k according to the pH at various temperatures can be found in table V. We can deduce $k_{20 °C}$ from the graphical representation of equation (1) by extrapolation (Fig. 3). Therefore, Arrhenius relationship (Eq. 1) gives us the activation energy (E_a) which is equal to 45.8 kJ mol^{-1}.

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Table III. C/C_0 ratio for various temperatures at specific time intervals for neohespiridin dihydrochalcone aqueous diluted solution (pH = 4.50).

Time (days)	90 °C C/C_o	70 °C C/C_o	50 °C C/C_o
0	1.000	1.000	1.000
1	0.982	–	–
2	0.953	–	–
3	0.924	–	–
4	0,896	–	–
5	0.869	0.946	0.982
6	0.843	–	–
7	0.817	–	–
8	0.792	–	–
9	0.768	–	
10	0.745	0.895	0.965
11	0.723	–	–
12	0.701	–	–
13	0.680	–	–
14	0.659	–	–
15	0.639	0.847	0.948
16	0.620	–	–
17	0.601	–	–
18	0.583	–	–
19	0.565	–	–
20	0.548	0.801	0.932
21	0.532	–	–
22	0.516	–	–
23	0.500	–	–
24		–	–
25		0.758	0.915
30		0.717	0.899
35		0.678	0.883
40		0.641	0.868
45		0.607	0.852
50		0.574	0.837
55		0.543	0.823
60		0.513	0.808
65		0.486	0.794

Table IV. Degradation rate constants (.95 confidence level) for neohesperidin dihydrochalcone (pH = 4.50) and half-time at various temperatures.

Temperatures (°C)	Degradation rate constants (10^{-3})	Half-time (days)
50	3.71 ± 0.25	195.41
70	10.06 ± 1.00	62.40
90	24.50 ± 2.28	23.00

Table V. Degradation rate constants for neohespiridin dihydrochalcone at various pH.

pH	Degradation rate constants (.95 confidence level)		
	k_{50} (10^{-3})	k_{70} (10^{-3})	k_{90} (10^{-3})
0.50	13.27 ± 0.73	37.66 ± 3.18	95.25 ± 7.54
2.50	5.10 ± 0.21	10.33 ± 1.28	19.43 ± 1.72
4.50	3.71 ± 0.25	10.06 ± 1.00	24.50 ± 2.28
6.00	9.60 ± 0.87	31.80 ± 2.87	93.15 ± 6.60

Table VI. $t_{90\%}$ according to pH, at 20 °C.

pH	$t_{90\%}$ (days)
0.50	49.56
2.50	72.31
4.50	164.07
6.00	91.51

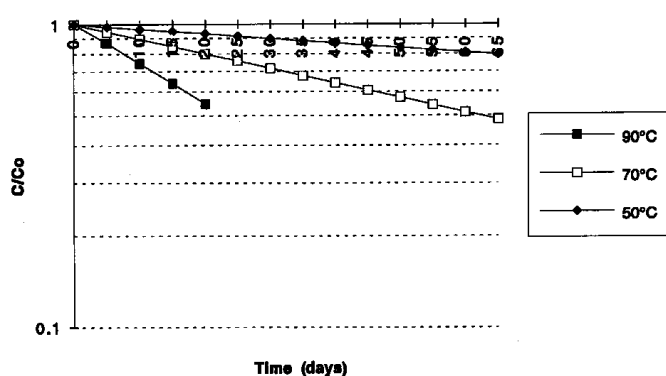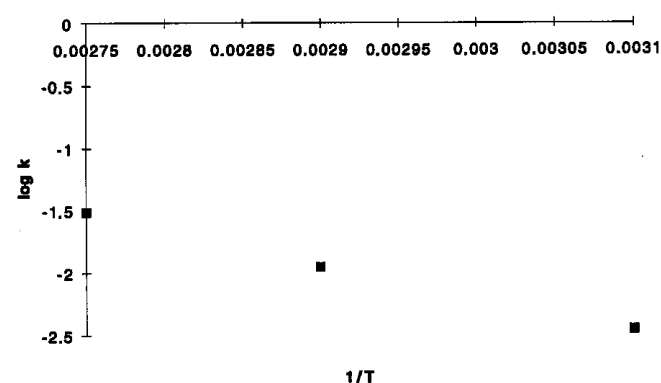**Fig. 2.** Thermodegradation of neohesperidin dihydrochalcone in aqueous diluted solution at pH 4.50, at 50, 70 and 90 °C.

Fig. 3. Arrhenius relationship log $k = f(1/T)$ concerning neohesperidin dihydrochalcone in aqueous diluted solution (pH = 4.50).

At 20 °C, it can be deduced $t_{90\%}$ for neohesperidin dihydrochalcone at various pH (Tab. VI).

Figure 4 allows us to conclude that neohesperidin dihydrochalcone is much stable at pH 4.50.

The values of E_a and $t_{90\%}$ for neohesperidin dihydrochalcone and aspartam [14] are very similar, so these two sweeteners have the same storage conditions in a range of pH from 0.5 to 6.
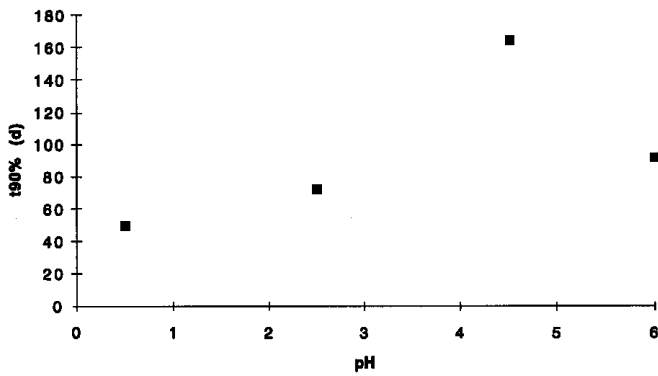
Fig. 4. Values of $t_{90\%}$ according to the pH for 3.26×10^{-5} M neohesperidin dihydrochalcone aqueous solutions.

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