

Kinetic Studies of Saffron (*Crocus sativus* L.) Quality Deterioration

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Kinetic studies on carotenoid loss and changes in other quality attributes of saffron were carried out under varying conditions of water activity (a_w) and temperature. The water sorption isotherms of saffron powders were also determined. Within the a_w range tested (0.11–0.75), both Brunauer–Emmett–Teller (BET) and Guggenheim–Anderson–Deboer (GAB) sorption models fit the data well and gave estimates for the monolayer values between 1.8 and 5.2 g of H₂O/100 g of solids, depending on temperature and the equation used. The glass transition temperature (T_g) of freeze-dried saffron carotenoid extracts, as detected by calorimetry (DSC), was very sensitive to the moisture content of the system; water plasticizes the amorphous matrix and thereby increases the mobility and reactivity of the reactants. Saffron carotenoid (mainly crocins) degradation followed first-order-like reaction kinetics, and it was strongly dependent on temperature and a_w . The dependence of the rate constant (k) for decoloration on a_w was different from that for other typical nonpolar carotenoids, resembling more the kinetic responses of water-soluble pigment degradation and nonenzymatic browning reactions. Carotenoid loss occurred only at temperatures above the T_g , presumably because of limited molecular diffusion in the matrix at sub- T_g temperatures. However, testing of the applicability of a Williams–Landel–Ferry (WLF)-governed dependence of k on temperature was not successful. Activation energies, calculated from Arrhenius-type plots, gave an average value of ~20 kcal/mol, typical of other deteriorative processes in food materials. At ambient temperature, an intermediate water activity ($a_w \sim 0.43$ –0.53) seemed to favor the development of aroma constituents (safranal) while maintaining a relatively low degradation rate for the carotenoids.

Keywords: Saffron quality; water activity; glass transition; kinetics; carotenoids; crocins; picrocrocins; safranal

INTRODUCTION

Saffron, the dried stigmas of the flower of *Crocus sativus* L. (Sampathu et al., 1984), is a spice appreciated worldwide for its unique bitter taste, pungent aroma, and coloring properties (Oberdieck, 1991). In recent years, the use of saffron in food applications has been steadily increasing, despite its high price, because of changes in consumer preference toward natural products (Knewstubb and Henry, 1988). The shelf life of saffron seems to be related to the fate of its pigments, the crocins, a group of water-soluble carotenoids (Raina et al., 1996), which are glycosyl esters of 8,8'-diapocrotene-8,8'-dioic acid (crocin) (Pfander and Wittwer, 1975a,b; Tarantilis et al., 1994).

Being highly unsaturated, carotenoids are prone to *trans* → *cis* isomerization (*cis* forms are less intensely colored) and oxidation reactions, the latter being the major cause of color fading of carotenoid-containing plant materials. The consequences of carotenoid degradation include losses of colorant power and nutritive value as well as the development of off-flavors (Rodriguez-Amaya, 1993). Depending on molecular structure, factors affecting the rate of carotenoid degradation include temperature, oxygen, light, moisture content/water activity, metals and other pro-oxidants, presence

of antioxidants, free-radical inhibitors, and the composition and physical structure of the sample (e.g., compact vs porous macrostructure) (Rodriguez-Amaya, 1993). These factors should be properly controlled to maximize color retention during storage. The degradation of carotenoids in dehydrated food systems has mainly been studied for β -carotene (Walter et al., 1970; Chou and Breene, 1972; Kanner et al., 1978; Arya et al., 1979a, b; Neto et al., 1981; Goldman et al., 1983; Lee et al., 1992) and, to a lesser extent, for other carotenoids of plant or animal origin (Rodriguez-Amaya, 1993). β -Carotene seems to be very labile upon exposure to light, oxygen, and heat, whereas its stability increases with increasing water activity (a_w) (Martinez and Labuza, 1968; Ramakrishnan and Francis, 1979; Arya et al., 1979a,b; Goldman et al., 1983). The protective effect of water on the oxidative losses of carotenoids is similar to that observed for oxidative deterioration of lipids. In most studies, values of a_w above the monolayer value and up to $a_w \sim 0.7$ seemed to provide maximum protection against oxidation of carotenoids (von Elbe, 1987). On the other hand, pH variations affect more the stability of polar carotenoids, such as bixin (principal pigment of annatto) and crocin, in various types of extracts (Najar et al., 1988; Tsimidou and Tsatsaroni, 1993). Although many studies have been devoted to the stability of carotenoids in model or native food systems (Chen and Gutmanis, 1968; Walter et al., 1970; Chou and Breene, 1972; Kanner et al., 1978; Arya et al.,

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1979a,b; Gordon and Bauernfeind, 1982; Goldman et al., 1983; Saguy et al., 1985; Berset and Marty, 1986; Najjar et al., 1988; Minguez-Mosquera and Jaren-Galan, 1995), relevant information on stability of saffron carotenoids during storage is rather limited (Mannino and Amelotti, 1977; Alonso et al., 1990, 1993; Tsimidou and Tsatsaroni, 1993; Orfanou and Tsimidou, 1995).

For carotenoid degradation reactions, one of the main points of contention concerns the kinetic model that best describes the experimental data. Several attempts have been made to kinetically treat carotenoid losses in solutions, dehydrated foods, or systems simulating dehydrated foods (Philip and Francis, 1971; Goldman et al., 1983; Haralampu and Karel, 1983; Saguy et al., 1985; Minguez-Mosquera and Jaren-Galan, 1995; Gloria et al., 1995). Pesek and Warthesen (1989) reported that, in an aqueous medium and under conditions of strong lighting, the kinetics of β -carotene degradation were of first-order. The same authors (Pesek and Warthesen, 1990) also established that the photoisomerization of β -carotene followed approximately zeroth-order kinetics. As pointed out in several of these studies (Goldman et al., 1983; Pesek and Warthesen, 1989; Pesek et al., 1990; Minguez-Mosquera and Jaren-Galan, 1995), the nature of the reaction medium has a very strong influence on pigment degradation; in anhydrous media decoloring of carotenoids seems to follow zeroth-order kinetics, whereas in an aqueous medium, the reaction is of first-order. In low-moisture or dehydrated food systems, diffusion of oxygen, as affected by the physical state and moisture content of the porous matrix, has a major effect on the magnitude of the induction period and on the overall rate of oxidation.

Acceleration of deteriorative changes in amorphous food materials has been attributed to the decreased viscosity and consequent increase in mobility when a system passes from a glassy (below glass transition temperature, T_g) to a rubbery (above T_g) state, as a result of increasing temperature and/or moisture content (Slade and Levine, 1991; Karel et al., 1993); water is regarded as a plasticizing agent, thus depressing the T_g of the food matrix (Slade and Levine, 1991). In recent studies, the physical state of low- and intermediate-moisture-content foods has been claimed as a major determinant of the diffusion rate of reactants, thus affecting the rate of deteriorative reactions (e.g., non-enzymatic or enzymatic browning) in foods (Slade and Levine, 1991; Karel and Saguy, 1991; Karmas et al., 1992; Buera and Karel, 1993; Roos and Himberg, 1994). Above T_g , the temperature dependence of the changes in reaction rates has been modeled using the Williams-Landel-Ferry (WLF) equation (Williams et al., 1955), instead of the Arrhenius relationship (Slade and Levine, 1991). The WLF equation was originally applied to relate the time required for mechanical relaxation processes to the difference between the temperature of the material and its T_g ($T - T_g$) and subsequently used to describe temperature-dependent changes in viscosity of sugar solutions (Soesanto and Williams, 1981), changes in time for sugar crystallization (Roos and Karel, 1990, 1991a), and changes in kinetics of many other deteriorative processes in foods (Levine and Slade, 1986; Karel et al., 1993; Slade and Levine, 1993).

The aim of the present study was mainly to investigate the kinetics of saffron carotenoid degradation under varying storage conditions (a_w and temperature). Observations were also made on relative changes in other quality attributes of saffron, i.e., to compounds respon-

sible for its bitter taste or aroma. Picrocrocin (4-(β -D-glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde) is the bitter component of saffron. One of its degradation products, safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde), is its major flavor compound (Zarghami and Heinz, 1971). A further objective was to explore whether the degradation rates are more closely related to a_w or to molecular mobility associated with the glass transition of the system. Kinetic studies reported herein are relevant to minimizing the undesired losses in color and other quality characteristics of saffron commercial products during storage.

MATERIALS AND METHODS

Sample. The saffron sample was a representative mixture of saffron stigmas and was kindly donated by a local cooperative of saffron producers (Cooperative de Safran, Crocos, Kozani, Greece). The sample was air-dried in the dark by the producers and was subsequently dried in a laboratory oven under vacuum at 40 °C. Portions of the sample used for the kinetic studies had a moisture content of 10.3%, while those used to generate true water sorption data were dried to a much lower moisture content of 4.0%.

Water Adsorption. Portions of approximately 1.5 g of dried sample were spread in weighing dishes and kept over saturated salt solutions in desiccators. Several salt solutions were used to achieve various relative humidities: LiCl, CH₃-COOK, MgCl₂·6H₂O, K₂CO₃, Mg(NO₃)₂·6H₂O, NaNO₂, and NaCl, which give a_w values (25 °C) of 0.11, 0.23, 0.33, 0.43, 0.53, 0.64, and 0.75, respectively (Rockland, 1960; Greenspan, 1977). The salts (reagent grade) were from Merck (Darmstadt, Germany). In each case, the a_w values for the salts were obtained from the literature (Rockland, 1960; Winston and Bates, 1960; Labuza, 1984). The closed desiccators were stored in incubators at three different temperatures, in the dark; the incubators were set at 25 °C (using a Gallenkamp cooled incubator), 40 °C (using a Memmert oven), and 60 °C (using a Heraeus oven). The saturated salt solutions were kept for 2 days before use at the above temperatures. The moisture content of the samples, after reaching equilibrium, was determined according to ISO 3632-2 (1993). Triplicate determinations were made for all equilibrium moisture contents of each sample at each temperature.

The a_w -moisture data were used to construct sorption isotherms. The data were fit to the Brunauer-Emmett-Teller (BET) (Brunauer et al., 1938) or the Guggenheim-Anderson-DeBoer (GAB) (van den Berg and Bruin, 1981) sorption isotherm models.

The BET model is described by eq 1:

$$a_w/(1 - a_w)m = 1/m_m K + a_w(K - 1)/m_m K \quad (1)$$

where m_m is the BET monolayer value and K is a constant. The constants m_m and K were calculated from the linear regression of the experimental data in the range of a_w examined.

The three-parameter GAB isotherm model is given by eq 2:

$$m/m_m = CK'a_w/(1 - K'a_w)(1 - K'a_w + CK'a_w) \quad (2)$$

where m_m is the monolayer value and C and K' are constants. For data-fitting purposes this model can be transformed into a second-order polynomial (eq 3):

$$a_w/m = \alpha(a_w^2) + \beta a_w + \gamma \quad (3)$$

from which the constants C and K' and the monolayer value

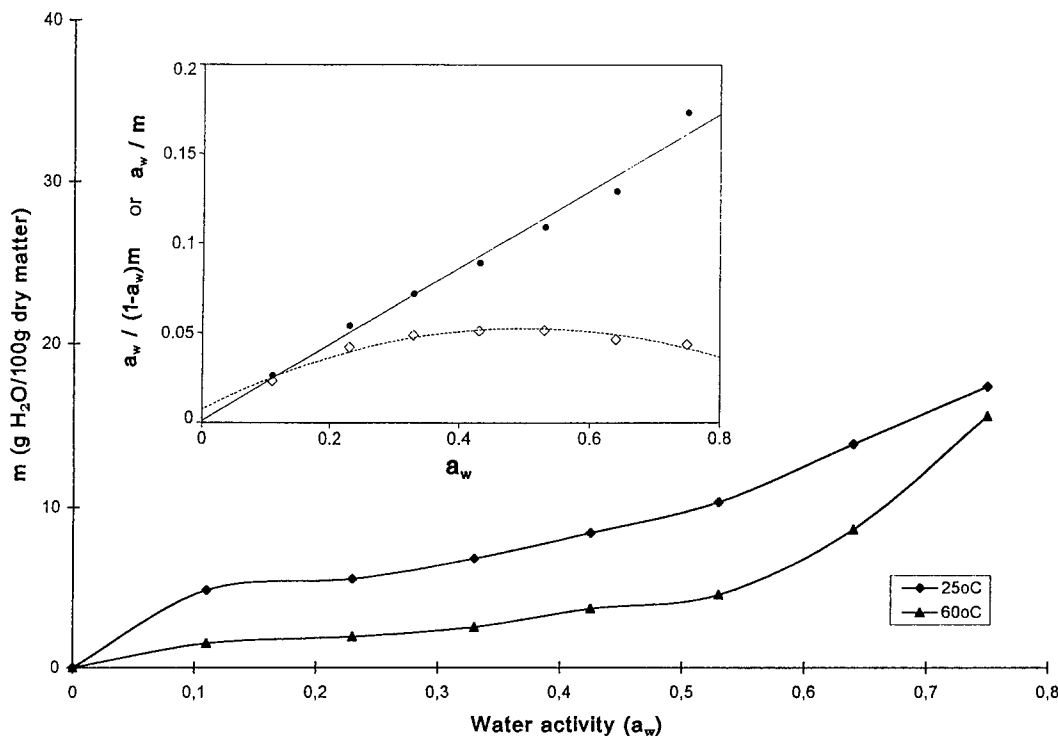


Figure 1. Sorption isotherms for saffron powder. Inset shows the BET (solid line) and the GAB (dotted line) adsorption models with the experimental data at 25 °C.

can be calculated using the following equations

$$m_m = \sqrt{-1/(4\alpha\gamma - \beta^2)}$$

$$K' = [\beta - (1/m_m)] / -2\gamma$$

$$C = 1/m_m\gamma K'$$

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Samples of powdered saffron (3 g), ground with a pestle and mortar and passed through a 500 μm mesh sieve, were uniformly distributed in Petri dishes (61 mm i.d.) and kept at the above-mentioned relative humidity conditions. The degradation of carotenoids was followed by periodic measurements of the coloring power of saffron aqueous extracts. The extracts were prepared according to the ISO 3632-2 method (1993). The repeatability of the extraction method was satisfactory ($CV \sim 0.8\%$, $n = 5$). The coloring power of the extract (related to the concentration of crocins), measured with a UV-vis spectrophotometer (Model 2000, Hitachi Ltd., Tokyo, Japan), was expressed as $E^{1\%}_{443\text{nm}}$, where $E = A_{443}/C_{(1\text{g}/100\text{cm}^3)}$, according to the above standard method; 443 nm was the λ_{max} recorded for crocins. Reaction rate constants and half-life values ($t_{1/2}$) for crocins degradation were determined by applying a first-order reaction model (Alonso et al., 1990; Tsimidou and Tsatsaroni, 1993). Assessment of changes in picrocrocin and safranal levels was made from the absorption data at 257 and 328 nm, respectively (ISO 3632-2, 1993).

Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) was used to determine the glass transition temperature (midpoint temperature of endothermic baseline shift) of saffron carotenoid extracts. The extracts were freeze-dried (Leybold-Heraeus GmbH & Co., Köln, Germany) to produce amorphous materials, which were stored over P_2O_5 at a temperature below their T_g . Small amounts (8–15 mg) of freeze-dried materials were placed in Mettler medium-pressure stainless-steel pans (ME 29990, 120 μL) and rehumidified at various relative humidities over saturated salt solutions in desiccators at 25 °C. The freeze-dried materials had a large surface area, and equilibration occurred within 5 days. After equilibration the DSC pans were hermetically sealed and analyzed with a PL DSC-Gold calorimeter (Polymer

Labs Ltd., Epsom, U.K.). The samples were heated at 5 °C/min under a continuous flow of N_2 gas.

Temperature Dependence of Reaction Kinetics. The dependence of reaction rate constants on temperature was modeled using the Arrhenius equation and a WLF-type equation. According to the Arrhenius equation (eq 4), a linear relationship exists between $\ln k$ and $1/T$:

$$k = k_0 \exp(-E_a/RT) \quad (4)$$

where R is the gas constant and E_a is the activation energy. The WLF-type temperature dependence is described by eq 5, which should give a straight line when $\ln k$ is plotted against $T - T_g$, when T_g is used as the reference temperature, T_{ref} (Slade and Levine, 1991; Roos and Karel, 1992):

$$\ln(k_{\text{ref}}/k) = -C_1(T - T_{\text{ref}})/[C_2 + (T - T_{\text{ref}})] \quad (5)$$

RESULTS AND DISCUSSION

Water Adsorption Behavior. The water vapor adsorption isotherms of saffron were of sigmoidal shape (Figure 1), typical of most food materials. A downward shift of the isotherms was observed from 25 to 60 °C, indicating that, at a constant moisture content, a_w increases with increasing temperature. Temperature affects the mobility of water molecules and the dynamic equilibrium between the vapor and adsorbed phases; i.e., an increase in temperature is unfavorable to water sorption. There was also no indication of crystallization, due to water plasticization, for any component in the sample, as evidenced by the shape of the isotherms (there were no discontinuities) and the lack of any melting endotherm on DSC analysis of the stored samples. Crystallization of amorphous sugars during water adsorption has often been observed in dehydrated food powders (Bushill et al., 1965; Labuza, 1984; Roos and Karel, 1990); i.e., due to water plasticization, the T_g is depressed below the storage temperature, thus

Table 1. Constants for BET and GAB Isotherm Models of Saffron Sorption Data (a_w Range: 0.11–0.75)

temperature (°C)	BET				GAB			
	n^a	m_m (g of H ₂ O/100 g)	K	r^2 ^b	m_m (g of H ₂ O/100 g)	K	C	r^2 ^c
25	7	4.65	222.5	0.98	5.19	0.94	27.1	0.95
40	7	4.48	20.5	0.97	3.85	1.09	440.1	0.94
60	7	3.61	2.5	0.52	1.82	1.19	27.0	0.89

^a Number of experimental data points. ^b For the linear regression $a_w/(1 - a_w)m = a + ba_w$. ^c For the quadratic regression $a_w/m = \alpha(a_w^2) + \beta a_w + \gamma$.

allowing crystallization of sugar components. The BET and GAB equations were tested for fit to the adsorption data within the a_w range of 0.11–0.75, and estimates of the constants are summarized in Table 1. In general, both equations fit reasonably well the adsorption data for saffron powders (Figure 1, inset), with the exception of the 60 °C results using the BET model (Table 1). The GAB isotherm gave good agreement with the experimental data for all temperatures ($r^2 > 0.89$). The GAB equation has been widely used to describe moisture sorption isotherms of many food systems up to a_w s of ~0.95 (van den Berg, 1984). The estimated monolayer values in the present study ranged between 1.82 and 5.19 g of H₂O/100 g of solids, depending on temperature and the equation used for estimation. Our data show that m_m decreased with increasing temperature, a trend also reported for other food systems (Labuza et al., 1985). Temperature-related shifts in a_w can have a practical effect on chemical reactivity and increasing rates of food deterioration processes (Labuza, 1975).

Estimated a_w data at constant moisture contents (within 5–15%), using the GAB model, are presented in Figure 2a, as $\ln a_w$ vs $1/T$. These plots were linear, implying that the data followed the Clausius–Clapeyron equation:

$$d(\ln a_w)/d(1/T) = -\Delta H_s/R$$

where T is absolute temperature, ΔH_s is excess heat of sorption at constant moisture content, and R is the gas constant. The enthalpic term, ΔH_s , is a measure of the binding energy between water and the food matrix. The slopes of the lines in Figure 2a, from which ΔH_s can be obtained, decreases with increasing moisture content. This suggests a decrease in the binding energy for water molecules. At 5% water content, the estimated ΔH_s is approximately 6.7 kcal/mol (Figure 2b), and it decreases sharply with increasing moisture, up to about 12.5%, where it starts to level off. Within this moisture content range, similar trends in the heat of sorption are exhibited by most food products (Kapsalis, 1987).

Glass Transition Temperature. Due to water plasticization effects, the T_g of amorphous food matrices decreases with increasing water content (Slade and Levine, 1991). Figure 3 shows the DSC scans for selected freeze-dried saffron extracts equilibrated in different relative humidity environments. The observed endothermal baseline shifts are typical of glass transition, indicating a progressive lowering of T_g with increasing a_w . The measured T_g values of the extracts were found to decrease linearly with increasing a_w : $T_g = 40.9 - 91.9a_w$ ($r^2 = 0.96$). The linearity between T_g and a_w allows prediction of the T_g of extracts exposed to various relative humidities. Similar linear relationships have been reported for sugars, maltodextrin-based food models, and some freeze-dried foods with high carbohydrate content (Slade and Levine, 1991; Roos and Karel, 1991b; Roos and Himberg, 1994). For several

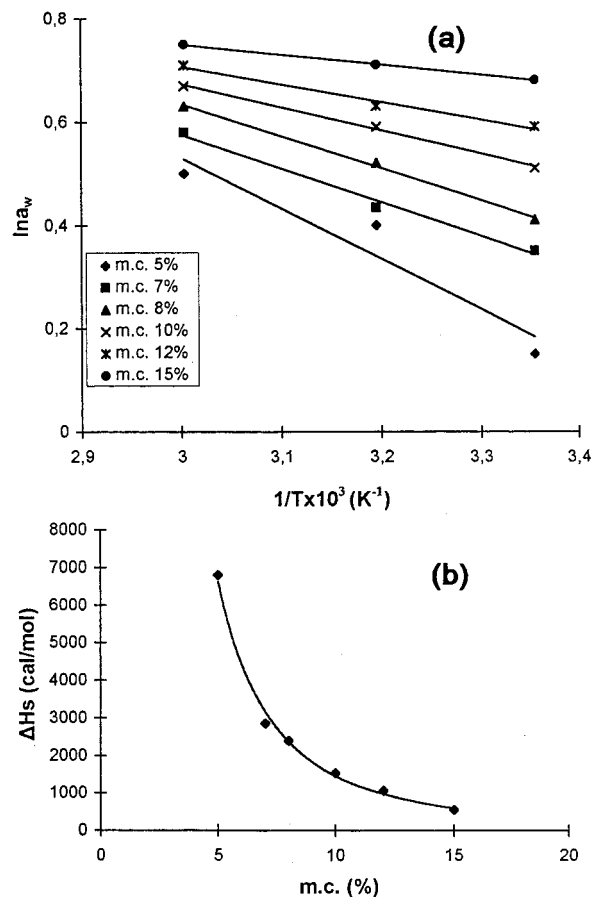


Figure 2. Relationship between a_w and temperature, according to the Clausius–Clapeyron equation (a), and excess heat of sorption (ΔH_s) as a function of moisture content (b).

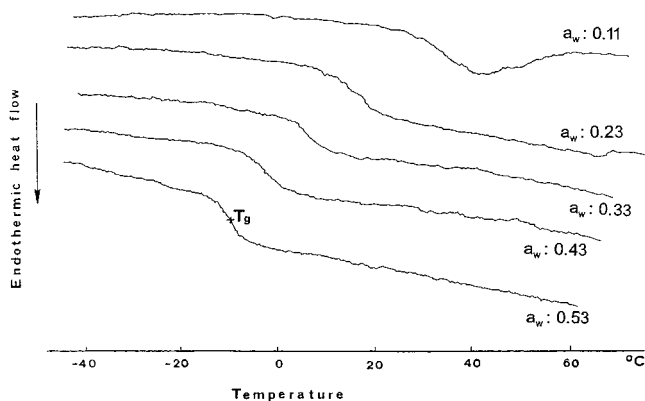


Figure 3. DSC thermal curves for amorphous (freeze-dried) saffron carotenoid extracts, after equilibration in different relative humidity environments (25 °C).

maltodextrin samples, Roos and Karel (1991a) have shown a constant slope of larger magnitude (−150) in the linear relationships of T_g vs a_w .

Degradation Kinetics of Saffron Carotenoid Pigments. The UV–visible spectra of aqueous extracts

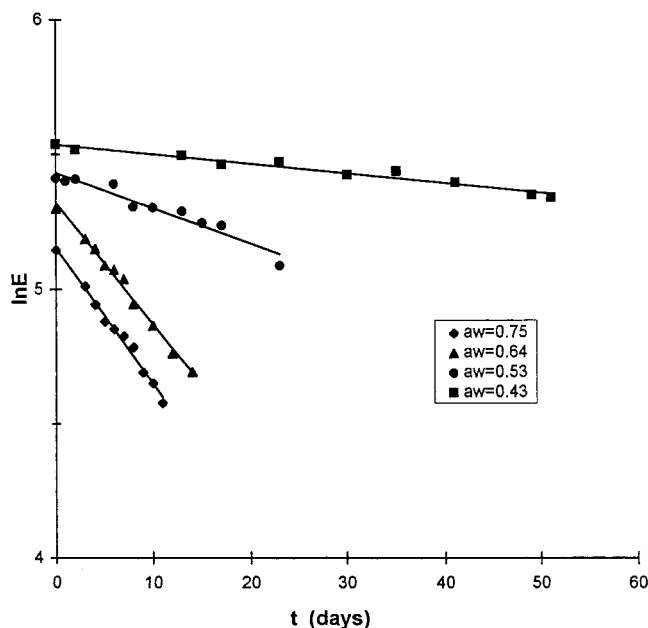


Figure 4. Degradation plots for saffron carotenoids (coloring intensity at $\lambda_{\max} = 443$ nm) following storage at 40 °C and in different relative humidity environments. Linear regression analysis was used to obtain the rate constants.

of saffron stigmas indicated a progressive decoloring of carotenoid pigments under all storage conditions employed. The spectra exhibited two major peaks at 257 and 443 nm and a minor peak at 328 nm. According to ISO specifications (ISO, 1993), absorption values at about 330 and 257 nm, expressed as $E_{\lambda}^{1\%}$, are related to safranal and picrocrocin, respectively, and used as aroma and bitterness indices; absorption at about 440 nm is related to crocins and used as the coloring-strength index of saffron. Although today it is accepted that the above maxima may be also due to the presence of other saffron components, such as *cis*-crocins or glycosidically linked moieties (Tarantilis et al., 1994; Orfanou and Tsimidou, 1996), the ISO specifications were chosen as quality indices for apparent reasons. The regression lines of $\ln E_{\lambda}^{1\%}$ vs time were linear, implying first-order reaction kinetics of coloring-strength loss (Figure 4). Similar kinetic responses were also reported by Alonso et al. (1990) for autooxidation of crocins in ground saffron samples, as well as by Tsimidou and Tsatsaroni (1993) for oxidation of saffron pigments in aqueous solutions. The values of the reaction rate constants and half-times ($t_{1/2}$), for color loss of the saffron samples stored at different temperatures and relative humidities, are summarized in Table 2.

Relative Changes in Picrocrocin and Safranal on Storage. In previous studies on autooxidation of

ground saffron (Alonso et al., 1990), it was found that the overall loss of bitterness was best described by a second-order reaction model. Analysis of the absorption data for picrocrocin loss during storage, with either first- or second-order reaction models, did show reasonably good fits, presumably due to the very slow kinetic responses. Nevertheless, some values for the relative retention of picrocrocin, in saffron samples stored under different conditions, are presented in Table 3. In the same table, the respective changes in safranal levels are also given. Considering that picrocrocin is degraded into safranal (Pfander and Schurtenberger, 1982), one of the main compounds of saffron responsible for its aroma, conditions that favor picrocrocin stability have an impact on flavor generation.

Reaction Rates in Relation to a_w . The results presented in Table 2 show that the rate of saffron carotenoid decoloring was affected by temperature and moisture content. First-order rate constants for crocin degradation, plotted against a_w (Figure 5), indicate an increase in rate with increasing a_w , particularly above the zone corresponding to the intermediate-moisture regime. This kinetic response is somewhat different from that reported in most studies concerning oxidation of carotenoids in low-moisture food systems. For β -carotene and other nonpolar carotenoids, the decoloration rate constants generally decrease and the half-life periods increase, with increasing a_w , up to an $a_w \sim 0.7$ –0.8 (Martinez and Labuza, 1968; Ramakrishnan and Francis, 1979; Goldman et al., 1983; von Elbe, 1987; Rodriguez-Amaya, 1993). The carotene loss in paprika powder and in an oleoresin–cellulose system was at a minimum at a_w s of 0.64 and 0.32, respectively (Kanner et al., 1978). Below and above these a_w levels, the rate of carotenoid destruction increased significantly. Similarly, carotenoids in dehydrated carrots were more stable at a_w of 0.32–0.57 (Arya et al., 1979b). Even for the water-soluble annato carotenoid, bixin, in an annato extract–microcrystalline cellulose model system, maximum stability was observed at intermediate/higher a_w s. The protective effect of water on oxidation of carotenoids has been explained in terms of water's direct effect on free radicals produced during the pigment's oxidation (von Elbe, 1987). The free-radical concentration can be reduced by their interaction with water molecules, a reduction that increases with the amount of water present in the system. In contrast to the findings from past studies, the kinetic data for oxidation of saffron carotenoids (Table 2) show an increasing rate with increasing a_w , within the range of 0.11–0.65. This behavior may be related to the higher water solubility of saffron carotenoids than those of other origin, favoring a greater access of dissolved oxygen to the pigments. Indeed, the moisture-content dependence of the oxida-

Table 2. Rate Constants and Half-Life Periods for Crocin Degradation in Saffron Powders, upon Storage under Different Conditions

a_w	25 °C			40 °C			60 °C		
	$k \times 10^3 \pm s_k \times 10^3$ (days ⁻¹)	$t_{1/2}$ (days)	r^2 (n) ^b	$k \times 10^3 \pm s_k \times 10^3$ (days ⁻¹)	$t_{1/2}$ (days)	r^2 (n) ^b	$k \times 10^3 \pm s_k \times 10^3$ (days ⁻¹)	$t_{1/2}$ (days)	r^2 (n) ^b
0.11	<i>a</i>			0.4 ± 0.0	1733	0.91 (10)	10.6 ± 0.6	65	0.96 (14)
0.23	<i>a</i>			0.8 ± 0.0	866	0.98 (9)	16.6 ± 0.5	42	0.98 (17)
0.33	<i>a</i>			1.7 ± 0.1	408	0.99 (12)	24.3 ± 0.6	29	0.99 (17)
0.43	0.4 ± 0.1	1733	0.65 (10)	3.6 ± 0.2	193	0.97 (15)	27.9 ± 0.8	25	0.93 (14)
0.53	1.5 ± 0.1	462	0.92 (12)	14.3 ± 0.4	48	0.99 (14)	33.6 ± 2.6	21	0.99 (14)
0.64	22.2 ± 0.4	31	0.99 (18)	44.5 ± 2.1	16	0.98 (11)	112.5 ± 2.3	6	0.99 (14)
0.75	6.6 ± 0.2	105	0.99 (13)	45.0 ± 4.2	15	0.92 (11)	210.9 ± 10.6	3	0.97 (13)

^a Calculation of reaction rate constants was not feasible, due to extremely slow reaction kinetics. ^b Correlation coefficients and number of data points for the regression lines.

Table 3. Relative Changes in Bitterness and Aroma Indices during Storage of Saffron

time (days)	a_w	bitterness (% retention) ^a	aroma (% increase) ^a
25 °C			
400	0.11	100	14
400	0.23	100	10
400	0.33	96	5
400	0.43	90	22
400	0.53	71	34
50	0.64	69	-7
40 °C			
500	0.11	91	11
500	0.23	83	17
250	0.33	77	13
250	0.43	65	20
50	0.53	77	13
15	0.64	85	1

^a Reported values are in relation to the initial levels of the two indices in saffron.

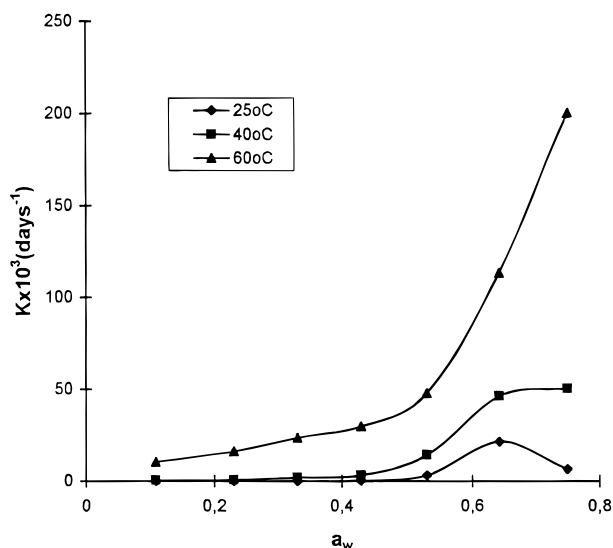


Figure 5. Degradation rates for crocins as a function of a_w . Degradation rates of saffron pigments resembled more that of reactions controlled by the mobility of reactants in foods, e.g., nonenzymatic browning, degradation of water-soluble pigments and vitamins (Leung, 1987; von Elbe, 1987). For those reactions, water accelerates the kinetics by imparting mobility to the reactants. On the other hand, an increase in water content above a certain level may decrease the rate by diluting the reactive species. In this context, the mobility factor dominates the kinetics at low a_w s, whereas the dilution effect becomes predominant in the high- a_w range. As a result, the rate may reach a maximum value at intermediate a_w s (~0.5–0.7) and then decrease with further increase in a_w . The kinetic responses observed at 40 °C, and particularly at 25 °C (rate maximum at 0.64 a_w), seem to concur with such a notion. At high temperatures (60 °C), there is a strong accentuation of pigment degradation with increasing a_w , implying that the mobility effect dominates the reaction kinetics.

Changes in bitterness and aroma indices (expressed as $E^{1\%}_{257\text{nm}}$ and $E^{1\%}_{328\text{nm}}$, respectively), as a function of a_w , showed that there was a decrease in bitter index values and a concomitant increase in aroma index values with increasing a_w . It would appear that aroma development in saffron during storage is favored in an intermediate relative humidity environment, as expected, considering that safranal is a hydrolysis product of picrocrocin (Pfander and Schurtenberger, 1982).

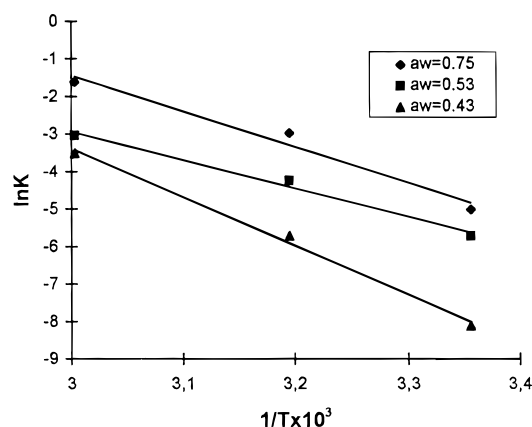


Figure 6. Arrhenius plots of rate constants for crocin degradation in saffron samples stored at different relative humidities.

Obviously, conditions favoring aroma development are not necessarily optimum for pigment stability (Figure 5).

Temperature Dependence of Degradation Rates.

The rates of deteriorative processes in food materials have often been modeled with the Arrhenius-type temperature dependence (eq 4). Plots of the experimental data, $\ln k$ vs $1/T$ (absolute temperature), showed separate lines for each a_w level (Figure 6). Similar behavior has been reported for nonenzymatic browning rates over a wide range of temperatures and at various water contents (Karmas et al., 1992; Roos and Himberg, 1994). In Figure 6, the slopes of the lines obtained by linear regression analysis gave an average activation energy (E_a) of 20 kcal/mol (83.6 kJ/mol). This value is in good agreement with values, ranging between 20 and 30 kcal/mol, reported in many previous studies on foods and other biological materials (Petriella et al., 1985; Labuza and Baisier, 1992; Roos and Himberg, 1994).

Moisture content or a_w alone cannot fully explain the relationship between water and chemical reactivity or stability of food materials (Karel et al., 1993; Slade and Levine, 1991). Constituent (reactant) mobility and the physical state of the food matrix also influence its susceptibility to chemical and physical deteriorative changes. These physical properties are directly related to the glass transition of foods and their constituents. For both polymers and low molecular weight amorphous materials, there is a large change in material properties on going through the glass transition. First, there is a large decrease in modulus (10^3 – 10^5) as a material passes from a glassy to a rubbery state. Second, functional behavior is greatly affected, as a result of dramatic acceleration of diffusive processes, as the T_g is exceeded; i.e., below T_g , molecules exhibit very low mobility, and on a practical time scale, they behave as if “frozen” in a highly viscous, nonequilibrium medium. This in turn affects all time-dependent processes related to the stability and quality attributes of the material. For food systems, in particular, there is a dramatic change in the rates of crystallization, chemical reactions, etc. (Slade and Levine, 1991). Using literature data from nonenzymatic browning studies on dehydrated vegetables, Karmas et al. (1992) concluded that the rate constants increased greatly after a certain temperature, mainly above $T - T_g \approx 40$ °C, was exceeded. Nevertheless, nonenzymatic browning was observed even at temperatures below T_g , but at a very slow rate, as reported by Karmas et al. (1992) and Roos and Himberg (1994). This was attributed to nonhomogeneous distri-

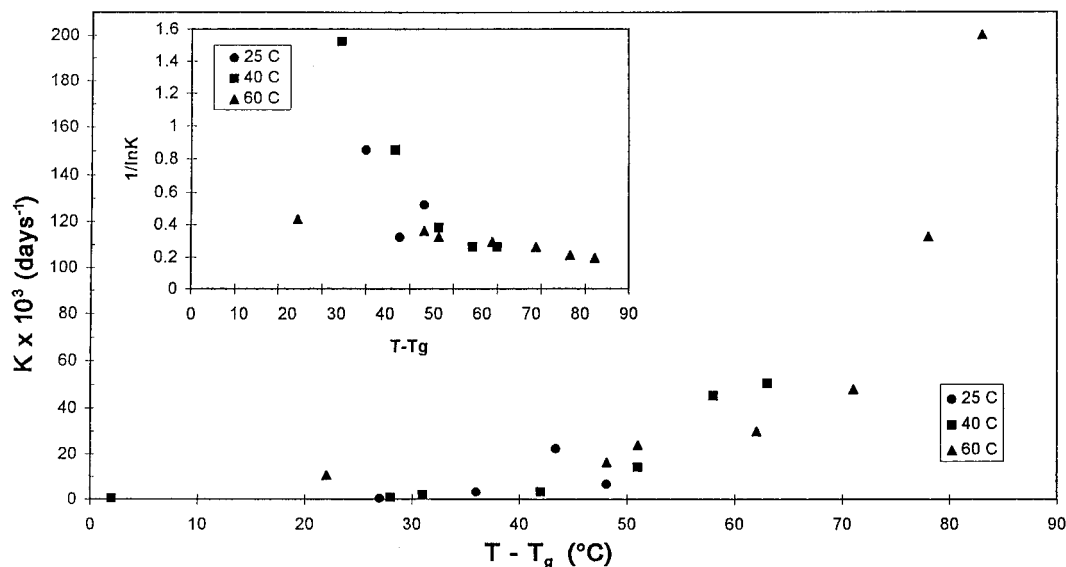


Figure 7. Temperature dependence of oxidation rates of crocins. Inset shows the corresponding WLF plot for the experimental data, $(\ln k)^{-1}$ vs $T - T_g$.

tribution of water in the food matrix, which would bring about nonuniform plasticization of the reactants. According to the glass transition theory, small molecules, such as water, act as plasticizing agents, causing a large depression in the T_g and thereby accelerating reaction kinetics. As suggested in numerous reviews by Slade and Levine (1991, 1993), the rate of chemical reactions that occur in materials that are in their rubbery state is best described by the kinetic approach developed by Williams et al. (1955), known as the WLF model (eq 5). In applying this model, the degradation rates of saffron carotenoids (crocins) were plotted as a function of $\Delta T = T - T_g$ (Figure 7). It is clear from this plot that the rate of oxidation, at each temperature studied, increased substantially, above the temperature vicinity of the glass transition. Below the T_g , oxidation seemed to be inhibited, probably because of diffusional limitations. However, an attempt to plot $(\ln k)^{-1}$ against $T - T_g$ (Figure 7, inset) did not give a single, universal line. Instead, the data were rather scattered, particularly in the low range of ΔT . It is worth noting, however, that in similar studies on the applicability of the WLF equation made by Roos and Himberg (1994) regarding nonenzymatic browning rates, using a food model system at chilling temperatures (5–20 °C), the temperature dependence of the reaction rates showed three distinct separate lines. According to Slade and Levine (1993), following the WLF model, the rates of reactions near T_g would increase by 4–5 orders of magnitude for a temperature change of 20 °C. In contrast, the corresponding Arrhenius rates would increase approximately 4-fold over the same temperature rise. As pointed out by Fennema (1996), it is only those reactions that are diffusion-limited (usually of low activation energies, 8–25 kJ/mol) which conform reasonably well to WLF kinetics. Apparently, the magnitudes of the observed rates of oxidation of saffron carotenoids, in the vicinity above the T_g (Figure 7), do not seem to support a WLF-governed dependence of k on temperature. However, unlike synthetic polymer systems for which the WLF relationship has been successfully applied, there are many factors influencing reactivity and stability of composite food matrices. For lipid oxidation, in particular, it is not clear whether the limited free volume associated with the glassy state is sufficient to restrict small reactant molecules such as oxygen from diffusing

through the amorphous matrix. Also, once in the rubbery state, where structural collapse of the metastable matrix might occur, there may be a counteracting effect to the expected increase in reaction rates as a result of higher reactant mobility. Collapse of the matrix would decrease the porosity and thereby lower the diffusion rate of oxygen to the interior of the product. This in turn could result in reduced oxidation rates, despite enhanced molecular mobility above the T_g . Moreover, any phase changes in the matrix (e.g., crystallization), in which oxidation and other deteriorative reactions occur, must be taken into account in analyzing and predicting kinetic changes within the rubbery state.

It can be concluded that stability of saffron carotenoids is dependent on the storage conditions employed. Both increasing temperature and relative humidity exert a strong influence on the degradation kinetics, accelerating the rate of pigment decomposition. Unlike the typical effect of a_w on lipid oxidation kinetics, where the rate increases below the monolayer a_w value (i.e., in the dry state), as well as above 0.3–0.4 a_w , oxidative degradation of saffron pigments increases with increasing a_w , within the range of 0.11–0.64 a_w . Water appears to have a plasticizing effect, lowering the T_g of the material and thereby enhancing the mobility of the reactants. Although the temperature dependence of degradation rates did not seem to follow a WLF-type response but rather appeared to show typical Arrhenius behavior, it is clear that pigment decomposition was inhibited at temperatures below the T_g of the matrix. The results of this study led to some practical hints about the processing and storage conditions required for producing and maintaining high-quality saffron products. The kinetics of saffron quality losses need to be further studied, by examining other factors that influence the oxidation of carotenoids or the changes in other quality attributes, e.g., light, nitrogen-flushed packaging, encapsulation in an inert matrix, presence of antioxidants, etc. Such studies will be important to establish optimum storage protocols to enhance and preserve saffron quality.

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