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Shelf Life Modeling of Photosensitive Food: The Case of Colored Beverages

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A new approach to shelf life modeling of photosensitive foods was developed taking into consideration the example of a saffron-containing yellow beverage highly prone to oxidative photobleaching. The beverage was exposed to different light levels at increasing temperatures. During exposure, samples were analyzed for bleaching rate, pigment content, and pigment degradation products. The results obtained clearly showed that shelf life testing of light-sensitive foods must take into proper account the effect of light. In addition, for these foods, shelf life models based on the sole accelerating effect of temperature may be misleading. By contrast, the concomitant exploitation of the accelerating effects of both light and temperature was used to develop and validate a simple model correctly predicting the shelf life of the beverage under actual storage conditions. The methodology proposed may allow solving of the difficult task of predicting shelf life of photosensitive foods usually marketed in the presence of light.

KEYWORDS: Light; photobleaching; ASLT; modelling; pigment oxidation; saffron

INTRODUCTION

Light is widely known to promote food degradation phenomena such as the oxidation of lipids, vitamins, and natural pigments, which results in the formation of unpleasant off-flavors, loss of nutritional value, and color fading (*1*). Exposure of photosensitive foods to the light of retail displays causes a quality depletion, whose extent is directly related to several factors, including light spectrum and intensity as well as exposure time and packaging material (*2*). Notwithstanding this awareness, for marketing reasons, photosensitive foods are commonly packed in see-through materials and exposed on highly enlightened shelves.

Despite the fact that a huge amount of literature data about the effect of light on food is available (*3–11*), no information is present about how to perform shelf life tests of light-sensitive products. In the common practice, even for light-sensitive foods, shelf life testing is carried out assessing food quality changes during storage, ignoring the effect of light. Such an approach may be fraught with risk, which often ends in dramatic shelf life underestimation. In addition, when dealing with relatively stable foods, the direct measurement of shelf life is slow and definitely not consistent with the short life span of products in competitive markets. For this reason, shelf life experiments are speeded up by testing food under temperatures higher than those usually experienced by the product. Data achieved at high temperatures are used to get proper predictive models, allowing one to quickly extrapolate shelf life at milder conditions. However, for photosensitive foods, temperature could not be the proper accelerating factor, since light-induced reactions may be scarcely temperature-dependent events. To this regard, Kristensen et al. (*9*) showed that riboflavin, a potent photosensitizer, was degraded on light exposure independently of storage temperature. Thus, it is evident that a new approach to shelf life assessment of light-sensitive food is needed. Such an approach could be based on the exploitation of light itself during shelf life assessment. Moreover, for photosensitive products, light could also represent an unconventional accelerating factor in shelf life studies. In other words, light could be used instead of or in concomitance with temperature to speed up degradation kinetics. To verify these hypotheses, the effect of light on the degradation kinetics of photosensitive foods should be evaluated.

In the present work, the capability of light in speeding up degradation reactions was investigated taking into consideration colored soft drinks, which are widely consumed all over the world. In particular, the basic requirement of soft drink attractiveness is an intense and stable color. As a consequence, for these products, photobleaching becomes a major concern during shelf life. On the basis of these considerations, to simulate a colored soft drink, a simple aqueous model system containing saffron as a colorant was prepared. Actually, saffron is a particularly interesting natural color, whose intense yellowness is mainly attributed to crocins, which are water-soluble glycosyl esters of the carotenoid crocetin. As a consequence, the quality of saffron-containing beverages is strictly related to the fate of these molecules, which are known to be highly photosensitive (*12–16*).

The saffron-containing aqueous model system was exposed to different light levels at increasing temperatures. During

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Table 1. Irradiance of the Fluorescent Tubes as a Function of Wavelength Range

wavelength (nm)	irradiance (mW/cm^2)
250−380	0.074
380−430	0.113
430−490	0.331
490−560	0.340
560−590	0.085
590−630	0.177
630−780	0.080

exposure, samples were analyzed for (i) color by a tristimulus colorimeter, (ii) crocin content by high-performance liquid chromatography (HPLC), and (iii) crocin degradation products by LC-MS. Color changes were used to obtain the bleaching rate under the different light/temperature conditions. Data were elaborated to develop a simple model predicting the beverage shelf life as a function of the light intensity to which it is exposed. In addition, the temperature dependence of the light effect on color fading was evaluated, and a shelf life predictive model accounting for the influence of both temperature and light was obtained.

MATERIALS AND METHODS

Sample Preparation and Storage. A commercial saffron powder was purchased. Saffron pigments were then isolated from the powder by methanol extraction. The solution was diluted with water to obtain the beverage model system containing 1.2×10^{-5} M crocin ($\epsilon = 1.33 \times 10^5$ L mol^{-1} cm^{-1}). Aliquots of 8 mL of the model system were introduced into 10 mL capacity vials and hermetically sealed with butyl septa and metallic caps (Carlo Erba, Milano, Italy). Additional samples were prepared by flushing nitrogen into the sample for 5 min before sealing. Preliminary trials showed that no weight changes occurred upon nitrogen flushing. The vials were stored into an incubator (Climacell 222, MMM Medcenter, Einrichtungen GmbH, Graefling, Germany) at 20 cm distance from the fluorescent tubes positioned vertically in the front door. No temperature changes were observed as a consequence of lightning. Fluorescent tubes SLI Activa-172 (34.2 W, Sylvania, SLI Lighting, Raunheim, Germany) were used. Irradiance of the fluorescent tubes was equal to 1.199 mW/cm^2 subdivided as reported in **Table 1**. The combined effect of different lighting levels (from 0 to 100% light intensity, corresponding, respectively, to 0 and 8100 Lx) and four temperatures (20, 30, 35, and 40 °C) was studied. For the validation test, a data set was obtained by storing the model system at 25 °C under increasing light conditions (from 0 to 100%).

Temperature. The sample temperature was measured (with an accuracy of ±0.2 °C) by a copper/constant thermocouple probe (type T thin thermocouple, Ellab, Denmark).

Illuminance. Illuminance was measured using a Colormaster 3F Light meter (Gossen, Neurnberg, Germany) and expressed in Lx.

Absorbance Spectra. The UV−vis spectra were recorded in the region 190−590 using a UV−vis spectrophotometer (Varian DMS 80, Mulgrave, Australia).

Color. At different lengths of time, samples were analyzed for b^* Hunter color parameters (yellow point) by a tristimulus colorimeter (Chomameter-2 Reflectance, Minolta, Osaka, Japan) equipped with a CR-200 measuring head and standardized against a white tile.

Bleaching Rate. The beverage bleaching was observed to follow a first order kinetic reaction, and the apparent bleaching rate constant (k, h^{-1}) was calculated by nonlinear regression. To evaluate the temperature dependence of k, the reparametrised Arrhenius equation was used as follows:

$$k = k_{\text{ref}} \cdot e^{\left(\frac{-E_a}{R}\right)\left(\frac{1}{T}-\frac{1}{T_{\text{ref}}}\right)} \quad (1)$$

where T is the absolute temperature (K), T_{ref} is the reference temperature, namely, the value corresponding to the mean of temperature values tested (30 °C), E_a is the activation energy (J mol^{-1}), k_{ref} is the pre-exponential factor or number of successful collisions, and R is the molar gas constant (8.31 J mol^{-1} K^{-1}). The reparametrised Arrhenius equation was chosen due to the fact that the experimental range of temperatures studied was very narrow as compared to the absolute temperature rango over which the Arrhenius equation would apply (*17*).

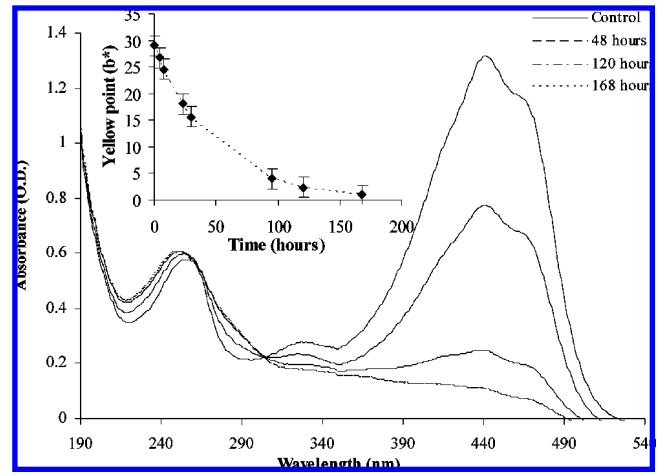

Figure 1. Absorption spectra of the saffron-containing beverage exposed to 100% light intensity at 20 °C for increasing times and relative changes in the yellow point (b^*) (inset).

E_a and k_{ref} were determined by linear regression to calculate the frequency factor:

$$k_o = e^{\left(\ln k_{\text{ref}} + \frac{E_a}{RT}\right)} \quad (2)$$

HPLC Analysis. HPLC determinations were carried out according to Vickackaite et al. (*16*). A HPLC system Varian ProStar (model 230, Varian Associates Ldt., Walnut Creek, CA) equipped with a photodiode array detector was used. For analytical purpose, an Econosil C18 (Altech, Altech Associates, Inc., Deerfield, IL, 250 mm length, 4.6 mm internal diameter, 10 μm particle size) column was used. The detection wavelength was 450 nm. The mobile phase delivered at a flow rate of 1 mL/min was acetonitrile−water (15:85 v/v) for the first minute; it was linearly changed for 24 min to acetonitrile−water (80:20 v/v) and then maintained for 6 min; at 30 min, it went back to the initial conditions. The flow rate was kept constant at 1.0 mL/min. Samples of 20 μL were injected.

LC-MS Analysis. A Finnigan liquid chromatograph−mass spectrometer (model LXQ, Thermo Electron Corp., San Jose, CA) was used. The same HPLC conditions previously reported were used, and the detection was in positive mode with the electron spray ionization (ESI) method.

Data Analysis. The results reported in this paper are the average of at least three measurements, and the coefficients of variation were found to be <3% for color and HPLC results and <10% for bleaching rate.

Analysis of variance (ANOVA) and least-squares linear regression were performed by Statistica for Windows (version 5.1, StatSoft Inc., Tulsa, OK). Significance of the differences among means was evaluated using the Tukey test. Regression significance was evaluated by considering determination coefficients (R^2) and probability value (p).

RESULTS AND DISCUSSION

An aqueous model system containing 1.2×10^{-5} M crocin was prepared to simulate a soft drink owing its yellow color to saffron. The yellowness of the freshly prepared beverage, expressed in b^* Hunter parameter, was 29.57 ± 1.91. The beverage was exposed to 100% light intensity at 20 °C. During storage, samples were analyzed for their absorption spectra (Figure **1**). Spectrophotometric analysis highlighted the presence of three major peaks at 250, 310, and 440 nm. Absorption at 440 nm is attributable to *trans*-crocins, which are the main compounds responsible for the coloring strength of saffron. Glycosidic bonds of crocins

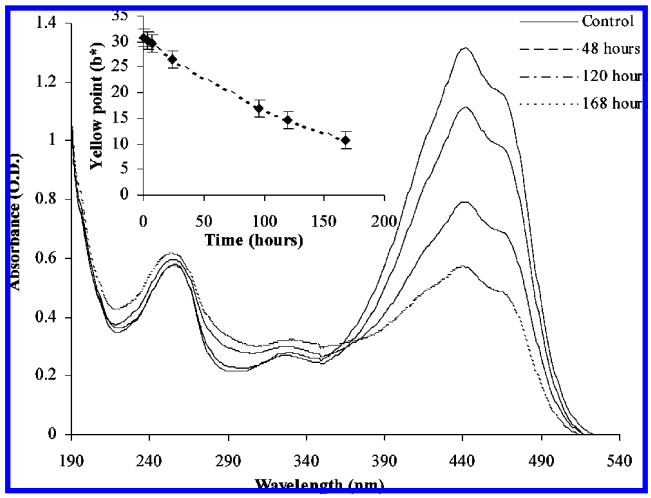

Figure 2. Absorption spectra of the saffron-containing beverage stored under dark at 20 °C for increasing times and relative changes in the yellow point (b^*) (inset).

are responsible for the 250 nm peak, while the 310 nm peak can be mainly attributed to *cis*-crocins (*12–14, 18, 19*). In fact, according to ISO specifications (*20*), absorption values at about 250 and 310 nm are also related, respectively, to picrocrocin and safranal, generally recognized as bitterness indexes of saffron. During storage under light, the intensity of *trans*-crocins (440 nm) and *cis*-crocins (310 nm) peaks decreased, leading to a progressive discoloration of the beverage, clearly evidenced by the changes in yellowness (b^* parameter, inset in **Figure 1**). In addition, an increase in absorbance at 250 nm was detected. According to the literature, such an increase would be related to the formation of crocin radicals and oxidized products (*21*). This hypothesis is also confirmed by the hypsochromic shift of the 250 nm peak. Actually, Quan Pham et al. (*22*) suggested that crocin oxidation would promote the loss of the conjugated carbon−carbon double bond system, leading to a shift to shorter wavelengths. These results suggest that the bleaching of saffron-containing beverages in the presence of light may proceed through reactions involving oxygen. In fact, further tests carried out on the beverage stored under light after removing headspace oxygen by nitrogen flushing showed no significant discoloration (data not shown).

Additional trials were carried out on saffron-containing beverages stored under dark at 20 °C (**Figure 2**). Spectrophotometric and colorimetric changes were similar to those detected under light, even if to a lower extent. However, it is noteworthy that the 310 nm peak, attributable to *cis*-crocins and safranal, was subjected to a slight increase, suggesting both a *trans* ↔ *cis* isomerization and the possible formation of safranal by cleavage of picrocrocin (*23, 24*). Such events probably occurred even under light but were counterbalanced by the concomitant degradation of these molecules upon lightening (**Figure 1**).

HPLC analysis was performed on the saffron-containing beverage stored for increasing times under 100% light intensity and under dark at 20 °C. The most important peaks appeared to be relevant to the *trans* and *cis* forms of crocetin di(β-D-gentiobiosyl) ester, having retention times of 11.91 and 14.71 min, respectively (*16*). Peak area values are reported in **Table 2**. It can be observed that *trans*- and *cis*-crocin peak area decreased upon storage under both light and dark conditions. However, analogously to what was previously observed (**Figure 1** and **2**), light promoted much higher

Table 2. *trans*- and *cis*-Crocetin Di(β-D-gentiobiosyl) Ester Peak Areas during Storage of the Saffron-Containing Beverage under 100% Light Intensity and under Dark[a]

	peak area (arbitrary units)			
	trans-crocetin di(β-D-gentiobiosyl) ester		*cis*-crocetin di(β-D-gentiobiosyl) ester	
storage time (days)	100% light	dark	100% light	dark
0	7499.991 a	7499.991 a	463.018 a	463.018 a
2	2853.957 b	5778.916 b	504.290 a	592.398 b
7	380.184 c	3147.568 c	81.783 b	258.007 c
9	284.546 d	2042.754 d	196.834 c	243.122 c

[a] Values in the same column followed by the same letter do not differ significantly ($p > 0.05$).

changes than those observed under dark, confirming the accelerating effect of light on crocin pigment bleaching.

It is noteworthy that a peak relevant to molecules absorbing at 250 nm was also detected at a retention time of 16.6 min. Such a peak could be reasonably attributable to crocin oxidation products, which are known to absorb in this wavelength range (**Figures 1** and **2**). To understand the mechanism of crocin bleaching under light, samples were analyzed by LC-MS.

The predominant signals in a freshly prepared saffron-containing beverage were *m/z* 675.44, *m/z* 837.57, and *m/z* 999.51. The first signal corresponded in mass to the crocetin di(β-D-glucosyl) ester, the second one to the crocetin (β-D-glucosyl-β-D-genziobiosyl) ester, while the third one can be attributed to the crocetin di(β-D-genziobiosyl) ester. Upon 7 days of light storage at 20 °C, LC-MS showed the signals *m/z* 709, *m/z* 871.6, and *m/z* 1033. The latter corresponded in mass to the hydroperoxyl form of the crocetin di(β-D-glucosyl) ester, crocetin (β-D-glucosyl-β-D-genziobiosyl) ester, and crocetin di(β-D-genziobiosyl) ester, respectively. These observations clearly suggest an oxidation process going on. Moreover, there were also signals of *m/z* 365 and 367, due to alcohol and keton forms obtained by cleavage of the monohydroperoxyl form of the crocetin di(β-D-glucosyl) ester. A similar mass spectrum was also detected for the saffron-containing beverage stored for 7 days under dark at 20 °C. The latter, however, differed from the enlightened one because it still contained the starting reagents (i.e., crocetin esters having mass *m/z* 675.44, *m/z* 837.57, and *m/z* 999.51).

Although saffron bleaching has been largely attributed to the isomerisations of the *trans*-crocins to the less colored *cis* isomers (*15*), these findings highlighted the critical role of oxidative reactions. In particular, it can be inferred that the color fading of the saffron-containing beverage is mainly attributable to the formation of the uncolored hydroperoxi-crocins, according to the pathway reported in **Figure 3**.

On the basis of these results, the light dependence of color fading of the saffron-containing beverage was evaluated. To this aim, samples were stored at 20 °C under increasing light conditions (from 0 to 100% of light intensity), and pseudo-first order rate constants ($R^2 > 0.97$, $p < 0.05$) were obtained (**Figure 4**).

As the light intensity increased, the bleaching rate of the saffron-containing beverage was faster, indicating that light exerts a dramatic effect on crocin oxidation. These observations clearly evidence that light can speed up quality depletion of photosensitive foods, such as saffron-containing beverages, thus strongly affecting their shelf life. Consequently, its role cannot be underestimated but is necessary to be taken into account to get accurate and reliable shelf life data. Moreover, the linear relation between bleaching rate and light intensity

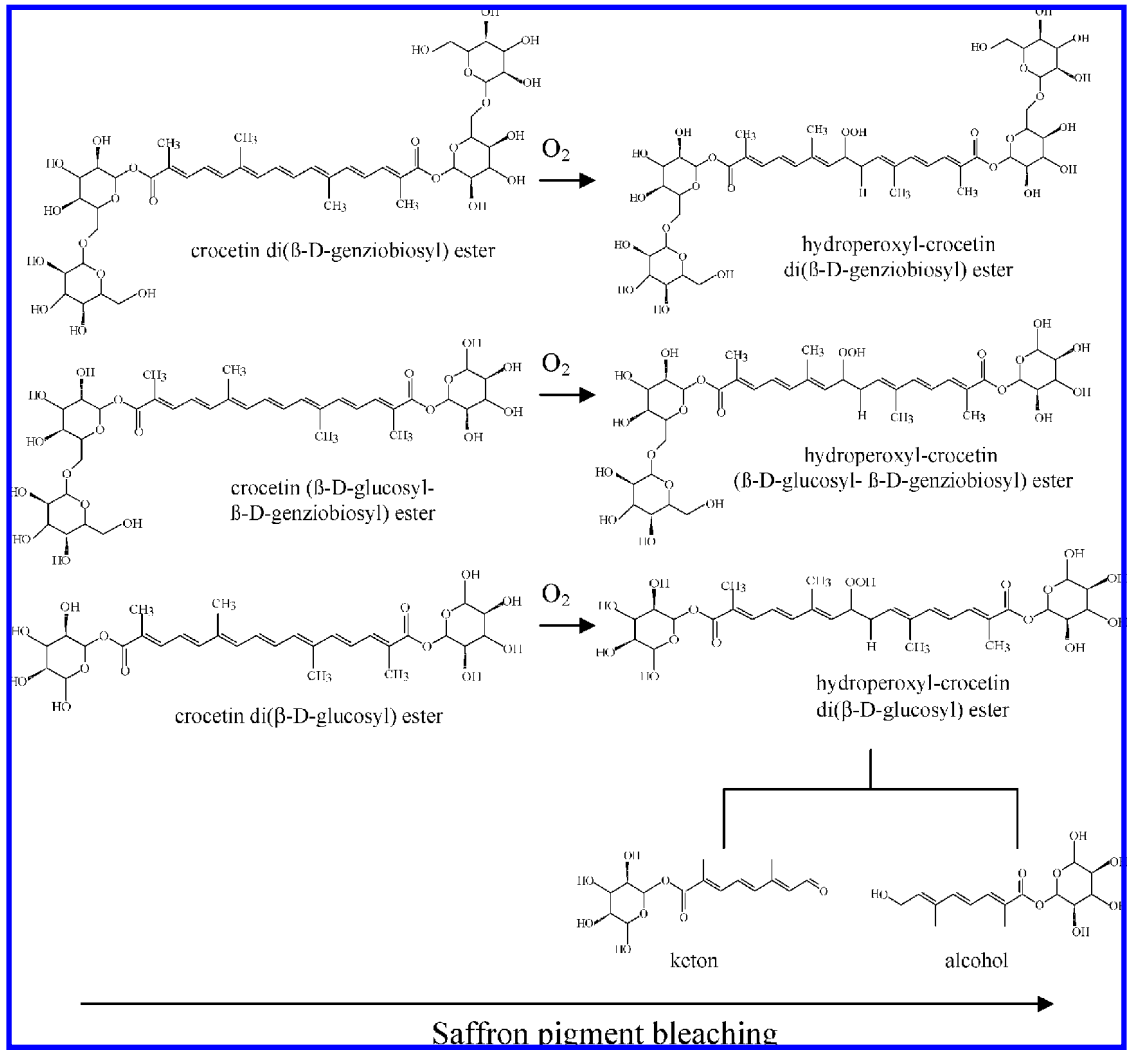

Figure 3. Bleaching pathway of the saffron-containing beverage.

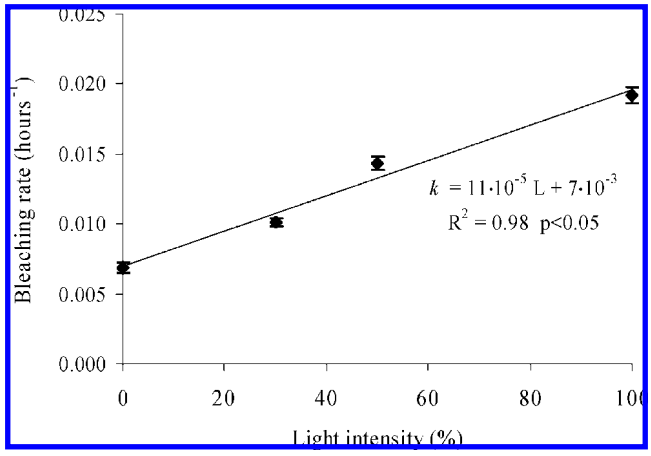

Figure 4. Pseudo-first order bleaching rate constants (k) of the saffron-containing beverage stored at 20 °C under increasing light intensity (L). Results of linear regression analysis are also shown.

indicates that light can be easily used as an accelerating factor in shelf life accelerated tests by measuring the bleaching rate under increasing light and then extrapolating the rate at milder conditions usually experienced by the product on the retail shelves.

In light of these considerations and according to the first order kinetics of bleaching, the general model for shelf life prediction of the saffron-containing beverage is

$$SL_{L,T} = \frac{\ln a - \ln b}{k_{L,T}} \quad (3)$$

where $SL_{L,T}$ is the shelf life at a the selected light intensity (L) and temperature value (T), a and b are the final (corresponding to the acceptability limit) and the initial color values, respectively, and $k_{L,T}$ is the rate constant.

For the considered beverage model system, having an initial b^* value of 29.57 and stored at 20 °C, the shelf life model is as follows:

$$SL_{L,20} = \frac{\ln a - \ln 29.57}{11 \times 10^{-5} L + 7 \times 10^{-3}} \quad (4)$$

Equation 4 represents a simple model allowing prediction of color fading of the beverage as a function of light intensity at room temperature. It can be observed that the only independent variables of the model are light intensity, which are the enlightened conditions experienced by the beverage on the retail shelves, and the acceptability yellowness limit, which is chosen by the company on the basis of its commercial policy.

It must be noted that shelf life models are traditionally based on the accelerating effect of temperature since most reactions

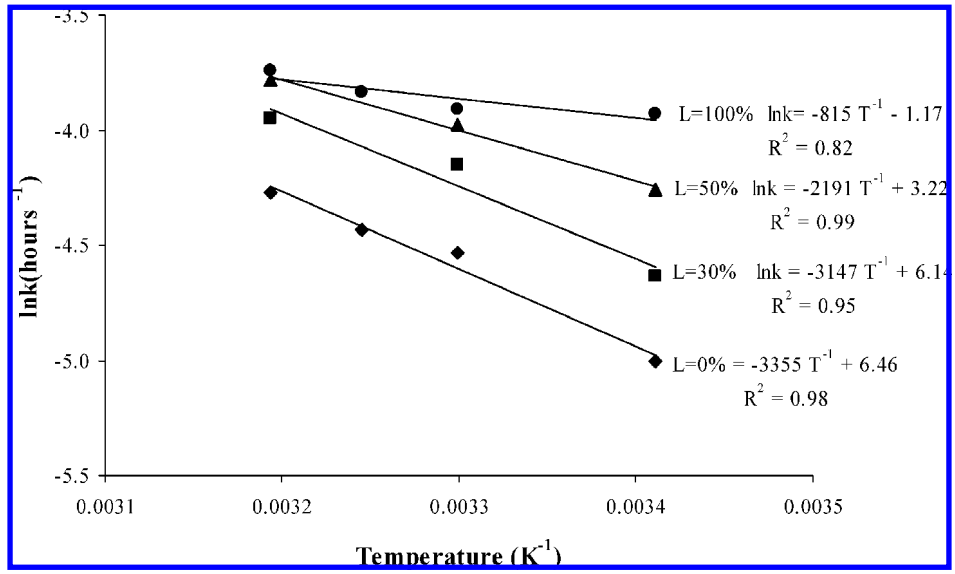

Figure 5. Arrhenius plot of pseudo-first order bleaching rate constants (k) of the saffron-containing beverage exposed to different light intensity (L).

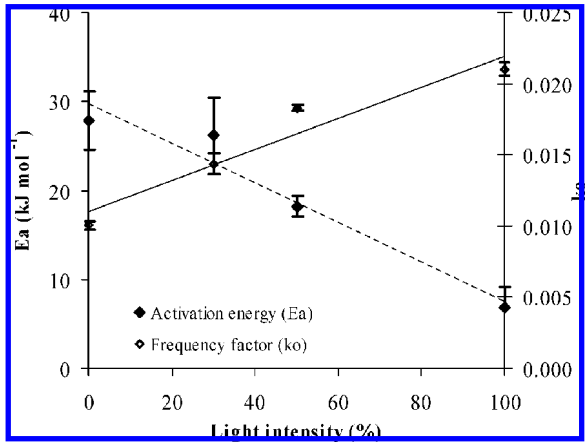

Figure 6. Activation energy (E_a) and frequency factor (k_o) values for the bleaching of the saffron-containing beverage exposed to increasing light intensity (%).

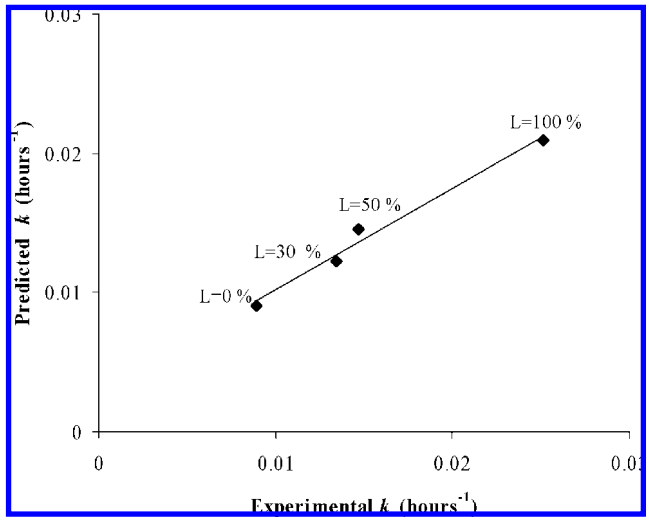

Figure 7. Pseudo-first order bleaching rate constants predicted by eq 5 as a function of experimental bleaching rates constants (k) of the saffron-containing beverage stored at 25 °C under increasing light conditions.

are strongly dependent on this environmental factor (*25–28*). However, it has been previously observed that light-induced reactions may be scarcely temperature-dependent events (*9*). To evaluate whether temperature can be used to accelerate food photodegradation or not, the saffron-containing beverage was stored in the presence of different light intensities at 30, 35, and 40 °C. The bleaching rates were evaluated, and results were plotted according to the Arrhenius model (**Figure 5** and eq 1), which is conventionally used to evaluate the temperature effect on reaction rates (*29–33*). The Arrhenius model led to a good prediction of temperature dependence of saffron bleaching under all lighting levels considered. However, while under dark, a strong temperature dependence of the beverage bleaching was observed; at increasing light intensity, temperature did not have such a marked effect. This result indicates that the accelerating effect of temperature is strongly affected by enlightened conditions. Thus, the temperature dependence of the quality depletion of the beverage stored under dark cannot be used to predict that of the same beverage stored in the presence of light.

In agreement with the Arrhenius equation, the activation energy (E_a) and frequency factor (k_o) were thus calculated (eq 2). The effect of light on E_a and k_o was then evaluated by plotting their values as a function of light intensity (**Figure 6**).

By regression analysis, it was possible to find the equation describing the light (L) dependence of activation energy (E_a) and frequency factor (k_o) ($R^2 > 0.92$, $p < 0.05$):

$$E_a = -223.37L + 29819.02 \quad (5)$$

$$k_o = 11 \times 10^{-5}L + 11 \times 10^{-3} \quad (6)$$

As it can be seen, E_a decreased with the increase in light intensity, confirming that light affected the temperature dependence of the reaction. Regarding k_o, it is noteworthy that its increase was limited within low values. This suggests that, despite their low number, all collisions between crocins and oxygen (**Figure 4**) are efficient enough to let bleaching proceed. On the basis of these considerations, it is useful to integrate into a single model the effect of both variables, light and temperature. Thus, eqs 5 and 6 were substituted into the Arrhenius equation:

$$k = (11 \times 10^{-5}L + 11 \times 10^{-3})e^{\left(\frac{223.37L+29819.02}{R}\right)\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)} \quad (7)$$

Such a model is particularly useful and versatile since it allows the prediction of the bleaching rate in different experi-

mental conditions. In fact, if the beverage is stored in the dark ($L = 0$) at increasing temperatures, the model is brought back to the Arrhenius equation. By contrast, if the beverage is stored at room temperature under increasing light intensity, the model returns to eq 4. Finally, model 7 allows prediction of the bleaching rate at any combination of temperature and light intensity within the experimental range considered.

To verify the reliability of the model proposed, a validation test on external data was carried out. In particular, a saffron-containing beverage stored at 25 °C under increasing light conditions was considered. k values were predicted by substituting light and temperature data in eq 7. Predicted k values were compared with those experimentally assessed (**Figure 7**). It can be observed that the proposed model allowed a very good prediction of the bleaching rate of the saffron beverage. In fact, regression analysis carried out between experimental and predicted data pointed out a very low standard error (8×10^{-3}), a determination coefficient (R^2) close to 1 ($p < 0.05$), and random residuals.

To achieve a shelf life model accounting for both the effect of light and the temperature on the quality depletion of the saffron beverage, eq 7 was substituted in eq 3. The general shelf life model results as follows:

$$SL_{L,T} = \frac{\ln a - \ln 29.57}{(11 \times 10^{-5} L + 11 \times 10^{-3}) e^{\left(\frac{223.37L + 28819.02}{R}\right)\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)}} \quad (8)$$

The results obtained clearly showed that shelf life testing of light-sensitive foods must take into proper account the effect of light. In addition, for these foods, shelf life models based on the sole accelerating effect of temperature may be tricky. By contrast, for photosensitive products, light was demonstrated to be a useful unconventional accelerating factor. The specific application was relevant to colored soft drinks, but the procedure adopted in this paper can definitely be extended to many other photosensitive foods. In fact, the approach based on the concomitant exploitation of the accelerating effects of both light and temperature may allow solving the difficult task of predicting shelf life of photosensitive food usually marketed in the presence of light. In addition, another advantage could be represented by the exploitation of light instead of temperature to quickly predict shelf life of foods, which, because of their chemical, physical, and chemico-physical nature, are not able to withstand high temperature shelf life tests (e.g., fresh foods and frozen foods).

LITERATURE CITED

(1) Beckbölet, M. Light effects on food. *J. Food Prot.* **1990**, *53*, 430–440.

(2) Lennersten, M.; Lingnert, H. Influence of wavelength and packaging material on lipid oxidation and colour changes in low-fat mayonnaise. *LWT-Food Sci. Technol.* **2000**, *33*, 253–260.

(3) Attoe, E. L.; Von Elbe, J. H. Photochemial degradation of betanine and selected anthocyanins. *J. Food Sci.* **1981**, *46*, 1934–1937.

(4) Bradley, D. G.; Min, D. B. Singlet oxygen oxidation of food. *Crit. Rev. Food. Sci. Nutr.* **1992**, *31*, 211–236.

(5) Rafsgaard, H. H.; Rasmussen, M.; Skibsted, L. H. Light sensitivity of colourants used in alcoholic beverages. *Lebensm. Unters. Forsch.* **1993**, *197*, 517–521.

(6) Solomon, O.; Svanberg, U.; Sahström, A. Effect of oxygen and fluorescent light on the quality of orange juice during storage at 8 °C. *Food Chem.* **1995**, 363–368.

(7) Andersson, K.; Lingnert, H. Influence of oxygen concentration and light on the oxidative stability of cream powder. *LWT-Food Sci. Technol.* **1998**, *31*, 169–176.

(8) Ramìrez, G.; Hough, G.; Contarini, A. Influence of temperature and light exposure on sensory shelf life of a commercial sunflower oil. *J. Food Qual.* **2001**, *24*, 195–204.

(9) Kristensen, D.; Hansen, E.; Arndal, A.; Tinderup, R. A.; Skibsted, L. H. Influence of light and temperature on the colour and oxidative stability of processed cheese. *Int. Dairy J.* **2001**, *11*, 837–843.

(10) Frankel, N. Photooxidation of unsaturated fats. Lipid Oxidation; The Oil Press, PJ Barnes & Associates: Bridgwater, England, 2005; pp 51−66.

(11) Pan, X.; Ushio, H.; Ohshima, T. Effect of molecular configurations of food colourants on their efficacies as photosensitizers in lipid oxidation. *Food Chem.* **2005**, *92*, 37–44.

(12) Tsimidou, M.; Tsatsaroni, E. Stability of saffron pigments in aqueous extracts. *J. Food Sci.* **1993**, *58*, 1073–1075.

(13) Tarantilis, P. A.; Polissiou, M.; Manfait, M. Separation of picrocrocin, cis-trans-crocins and safranal of saffron using high-performance liquid chromatography with photodiode-array detection. *J. Chromatogr. A* **1994**, *664*, 55–61.

(14) Orfanou, O.; Tsimidou, M. Evaluation of the colouring strength of saffron spice by UV−vis spectrometry. *Food Chem.* **1996**, *57*, 463–469.

(15) Tsimidou, M.; Biliaderis, C. G. Kinetic studies of saffron (*Crocus sativus* L.) quality deterioration. *J. Agric. Food Chem.* **1997**, *45*, 2890–2898.

(16) Vickackaite, V.; Romani, A.; Pannacci, D.; Favaro, G. Photochemical and thermal degradation of a naturally occurring dye used in artistic painting. A chromatographic, spectrophotometric and fluorimetric study on saffron. *Int. J. Photoenergy* **2004**, *6*, 175–183.

(17) Van Boekel, M. A. J. S. Statistical aspects of kinetic modeling for food science problems. *J. Food Sci.* **1996**, *61* (3), 477–486.

(18) Van Calstrein, M. R.; Bissonnette, M. C.; Cormier, F.; Dufresne, C.; Ichi, T.; Leblanc, J. C. Y.; Perreault, D.; Roewer, I. Spectrosopic characterization of crocetin derivatives from *Crocus sativus* and *Gardenia jasminoides*. *J. Agric. Food Chem.* **1997**, *45*, 1055–1061.

(19) Lonzano, P.; Castellar, M. R.; Simancas, M. J.; Iborra, J. L. Quantitative high-performance liquid chromatographic method to analyse commercial saffron (*Crocus sativus* L.) products. *J. Chromatogr. A* **1999**, *830*, 477–483.

(20) ISO Saffron (*Crocus sativus* Linnaeus): (a) ISO-3632-1-1993 Part 1: Specification; (b) ISO-3632-2-1993 (E) Part 2: Test methods. International Organization for Standardization: Geneva, 1993.

(21) Jorgensen, K.; Skibsted, L. H. Carotenoid scavenging of radicals. Effect of carotenoid structure and oxygen partial pressure on antioxidative activity. *Z. Lebensm.-Unters. Forsch. A* **1993**, *196*, 423–429.

(22) Quan Pham, T.; Cormier, F.; Farnworth, E.; Han Tong, V.; Van Calstrein, M. R. Antioxidant properties of crocin from *Gardenia jasminoides* Ellis and study of the reactions of crocin with linoleic acid and crocin with oxygen. *J. Agric. Food Chem.* **2000**, *48*, 1455–1461.

(23) Iborra, J. L.; Castellar, M. R.; Cánovas, M.; Manjón, A. TLC preparative purification of picrocrocin, HTCC and crocin from saffron. *J. Food Sci.* **1992**, *57* (3), 714–716.

(24) Zoughagh, M.; Ríos, A.; Varcárcel, M. Determination of total safranal by in situ acid hydrolysis in supercritical fluid media: application to the quality of commercial saffron. *Anal. Chim. Acta* **2006**, *578*, 117–121.

(25) Ragnarsson, J. O.; Labuza, T. P. Accelerated shelf-life testing for oxidative stability on foods—A review. *Food Chem.* **1977**, *2*, 291–308.

(26) Labuza, T. P.; Schmidl, M. K. Accelerated shelf life testing of foods. *Food Technol.* **1985**, *39*, 57–64.

(27) Waterman, K. C.; Adami, R. C. Accelerating testing: prediction of chemical stability of pharmaceuticals. *Int. J. Pharm.* **2005**, *293*, 101–125.

(28) Corradini, M. G.; Peleg, M. Shelf life estimation from accelerated data. *Trends Food Sci. Technol.* **2007**, *18*, 37–47.
(29) Arrhenius, S. A. *Larobok I Teoretisk Elektrokeni*; Quando&Handel: Leipzig, 1901.
(30) Labuza, T. P.; Riboh, D. Theory and application of Arrhenius kinetics to the prediction of nutrient losses in food. *Food Technol.* **1982**, *10*, 66–74.
(31) Calligaris, S.; Manzocco, L.; Conte, L. S.; Nicoli, M. C. Application of a modified Arrhenius equation for the evaluation of oxidation rate of sunflower oil at subzero temperatures. *J. Food Sci.* **2004**, *69* (8), 361–366.
(32) Manzocco, L.; Calligaris, S.; Nicoli, M. C. Modeling bleaching of tomato derivatives at subzero temperatures. *J. Agric. Food Chem.* **2006**, *54* (4), 1302–1308.
(33) Manzocco, L.; Nicoli, M. C. Modelling the effect of water activity and storage temperature on chemical stability of coffee brews. *J. Agric. Food Chem.* **2007**, *55* (16), 6521–6526.

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