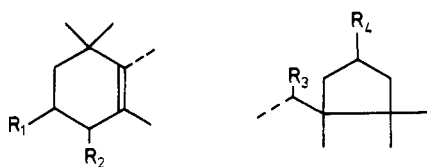
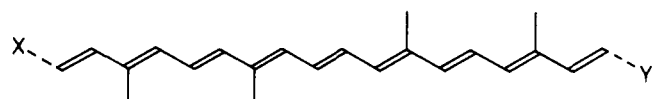


Photocatalyzed Oxidation of Paprika Pigments

Joseph Carnevale, Edward R. Cole,* and George Crank

The carotenoid pigments of paprika powder undergo photocatalyzed oxidation on exposure to fluorescent light at a rate dependent on the intensity of illumination. The rate of pigment degradation can be closely correlated with change in surface color. Relationships have been established which enable predictions of stability for various exposure conditions. The process shows an induction period, is subject to inhibition by antioxidants, and presumably involves free-radical reactions. As the light absorbing species the carotenoids catalyze their own degradation. Attention is drawn to the differing roles of carotenoids in photochemical processes according to whether the pigment functions as energy transducer or absorbing photoenergy in the presence of oxygen catalyzes self-decomposition.

The spice paprika, prepared from the dried ground fruits of *Capsicum annum* or *Capsicum frutescens*, and often referred to as red pepper, is used as a color and flavor additive to foods. Its deep red color, upon which rests much of its commercial value, is due to a complex mixture of carotenoids in which capsanthin and capsorubin are present in the greatest amounts. Other carotenoids include hydrocarbons such as β -carotene and its variously oxygenated derivatives including cryptoxanthin, zeaxanthin, and violaxanthin. Extensive studies have demonstrated the complexity of the mixture of pigments of paprika and bell peppers (Cholnoky, 1937, 1939, 1954; Garcia, 1951; Benedek, 1957; Cholnoky et al., 1958; Sacetta, 1960; Curl, 1962, 1964). More recent work on paprika oleoresin has shown that 37-54 pigments may be present, depending on the mode of preparation (extraction of unbleached or bleached paprika), of which only 21 and 33, respectively, could be completely or even tentatively identified (De La Mar and Francis, 1969). The major pigments capsanthin and capsorubin, together with cryptoxanthin and ze-



β -carotene	A	A
capsanthin	B	C
capsorubin	C	C
A, $R_1 = H, R_2 = H$		
B, $R_1 = OH, R_2 = H$		
C, $R_3 = O, R_4 = OH$		

xanthin, have been shown to be present as fatty acid esters (Zechmeister and Cholnoky, 1934), with capsanthin occurring as the dilaurate ester (Philip et al., 1971).

Paprika also contains 10-14% fat (Lease and Lease, 1956a) with a high degree of unsaturation (Philip et al., 1971). The fatty acid distribution in fat from whole paprika and pods, respectively, has been found to be of the order: linoleic acid, 66 and 45%; palmitic acid, 12 and 19%; oleic acid, 12 and 14%; and linolenic acid, 5 and 17%. Small quantities of shorter chain saturated myristic and

lauric acids were present with traces only of capric and stearic acids.

The long conjugated unsaturation of carotenoids renders them prone to oxidative degradation in the solid state illustrated by breakdown of lycopene at 50 °C (Cole and Kapur, 1957), as well as in solution. Atmospheric oxygen has been found to be mainly responsible for the destruction of carotenoids in dried alfalfa (Ogden, 1956). It has also been shown that oxidation of capsanthin by molecular oxygen at 40 °C in the solid states gives a number of ketocarotenoids such as capsanthone, 3-ketokryptocapsone and 3-keto- β -apo-8'-carotenal, indicating that the oxidation reactions primarily involved the hydroxyl groups, and scission of the chain at the carbon-carbon bond α to the in-chain carbonyl group (Philip and Francis, 1971). The oxygen absorption curve showed no induction period, from which it was concluded that the reaction mechanism did not involve autoxidation. The rates of oxidation of carotenes in hydrocarbon solvent systems have been extensively studied (Bickoff et al., 1955; Budowski and Bondi, 1960; El-Tinay and Chichester, 1970; Ramakrishnan, 1974).

Because of the association of carotenoids and fat in nature, considerable attention has been given to the concept of coupled oxidations incidental to the autoxidation of fat. It is on this point that the role of the unsaturation of the fat is not clear. Some reports have shown that increased unsaturation gives increased carotenoid stability (McDonald, 1933; Mitchell et al., 1954; Bickoff et al., 1955), while contrary reports have appeared claiming that unsaturated fats decrease stability (Budowski and Bondi, 1960; Lime, 1969; Blain and Shearer, 1963).

Similarly, the role of β -carotene in photochemical oxidation of unsaturated fats is also not conclusive. On the one hand, it has been shown that the pigment catalyzes an increased rate of fat autoxidation (Emanuel and Lyaskovskaya, 1967), but on the other, more recent reports suggest an inhibiting effect (Clements et al., 1973; Sattar et al., 1976). Factors such as the free radical character of the photocatalyzed loss of β -carotene from solutions in fatty acid esters, its inhibition by antioxidants, and interpretation as a self-catalyzed destructive reaction have been examined (Carnevale et al., 1979).

These factors have now been examined with respect to paprika powder, where a different type of substrate is involved with a different set of carotenoid pigments. A particular feature of the present investigation is the degree of correlation between loss of surface color and of carotenoids in photocatalyzed oxidations.

EXPERIMENTAL SECTION

Materials and Apparatus. Ground Hungarian paprika was obtained from Paprika Trading Co., N.S.W. All solvents used were analytical grade. Surface color measurements were carried out on a Hunter Lab 25D color

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Table I. Light Intensity at Sample Surface at Levels Used for Photooxidation of Paprika Pigments

level	distance from light, mm	light intensity, lux
1	175	6564
2	313	3766
3	460	2367
4	600	1668

difference meter. The instrument was calibrated on the pink tile with predetermined L , a , and b values. Light intensities were measured with an EEL Light Master Photometer, calibrated in lumens/square foot. Illuminance values are reported in units of lux, where lux = lumen/square foot \times 10.76.

PROCEDURES

Measurements of Surface Color Change. Rates of color change at room temperature were made on paprika powder (5.0 g accurately weighed) in a petri dish (50-mm diameter) pressed gently with a stopper (48-mm diameter) so as to form a smooth, firm, even layer of material (4.3 mm thick). A set of sample dishes was prepared from one batch of well-mixed paprika powder, and the Hunter L , a , and b values were determined. At least three measurements of these parameters on each sample were made since variations due to irregularities in the surface of the powder and the position of the sample in the light beam slightly affected the reproducibility of measurements. The samples were rotated in the instrument between measurements.

Exposures. Dishes were placed in the center of a tray (110 \times 12 \times 5.5 cm) provided with a sealable glass cover (held in position with a series of G clamps) and a gas inlet/outlet at opposite ends of the tray to maintain a slow flow of moist oxygen over the samples. Light was provided by two parallel 40-W cool white fluorescent tubes.

Photooxidations were carried out at different distances from the light source as shown in Table I, which also records the light intensity at the surface of the sample (under the glass). Since there was a slight drop in intensity output from the ends of the fluorescent tubes, samples were placed at the center portion of the tray where variation was minimal.

In a similar manner, dishes containing a smaller amount of paprika powder (1.5 g, 1.3-mm thickness) were exposed to light plus oxygen.

Hunter L , a , and b values for each sample were measured at appropriate intervals during exposure and immediately after withdrawal from the apparatus. Changes in surface color were measured in terms of ΔE , where $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$, and a/b ratio.

Dissection of Light/Oxygen Effects. A control experiment, in which paprika powder was stored in an atmosphere of oxygen in the dark, was run concurrently with the photocatalyzed oxidations.

Samples were also exposed in a series of individual trays (9.5 \times 9.5 \times 2.5 cm) each provided with a gas tight glass cover and a gas inlet/outlet to maintain a positive pressure of moist nitrogen gas (approximately 30 mL/min). The use of individual sample trays in this experiment, as distinct from the larger trays used for the oxygen atmosphere, allowed the removal of samples for surface color measurements without permitting access of air. The samples were exposed to light at level 2 (3766 lux light intensity). After completion of the series of exposures in a nitrogen atmosphere, the samples were transferred to the larger tray (110 \times 12 \times 5.5 cm) for exposure at the same level in an oxygen atmosphere.

Antioxidant Protection. The effect of the antioxidant ethoxyquin at concentrations of 0.1, 0.02, and 0.005% was measured on samples exposed at level 1 (6564 lux intensity). The antioxidant was incorporated into the paprika by a stepwise dilution process. A concentrate on paprika (5.00%) was prepared by applying a solution of ethoxyquin (10%) in acetone to the powder, mixing during the addition, and subsequently removing solvent under vacuum at room temperature. Dilutions of this with more powder gave the lower concentrations. This method of incorporation minimized disturbing effects on the nature of the surface of the paprika.

Pigment Losses. Photooxidized paprika powder (5-g and 1.5-g samples) each exposed to light intensities 3766 lux and 1668 lux was extracted with acetone (9 \times 10 mL) until the extract was colorless. The combined extracts were diluted to 100 mL. An aliquot (10.0 or 25.0 mL, depending on whether 5.00- or 1.50-g samples were used) was further diluted to 100 mL with acetone. From the visible spectra of these solutions absorbances at 440, 450, 460, and 478 nm were determined. Control samples (3 \times 5.00 g or 3 \times 1.50 g) of unoxidized paprika powder, from the same batch as that used for the oxidation experiments, were similarly extracted and absorbances of the final solutions were measured at the same wavelengths. Loss of total extractable pigment from the oxidized samples at each wavelength was calculated by reference to the average value obtained for the unoxidized material at the same wavelength.

Losses of total pigments have been calculated as percentage loss on total sample as well as percentage losses for the surface depth of 1 mm, assuming that all loss occurred in the surface layer to this depth.

Effect of Photooxidation on the Color of Pigment-Free Paprika Powder. The effect of photooxidation on changes in nonacetone extractable pigments (5.0-g paprika samples) was determined by returning the dried depigmented samples to their respective sample dishes, smoothing to an even layer as before, and then measuring L , a , and b values.

RESULTS AND DISCUSSION

A statistical summary of variations in measurement of Hunter L , a , and b values of sample dishes prepared from one batch of well-mixed paprika powder was prepared. Since the mean L , a , and b values for the individual dishes were significantly different from the overall mean L , a , and b values, each separate dish required measurement before and after exposure to light.

In the first stage gradual darkening of the surface occurred, indicated by change from the characteristic deep red color to a red brown. Finally the surface lightened to a yellow brown color, devoid of all redness. The speckled and slightly roughened appearance of the powder was due to difference in the types of tissue present.

Results for loss of surface color of samples exposed at levels 1-4 are summarized in Figure 1, expressed as ΔE values, and in Figure 2, expressed as change in a/b ratio.

The change in surface color in the early stages in not linear with time, but involves a lag phase leading to a period of more rapid losses where the relationship is partially linear.

The rate of change of ΔE /hour (K_1), calculated from the linear portion of curves 1, 2, 3, and 4 of Figure 1, gives a linear relationship to the illumination (E), fitting the regression equation

$$K_1 = 3 \times 10^{-5} E$$

with correlation coefficient 0.999. In view of the color

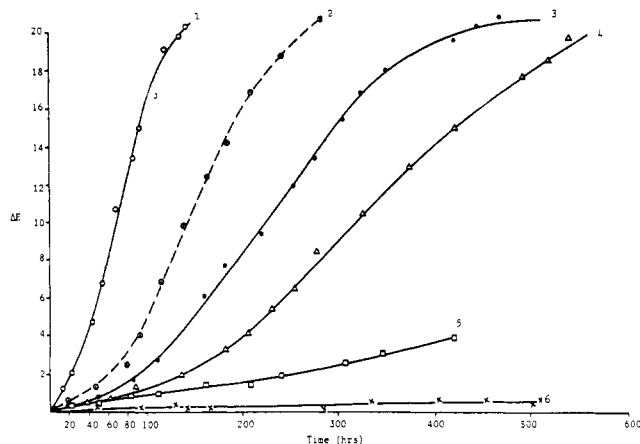


Figure 1. Surface color change (ΔE) of paprika: in an oxygen atmosphere exposed to 6564, 3766, 2367, 1668, and 0 lux (curves 1, 2, 3, 4, and 6, respectively); in nitrogen atmosphere exposed to 3766 lux (curve 5).

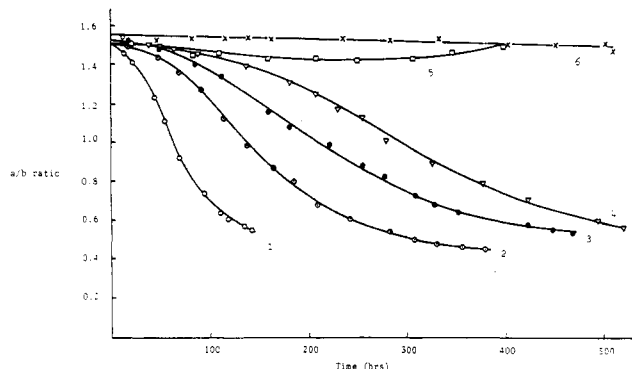


Figure 2. Change in the a/b ratio of paprika: in oxygen atmosphere exposed to 6564, 3766, 2367, 1668, and 0 lux (curves 1, 2, 3, 4, and 6, respectively); in nitrogen atmosphere exposed to 3766 lux (curve 5).

changes mentioned previously, it follows that it is not possible to derive a similar relationship for the a/b ratio.

Since the relationship between illumination and intensity of the light (I) is given by the ratio I/r^2 , where r is the distance to the light source, it would be expected that the log rate of change of ΔE ($\log K_1$) would be related to the log distance ($\log r$) from the light source. Indeed a linear relationship was found to fit the regression equation

$$\log K_1 = -1.070 \log r + 1.708$$

with correlation coefficient 0.998.

The induction periods for the different levels of illumination, determined by using the double tangent technique on the curves of Figures 1 and 2, are shown in Table II. Agreement between the two sets of results was reasonable, considering the fact that two different parameters were being used. Plots of log induction times (T_1 and T_2) against log illumination ($\log E$) gave linear relationships expressed by nearly identical regression lines:

$$\log T_1 = -1.130 \log E + 5.852$$

$$\log T_2 = -1.147 \log E + 5.880$$

These results mean that it becomes possible to predict the rate of photodegradation of surface color from a knowledge of either the level of illumination or the distance from a light source of known intensity. The induction period for the oxidation can also be predicted from a knowledge of the same parameters.

Change in sample size, to 1.50 g, and exposure to 3766 lux gave similar changes in ΔE , showing that change in surface color is independent of sample size at a given il-

Table II. Comparison of Induction Periods for the Photooxidative Degradation of Surface Color from ΔE and a/b Ratio

illumination, lux	induction period (T_1) ^a for ΔE (Figure 1)	induction period (T_2) ^a for a/b ratio
6564	33	31
3766	73	63
2367	101	100
1668	165	152

^a Hours.

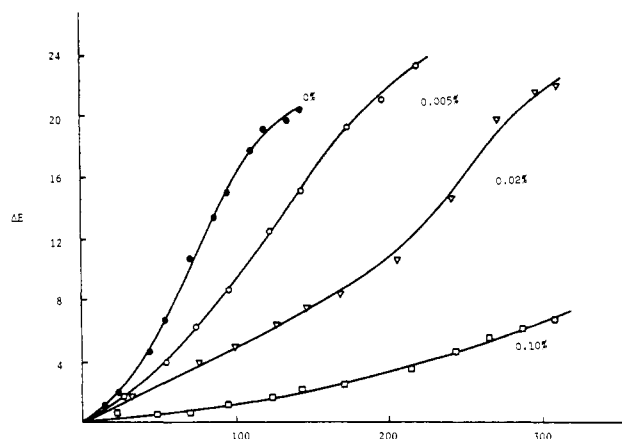


Figure 3. Ethoxyquin protection of paprika surface color of sample exposed to 6564 lux in oxygen.

lumination, thus confirming the phenomenon as a surface change only, without penetration to the interior of the sample.

The extent of separate contributions of oxygen and light to color changes as ΔE and a/b ratio are small compared to those produced by the combination (Figures 1 and 2).

Exposure in Nitrogen Atmosphere. Samples previously exposed to light in a nitrogen atmosphere when exposed to 3766 lux in oxygen gave color loss curves practically identical with those obtained on direct exposure, especially for exposure times less than 200 h, indicating that any loss of pigments during exposure to light in nitrogen had little effect on the mechanism for the photooxidative losses. The association of photooxidation with incident illumination is clear.

Protection by Antioxidant. Ethoxyquin has been found one of the most effective antioxidants for the protection of paprika color against losses during storage (Lease and Lease, 1956b). It is also effective against color degradation by ultraviolet light and direct sunlight (Chen and Gutmanis, 1968).

At 0.1% concentration very efficient inhibition to pigment loss was given in samples exposed at level 1 (6564 lux). Even with 0.005% concentration a significant increase in the induction period was obtained together with retardation in the rate of color loss (Figure 3).

Expression of Pigment Losses. It is possible to discuss pigment losses in several contexts, as a fraction of the total pigment content of the sample or as loss per unit area of the surface for a given depth.

Although spectra were surveyed over the visible range with particular attention given to absorptions at 440, 450, 460, 478 nm, the uniformity of changes at these wave lengths means that changes at 450 nm represents the extent of change at the other wavelengths. From measurement at 450 nm expressed as a fraction of the total pigment of a 5-g sample, the loss is only 12% even in a sample with completely bleached surface. The loss appears as a bigger

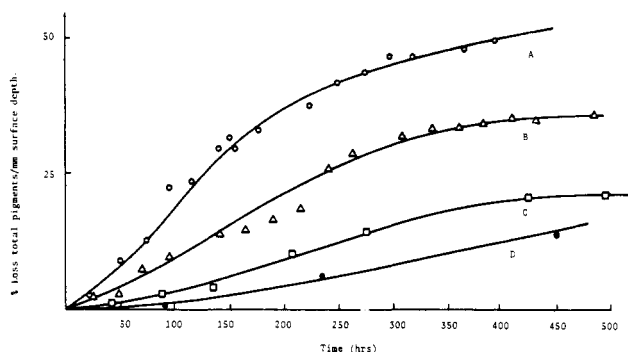


Figure 4. Loss of total pigments from paprika samples exposed to light in oxygen atmosphere: (A, C, D) 5.0-g samples exposed to 3766, 1668, and 0 lux, respectively; (B) 1.5-g samples exposed to 3766 lux.

Table III. Linear Correlation of Total Pigment Losses (as Percentage of First Millimeter of Surface) and ΔE Value and a/b Ratio of Samples Exposed to Varying Illumination^a

		illumination, lux		
		3766, 5.0-g sample	3766, 1.5-g sample	1668, 5.0-g sample
for ΔE value	m	2.27	1.33	1.59
	b	-0.89	2.07	0.28
	r	0.981	0.983	0.978
for a/b ratio	m	-42.24	-29.96	-30.12
	b	62.77	45.02	45.58
	r	0.964	0.971	0.983

^a Data were fitted to the equation $Y = mX + b$ and r is the regression coefficient.

fraction (30%) when the smaller 1.5-g sample is used.

A more realistic presentation of the results is given if calculations are expressed as loss per unit area per 1-mm depth (Figure 4).

Results expressed in this manner for the 5-g sample then approximate closely those for the 1.5-g sample where the total depth was 1.3 mm.

The rate of loss curves of total pigments at a given illumination are similar to those for the rate of surface color loss. Indeed the high degree of correlation between changes in ΔE and a/b ratio is shown on Table III.

Therefore the values ΔE and a/b ratio may be used to express loss of pigment under specified illumination.

The rate curves for the loss of color indicate an autocatalytic reaction with an induction period dependent on the intensity of light and susceptible to protection by an antioxidant. Thus a free radical type reaction is suggested. In this respect the process is identical with that previously found for solutions of β -carotene in fatty acid methyl esters (Carnevale et al., 1979). Although present discussion concerns an apparently solid material, the paprika pigments may be regarded as occurring also in fat solution. By analogy with the β -carotene solutions, it could therefore be presumed that a degree of protection is offered by the unsaturation of the fat (Rawls and Van Santen 1970a, b).

However, attention must be drawn to the unique contrasting roles of carotenoids in photochemical processes. On the one hand it has been established that β -carotene functions as a protective agent in plant tissues, acting as energy transducer without loss of pigment in dissipating the energy of singlet oxygen formed in limited amounts as a product of chlorophyll-sensitized absorption of photoenergy. On the other hand, where the carotenoids themselves function as the energy absorbing species in simpler media, and with greater availability of oxygen, the pigments promote their own destruction.

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Received for review December 3, 1979. Accepted April 4, 1980.