Time-Dependent Postirradiation Oxidative Chemical Changes in Dehydrated Egg Products

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Radiation-induced oxidative chemical changes in whole egg and egg yolk powder were followed in time after irradiation as a function of dose, dose rate, and storage atmosphere. In evacuated samples of whole egg powder the decay of lipid hydroperoxides (LOOH) was pseudo-first order ($k = 0.088$ day^{-1}), while carotenoids did not decay at all. In the presence of air both lipid hydroperoxides and carotenoids decayed during postirradiation storage. The decay of LOOH could be treated by dispersive kinetics with the measure of dispersion, $\alpha = 0.51 \pm 0.05$, independent of dose, and the effective lifetime τ inversely related to dose. The decay of carotenoids could also be treated by dispersive kinetics, with the values of α decreasing with increasing dose. The effective lifetimes of carotenoids did not depend on dose in samples irradiated in vacuum. In samples irradiated and stored in air the effective lifetimes decreased with dose, faster in egg yolk than in whole egg powder. The complex nature of postirradiation kinetics in solid food systems is discussed.

INTRODUCTION

Egg is a basic raw material used in many areas of the food industry (Horn, 1977). Dried egg has the additional advantages of easier handling, lighter weight, and longer storage time, while retaining excellent functional properties. However, dehydrated eggs also present a number of microbiological and chemical problems. Drying does not necessarily kill the entire pathogenic microflora, and additional microbicidal treatment is often necessary (Bomar, 1970).

Chemical problems with dehydrated eggs are often caused by the large surface area, which makes dried products particularly susceptible to oxidation (Hubbard et al., 1989). Exposure of egg powder to agents promoting oxidative deterioration is usually avoided by adhering to the principles of good manufacturing practice; however, deliberate exposures to heat (ICMSF, 1980) or radiation (Farkas, 1988) are needed to pasteurize or sterilize foods.

Drying and irradiation are intended to improve the stability and hygienic status of egg, respectively, as well as the status of the food products which contain it. On the other hand, radiation-induced oxidative chemical changes may persist in dry egg after the irradiation has ended, causing undesirable organoleptic changes. Thus, the temporal stability accomplished by drying becomes compromised by the means to accomplish the hygienic quality. Often, a delicate balance of risk and benefit must be considered when solutions made available by technology are evaluated.

The consideration of risks and benefits involved in irradiating foods has resulted in the recommendation made by the Joint Expert Committee on Food Irradiation (JECFI, 1980) that the wholesomeness of irradiated foods should be evaluated on the basis of radiation chemistry criteria. This requires quantitative knowledge of radiation-induced chemical changes in irradiated foods.

We have shown earlier that a 2.4-kGy dose of γ irradiation was adequate for a *Salmonella* inactivation factor of 10^3 in egg powder (Matić et al., 1990) without adverse effects on the organoleptic properties of the product, which begin to deteriorate above 3 kGy in air (Katušin-Ražem et al., 1989). However, some peroxidation was unavoidable on irradiation, even under vacuum (Katušin-Ražem et al., 1992).

Considering the postirradiation persistence of free radicals in irradiated egg powder (Diehl, 1972) and postirradiation changes of lipid peroxides in irradiated solid food model systems (Wills, 1980), it is conceivable that radiation-induced hydroperoxides in dry egg products will also continue to change after irradiation. This work deals with the time dependence of lipid hydroperoxides (LOOH) in irradiated dry whole egg and egg yolk powder, as well as with the hydroperoxide-mediated postirradiation changes of carotenoids. These changes were followed as a function of dose, dose rate, and storage atmosphere, and the complex nature of postirradiation kinetics is discussed.

MATERIALS AND METHODS

Materials. Commercial samples of whole egg powder and egg yolk powder were obtained from two manufacturers of dehydrated food products, several batches from each.

Determination of Lipids. The amount of lipids in samples was determined by extraction with a 2:1 mixture of chloroform–methanol in a Soxhlet apparatus or by shaking with a cold deaerated solvent mixture (Warren et al., 1988). Dry whole egg samples contained between 42 and 51% lipids, while dry egg yolk contained about 61% lipid. The profile of fatty acids in the lipidic component was determined by gas chromatography of methylated fatty acids on a 2-m, 3 mm i.d., column filled with 15% OV 275 on Chromosorb WAW 80–100 at 170 °C with 13 mL/min N_2 and flame ionization detector (FID) (Christie, 1982). Retention times were determined with authenthic compounds. Quantitation was carried out using the methyl ester of heptadecanoic acid as an internal standard and assuming the FID response factor 1 for fatty acid methyl esters relative to the standard. The major unsaturated fatty acids, oleic and linoleic, were between 21% in whole egg and 29% in egg yolk and between 2.5% in whole egg and 4.2% in egg yolk, respectively.

To account for the variability of the contents of lipids among the samples, the level of lipid hydroperoxides was expressed per unit mass of lipid (L) (millimoles of LOOH per kilogram of L).

Determination of Carotenoids. Carotenoids were determined in a $CHCl_3$–MeOH extract by spectrophotometry against a blank (an extract of whole egg powder irradiated with 20 kGy or of egg yolk powder irradiated with 40 kGy to destroy the characteristic absorption of carotenoids). The absorbance of the longest wavelength peak of carotenoids at 478 nm was used.

Sample Preparation, Irradiation, and Dosimetry. Samples weighing 10–50 g were sealed in polyethylene pouches in the presence of air. Samples to be irradiated and stored in vacuum were evacuated and sealed in laminated aluminum pouches using

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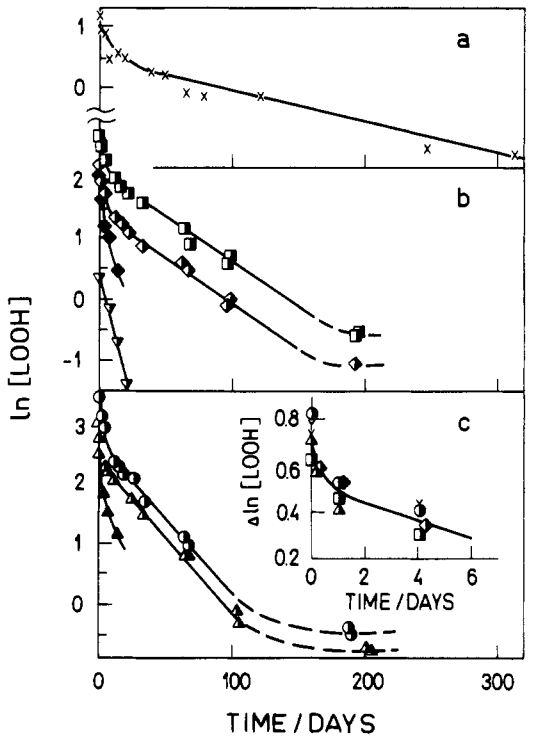

Figure 1. Decay of LOOH in dry egg products irradiated with (a) 2, (b) 4, and (c) 10 kGy. (Half-solid symbols and ×'s) Whole egg powder; (solid symbols) egg yolk powder. (Squares and circles) 0.4 Gy/s; (diamonds and triangles) 4 Gy/s; (inverted triangles) evacuated samples. [Insert in (c)] Difference between the actual [LOOH] and the values obtained by extrapolation of the pseudo-first-order decay to short times.

an industrial vacuum packaging machine (e.g., for coffee). Irradiations were carried out with ^{60}Co γ rays in a panoramic irradiator and in a well-type irradiator, both constructed by the Ruđer Bošković Institute. The dose rates varied from 4 Gy/s in the panoramic irradiator to 0.4 Gy/s in the well-type irradiator, as determined by the ethanol–chlorobenzene dosimetry (Ražem et al., 1985). Irradiated samples were stored in the dark.

Determination of Lipid Hydroperoxides. Hydroperoxides formed by irradiation were determined by spectrophotometry of the ferric thiocyanate complex. Ferrous iron was first oxidized by LOOH, and the formed ferric ions complexed with thiocyanate, which yields an intense red color (Tsoukalas and Grosch, 1977). Our modification of this method consisted mainly of the development of the ferric thiocyanate complex and its measurement directly in the extraction medium, a deaerated $CHCl_3$–MeOH (2:1) mixture, where it was characterized by λ_{max} = 485 nm and ϵ_{485nm} = 13 710 dm^3 mol^{-1} cm^{-1}) (Mihaljević, Katušin-Ražem, and Ražem, unpublished results). Some more commonly used methods for the determination of LOOH, such as the thiobarbituric acid (TBA) test or the measurement of conjugated dienes, could not be used because the major unsaturated fatty acid in egg, oleic acid, yields no response to these methods.

RESULTS

Lipid hydroperoxides induced by irradiation in dry whole egg and egg yolk decayed with time after the irradiation. The rate of decay depended on the presence of oxygen. The analysis of the decays in various samples in terms of the first-order kinetics is given in Figure 1. The decay in whole egg powder irradiated and stored in the presence of air can be separated into three intervals: the first initial interval was fast decay, the intermediate interval was pseudo-first-order decay, and the final interval was very slow decay.

The pseudo-first-order mode of LOOH decay lasted long enough to establish its pseudo-first-order character with some certainty: 2.5 half-life periods in samples irradiated with 2 kGy and 4 half-life periods in samples irradiated with 4 and 10 kGy, respectively. The rates of the pseudo-first-order decays depended on the total absorbed dose: at larger doses the decay was faster, as seen in Figure 1. They did not, however, depend on the dose rate. The pseudo-first-order rate constants were 0.0058 day^{-1} at 2 kGy, 0.0166 day^{-1} at 4 kGy, and 0.0256 day^{-1} at 10 kGy.

The level of remaining LOOH approached in the interval of the final very slow decay depended on the dose and dose rate; it was higher at higher doses and lower dose rates (for the given dose). Throughout the decay of hydroperoxides in samples irradiated with the same dose no crossover occurred: samples that contained the higher level of LOOH initially also retained it finally.

The separation of the initial fast decays by subtracting the pertaining underlying pseudo-first-order decays showed that the fast decaying parts were also independent of the dose rate and apparently independent of the dose (Figure 1c, insert).

It is seen in the insert to Figure 1c that the initial decay itself can be further resolved: it also consists of the fast decaying part and a pseudo-first-order decaying part (k = 0.038 day^{-1}). Apparently the decay of LOOH in irradiated dry whole egg is a complex process which cannot be characterized by a single rate constant; it should be better characterized by the time-dependent rate constant.

The decay of LOOH in egg yolk irradiated and stored in air was considerably faster than that in whole egg powder and apparently also proceeded with a time-dependent rate constant. On the other hand, the decay of LOOH in whole egg irradiated in vacuum and stored in vacuum appeared to be a pure pseudo-first-order process (k = 0.088 day^{-1}), with no sign of leveling off as it approached the background level of LOOH inherent to whole egg powder.

The analysis of the time-dependent decay of LOOH in whole egg powder irradiated and stored in the presence of air has been attempted here in terms of the dispersive kinetics (Plonka, 1988). The first-order reaction kinetics with a time-dependent rate constant has the form

$$[\mathrm{LOOH}]/[\mathrm{LOOH}]_0 = \exp[-(t/\tau)^{\alpha}] \quad (1)$$

where [LOOH] and $[\mathrm{LOOH}]_0$ denote the levels of LOOH at time t and time zero, respectively, and τ is the effective lifetime required for the LOOH level to decrease to $1/e$ of the initial value (approximately 37%).

$$\tau = (\alpha/B)^{1/\alpha} \quad (2)$$

The value of α has been related to the dispersion of reactivities; in heterogeneous systems it is proportional to the fractal dimension (Plonka, 1991).

Taking the logarithm of the ln of the eq 1, the equation of the straight line is obtained

$$\log(\ln[\mathrm{LOOH}]_0/[\mathrm{LOOH}]) = \alpha \log t - \alpha \log \tau \quad (3)$$

of which α is the slope and $\alpha \log \tau$ the intercept.

Experimental results for whole egg irradiated and stored in air calculated in this way are shown in Figure 2. The pertaining numerical values of α and τ are given in Table I. The effective lifetime decreased with increasing dose and amounted to 44 days at 2 kGy, about 20 days at 4 kGy (5 days for yolk), and 10 days at 10 kGy (7 days for yolk). The slopes α were rather uniform, the average of seven values being 0.51 ± 0.04.

Although apparently a pseudo-first-order process, the decay of LOOH in evacuated whole egg samples was also amenable to the same type of analysis. As expected, it yielded a value of α close to 1 (0.912).

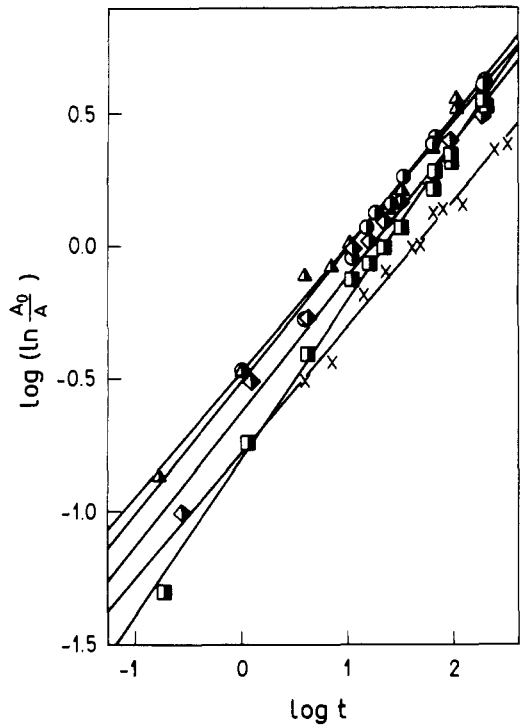

Figure 2. Dispersive kinetics treatment of the data for whole egg powder from Figure 1. Symbols for doses and dose rates are the same as in Figure 1.

Table I. Dispersive Kinetics Parameters of the Postirradiation Decay of Lipid Hydroperoxides in Irradiated Whole Egg and Egg Yolk Powder

	whole egg under atmosphere of (irr/stor)						egg yolk under atmosphere of air/air (irr/stor) at dose rate of 0.4 Gy/s	
	vac/vac at dose rate of 4 Gy/s		vac/air at dose rate of 4 Gy/s		air/air at dose rate of 0.4 Gy/s			
dose, kGy	α	τ, days	α	τ, days	α	τ, days	α	τ, days
2			0.474	44				
4			0.510	17	0.589	24	0.523	5
4.6	0.912	13						
10			0.473	10	0.505	10	0.469	7

Carotenoids in evacuated samples which were kept under vacuum after irradiation did not decay at all during the storage period of 100 days (not shown).

Carotenoids in samples which were irradiated under vacuum and subsequently kept in equilibrium with air displayed a behavior similar to the decay of LOOH: at least two pseudo-first-order decay periods could be observed (Figure 3). Only unirradiated samples kept in equilibrium with air showed a single slow pseudo-first-order decay of carotenoids throughout the studied time interval ($k = 6.9 \times 10^{-4}$ day^{-1}). Although the shape of the kinetic curve for unirradiated samples does not justify the dispersive kinetics treatment of these data, such treatment is nevertheless possible and is shown with data for irradiated samples in Figure 4. The increase of dose resulted in the increasing dispersion of the reactivities which are responsible for the depletion of carotenoids, as evidenced by the departure of parameter α from unity: from 0.907 for unirradiated samples to 0.446 for samples irradiated with 9 kGy (Table II).

Irradiation of whole egg powder in air, as compared to irradiation in vacuum, resulted in the larger immediate destruction of carotenoids (Katušin-Ražem et al., 1992) as well as in their faster postirradiation decay (Figure 5). The terminal levels of carotenoids were too low to observe the time dependence of the decay rate constant over an extended interval of time. Decays observed at lower doses seemed to be pseudo-first-order processes, while those at higher doses displayed a time dependence of the rate constant.

Nevertheless, it was possible to apply dispersive kinetics treatment to all data (Figure 6) and to observe the same

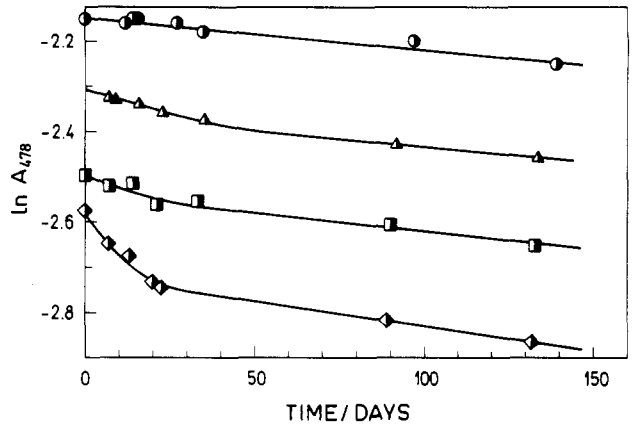

Figure 3. Decay of carotenoids in whole egg samples irradiated under vacuum and stored in air. (Circles) 0 kGy; (triangles) 3 kGy; (squares) 6 kGy; (diamonds) 9 kGy.

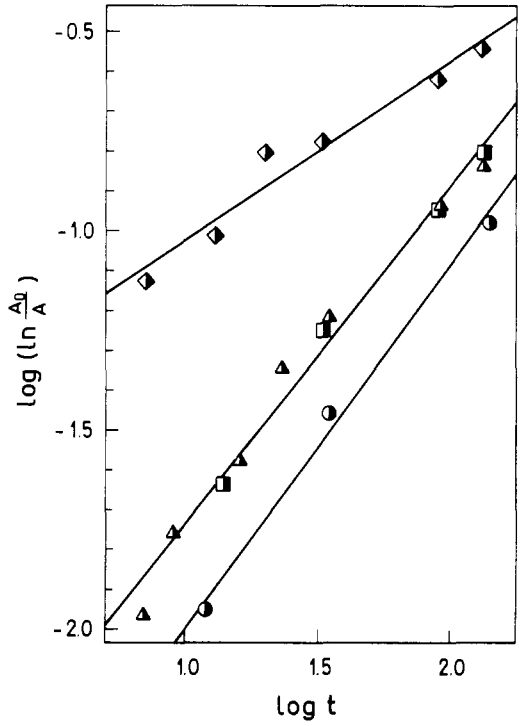

Figure 4. Dispersive kinetics treatment of the data from Figure 3. Symbols for doses are the same as in Figure 3.

Table II. Dispersive Kinetics Parameters of the Postirradiation Decay of Carotenoids in Irradiated Whole Egg and Egg Yolk Powder

	whole egg under atmosphere of (irr/stor)				egg yolk under atmosphere of air/air (irr/stor) at dose rate of 4 Gy/s	
	vac/air at dose rate of 4 Gy/s		air/air at dose rate of 4 Gy/s			
dose, kGy	α	τ, days	α	τ, days	α	τ, days
0	0.907	1587				
2			0.672	1224	0.873	531
3	0.838	1194				
4			0.602	377	0.859	166
5					0.637	94
6	0.831	1200	0.437	210		
9	0.446	1957				

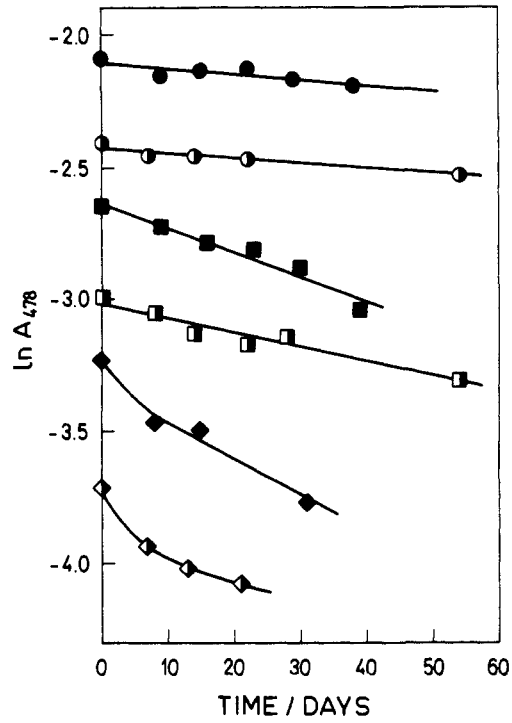

Figure 5. Decay of carotenoids in dry whole egg (half-solid symbols) and egg yolk (solid symbols) irradiated and stored in equilibrium with air. (Circles) 2 kGy; (squares) 4 kGy; (diamonds) 5 kGy (yolk) and 6 kGy (whole egg).

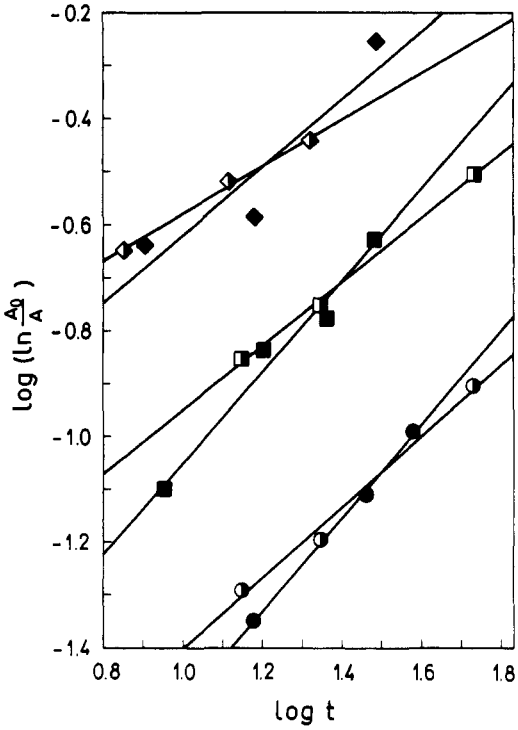

Figure 6. Dispersive kinetics treatment of the data from Figure 5. Symbols for doses are the same as in Figure 5.

regularity (Table II)—increasing the dose decreases the values of α: from 0.672 at 2 kGy to 0.602 at 4 kGy and to 0.437 at 6 kGy. The same considerations apply to egg yolk powder, where a decrease of α from 0.873 at 2 kGy to 0.637 at 5 kGy was observed.

The effective lifetimes of carotenoids did not depend on dose in samples irradiated in vacuum. In samples irradiated and stored in air, the effective lifetimes of carotenoids decreased with dose, and this decrease was faster in egg yolk than in whole egg.

DISCUSSION

It has been known for a long time that lipid hydroperoxides formed in foods and food model systems by irradiation undergo a change with time after the end of irradiation. The time course of the postirradiation change was first an increase and then a decrease of the level of LOOH. This type of change was observed in fish (Snauwert et al., 1977), as well as in solid food model systems (Gower and Wills, 1986; Wills, 1980). The decrease of LOOH was explained by the loss of volatile compounds with time, which would have otherwise reacted with TBA (Gower and Wills, 1986). In recognition of these problems, it was required that the time elapsed between the irradiation and analysis should be indicated (Diehl and Kim, 1981). In the present work the measure of LOOH level is the ability to oxidize ferrous iron, which shall be further referred to directly as LOOH.

In the absence of air, low levels of LOOH formed (1.5 mmol of LOOH/kg of L) disappear in whole egg powder either by recombination with some species in sufficient excess for the reaction to be pseudo-first order or by a genuine first-order decay. Candidate species for the destruction of LOOH could be free radicals which should be in a large excess of 1.5 mmol/kg of L. A 10-fold excess would require that the radiation–chemical yield of radicals $G(R^{\bullet})$ (μmol J^{-1}) be in excess of

$$G(R^{\bullet}) = \frac{(1.5 \times 10^{-2}\ \text{mol/kg of L}) \times 0.4\ \text{kg of L/kg}}{4000\ \text{J/kg}} = 1.5\ \mu\text{mol/J}$$

and a very long lifetime, conditions not likely to exist. For example, steady-state radical concentration in lyophilized lysozyme irradiated in air with 10–20 kGy was of the order of 10^{-9} spins/kg of protein (Schaich, 1980).

In the presence of air the decays of LOOH in whole egg and egg yolk powder become strongly time dependent. While increasing the dose decreases the effective lifetime of LOOH, it has a very small effect on the dispersion of reactivities, i.e., on the values of α. We understand it as a relative insensitivity of the geometrical factors to irradiation, both below and over the breakpoint dose of 2.5 kGy, at which chain oxidation starts (Katušin-Ražem et al., 1992). We maintain that the decrease of reactivity with time is related to the subsequent depopulation of the more reactive (decomposition-prone) LOOH molecules embedded in the powder matrix. The decrease of the effective lifetime with increasing dose is understood to be the result of increasing the spatial density of events leading to the depletion of LOOH.

The inverse dose-rate effect on the buildup of LOOH was explained (Katušin-Ražem et al., 1992) by fewer free radicals being formed in any segment of space and time at lower dose rate, leaving the radicals already formed to react with neighboring molecules, and thus propagate the chain, rather than to recombine with other radicals. Once formed, the decomposition of LOOH would be governed by the density of reactive species (proportional to the total dose), which would explain the lack of the dose-rate effect on the postirradiation kinetics of LOOH.

This requires that reactive species which participate in the destruction of LOOH be different from the primary free radicals formed by irradiation. The ESR evidence tells us that free radicals formed in the absence of lipids (pure egg white) are different from and more numerous than those formed in either whole egg or egg yolk (Katušin-Ražem et al., 1992). The presence of lipids facilitates both the transformation and the loss of free radicals, and we

believe that the faster decay of LOOH in egg yolk is also the consequence of these effects.

The amount of carotenoids in dehydrated egg products is rather low (about 46 μmol/kg of whole egg powder and about 63 μmol/kg of egg yolk powder), so that little direct radiation-induced decomposition occurs, as already observed in evacuated samples. Indirect action of radiation on carotenoids is due to the action of radiation-induced lipid hydroperoxides (Katušin-Ražem et al., 1992). It is reasonable to assume that the postirradiation decay of carotenoids is also related to the presence and reactivity of LOOH. It must also be related to the presence of oxygen, because a slow pseudo-first-order decrease of carotenoids ($k = 6.9 \times 10^{-4}$ day^{-1}) was observed in unirradiated samples exposed to air, where the background level of LOOH was 0.11 mmol of LOOH/kg of L (Katušin-Ražem et al., 1990), and not in evacuated and subsequently irradiated samples, where the level of LOOH was 1.5 mmol/kg of L, an order of magnitude higher than that in unirradiated samples due to the adsorbed oxygen remaining after the removal of headspace oxygen.

There is a large excess of LOOH over carotenoids in all irradiated samples: up to 30 times in evacuated samples and up to 3000 times in equilibrium with air. Nevertheless, dispersive kinetics treatment seems to be more generally applicable than the pseudo-first-order treatment. The observed decrease of α with increasing dose is in agreement with the behavior of α observed in LOOH decay. This would be expected if α were characteristic of the medium proportional to the fractal dimension (Plonka, 1991).

The effective lifetimes of carotenoids were about 30 times longer than those of LOOH at corresponding doses. However, taking into account that concentrations of carotenoids were about 30–3000 times lower as compared to those of LOOH, the decay of carotenoids normalized to the number of moles turns out to be comparable to or faster than that of LOOH.

It is interesting to note that oxidative decoloration of surface β-carotene was characterized by the same pseudo-first-order rate constant, 0.088 day^{-1}, in a low-moisture food model system (Chou and Breen, 1972), as well as in potato flakes, $k = 0.083$ day^{-1} (Neto et al., 1981). At the same time, bound β-carotene in potato flakes decayed at a pseudo-first-order rate of 8.6×10^{-4} day^{-1} (Neto et al., 1981), as compared to 6.9×10^{-4} day^{-1} in our unirradiated samples.

It would be presumptuous to hypothesize about the mechanisms of the temporal changes of LOOH and carotenoids without identifying the products of these changes and without accounting for the materials balance. These tasks would be extremely difficult in a complex natural material such as an egg. Therefore, the discussion was necessarily limited to phenomenology. Little more has been possible with other kinetic approaches to the oxidative deterioration of foods (Labuza, 1984; Labuza and Ragnarsson, 1985; Özilgen and Özilgen, 1990). We expect that a more comprehensive discussion of the observed phenomena would be possible under controlled conditions reproduced in a synthetic model system, which will be the subject of our subsequent work.

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