Light-Induced Changes in an Aqueous β-Carotene System Stored under Halogen and Fluorescent Lamps, Affected by Two Oxygen Partial Pressures

Sara Limbo,* Luisa Torri, and Luciano Piergiovanni

Department of Food Science and Microbiology, University of Milan, Via Celoria 2,
20133 Milan, Italy

The aim of this work was to investigate the reaction kinetics of β-carotene in an aqueous medium as a function of exposure to commercial lights (halogen and fluorescent sources) and oxygen partial pressures. The evolution of the pigment concentration, the changes in color, and the accumulation of a volatile compound (β-ionone) were monitored during storage. The kinetics of degradation were mathematically modeled to compare the effects of lighting conditions and oxygen partial pressures. Lighting was also a critical variable in the presence of a low oxygen partial pressure (5 kPa), and in these conditions, the β-carotene degraded completely during storage, even if more slowly than at 20 kPa of O_2. The pigment degradation was correlated to illuminance and UVA irradiance values, but the different decay rates of the fluorescent lamps were explained by the differences in the blue region of the energy emission spectra. A halogen lamp gave minor negative effects on β-carotene degradation.

INTRODUCTION

Food color and appearance are important visually perceived factors that contribute to customer selection (*1*). To fulfill the consumer's desire to visually appraise the product and to convince the potential buyer about the quality of the food prior to the purchase, transparent packaging materials are often used (*2*−*5*). During distribution and at the retail level, the clear packages are exposed to natural and artificial light, which may deteriorate the food quality and marketability, depending on the photosensitivity of the product. Moreover, cut, sliced, or grated foods are more susceptible to light-induced damage since they expose a larger surface area to light (*4*, *5*). In food and beverages, photochemical degradation occurs almost exclusively on the surface since level light penetration is very low, in particular when the products have a compact texture (*6*). In fact, the matrix influences the penetration level of light and, therefore, the rate of light-induced reactions. Generally, the scattering intensity of light at wavelength λ by a particle of diameter r is proportional to r^6/λ^4 and increases with a higher number of particles (*5*, *7*).

In general, light exposure causes the deterioration of lipids, vitamins, proteins, and natural pigments, which results in off-flavors, loss of flavor and nutrients, and color fading (*3*, *8*). The most harmful wavelengths are usually in the ultraviolet part of the spectrum, just below the visible range. This is due to the high-energy content, which is capable of splitting certain chemical bonds (*6*, *9*). The effects of light on the degradation of packaged foods have been widely investigated especially when lipidic substrates are involved (*10*); attention has therefore been focused on fatty foods, which contain pigments (milk, cheese, butter, yogurt, meat and meat products, oils, etc.) (*5*, *11*, *12*). The effect of light on the lipid oxidation and flavor stability may be attributed to photolytic free radical autoxidation and/or photosensitized oxidation (*5*, *13*, *14*). Photolytic free radical autoxidation is the production of free radicals, primarily from lipids, during exposure to far-UV and high-energy light, with intensities such as 10000−100000 lux (*5*, *14*, *15*). In the same way, light in the wavelength range of the visible spectrum might cause considerable problems, especially when the product contains photosensitizer molecules such as chlorophylls, carotenoids, flavonoids, anthocyanins, betaines, etc. These components, in fact, are characterized by their conjugated double-bond system. When light energy is absorbed, the sensitizer is converted into a triplet excited state, which can interact easily with another molecule, producing free radicals, or with molecular oxygen, leading to the formation of highly reactive singlet oxygen. The reaction rate of singlet oxygen with some food components is much greater than that of triplet oxygen: In fact, singlet oxygen can attack double bonds directly; for example, its reactivity with linoleic acid is about 1450 times faster than that of ground triplet state oxygen (*13*).

Photodegradative reactions might also take place in aqueous systems that do not contain fats but photosensitizer compounds such as natural pigments or artificial colorants (*16*−*19*). Among the numerous compounds available to the food industry, the

* To whom correspondence should be addressed. Tel: +39 02 50316659. Fax: +39 02 50316672. E-mail: sara.limbo@unimi.it.

Light-Induced Changes in an Aqueous β-Carotene System

J. Agric. Food Chem., Vol. 55, No. 13, 2007 **5239**

pigments belonging to the carotenoids group are widely used for coloring foods and beverages and also for increasing their nutrient stability. In fact, the increased awareness of healthy eating has led the consumer to prefer foods with a high nutritional value. For these reasons, natural substances are often added to many foods to make them healthier and more appealing. In particular, β-carotene has drawn considerable attention because of its role as an antioxidant that could reduce the risk of certain types of cancer and cardiovascular diseases. β-Carotene is a critical substance because it can act as pro/antioxidant as a function of some variables and it is sensitive to pH, temperature, and oxygen. Light also plays an important role in β-carotene degradation because it is a photosensitive molecule, although many foods and beverages that contain β-carotene are sold in stores where artificial light is used for illumination. In this case, not much information is available on the specific effects of light, whereas the effects of temperature are frequently studied (*10*, *16*, *18*). Generally, food photosensitivity is influenced by a number of factors: concentration of oxygen in the head space of the pack and dissolved in the product, oxygen permeability of the packaging material, storage temperature, exposure time to light, distance between the product and the light source, spectral emission and intensity light, transmittance level of packaging material, reflectance, transmittance, and absorbance spectra of the food product (*5*). For these reasons, not only the processing parameters but also the packaging materials and storage conditions must be selected and controlled to reduce degradation. A large number of works are focused on the effects of monochromatic lights (*20*−*23*), but only few evaluate the exposure under real light sources (*14*, *24*−*26*). For this reason, the aim of this work was to investigate the effects of various typologies of lamps used by different commercial retail stores to illuminate food products on carotenoids. In this way, the reaction kinetics of β-carotene in an aqueous medium as a function of exposure to commercial light sources with different spectral emission were studied. In these conditions, the effects of two different oxygen partial pressures were also investigated.

MATERIALS AND METHODS

Samples Preparation. A water dispersible β-carotene powder (Roha Caleb Ltd., United Kingdom) was used to prepare an aqueous solution at a concentration of 18 μg mL^{-1}. The powder consisted of β-carotene (10% w/w) solubilized in genetically modified free vegetable oil embedded in the form of very fine droplets coated with a matrix composed of dextrin and glucose (particle size: minimum, 98% smaller than 200 μm; maximum, 8% smaller than 50 μm). The powder contained calcium phosphate (E341) as an anticaking agent that assured the stability of the fine emulsion during the experimental time. In fact, suspended particles were never visible in samples, and the opacity (measured as absorbance at 660 nm) (*27*, *28*) did not increase over 10 days of storage.

The solution was buffered at pH 7.2 with citric acid−Na$_2$HPO$_4$, and 0.5 mL of ethanol (98% v/v) was added to prevent microbial growth during storage. The samples were composed of 10 mL of solution contained in 22 mL glass vials (clear in the UV−vis region of the spectrum) inside of which two oxygen partial pressures were created. A set of vials was hermetically closed under 20.9 kPa of oxygen to simulate packaging in air, while another set was conditioned under 5 kPa of oxygen using an automatic packaging machine (Tecnovac S100 DGT gas) connected to a gas mixer (PBI Dansensor MAP mix 9000) to simulate oxygen residual after modified atmosphere packaging. The vials were then hermetically closed with PTFE/butyl septa and aluminum crimp tops.

Storage Experiment and Light Exposure. Each set of vials was stored horizontally on black turning plates inside thermostated and

Table 1. Technical Characteristics of the Lamps Used for Lighting Samples

lamp typology	commercial name	color temp (K)	illuminance (lux)	UVA irradiance (Wm^{-2})
fluorescent				
cool white	Osram Dulux El Longlife 30W/840	4000	3300	0.065
warm white	Osram Dulux T 26W/830	3000	2700	0.052
halogen	Osram Decostar 51S Std 20W 36°		1800	0.017

ventilated cabinets for 11 days at 33 ± 2 °C. Data loggers (Smart Reader SR04) were used to verify the real temperature. During storage, the samples were exposed to constant illumination produced by different light sources, and the same number of samples were also conditioned in the darkness. The lamps and the conditions of lighting used in the storage experiments were selected after a census in different large-scale retail stores in Milan and on the outskirts of the city. Details of this part of the experiment are reported elsewhere (*29*). In particular, a cool white lamp (Osram Dulux El Longlife 30W/840), a warm white lamp (Osram Dulux T 26W/830), and a halogen lamp (Osram Decostar 51S Standard 20W 36°) were used. The lamps were installed inside the cabinets and placed 18 cm above the samples. To avoid light-reflecting phenomena, the internal walls of the cabinets were tinted black. For each selected light, illuminance and irradiance in the visible and UVA regions were measured under real storage conditions at the level of the samples (**Table 1**).

Determination of β-Carotene Concentration and Color Evolution of the Solutions. Samples were withdrawn in triplicate for analysis at predetermined times. The pigment degradation was controlled by monitoring the residual β-carotene and the color change.

The amount of β-carotene was determined by measuring absorbance at the λ$_{max}$ (479 nm) using an UV−visible spectrophotometer (Perkin-Elmer Lambda 650). This method of monitoring carotene oxidation is well-established and has been used previously in many studies (*30*, *31*). From a stock solution of β-carotene, 13 standard solutions were prepared by successive dilution with concentrations ranging from 1 to 22 μg mL^{-1}. To construct the calibration curve, all of the standard solutions were prepared and analyzed in triplicate. The evolution of the color of the samples was monitored by means of the CIE *L**a*b* parameters calculated by the software Color version 3.00 (Perkin-Elmer Instruments) from the transmittance spectrum acquired in the visible region (380−780 nm).

Isolation and Determination of β-Ionone. The production of a volatile compound (β-ionone) by means of the headspace solid-phase microextraction (HS-SPME) technique was evaluated. The SPME device was from Supelco Co. (Bellefonte, PA). Prior to the sample headspace exposure, the 10 mm fiber coated with 100 μm polydimethylsiloxane was thermally conditioned by its insertion into the gas chromatography (GC) injector port at 250 °C for 30 min, as recommended by the manufacturer. Moreover, each day, the fiber was activated by inserting it into the GC injector at 250 °C for 20 min.

For each extraction, the vial containing 10 mL of β-carotene aqueous solution was thermostated for 3 min at 50 °C in a water bath, submerging it only as far as necessary to submerge the liquid phase of the sample to help keep the SPME fiber cool. Then, the fiber was manually inserted into the sample vial at the above-mentioned temperature for an adsorption time of 30 min.

The volatile compounds extracted by HS-SPME procedure were identified using a gas chromatograph coupled to a mass selective detector. In particular, the GC instrument (model Autosystem XL, Perkin-Elmer Inc., United States) was equipped with a polar capillary column (SupelcowaxTM-10 30 m × 0.25 mm i.d. × 0.25 μm film thickness). Helium was used as the carrier gas at a flow rate of 1 mL/min. The volatile compounds were desorbed in the GC injector in splitless mode at 250 °C for 2 min (preliminary studies indicated that this exposure time permitted the fiber to be clean). The injector and transfer line temperatures were 250 and 280 °C, respectively, and the oven temperature program was as follows: 40 °C increased to 150 °C at 10 °C/min and to 190 °C at 5 °C/min.

5240 *J. Agric. Food Chem.,* Vol. 55, No. 13, 2007

Limbo et al.
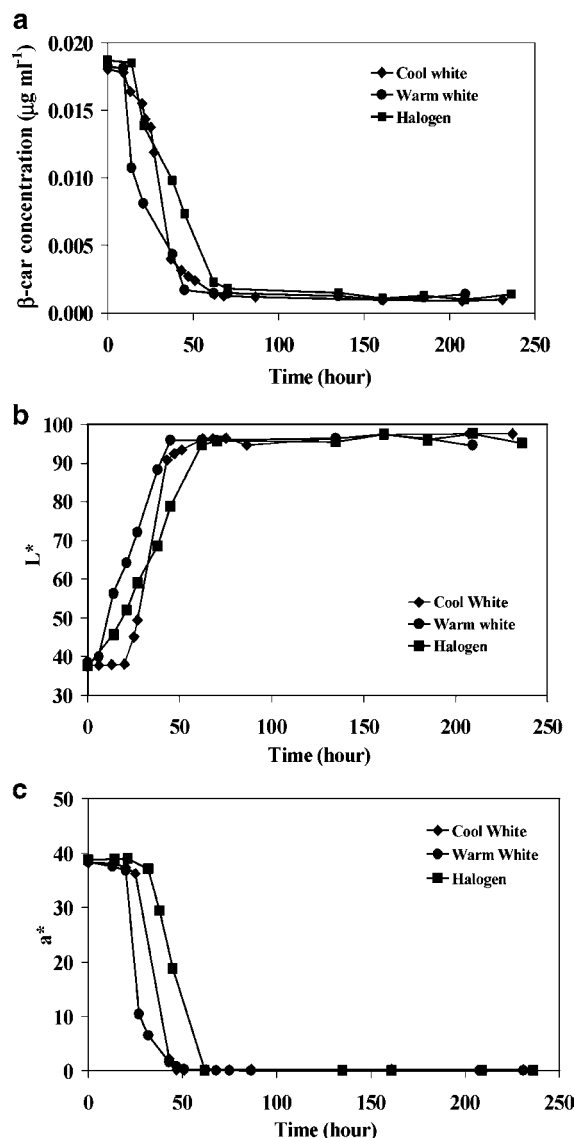
Figure 1. Sigmoid shape of some β-carotene concentration (**a**), L^* (**b**), and a^* (**c**) curves during storage at 20 kPa of oxygen.

The quadrupole mass detector (model Turbo Mass, Perkin-Elmer Inc.) was operated in the electron ionization mode at a voltage of 70 eV. The ion source temperature was set at 180 °C. The electron impact spectra were recorded at 1 s/scan in the mass range of 40−620 m/z. The peak of β-ionone was identified via a comparison with the NIST mass spectral library (version 1.7, 1999) and the retention time of the chemical standard ($\geq$95.0% Fluka, Switzerland).

RESULTS AND DISCUSSION

The effects of three selected lamps (cool white, warm white, and halogen) on the stability of β-carotene were performed by monitoring its concentration and color changes during time. It was evident that the changes in the β-carotene concentration and also those in the L^* and a^* CIE parameters had a sigmoid shape, as shown in **Figure 1** for the storage at 20 kPa of oxygen. An induction time was evident, and the decay rate did not decline immediately but decreased up to a minimum before steadily reaching an asymptotic level (**Figure 1a**).

The same trend was obtained for the evolution of the color coordinate a^*, as it can be seen in **Figure 1c** referred to the 20 kPa of oxygen. The L^* values (**Figure 1b**), after the induction time, increased up to a maximum before reaching the asymptotic level. A different behavior was observed for the b^* values (data

not shown): In fact, in all of the lighting experiments, the induction phase was not evident and a maximum was observed after a few hours from storage and before its decrease up to a constant value. For this reason, the b^* data will not be discussed in this paper.

From a literature review, it has been noted that the degradation of this carotenoid follows different order kinetic models, as a function of the type of β-carotene, the substrate compound involved, and, also, the lighting conditions. For example, Jarén-Galàn and Minguez-Mosquera (*32*) reported that β-carotene degradation during time in phosphate buffer under an illuminance of 1000 lux was fitted by a first-order kinetic model; however, in this work, the authors gave no information about the typology of the lamp and the conditions of expositions. On the other hand, Gao and co-worker (*33*) observed a zero-order kinetic for β-carotene in dichloromethane solution when irradiated with near-UV and visible light. These last findings were in agreement with the conclusions indicated in other papers about the effect of ozone, oxygen exposures, and heat treatment on carotenoid degradation in an aqueous medium (*16, 18*). Even if the substrate used in these experiments was the same as ours (i.e., water), the experiments were carried out without considering the light exposition. The sigmoid pattern has been noticed in the literature where many decay data have been described by a number of so-called sigmoid models, such as the Logistic, Gompertz, Richards Morgan, and Weibull models (*34−36*). In particular, the sigmoid trend was also found both in the degradation of color pigments (mostly carotenoids) in dehydrated and ground chili peppers during 12 months of storage (*37*) and in the overall degradation pattern of the vitamin A palmitate-only sample at ambient temperature (*38*).

In this work, the Gompertz model as modified by Zwietering et al. (*39*) (eq 1), adapted to both growth and decay kinetics, was chosen to describe the sigmoid behavior of the β-carotene concentration and the L^* and a^* changes, to obtain information that made it possible to compare the effects of both light exposure and oxygen partial pressure.

$$\log (C/C_0) = \pm A \times \exp\left\{-\exp\left\{\left[(\mu \times 2.7182) \times \frac{\lambda - t}{A}\right] + 1\right\}\right\} \quad (1)$$

where C is the β-carotene concentration (or L^* and a^* value) at time t, C_0 is the β-carotene concentration (or L^* and a^*) value at time 0, A is the increase (or decrease) in log C/C_0 between the maximum and the minimum value achieved at the stationary phase and time 0, μ is the maximal increase or decrease rate [$\Delta\log(C/C_0)$/h], λ is the induction time (h), and t is the time (h).

Table Curve 2D Software Version 4 (Jandel Scientific Software) was used for the nonlinear regression analysis and to determine the parameters of the modified Gompertz equation.

The goodness of fit of the experimental data was evaluated by means of the adjusted R^2 and the root-mean-square error (RMSE, eq 2), which measures the average deviation between the observed and the fitted values. The small RMSE value indicates a better fit of data for that model (*40*)

$$\text{RMSE} = \sqrt{\sum \frac{(\text{fitted} - \text{observed})^2}{n - p}} \quad (2)$$

where n is the number of observations and p is the number of parameters to be estimated. The results are shown in **Table 2**.

Light-Induced Changes in an Aqueous β-Carotene System

J. Agric. Food Chem., Vol. 55, No. 13, 2007 **5241**

Table 2. R^2 and RMSE Values of the Data Fitting for All Tested Conditions

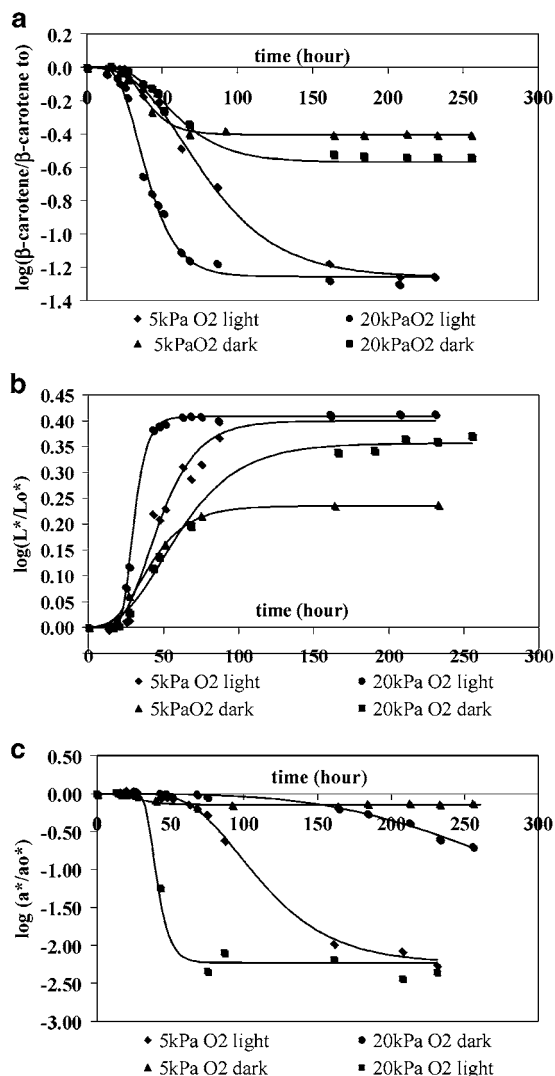| oxygen (kPa)	β-carotene concentration		L^*		a^*	
	R^2	RMSE	R^2	RMSE	R^2	RMSE
dark						
20	0.988	0.028	0.998	0.013	0.994	0.023
5	0.989	0.021	0.979	0.023	0.989	0.076
cool white						
20	0.993	0.045	0.999	0.004	0.994	0.099
5	0.997	0.030	0.981	0.025	0.997	0.044
warm white						
20	0.989	0.028	0.994	0.012	0.971	0.251
5	0.997	0.030	0.975	0.331	0.997	0.045
halogen						
20	0.998	0.033	0.998	0.013	0.994	0.023
5	0.986	0.059	0.979	0.023	0.989	0.076

The adjusted R^2 higher than 0.970 and the low values of RMSE suggested a good correspondence between the experimental and the predicted data both at 5 and 20 kPa of oxygen. So, the β-carotene degradation of the aqueous solution was properly described in terms of the sigmoid model, as shown in **Figure 2**, where, for example, the concentration decrease, the L^*, and the a^* changes during the exposition under the cool white lamp and in the dark are shown.

The modeling of the experimental data made it possible to study the acceleration induced by light exposition with respect to dark storage and the effects of the oxygen partial pressure on the photoxidation of the pigment.

All data (induction time and maximum rate) are shown in **Table 3** for the storage at 20 and 5 kPa of oxygen. To quantify the effect of light exposure in the acceleration of the β-carotene degradation (both in terms of concentration, L^*, and a^* changes) for the same oxygen partial pressure, a ratio between the induction time estimated under illumination (λ_l) and that estimated in the dark (λ_d) was calculated and expressed as λ_l/λ_d; the same calculus was performed on the rates values (μ_l/μ_d). The results are shown in **Table 4**.

At 20 kPa of oxygen (**Table 4**), for all of the analyzed indexes, the induction times were higher in the dark than under illumination: In fact, the ratios (λ_l/λ_d) were always lower than 1. The light exposure accelerated the maximum reaction rates as shown by the ratios (μ_l/μ_d), which were all higher than 1.

The presence of an induction phase is probably due to the antioxidant activity of β-carotene, which, acting both as a physical quencher and a radical trapping, could be able to protect itself from oxidation for a certain time (16, 31, 38). The induction time could also be due to the protective effect of the thin coating of the fine droplets that surrounded the β-carotene solubilized in a vegetable oil. This protective effect was found for microcrystalline cellulose and also for starch used as a supporter of β-carotene, as described by Goldman et al. (41). Moreover, oxygen is more soluble in nonpolar lipids than in aqueous system (13); for this reason, the low water solubility of oxygen could also induce a slack in the photoxidation. The fact that the induction times were always longer and the maximum reaction rates lower in the dark than under illumination might be explained by the different mechanisms involved in the β-carotene degradation. In fact, in the dark, the oxidation of β-carotene does not follow a photodegradative route (with the production of singlet oxygen) but a radical pathway that can be induced by temperature (equal to 33 °C in this experiment) (42) and radical starters (metals or probably other

Figure 2. β-Carotene concentration (**a**), L^* (**b**), and a^* (**c**) data for the cool white and dark storage, modeled with the modified Gompertz equation.

contaminants present in water or in the powder). Instead, in lighted conditions, it can suppose that two parallel reactions take place: One is a radical oxidation, and the other is a photoxidation that follows prevalently a type II pathway, where the excited triplet sensitizer may react with the triplet oxygen to form singlet oxygen and singlet sensitize (13). Generally, the rate of singlet oxidation is much greater than that of atmospheric triplet (therefore, radicalic) oxidation (13), explaining the obtained data.

The comparison among the effects of the different lamps was more evident considering the maximal rates values than the induction times. In fact, the length of the induction times was not dependent on the lighting conditions while the rates were influenced by the illuminance and UVA irradiance data measured during the storage experiment (**Tables 1** and **3**). In other words, the highest rates of changes were found for the cool white lamp, while the lowest rates were found for the halogen lamp.

It was interesting to note that the highest degradation rates were found under illumination with the two lamps less different in terms of illuminance and UVA irradiance. To better investigate these results, the relative spectral distribution of the energy was acquired using an optical probe connected to a detector simulating the real illumination geometry of the cabinets.

Table 3. Induction Times (λ) and Maximum Reaction Rates (μ) under Lighting and in Darkness at 20 and 5 kPa of Oxygen[a]

lamp typology	h	β-carotene concn	L^*	a^*	h^{-1}	β-carotene concn	L^*	a^*
				20 kPa				
cool white	λ_l	20.89 c	22.30 c	20.05 a	μ_l	0.0355 d	0.0196 d	0.1000 d
warm white	λ_l	7.80 a	2.50 a	30.41 b	μ_l	0.0264 c	0.0137 c	0.0696 c
halogen	λ_l	18.20 b	7.44 b	44.12 c	μ_l	0.0209 b	0.0088 b	0.0416 b
dark	λ_d	26.00 d	24.80 d	148.00 d	μ_d	0.0091 a	0.0049 a	0.0080 a
				5 kPa				
cool white	λ_l	31.00 c	23.10 c	63.10 b	μ_l	0.0136 b	0.0076 c	0.0701 c
warm white	λ_l	14.30 a	3.30 a	69.34 c	μ_l	0.0123 b	0.0046 ab	0.0180 b
halogen	λ_l	12.00 a	3.30 a	173.02 d	μ_l	0.0095 a	0.0040 a	0.0055 a
dark	λ_d	23.78 b	16.50 b	13.60 a	μ_d	0.0135 b	0.0050 b	0.0050 a

[a] For each column, different letters indicate statistically different groups ($p < 0.05$).

Table 4. Ratios of Induction Times (λ) and Maximum Reaction Rates (μ) under Lighting and Dark

lamp typology	ratio	β-carotene concn	L^*	a^*	ratio	β-carotene concn	L^*	a^*
				20 kPa				
cool white	λ_l/λ_d[a]	0.80 ± 0.02	0.90 ± 0.05	0.13 ± 0.06	μ_l/μ_d[b]	3.90 ± 0.04	4.00 ± 0.02	12.50 ± 0.12
warm white	λ_l/λ_d	0.30 ± 0.05	0.10 ± 0.02	0.20 ± 0.04	μ_l/μ_d	2.90 ± 0.03	2.80 ± 0.09	8.70 ± 0.15
halogen	λ_l/λ_d	0.70 ± 0.02	0.30 ± 0.06	0.30 ± 0.07	μ_l/μ_d	2.30 ± 0.04	1.80 ± 0.07	5.20 ± 0.09
				5 kPa				
cool white	λ_l/λ_d [1]	1.30 ± 0.12	1.40 ± 0.06	4.60 ± 0.85	μ_l/μ_d [2]	1.00 ± 0.12	1.10 ± 0.05	4.00 ± 0.92
warm white	λ_l/λ_d	0.60 ± 0.48	0.20 ± 0.45	5.10 ± 0.32	μ_l/μ_d	0.91 ± 0.12	0.92 ± 0.17	3.60 ± 0.98
halogen	λ_l/λ_d	0.50 ± 0.09	0.20 ± 0.08	12.70 ± 0.14	μ_l/μ_d	0.85 ± 0.18	0.81 ± 0.14	1.10 ± 0.98

[a] Ratio between the induction time estimated under light (λ_l) and in the dark (λ_d). [b] Ratio between the rate estimated under light (μ_l) and in the dark (μ_d).

The difference between the two lamps is well-highlighted in **Figure 3**, which shows the ratio between the two relative spectra. The cool white had a higher emission in the blue region of the spectrum where the β-carotene absorbs. This might explain, together with the illuminance and UVA irradiance values, the highest modifications of the aqueous solution exposed under the cool white lamp.

At 5 kPa of oxygen (**Table 3**), the interpretation of induction times as a function of the storage condition was not simple, as also found at 20 kPa. In particular, for the warm white and halogen lamps, the induction times of the β-carotene concentration and L^* curves were shorter than in the dark (i.e., the ratios were lower than 1 as shown in **Table 4**), meaning that the illumination accelerated the start of oxidation. On the contrary, this situation was not verified during the exposition of the model system under the cool white lamp, and the induction times for the carotenoid concentration and L^* were higher than in the dark (ratio higher than 1). The ratios of the maximum reaction rates for β-carotene and L^* indexes with respect to the dark (μ_l/μ_d) were quite close to 1, suggesting that the radical reactions seemed to prevail both under light and under dark. In lighted samples, the availability of low oxygen combined with the low water solubility of singlet oxygen could have favored the type I pathway of the photoxidation, where the excited triplet sensitizers that were formed after the absorption of visible and ultraviolet radiation energy acted as a photochemically activated free radical initiator for a radical compound and reacted with the triplet oxygen to form peroxy radical. Generally, as the oxygen in a system becomes depleted, the type I mechanism is favored (*13*).

In the case of the redness/greenness parameter (a^*) at 5 kPa of oxygen, both the induction and the rate ratios were higher than 1 for all of the selected lamps (**Table 4**): This means that the modifications described by this component of the color were detected more rapidly in the dark but the rates of reactions were

Table 5. Times at Which Correspond the Maximum and Minimum of the Second Derivative of β-Carotene, L^*, and a^* Curves[a] at 5 kPa of Oxygen and Values Achieved at the Stationary Phase (A)

β-carotene concn		L^*		a^*	
max d^2y/dt^2 (h)	A	min d^2y/dt^2 (h)	A	max d^2y/dt^2 (h)	A
		dark			
50 a	0.40 a	51 a	0.23 a	70 a	0.14 a
		cool white			
97 b	1.26 b	54 b	0.39 b	112 b	2.93 c
		warm white			
98 b	1.26 b	56 b	0.40 b	122 b	2.24 c
		halogen			
189 c	1.42 c	81 c	0.39 b	209 c	1.00 b

[a] For each column, different letters indicate statistically different groups ($p < 0.05$).

higher during lighting. This situation can be explained assuming that at low oxygen partial pressures the effect of light and temperature could be limited to causing *cis—trans* isomerization (*36*), which probably did not significantly modify the red components of the solution for the time corresponding to the induction phase.

From these data, it might seem that exposition to light did not have a great effect at 5 kPa of oxygen, while the limiting factor was represented by the oxygen. However, considering the maximum of the second derivative of the β-carotene concentration and a^* curves or the minimum of L^* curves, it was possible to check when the reaction rates decreased drastically, that is, when the reaction reached the asymptotic level (**Table 5**). It was evident that the reaction tended to exhaust more rapidly in the dark but without the complete degradation of the reactant, as the values of the increase (or decrease) in log C/C_0 between time 0 and the maximum or
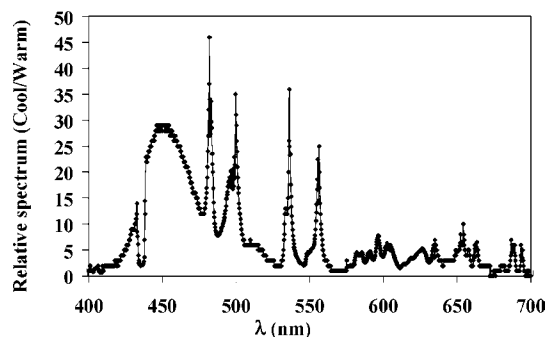
Light-Induced Changes in an Aqueous β-Carotene System

J. Agric. Food Chem., Vol. 55, No. 13, 2007 **5243**

Figure 3. Ratio between the relative emission spectrum of cool and warm white lamps.

minimum value achieved at the stationary phase (parameter A in eq 1) show.

Another consideration may be done; the degradation of the β-carotene expressed as the decrease in concentration was also described by the lightness changes (L^*), as demonstrated by the similar rate ratios at both 5 and 20 kPa (**Table 4**).

Comparing the results in the darkness, the maximum rate of β-carotene degradation at 5 kPa was about 1.5 times higher than that measured at 20 kPa (**Table 3**). Moreover, also, the induction times were longer at 20 than at 5 kPa of oxygen for all of the analyzed indexes. This behavior could be explained assuming that neighboring O_2 molecules, when in liquid form, have a negative exchange energy and thus tend to align antiparallel with each other. So, at high concentrations, this fact could have reduced, for a certain time, the amount of oxygen able to interact with the fine microemulsion structure of the water dispersible β-carotene. In fact, in a previous work, Goldman and al. (*41*) found that the presence of oxygen in the headspace (without light exposure) was a crucial factor in β-carotene degradation but the limiting factor in the oxidation of the dispersed pigment was the adsorbed oxygen content.

During storage, the formation of a volatile compound was also investigated. In fact, the oxidation of carotenoids generally involves epoxidation, the formation of apocarotenoids, and hydroxylation. Subsequent fragmentations result in a series of compounds of low molecular masses and volatiles in nature. In the case of β-carotene, compounds such as β-ionone, β-cyclocitrale, damascenone, and dihydroactinidiolide are considered as degradative volatile products (*43, 44*), which contribute to the aroma/flavor, desirable in tea and wine (*45*) but undesirable in other foods such as dehydrated carrot and some soft drinks (*46*). The formation of volatile compounds by thermal degradation of the carotenoids during heat treatment of vegetables products has been mentioned by some authors (*18, 19*). On the contrary, the effects of light and oxygen have been studied probably to a lesser extent, and no references were found.

Figure 4 shows the evolution of β-ionone, at 20 and 5 kPa of oxygen, during storage under the illumination of the three selected lamps. It is noted that at 5 and 20 kPa of oxygen the trend was the same for all of the storage conditions tested: The β-ionone level (expressed as area unit) rose up to a maximum and then decreased in time (**Figure 4a,b**). It was also evident that the maximum of β-ionone accumulation was reached later in darkness conditions than under light, and in the former case, the amounts accumulated were always lower (**Figure 4c**). In particular, this phenomena was more noticeable at 5 kPa of oxygen than at 20 kPa.

It was expected that higher oxidation rates of β-carotene would result in the major formation of β-ionone, but this compound accumulated at lower levels in those samples exposed
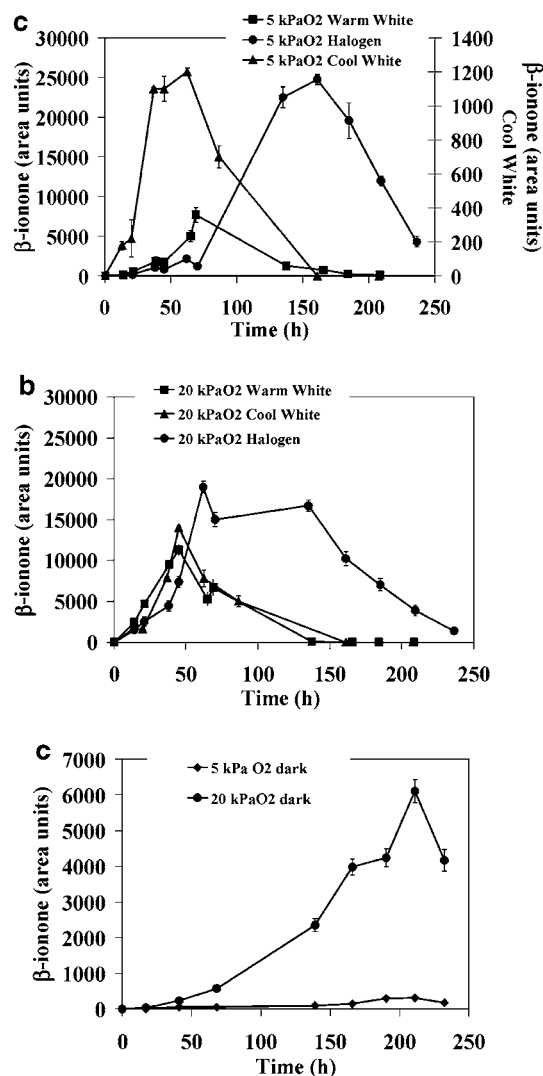

Figure 4. Evolution of β-ionone during exposition under the three lamps at 20 (**a**) and 5 kPa (**b**) and in darkness (**c**).

to the fluorescent lamps. Both at 5 and 20 kPa of oxygen, the halogen lamp, which gave the lowest rates of degradation in terms of β-carotene concentration and lightness changes, induced the highest β-ionone accumulation, especially at 5 kPa of oxygen. This was probably due to the minor β-ionone degradation during the exposition under this light. In fact, as other authors have sustained (*18, 47*) in a highly reactive environment (like this one where light and oxygen were present), rapid isomerization and oxidation of the degradative products were observed. An arbitrary mathematical model (a peak function) was applied to describe the evolution of β-ionone to calculate the first and the second time derivatives from which it was possible to estimate the time related to the maximum rate and the acceleration of the volatile production phenomenon. At 20 kPa, the time of maximum acceleration of the production of β-ionone coincided with the induction time calculated from the fitting of the redness/greenness experimental data. In fact, when the redness/greenness index started to decrease, the rate of the β-ionone production tended to increase. At 5 kPa, the maximum of the β-ionone curves coincided with the estimated induction time for a^* curves (**Table 6**). In other words, this color index (a^*) was able to describe the β-carotene degradation giving information about the transit of the reaction from the nonvolatile (and colored) to volatile (and colorless) compounds.

5244 *J. Agric. Food Chem.,* Vol. 55, No. 13, 2007

Limbo et al.

Table 6. Times at Which Correspond the Maximum of the First and Second Derivative of the β-Ionone Curves[a]

	20 kPa O_2		5 kPa O_2	
lamp typology	max d^2y/dt^2 β-ionone (h)	induction time a^* (h)	max dy/dt β-ionone (h)	induction time a^* (h)
cool white	21.0 a	20.0 a	62.1 a	63.1 a
warm white	32.4 b	30.4 b	69.0 a	69.3 a
halogen	43.2 c	44.1 c	175.9 b	173.0 b

[a] For each column, different letters indicate statistically different groups ($p < 0.05$).

In conclusion, the aqueous solution of β-carotene had the advantage of simplicity for testing the effects of both light exposure and oxygen partial pressure. The modeling of the experimental data made it possible to estimate the rate of decay of some quality indexes, such as the β-carotene concentration and the color of the solution. Nevertheless, the kinetic model of aqueous β-carotene as affected by light, oxygen, and other factors needs further study, and the approach of a multiresponse analysis could allow one to obtain a more realistic kinetic model and thus a deeper insight in reaction mechanisms. In this study, it appeared that lightness index (L^*) seemed to be a good indicator of the decrease in β-carotene concentration, while the changes in redness/greenness index (a^*) coincided with the production of the volatile compound β-ionone. This means that the colorimetric measurement is able to describe the molecular changes of β-carotene during oxidation.

Lighting was also a critical variable in the presence of a low oxygen partial pressure. In fact, even if more slowly than at 20 kPa of O_2, the β-carotene degraded during storage. The halogen lamp had minor degrading effects on the β-carotene solution but allowed the highest accumulation of β-ionone: If this result were verified in a real food system, their use should be recommended for the lighting of products rich in carotenoids but not sensitive to sensorial modifications. The knowledge of the emission spectra of the light sources contributed to explaining the different decay rates of the fluorescent lamps that resulted higher using the cool white light.

From a technological point of view, the packaging materials performances and the modified atmosphere technique need to be optimized to reduce the oxygen content in photosensitive foods. On the other hand, the light sources in the retail stores have to be chosen with care and not just on the basis of cost, efficiency, durability, and aesthetic factors.

LITERATURE CITED

(1) Barbut, S. Effect of retail lights on acceptability of salami. *Meat Sci.* **2003**, *66*, 219−223.

(2) Krawczyk, T. Retail oil packaging. *Inform* **2000**, *11*, 932−942.

(3) Thron, M.; Eichner, K.; Ziegleder, G. The influence of light of different wavelenghts on chlorophyll-containing foods. *Lebensm.-Wiss. Tecnol.* **2001**, *34*, 542−548.

(4) Juric, M.; Bertelsen, G.; Mortensen, G.; Petersen, M. A. Light-induced colour and aroma changes in sliced, modified atmosphere packaged semi-hard cheeses. *Int. Dairy J.* **2003**, *13*, 239−249.

(5) Mortensen, G.; Bertelsen, G.; Mortensen, B. K.; Stapelfeldt, H. Light-induced changes in packaged cheeses−A review. *Int. Dairy J.* **2004**, *14*, 85−102.

(6) Bosset, J. O.; Gallmann, P. U.; Sieber, R. Influence of light transmittance of packaging materials on the shelf-life of milk and dairy products−A review. In *Food Packaging and Preservation*; Mathlouthi, M., Ed.; Blackie Academic & Professional: London, United Kingdom, 1992; pp 222−265.

(7) Parker, C. A. *Photoluminescence of Solutions*; Elsevier: Amsterdam, The Netherlands, 1968.

(8) Bekbölet, M. Light effects on food. *J. Food Prot.* **1990**, *53*, 430−440.

(9) Brown, W. E. In *Plastic in Food Packaging*; Hughes, H. A., Ed.; Marcel Dekker: New York City, New York, 1992; Chapter 1, pp 1−40.

(10) Liu, M. H.; Chen, B. H. Relationship between chlorophyll a and β-carotene in a lipid-containing model system during heating. *Food Chem.* **1998**, *61*, 41−47.

(11) Kalua, C. M.; Bedgood, D. R.; Bishop, A. G.; Prenzler, P. D. Discrimination of storage conditions and freshness in virgin olive oil. *J. Agric. Food Chem.* **2006**, *54*, 7144−7151.

(12) Andersen, C. M.; Vishart, M.; Holm, V. K. Application of fluorescence spectroscopy in the evaluation of light-induced oxidation in cheese. *J. Agric. Food Chem.* **2005**, *53*, 9985−9992.

(13) Min, D. B.; Boff, J. M. Chemistry and reaction of singlet oxygen in foods. *Comp. Rev. Food Sci. Food Saf.* **2002**, *1*, 58−72.

(14) Lennersten, M.; Lingnert, H. Influence of different packaging materials on lipid oxidation in potato crisps exposed to fluorescent light. *Lebensm.-Wiss. Tecnol.* **1998**, *31*, 162−168.

(15) Haisman, D. R.; Groenendijk, C. B. M.; O'Sullivan, G. J. The effect of light on flavour and nutritional content of milk. Paperboard vs. plastic containers. *Food Technol. N. Z.* **1992**, *27*, 16−20.

(16) Henry, L. K.; Pupistari-Nienaber, N. L.; Jarèn-Galàn, M.; Van Breemen, R. B.; Catignani, G. L. Effect of ozone and oxygen on the degradation of carotenoids in an aqueous model system. *J. Agric. Food Chem.* **2000**, *48*, 5008−5013.

(17) Jung, M. Y.; Lee, K. H.; Kim, S. Y. Riboflavin-sensitized photochemical changes in β-lactoglobulin in an aqueous buffer solution as affected by ascorbic acid. *J. Agric. Food Chem.* **2000**, *48*, 3847−3850.

(18) Kanasawud, P.; Crouzet, J. C. Mechanism of formation of volatile compounds by thermal degradation of carotenoids in aqueous medium. 1. β-carotene degradation. *J. Agric. Food Chem.* **1990**, *38*, 237−243.

(19) Kanasawud, P.; Crouzet, J. C. Mechanism of formation of volatile compounds by thermal degradation of carotenoids in aqueous medium. 2. Lycopene degradation. *J. Agric. Food Chem.* **1990**, *38*, 1236−1242.

(20) Moller, J. K. S.; Nannerup, L.; Skibsted, L. H. Effect of carbon dioxide on autoxidation and photooxidation of nitrosylmyoglobin. *Meat Sci.* **2005**, *69*, 71−78.

(21) Lennersten, M.; Lingnert, H. Influence of wavelength and packaging material on lipid oxidation and colour changes in low-fat mayonnaise. *Lebensm.-Wiss. Tecnol.* **2000**, *33*, 253−260.

(22) Hansen, E.; Skibsted, L. H. Light-induced oxidative changes in a model dairy spread. Wavelength dependence of quantum yields. *J. Agric. Food Chem.* **2000**, *48*, 3090−3094.

(23) Jespersen, L.; Strømdahl, L. D.; Olsen, K.; Skibsted, L. H. Heat and light stability of three natural blue colorants for use in confectionery and beverages. *Eur. Food Res. Technol.* **2005**, *220*, 261−266.

(24) Hiesberger, J.; Luf, W. Oxidation of cholesterol in butter during storage−Effects of light and storage. *Eur. Food Res. Technol.* **2000**, *211*, 161−164.

(25) Lin, C. H.; Chen, B. H. Stability of carotenoids in tomato juice during storage. *Food Chem.* **2005**, *90*, 837−846.

(26) Chen, B. H.; Huang, J. H. Degradation and isomerization of chlorophyll a and β-carotene as affected by various heating and illumination treatments. *Food Chem.* **1998**, *62*, 299−307.

(27) Taherian, A. R.; Fustier, P.; Ramaswamy, H. S. Effect of added oil and modified starch on rheological properties, droplet size distribution, opacity and stability of beverage cloud emulsions. *J. Food Eng.* **2006**, *77*, 687−696.

(28) Dlużewska, E.; Panasiewicz, M.; Leszczyński. Effect of gum Arabic and modified starch on stability of beverage emulsions. *Electr. J. Pol. Agric. Univ. Food Sci. Technol.* **2004**, www.ejpau.media.pl/volume7/issue2/food/art-10.html.

Light-Induced Changes in an Aqueous β-Carotene System

J. Agric. Food Chem., Vol. 55, No. 13, 2007 **5245**

(29) Torri, L.; Limbo, S.; Piergiovanni, L. Indagine sulle condizioni di illuminazione dei prodotti alimentari nei punti vendita della grande distribuzione organizzata. *Ind. Aliment.* **2007**, in press.

(30) Everett, S. A.; Dennis, M. F.; Patel, S.; Maddix, S. C.; Kundu, R. L.; Willson, J. Scavenging of nitrogen dioxide, thiyl, and sulfonyl free radicals by the nutritional antioxidant β-carotene. *J. Biol. Chem.* **1996**, *271*, 3988−3994.

(31) Woodall, A. A.; Wai-Ming Lee, S.; Weesie, R. J.; Jackson, M. J.; Britton, G. Oxidation of carotenoids by free radicals: Relationship between structure and reactivity. *Biochim. Biophys. Acta* **1997**, *1336*, 33−42.

(32) Jarén-Galàn, M.; Mìnguez-Mosquera, M. I. β-carotene and capsanthin co-oxidation by lipoxygenase. Kinetic and thermodynamic aspects of the reaction. *J. Agric. Food Chem.* **1997**, *45*, 4814−4820.

(33) Gao, G.; Deng, Y.; Kispert, L. D. Photoactivated ferric chloride oxidation of carotenoids by near-UV to visible light. *J. Phys. Chem. B* **1997**, *101*, 7844−7849.

(34) Manso, M. C.; Oliveira, F. A. R.; Oliveira, J. C.; Frias, J. M. Modelling ascorbic acid thermal degradation and browning in orange juice under aerobic conditions. *Int. J. Food Sci. Technol.* **2001**, *36*, 303−312.

(35) Sakai, Y.; Watanabe, H.; Takai, R. A kinetic model for oxidation of ascorbic acid and beta-carotene. *J. Food Process. Preserv.* **1987**, *11*, 197−207.

(36) Kebede, E.; Mannheim, C. H.; Miltz, J. Ascorbic acid retention in a model food packed in barrier plastic trays and cans. *Lebensm.-Wiss. Technol.* **1998**, *31*, 33−37.

(37) Chen, S. L.; Gutmanis, F. Auto-oxidation of extractable color pigments in chili pepper with special reference to ethoxyquin treatment. *J. Food Sci.* **1968**, *33*, 274−280.

(38) Kim, Y. S.; Strand, E.; Kickmann, R.; Warthesen, J. Degradation of vitamin A palmitate in corn flakes during storage. *J. Food Sci.* **2000**, *65*, 1216−1219.

(39) Zwietering, M. H.; de Koos, J. T.; Hasenack, B. E.; van't Riet, K. Modelling of the bacterial growth curve. *Appl. Environ. Microbiol.* **1990**, *56*, 1875−1881.

(40) Buzrul, S.; Alpas, H. Modeling the synergistic effect of high pressure and heat on inactivation of *Listeria innocua*: A preliminary study. *FEMS Microbiol. Lett.* **2004**, *238*, 29−36.

(41) Goldman, M.; Horev, B.; Saguy, I. Decolorization of β-carotene in model systems simulating dehydrated foods. Mechanism and kinetic principles. *J. Food Sci.* **1983**, *48*, 751−754.

(42) Martin, H. D.; Ruck, C.; Schidnt, M.; Sell, S.; Beutner, S.; Mayer, B.; Walsh, R. Chemistry of carotenoid oxidation and free radical reactions. *Pure Appl. Chem.* **1999**, *12*, 2253−2262.

(43) Lewinshon, E.; Sitrit, Y.; Bar, E.; Azulay, Y.; Meir, A.; Zamir, D.; Tadmor, Y. Carotenoid pigmentation affects the volatile composition of tomato and watermelon fruits, as revealed by comparative genetic analyses. *J. Agric. Food Chem.* **2005**, *53*, 3142−3148.

(44) Waché, Y.; Bosser-DeRatuld, A.; Lhuguenot, J. C.; Belin, J. M. Effect of cis/trans isomerism of β-carotene on the ratios of volatile compounds produced during oxidative degradation. *J. Agric. Food Chem.* **2003**, *51*, 1984−1987.

(45) Ravichandran, R. Carotenoid composition, distribution and degradation to flavour volatiles during black tea manufacture and the effect of carotenoid supplementation on tea quality and aroma. *Food Chem.* **2002**, *78*, 23−28.

(46) Rodriguez-Amaya, D. B. Changes in carotenoids during processing and storage of foods. *Arch. Latinoam. Nutr.* **1999**, *49* (3 Suppl. 1), 38S−47S.

(47) Mordi, R. C.; Walton, J. C. Oxidative degradation of β-carotene and β-apo-8′-carotenal. *Tetrahedron* **1993**, *49*, 911−928.