Light-Induced Oxidative Changes in a Model Dairy Spread. Wavelength Dependence of Quantum Yields

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Light-induced formation of lipid peroxides in a water-in-oil emulsion based on purified rape-seed oil (80%) was found to increase with decreasing wavelength and to have the (apparent) quantum yields $(1.1 \pm 0.1) \times 10^{-3}$ for 436 nm, $(2.6 \pm 0.1) \times 10^{-3}$ for 405 nm, and $(4.5 \pm 0.4) \times 10^{-3}$ for 366 nm irradiation, as determined after 12 h of exposure to monochromatic light of an approximate intensity of 10^{18} quanta·min^{-1}·mL^{-1} and related to total light absorption. Riboflavin (0.8 ppm) had no effect on lipid peroxidation, but photodegraded with a quantum yield ($(1.5 \pm 0.3) \times 10^{-5}$ for 436 nm, $(1.7 \pm 0.2) \times 10^{-5}$ for 405 nm and $(1.39 \pm 0.09) \times 10^{-5}$ for 366 nm irradiation) independent of irradiation wavelength. β-Carotene was only photodegraded to a minor extent, but protected riboflavin against photodegradation and the lipids against peroxidation for 436 and 405 nm irradiation (reduction in quantum yield three times for 4.5 ppm β-carotene for lipid oxidation and more for riboflavin degradation), but not for 366 nm irradiation, where β-carotene has an absorption minimum.

Keywords: *Dairy spread; photooxidation; lipid hydroperoxides; riboflavin; β-carotene*

INTRODUCTION

Light-induced processes in dairy products are mainly oxidations, which lead to formation of off-flavors, loss of nutrients, and formation of oxidation products, some of which are suspected to be toxic (Bekbölet, 1990). Since only absorbed light can initiate chemical reactions, a comparison of the spectral distribution of the light to which a product is exposed with the absorption spectra of sensitive components and the wavelength dependence of the quantum yield is central for prediction of light-induced damage and for design of protective packaging material (Sattar & deMan, 1975). Photosensitizing compounds, such as riboflavin and chlorophyll, need also to be taken into account since they can absorb light and either transform ground-state oxygen into the very aggressive singlet oxygen or initiate free radical processes in the product (Aurand et al., 1977).

Dairy spreads in which butterfat is partly substituted by vegetable oil are increasing their market share. Such products are more vulnerable to oxidations than butter, since the vegetable oil contains more unsaturated lipid than butter fat. The vegetable oil may also contain chlorophyll or chlorophyll degradation products, which in the dairy spread together with riboflavin from the milk may act as a photosensitizer for cholesterol oxidation and for oxidation of the unsaturated plant lipids (Luby et al. 1986).

Aluminum films have been used for wrapping of dairy spreads and butter since they effectively exclude light. However, environmental concerns make the use of other packaging material of interest, although such material not as effectively as aluminum foil excludes light from the products. It is accordingly important to determine the wavelength dependence of light-induced oxidation processes in order to design wrapping materials that effectively exclude the most harmful wavelength regions from the product. In the present study, we have determined quantum yields for formation of lipid peroxides in a model dairy spread for three wavelengths important in fluorescent light used for retail display and have also considered the influence of the photosensitizer riboflavin and the singlet oxygen quencher β-carotene. Quantum yields at these spectral wavelengths should, together with the absorption spectra of the sensitive compounds in a product, provide the background for design of optimal packaging material.

MATERIALS AND METHODS

Materials. For the chemical analysis, analytical-grade chemicals and solvents were used. Water was purified through a Millipore Q-plus purification train (Millipore Corp., Bedford, MA). The food-grade rape-seed oil was Colza from Aarhus Olie A/S, Denmark. Riboflavin (R-4500) from Sigma (St. Louis, MO) and β-carotene (in sealed ampules) from Roche A/S (Hvidovre, Denmark) was used without further purification.

Purification of Oil. A triacylglycerol fraction was made by purification of the food-grade rape-seed oil by a principle described by Lampi et al. (1992) using a glass column ($l = 25$ cm and $d = 4$ cm). The column was filled with two portions of 18 g of thermally activated Silica (100−200 mesh, Sigma, St. Louis, MO, washed three times with water followed by a methanol washing prior to heating at 100 °C for ∼10 h followed by 200 °C for ∼10 h), separated by 9 g of a mixture (1:2) of Celite 545 (Struers KEBO Lab, Albertslund, Denmark) and activated charcoal (Norit, Amersfoort, The Netherlands) and 36 g of a mixture (1:2) of Celite 545 and powdered sucrose (Danisco Sugar, Copenhagen, Denmark). Oil (90 g) was dissolved in an equal amount of hexane (HPLC-grade, Labscan Ltd., Dublin, Ireland), during and after the purification, the column and oil were protected against light. Hexane was removed by rotary evaporation at 40 °C after purification. The fatty acid distribution, peroxide value, and tocopherol content of the oil prior to and after purification is shown in Table 1.

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Table 1. Fatty Acid Distribution[a], Peroxide Value and Tocopherol Content in Food-Grade Rape-Seed Oil prior to and after Purification[b]

	food-grade rape-seed oil	purified
palmitic acid [%]	5.0	5.1
palmitoleic acid [%]	0.3	0.3
stearic acid [%]	1.2	1.3
oleic acid [%]	59.2	60.5
linoleic acid [%]	21.2	20.8
linolenic acid [%]	10.3	9.2
eicosan acid [%]	1.2	1.2
peroxide value [meq/kg]	0.8 ± 0.02	$\leq 0.1 \pm 0.002$
γ-tocopherol [ppm]	13.9 ± 0.6	2.3 ± 0.1
δ-tocopherol [ppm]	359 ± 14	57 ± 2
α-tocopherol [ppm]	262 ± 10	11.4 ± 0.5

[a] Percent of total fatty acid content. [b] Standard deviation of fatty acid content is 0.1.

The food-grade oil contained traces of chlorophyll as determined spectrophotometrically, but in the purified oil, no indication of presence of chlorophyll was seen.

Dairy Spread Model. Eight different emulsions were made varying the following three factors: (i) food-grade rape-seed oil or purified rape-seed oil; (ii) presence or absence of riboflavin (0.80 ppm); and (iii) presence or absence of β-carotene (4.5 ppm). In each case, 80% (w/w) rape-seed oil, 1% emulsifier (Dimodan CP, Danisco Ingredients, Denmark) and fully hydrogenated sun flower oil (1%) were mixed and melted in water bath at 75 °C. Slowly, while being homogenized (Ultra Turrax T25), 18% water was added, and the mixture was further homogenized for 3 min. β-Carotene was added to the oil phase, and riboflavin was dissolved in the water prior to homogenization. To confirm that the continuous phase in the emulsion consisted of oil, a few drops of an "Oil red O" solution (Sigma, St. Louis, MO, USA; 0.7 g/100 mL of propan-2-ol) were added to samples of the emulsions. A uniformly colored emulsion with droplet of uncolored water was observed, confirming that the emulsion is a water-in-oil emulsion.

Photolysis. Monochromatic light (366, 405, or 436 nm) was selected from an Osram 100/2 HBO high-pressure Hg lamp (line spectrum) mounted as part of an optical train, which also included a light condenser, a heat filter, an interference filter, and lenses focusing the light into a thermostated (23 ± 2 °C) cell ($l = 5$ cm) containing the emulsion, which was stirred by a Teflon-coated magnetic bar. All optical components and cells were made of quartz (Spindler und Hoyer, Göttingen, Germany). Light intensities at each of the three wavelengths used in the experiment were determined by ferrioxalate actinometry (Hatchard & Parker, 1956) prior to and after each photolysis series.

Chemical Analysis. To separate the two phases of the emulsions following exposure to monochromatic light, the samples were centrifuged for 30 min at 2900*g*. The oil and water samples were then stored separately in dark at −30 °C until analysis.

Peroxide Value: A method described by Shanta and Decker (1994) based on oxidation of iron(II) was used to determine the peroxide value in the oil phase, and the absorbance of the samples was measured using a Varian Cary 3 spectrophotometer (Varian, Palo Alto, CA).

Conjugated Dienes: The spectrophotometric method described by Corongiu and Banni (1994) was used to yield relative concentrations of conjugated dienes using the second-derivative spectrum. Approximately 10 mg of oil phase was weighed accurately (m_{oil}) in a test tube with screw cap, to which 3.00 mL of cyclohexane (HPLC grade) was added. From the second derivative of the absorbance at the absorption minimum at 242 nm and at the absorption maximum at 252 nm, the relative content of conjugated dienes (in inverse grams) was calculated using the following expression:

$$[\text{conj. dienes}] = \frac{A''_{252} - A''_{242}}{m_{oil}}$$

Tocopherols: A 50-mg oil-phase aliquot was dissolved in 5.00 mL of ethanol (96%). Quantification of tocopherols in the samples was performed with reversed-phase HPLC Shimadzu 10A (Shimadzu, Kyoto, Japan) using a Chromosphere 250 mm × 4.6 mm, 5 μm C_{18} column (Chrompack, Melburg, The Netherlands) with methanol as the mobile phase with fluorescence detection (Waters 470, Milford, MA) using excitation at 292 nm and emission at 330 nm. Integration was performed by the LC10 software (Shimadzu, Kyoto, Japan), and the concentrations of the homologues tocopherols were determined using external standards (Merck, Darmstadt, Germany); values reported are means of two determinations.

β-Carotene: Approximately 0.45 g of accurately weighed oil phase was dissolved in 3.00 mL of chloroform, and the absorbance was measured (HP 8452A Diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA)) at the absorption maximum for β-carotene dissolved in chloroform at 460 nm (Craft & Soares, 1992). The standard curve was based on β-carotene (in five different concentrations) dissolved in the same oil.

Riboflavin: The content of riboflavin in the samples was determined using the AOAC method 970.65 as described by Nielsen (1994), autoclave treatment was not needed because of the low content of proteins. Fluorescence was determined at a Shimadzu RF-5001 PC spectrofluorometer (Shimadzu, Kyoto, Japan).

Statistical Analysis. The effect of the experimental factors on the apparent quantum yields (Φ_{app}) for each sample, and each chemical analysis were analyzed using the procedure ANOVA in the statistical package SAS (SAS Inc, Cary, NC). All experiments were repeated independently with double analyses.

RESULTS

Light was found to induce oxidation in the dairy spread model studied. Food-grade rape-seed oil gave a product which was slightly more sensitive to light exposure, at least for the wavelengths where traces of chlorophyll may absorb when compared to the product based on purified rape-seed oil. While the purified rape-seed oil had a fatty acid distribution practically unaltered compared to the food-grade oil, the tocopherol content was lowered significantly for the purified oil (Table 1). The low content of tocopherols and of chlorophyll (and chlorophyll degradation products) in the dairy spread model based on purified oil made this product more suitable for determination of more fundamental constants characteristic for photoinduced lipid oxidation and riboflavin degradation. In contrast, the spread model made with the food-grade oil had a composition closer to real products, and the constants determined for this latter product may be of more practical value. Results from both series of experiments will be discussed.

Only light absorbed can cause chemical reaction, and the proportionality factor between the light absorbed (I_{abs} in quanta per minute per milliliter) and rate of the resulting chemical reaction (dC_i/dt in molecules per minute per milliliter) is known as the quantum yield which also is defined as

$$\Phi = \frac{\text{molecules reacted}}{\text{photon absorbed by reacting compound}} = \frac{\Delta C_i}{Q_i} \quad (1)$$

where ΔC_i is the change in concentration of the specified compound resulting from absorption of the number of photons by this compound, Q_i. For an emulsion like the one used as a model for a dairy spread, most of the light

Table 2. Apparent Quantum Yields[a] Based on Total Light Absorption for Formation of Peroxides and Degradation of Riboflavin in a Model Dairy Spread[b] Exposed to Monochromatic Light at 23 °C for 12 and 24 h

λ_{irr}[c] [nm]	β-carotene [ppm]	riboflavin [ppm]	stripped oil		food-grade oil	
			$\Phi_{12\ h}$	$\Phi_{24\ h}$	$\Phi_{12\ h}$	$\Phi_{24\ h}$
			(A) Peroxide			
366	0	0	$(4.5 \pm 0.4) \times 10^{-3}$	$(2.9 \pm 0.06) \times 10^{-3}$	$(3.3 \pm 0.9) \times 10^{-3}$	$(6.0 \pm 0.5) \times 10^{-3}$
366	0	0.8	$(3.8 \pm 0.5) \times 10^{-3}$	$(2.9 \pm 0.2) \times 10^{-3}$	$(7.3 \pm 2.2) \times 10^{-3}$	$(5.0 \pm 0.001) \times 10^{-3}$
366	4.5	0	$(4.3 \pm 1.0) \times 10^{-3}$	$(2.2 \pm 0.3) \times 10^{-3}$	$(4.5 \pm 1.9) \times 10^{-3}$	$(4.6 \pm 0.09) \times 10^{-3}$
366	4.5	0.8	$(4.1 \pm 1.6) \times 10^{-3}$	$(2.9 \pm 1.0) \times 10^{-3}$	$(2.9 \pm 0.05) \times 10^{-3}$	$(5.5 \pm 0.4) \times 10^{-3}$
405	0	0	$(2.6 \pm 0.08) \times 10^{-3}$	$(1.9 \pm 0.3) \times 10^{-3}$	$(5.4 \pm 2.0) \times 10^{-3}$	$(6.5 \pm 2.1) \times 10^{-3}$
405	0	0.8	$(2.7 \pm 0.07) \times 10^{-3}$	$(2.0 \pm 0.3) \times 10^{-3}$	$(6.9 \pm 0.05) \times 10^{-3}$	$(4.8 \pm 0.6) \times 10^{-3}$
405	4.5	0	$(8.7 \pm 1.8) \times 10^{-4}$	$(6.8 \pm 0.3) \times 10^{-4}$	$(4.2 \pm 2.0) \times 10^{-3}$	$(4.1 \pm 0.3) \times 10^{-3}$
405	4.5	0.8	$(1.2 \pm 0.009) \times 10^{-3}$	$(1.5 \pm 0.3) \times 10^{-3}$	$(4.5 \pm 1.2) \times 10^{-3}$	$(4.0 \pm 0.6) \times 10^{-3}$
436	0	0	$(1.1 \pm 0.04) \times 10^{-3}$	$(1.2 \pm 0.4) \times 10^{-3}$	$(5.8 \pm 0.04) \times 10^{-3}$	$(3.2 \pm 1.5) \times 10^{-3}$
436	0	0.8	$(1.6 \pm 0.03) \times 10^{-3}$	$(1.3 \pm 0.1) \times 10^{-3}$	$(5.4 \pm 0.1) \times 10^{-3}$	$(3.5 \pm 0.1) \times 10^{-3}$
436	4.5	0	$(3.8 \pm 0.04) \times 10^{-4}$	$(4.5 \pm 0.08) \times 10^{-4}$	$(2.8 \pm 1.7) \times 10^{-3}$	$(2.9 \pm 0.09) \times 10^{-3}$
436	4.5	0.8	$(5.1 \pm 1.1) \times 10^{-4}$	$(5.7 \pm 1.2) \times 10^{-4}$	$(3.1 \pm 1.4) \times 10^{-3}$	$(2.7 \pm 0.1) \times 10^{-3}$
			(B) Riboflavin			
366	0	0.8	$(1.4 \pm 0.1) \times 10^{-5}$	$(1.1 \pm 1.6) \times 10^{-5}$	$(2.0 \pm 0.4) \times 10^{-5}$	$(7.3 \pm 4.9) \times 10^{-6}$
366	4.5	0.8	$(1.7 \pm 0.7) \times 10^{-5}$	$(7.5 \pm 0.4) \times 10^{-6}$	$(1.4 \pm 0.4) \times 10^{-5}$	$(9.0 \pm 1.7) \times 10^{-6}$
405	0	0.8	$(1.7 \pm 0.3) \times 10^{-5}$	$(1.3 \pm 0.3) \times 10^{-5}$	$(6.2 \pm 5.9) \times 10^{-6}$	$(4.7 \pm 0.8) \times 10^{-6}$
405	4.5	0.8	$(1.9 \pm 2.7) \times 10^{-6}$	$(4.8 \pm 0.7) \times 10^{-6}$	$(8.2 \pm 3.4) \times 10^{-6}$	$(2.8 \pm 3.7) \times 10^{-6}$
436	0	0.8	$(1.5 \pm 0.5) \times 10^{-5}$	$(9.4 \pm 1.2) \times 10^{-6}$	$(1.5 \pm 0.3) \times 10^{-5}$	$(8.7 \pm 1.9) \times 10^{-6}$
436	4.5	0.8	~ 0	$(2.2 \pm 3.0) \times 10^{-6}$	$(6.2 \pm 7.1) \times 10^{-6}$	~ 0

[a] Each figure is calculated from two independent determinations in both of which each analysis was done in triplicate and given with standard deviations. Samples left in the dark for the same period of time showed only insignificant changes in peroxide value or riboflavin degradation. [b] 80% stripped rape-seed oil or food-grade rape-seed oil in a water-in-oil emulsion. [c] Light intensities were approximately 7.72×10^{16} quanta·min^{-1}·ml^{-1} for 366 nm, 7.34×10^{16} for 405 nm and 1.25×10^{17} for 436 nm. The exact value was determined by ferrioxalate actinometry after each group of photolysis experiments.

in the UV−near-UV region is absorbed ($A_{irr} > 1$), and the following approximation becomes valid:

$$I_{abs} = I_0(1 - 10^{-A_{irr}}) \approx I_0 \quad (2)$$

and the intensity of light as determined by actinometry, I_0, can be used for calculation of the quantum yield. However, it is not possible to calculate the fraction of the light absorbed by the i-th compound, $I_{abs}(i)$, from the total light absorbed by the emulsion using the equation valid for simple solutions:

$$I_{abs}(i) = \frac{lC_i\epsilon_{irr}(i)}{A_{irr}} \quad (3)$$

In eq 3, the molar absorptivity, $\epsilon_{irr}(i)$, for the i-th compound together with the concentration of this compound, C_i, the light path, l, and the total absorbance at the wavelength of irradiation, A_{irr}, in principle form the basis for assignment of light absorption to individual compounds. Such an assignment is not possible due to lack of additivity of absorption by the individual components in a heterogeneous system such as the nontransparent emulsion. In contrast, apparent quantum yields based on total light absorption may be used for comparison of photoinitiated processes in comparable systems:

$$\Phi_{app} = \frac{\text{molecules absorbed}}{\text{photons absorbed by emulsion}} = \frac{\Delta C'_i N_A V}{Q_{total}} \quad (4)$$

$\Delta C'_i$ is the change in concentration of the reacting compound determined by a chemical analysis, N_A is Avogadro's number, V is the volume of the reaction system which has to be well-stirred in order to ensure homogeneity with respect to reacted/nonreacted material, and Q_{total}, the total number of photons absorbed by the system, is calculated from

$$Q_{total} = I_{abs}t \approx I_0 t \quad (5)$$

for the time of light exposure, t. Since the concentration of the reacting compound in any specific reaction is declining on exposure to light and the fraction of light absorbed by this species also is becoming relatively smaller, the apparent quantum yield will, in contrast to quantum yield as defined by eq 1, be smaller by prolonged time of exposure to light, t.

Apparent quantum yields for formation of lipid hydroperoxides and degradation of riboflavin (when present) are presented in Table 2 for eight different model dairy spreads (purified or food-grade rape-seed oil, each with or without addition of riboflavin and each with or without addition of β-carotene in concentrations typical for an average dairy spread), each for two exposures to monochromatic light of each of three wavelengths of importance in standard fluorescent light. For each of the forty-eight combinations of conditions, the apparent quantum yields presented are mean values from two experiments. The quantum yields are based on the peroxide values, but the conjugated dienes gave qualitatively the same results (Table 3). Emulsions kept in the dark at 23 °C for the same reaction time showed insignificant formation of lipid hydroperoxides and no degradation of riboflavin. Therefore, correction for "dark reactions" was not important. It should be noted that the apparent quantum yields are not absolute values in the sense that they can be applied to other types of product but only serve the purpose of comparison in the actual spread.

During the light exposure, β-carotene degradation was found only in the spread based on food-grade oil and with an average quantum yield as low as 1.6×10^{-6}, and correction for β-carotene photodegradation was not employed for calculation of efficiencies of photoinduced lipid oxidation and riboflavin degradation. Degradation of the tocopherols was likewise found to be unimportant although slightly influenced by the presence of riboflavin, as may be seen from Table 3. From the effect of

Table 3. Concentration of Conjugated Dienes and Peroxide Values for Model Dairy Prepared from Purified Rape-Seed Oil and Tocopherol Content of Model Dairy Prepared from Food-Grade Oil Illuminated for 24 h

			purified product		food-grade product percent of tocopherols left		
wavelength [nm]	β-carotene	riboflavin	conjugated dienes[a]	peroxide value[b]	δ[c]	γ	α
366	−	−	1.41×10^{-4}	2.00	99	93	90
366	−	+	1.40×10^{-4}	2.04	99	92	66
366	+	−	1.33×10^{-4}	1.64	101	92	91
366	+	+	1.33×10^{-4}	1.99	102	93	90
405	−	−	1.34×10^{-4}	1.54	100	93	91
405	−	+	1.36×10^{-4}	1.58	101	79	93
405	+	−	1.16×10^{-4}	0.78	99	92	92
405	+	+	1.22×10^{-4}	1.20	100	93	93
436	−	−	1.20×10^{-4}	1.26	102	93	90
436	−	+	1.38×10^{-4}	1.36	97	94	93
436	+	−	1.21×10^{-4}	0.53	103	94	88
436	+	+	1.16×10^{-4}	0.68	100	94	95

[a] Relative scale. [b] In millimoles of peroxide per kilogram of sample. [c] The fluctuating values of δ-tocopherol is due to the low content of this specie. The concentration is close to the detection limit.

the experimental factors on the apparent quantum yields, a statical analysis warranted the following conclusions to be drawn.

For lipid peroxidation, as monitored by the peroxide value and conjugated dienes, the nature of the oil, the wavelength of the light, and the presence or absence of β-carotene all had a significant influence on the quantum yields with a interaction between nature of oil and wavelength of irradiation ($p = 0.001$). Presence or absence of riboflavin was on the other hand not found to have significant influence on lipid peroxidation. Wavelength of light was concluded to be the most significant single factor for the light-induced lipid peroxidation, with 366 nm light being most damaging ($p = 0.1$), and with 405 and 436 nm light having comparable efficiencies. The spread based on rape-seed oil was more sensitive to light than the spread based on stripped oil ($p = 0.001$). β-Carotene was found to decrease the quantum yield significantly ($p = 0.001$).

Riboflavin degradation was, besides time of light exposure, influenced by the wavelength of the light and by the presence or absence of β-carotene with a significant interaction between these two factors ($p = 0.05$). Again, the two wavelengths, 405 and 436 nm, had comparable effect while 366 nm light being more damaging ($p = 0.0001$). β-carotene protected riboflavin significantly ($p = 0.05$) against light-induced degradation. These conclusions were found valid both for spread based on food-grade oil and on stripped oil, and the nature of the oil had no effect on the photodegradation of riboflavin.

DISCUSSION

Riboflavin is an important nutrient of milk products but is also a photosensitizer which induces off-flavor in milk on exposure to sunlight or fluorescent light during retail display (Jung et al., 1998). Two limiting mechanisms may be active during the photosensitized damage to proteins leading to the "burnt feather" note and to lipids subsequently leading to the "cardboardlike" off-flavor (Sattar & deMan, 1975; Marsili, 1999). The action of riboflavin is, however, not cleanly "photodynamic" as riboflavin as sensitizers is gradually being degraded with light exposure (Korycka-Dahl & Richardson, 1978).

One mechanism involves formation of singlet oxygen through deactivation of triplet state riboflavin by oxygen formed by intersystem crossing of excited-state singlet riboflavin, Figure 1. Singlet oxygen can add to unsaturated lipids to form lipid hydroperoxides or may be deactivated by quenchers such as β-carotene (Aurand et al., 1977). This so-called type II photooxidation is in contrast to the other mechanism where triplet riboflavin will act as an oxidant abstracting one electron from an oxidation substrate initiating free radical chain processes in lipids and proteins. Upon reoxidation of the riboflavin anion radical as shown in Figure 1, or of the fully reduced form, superoxide anion is formed which may initiate further oxidative degradation (Korycka-Dahl & Richardson, 1978). β-Carotene does not directly interfere with this so-called type I photooxidation. Our observation can be fully understood on the basis of type I sensibilization although formation of singlet oxygen cannot fully be excluded.

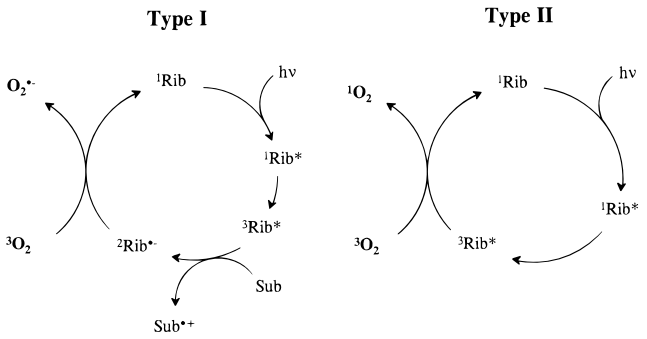

Figure 1. Two limiting mechanisms for riboflavin-sensitized oxidation of relevance for the dairy spread model. ^{1}Rib* is formed by absorption of a photon forming the triplet excited state, ^{3}Rib*, by intersystem crossing. ^{3}Rib* is subsequently deactivated by ground-state oxygen (type II) or reacting with reductant by electron transfer (type I).

For the present food model, lipid peroxidation was initiated by light and UV light was more harmful than visible light. However, riboflavin had no effect on the light-induced lipid oxidation. Degradation of the tocopherols in the spread based on food-grade oil was likewise found to be insignificant. The presence of a higher concentration of tocopherols in the food-grade oil made the pattern observed less clean in the spread based on this oil. β-Carotene was found to protect the lipid against light-induced oxidation both in the presence and in the absence of riboflavin. Notably, this protection was not significant for 366 nm light, and when comparing the absorption spectra of β-carotene and riboflavin for the concentrations used, it is seen that β-carotene more effectively absorbs light at 405 and 436

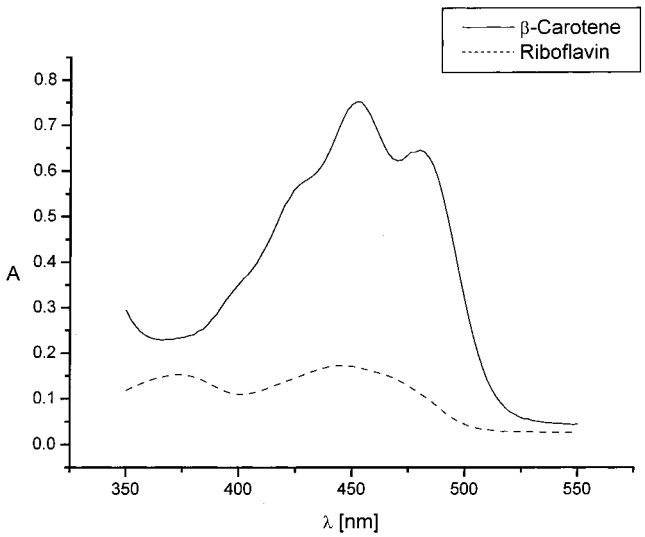

Figure 2. Absorption spectra (light path 1 cm) of riboflavin and β-carotene in concentrations used in the model dairy spread. Riboflavin (0.80 ppm) dissolved in water and β-carotene (4.5 ppm) dissolved in stripped rape-seed oil.

nm than at 366 nm (Figure 2). It is suggested that β-carotene protects lipids through an inner-filter mechanism by absorbing a significant fraction of the light and reducing chemical reactions. A similar conclusion was reached for the protection of olive oil against light-induced oxidation by β-carotene (Fakourelis et al. 1987).

Riboflavin upon absorption of light did not seem to form singlet oxygen in the present food model, or any singlet oxygen formed was not sufficient for inducing lipid peroxidation. For milk riboflavin, sensitized oxidation of protein is faster than that of lipids; i.e., the burnt feather off-flavor is recognized prior to the cardboardlike off-flavor (Sattar & deMan, 1975). This observation could also indicate the importance of sensitizer and substrate being located in the same phase, especially in a high-viscosity product like the present spread model (Conn et al. 1991).

Type I sensitization could, however, also be important and account for the photooxidation of riboflavin (Skibsted, 2000). Again, β-carotene was found to protect against 405 and 436 nm irradiation, but not against 366 nm irradiation. Since β-carotene is not scavenging the superoxide anion present in the water phase, the protection against light of the two wavelengths where both riboflavin and β-carotene absorb significantly (Figure 2) can also be accounted for by an inner-filter mechanism.

In conclusion, β-carotene has been found to have an important function as an inner filter in a model dairy spread where it protects riboflavin against light-induced degradation and the lipid against photooxidation. When formulating such products, it could be important to use milk fat with a high β-carotene content, and when designing packaging material, it seems to be important to exclude light of wavelengths not absorbed by β-carotene, such as the 366 nm Hg-line present in many fluorescent tubes.

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