JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Effect of Naturally Occurring Tetrapyrroles on Photooxidation in Cow's Milk

Diego Airado-Rodríguez,*[,†,§] Natthorn Intawiwat,[†,#] Josefine Skaret,[†] and Jens Petter Wold[†]

[†]Nofima Mat AS, Osloveien 1, N-1430 Ås, Norway

[§]Department of Analytical Chemistry, Faculty of Sciences, University of Extremadura, Avenida de Elvas s/n, 06006 Badajoz, Spain

[#]Department of Chemistry, Biotechnology and Food Sciences, Norwegian University of Life Sciences, NO-1432 Ås, Norway.

ABSTRACT: The objective of this work was to better understand the photosensitizing effect of riboflavin versus naturally occurring tetrapyrroles in cow's milk. This was done by exposure of milk samples to blue light (400−500 nm), which is absorbed by riboflavin and tetrapyrroles, orange light (575−750 nm), which is absorbed by tetrapyrroles but not riboflavin, and white light, which contains the entire visible region. The milk was exposed to about 1.6 W/m^2 in 20 h, and two different light sources were tested: HMI lamp and fluorescent light tubes used for commercial display. Sensory analysis showed that wavelengths longer than 575 nm induced significantly more off-flavors than wavelengths shorter than 500 nm. By fluorescence spectroscopy it was observed that tetrapyrroles, in particular, chlorophyllic compounds, were degraded more by orange light than by blue and that the degree of degradation correlated closely with the formation of sensory off-flavors. The fluorescent agent Singlet Oxygen Sensor Green (SOSG) was used to monitor the formation of singlet oxygen under the different light exposure conditions, and the method verified that singlet oxygen was formed in large proportions in milk exposed to wavelengths longer than 575 nm, presumably with minor or no involvement of riboflavin. The results suggest that cholorophyllic compounds are responsible for a major part of photooxidation in milk. It is also suggested that β-carotene protects against photooxidation under blue light because it absorbs a major portion of the light below 500 nm and thereby reduces reactions with photosensitizers.

KEYWORDS: milk, photooxidation, photosensitizers, Singlet Oxygen Sensor Green (SOSG), front-face fluorescence, sensory analysis

INTRODUCTION

It is well-known that milk is susceptible to photosensitized oxidation. It is therefore important to protect milk against light during transport and storage to avoid formation of off-flavors and shortened shelf life. Much work has been undertaken to explain the mechanisms of photooxidation in milk and also to determine harmful and less harmful light wavelength regions.[1,2] Nevertheless, the present knowledge about the main photochemical reactions in milk is still not conclusive or even sufficient to give well-founded practical advice to the industry on how to minimize photooxidation through packaging and retail. Only one thing seems to be certain; the longest shelf life is obtained by complete blocking of all light, from ultraviolet radiation (UV) through the entire visible region including red light.[3]

In 1946 Josephson reported the results from an interesting study.[4] He exposed milk to light of different wavelengths and measured the resulting photodegradation of riboflavin (vitamin B_2) as well as the formation of sensory sunlight flavor. He reported that riboflavin was degraded by violet and blue light below 500 nm, whereas orange light in the 590−630 nm region induced the strongest sunlight flavor. To avoid formation of off-flavors, complete blocking of all wavelengths below 750 nm was necessary. This sensory response did not correspond with a purely riboflavin-sensitized oxidation. He also reported that degradation of riboflavin occurred 0.3 in. into the milk, whereas the sunlight flavor was generated 0.6 in. into the milk. On the basis of these findings he concluded that orange and red light of wavelengths in the 590−630 nm region was responsible for the formation of sunlight flavor in milk.

Despite these reported results, the overall accepted view the past decades has been that riboflavin is the responsible photosensitizer in milk, and as a result of this, blue light and UV have been regarded as the most harmful radiation with regard to photooxidation.[1] There are, however, some reports that point in other directions. Several packaging materials combining color pigment and UV block have been studied in relation to their light transmittance properties and prevention of photooxidation in pasteurized milk.[3,5−10] One overall finding in these studies is that UV block as well as blocking of blue light below 500 nm reduces oxidation, but it is not sufficient to avoid oxidation. Visible light, also yellow and red light, induces photooxidation in milk and dairy products.

Milk is a very complex liquid containing myriad compounds that can take part in the photoreactions. Photooxidation takes place either by photolytic autoxidation or by photosensitized oxidation. Photolytic autoxidation consists in the production of free radicals primarily from lipids during exposure to UV light.[11] This type of reaction proceeds by normal free radical chain reactions.[12] However, direct interaction of UV light with lipids in foods is minimal and, thus, not a primary concern.[12] Photosensitized oxidation occurs in the presence of photosensitizers.[11] These compounds can absorb visible or near-UV light to become electronically excited. Photosensitizers have two excited states:

Received: November 2, 2010
Accepted: January 27, 2011
Revised: January 26, 2011
Published: March 15, 2011

singlet and triplet. The triplet excited state has a longer lifetime and initiates oxidation. Photooxidation by a photosensitizer can proceed through either type I or type II reactions. Type I reactions proceed through a free radical mechanism, whereas in type II reactions, the sensitizer reacts with oxygen to produce singlet oxygen ($^1O_2$), which is highly reactive. These reactions can occur at the same time, in a competitive fashion,[13] and this has been observed in milk.[14] However, at low oxygen concentrations, the type I reactions are most efficient.[15] In terms of concentration, riboflavin is the most prominent photosensitizer in milk. Recently, a more complete list of photosensitizers in dairy products was proposed by Wold et al.,[16] including tetrapyrroles, which seem to be actively involved in the photosensitized oxidation of milk.[10] These are riboflavin, protoporphyrin IX (PpIX), hematoporphyrin, a chlorophyll *a*-like compound, and two unidentified tetrapyrroles. The concentrations of these compounds seem to vary with fat content. According to fluorescence spectroscopy, butter has higher concentrations of tetrapyrroles than cheese and sour cream and a considerably higher concentration than milk, whereas the opposite is the case for riboflavin, which is water-soluble.[17] To understand photoreactions in milk, it is important to take into account the presence of β-carotene, one of the major chromophores in milk, which is also an effective scavenger of radicals and singlet oxygen.[18,19]

Monitoring the formation of singlet oxygen as a function of light exposure can be a valuable tool for understanding photoreactions in milk. Recently, a new $^1O_2$ fluorescent probe has been released, under the trade name Singlet Oxygen Sensor Green (SOSG) reagent.[20] This probe is highly selective for $^1O_2$, and unlike other available fluorescent $^1O_2$ detection reagents, it does not show any appreciable response to hydroxyl radical ($^{\bullet}OH$) or superoxide ($^{\bullet}O_2^-$). SOSG emits weak blue fluorescence peaking at 395 and 416 nm for excitation at 372 and 393 nm. After reaction with $^1O_2$, it emits a green fluorescence similar to that of fluorescein (excitation/emission maxima ~ 504/525 nm).[20] The photometric and fluorescent spectral properties of SOSG before and after reaction with $^1O_2$ have been reported by Flors et al.[21] An apparent limitation with this probe with regard to photooxidation studies is that it might act as a $^1O_2$ photosensitizer itself under exposure to radiation (UV and some visible wavelengths).[22]

The objective of this work was to better understand the photosensitizing effect of riboflavin versus naturally occurring tetrapyrroles in cow's milk. This was done by exposure of milk samples to blue light, which is absorbed by riboflavin and tetrapyrroles, orange light, which is absorbed by tetrapyrroles (but not riboflavin), and white light, which contains the entire visible region. Sensory analysis was used to measure formation of off-flavors and off-odors. Fluorescence spectroscopy was used to monitor the photodegradation of the photosensitizers in milk with emphasis on riboflavin, PpIX, and chlorophyllic compounds. The fluorescent agent SOSG was used to measure the amount of singlet oxygen formed under the different light exposure conditions.

MATERIALS AND METHODS

Experimental Design. The purpose of the experimental design was to study differences in photooxidation in milk induced by wavelengths shorter than 520 nm (violet and blue) and longer than 550 nm (yellow, orange, red), as well as white light consisting of all visible wavelengths. In this section an overview of the experimental design is given. Details on materials and methods follow in the next sections.

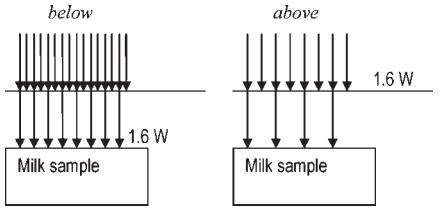

Figure 1. Light intensity was adjusted to about 1.6 W/m^2 either below the filters or above the filters. In the case of adjustment below, the intensity at the milk surface was around 1.6 W/m^2. In the case of adjustment above, the intensity at the milk surface was approximately 1.6 W/m^2 or less, depending on the film properties.

Milk from the same batch was packed in plastic trays and sealed with transparent film. The headspace was either air or nitrogen. The trays were covered with colored filters (blue, orange, and gray), and the light intensity was adjusted in two ways: (1) The intensity was adjusted to be about 1.6 W/m^2 at the milk surface for all storage conditions. This setup is denoted "below" because the intensities were adjusted to be equal below the colored filters (Figure 1), which enables comparison of the photooxidative effect of the different wavelength regions. (2) The intensity was adjusted to be about 1.6 W/m^2 above the colored filters. This setup is denoted "above" (Figure 1) and enables comparison of the particular filters used.

The samples were stored and exposed to light according to columns 1−3 in Table 2. Two different headspace atmospheres, three light filters, the two different light intensity adjustment procedures, as well as milk stored in the dark with air and nitrogen, were included in the design. Two replicates of each experimental point resulted in a total of 28 samples. Storage time was 20 h.

Two sets of experiments were executed according to this design, using two different light sources: (i) metal halide lamps (HMI) with a relatively equal intensity over all wavelengths and (ii) fluorescent light tubes commonly used for display of dairy products.

After storage, the milk samples were immediately profiled by a sensory panel focusing on attributes connected to light-induced oxidation.

In the experiment with the HMI lamps, milk samples with the agent SOSG were also included to study the formation of singlet oxygen under the different conditions. The properties of SOSG in light-exposed milk had been studied in advance, and this study is also described and reported below.

Packing and Light Exposure of Milk Samples. Commercially produced, homogenized, pasteurized bovine milk with 3.9% fat content, packed in gable-top cartons, was obtained from a local dairy company (Tine, Oslo, Norway). The milk was from a single batch and stored at 4 °C in the dark before being repacked in plastic trays or vials.

Milk aliquots (230 mL) measured with sterilized gradual flasks were placed in white, sterilized, high-density polyethylene (HDPE) trays (5.3 × 9.2 × 9.2 cm; Promens AS, Kristiansand, Norway). Each of these trays was packed in black amorphous polyethylene terephthalate (A-PET)/PE thermoformed trays (amorphous A-PET/PE sheets were manufactured by Wipak (Nastola, Finland) and thermoformed by Jihå Plast AB (Karlskoga, Sweden)). The thermoformed trays (14.5 × 20.5 × 7.5 cm) were sealed with a top web consisting of PET/PE/ethylene vinyl alcohol/PE (Wipak) using a 511VG tray-sealing machine (Polimoon, Kristiansand, Norway). The milk contained in the white tray was used for sensory evaluation. In addition, one vial (Ø = 34 mm) with milk was also placed in each of the black trays, intended for front-face fluorescence spectroscopy measurements. The depth of the milk in these vials was the same as in the white trays for sensory analysis.

The gas in the headspace was air or N_2 (0.03 ± 0.03% O_2), according to the experimental design. To ensure proper sealing and no leakage

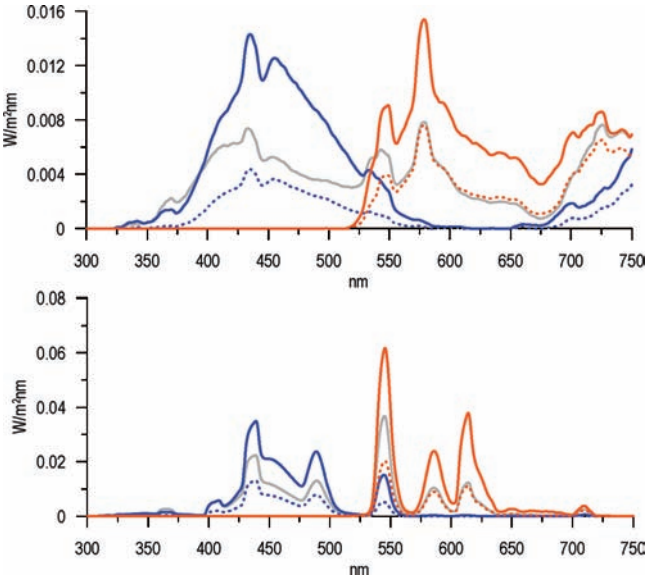

Figure 2. Transmitted light intensity as a function of wavelength for clear (gray line), blue (blue line), and orange (orange line) films, under metal halide (HMI) lamp (top) and fluorescent light tubes (bottom). Solid and dashed lines correspond to the different light intensity adjustments, below or above, respectively. For the clear film (white light) these curves were identical for the two situations.

during storage, the gas composition (% O_2 and % CO_2) in the headspace was measured using an O_2/CO_2 analyzer (CheckMate 9900 O2/CO2, PBI Dansensor A/S, Denmark). The oxygen content was checked at 4 °C directly after sealing and after 20 h of storage.

Orange transparent film based on PET (Ciba Specialty Inc., Basel, Switzerland) transmitting light from about 520 to 750 nm was used to obtain orange light (Figure 2). Blue light was obtained by using a plastic film manufactured by Rosco (Stamford, CT) under the trade name "69 Super Brilliant Blue", transmitting light between 300 and 580 nm (Figure 2). These colored films were placed on the top of the trays. Gray films were used together with the colored to adjust the light intensity in each tray to the desired values. Gray films used: 1/2 stop (3415, Rosco N.15), one stop (3402, Rosco N.3), and two stops (3403, Rosco N.6).

All light intensity adjustments and light exposure experiments were carried out in a cold-storage chamber at 4 °C. The exposure time was 20 h under the two assayed light sources (described below), and the samples were analyzed immediately after exposure.

Metal Halide Lamps. Two broadband 575 W metal halide lamps (Osram HMI 575W/SE, Munchen, Germany), which have a relatively flat emission spectrum in the visible and near-UV region, were used for the first set of experiments. The light intensity was measured by a calibrated spectrometer (Apogee Spectroradiometer, Apogee Instruments Inc., Roseville, CA), which was integrated in the region from 300 to 750 nm. The two types of light intensity adjustment ("above" and "below") were done according to Figure 1. For the "below" case the light intensity was adjusted to about 1.6 ± 0.1 W/m^2 at the milk surface but below the colored filters. For the "above" case, the light intensity was adjusted to about 1.6 ± 0.1 W/m^2 at the upper surface of the colored films. The resulting light intensity at the milk surface was also measured.

Fluorescent Light Tubes. Fluorescent light tubes (Aura Ultimate long life 830, 36-W, Aura Light International AB, Sweden) were used in the second experiment. The light intensity was adjusted as explained above.

Figure 2 shows the light intensity at the milk surface as a function of wavelength for the different films, light sources, and intensity adjustments.

Monitoring Singlet Oxygen by SOSG. For all experiments, analytical reagent grade chemicals and solvents were used. Ultrapure water was obtained from a Millipore Milli-Q System. Buffer solution citric acid/sodium citrate (total concentration = 0.01 M) was prepared by dissolving the suitable amount of citric acid monohydrate (Merck KGaA, Darmstadt, Germany) with water and adjusting the pH of the resulting solution to 6.7 by the addition of small volumes of diluted sodium hydroxide (Merck KGaA).

The probe SOSG was purchased from Invitrogen (Eugene, OR). It was provided in vials of 100 μg. It was stored desiccated and protected from light at ≤ -20 °C. Stock solutions of $\sim 5 \times 10^{-3}$ M were prepared in methanol by dissolving the contents of one 100 μg vial in 33 μL of methanol. Working solutions were prepared immediately before use, by dilution with ultrapure water.

Concentrations of SOSG around 2 μM in milk were enough to detect green fluorescence of sufficient intensity. SOSG stock solution was diluted with ultrapure water to a final concentration of 159.7 μM. Six milliliters of this diluted solution was blended with 480 mL of milk, giving a concentration of 1.97 μM SOSG in milk. Another aliquot of milk containing identical proportions of methanol and water, free of SOSG, was prepared to obtain batches of milk with and without SOSG in the same conditions.

In the experiment carried out under the HMI lamp, a vial (Ø = 22 mm) filled with milk with SOSG and another vial (Ø = 34 mm) containing milk without SOSG were packed and exposed to light, according to the experimental design, together with milk for sensory analysis. The depth of milk in vials was the same as in the trays intended for sensory analysis. Further fluorescence measurements were performed on 15 mL milk aliquots.

A control experiment to examine how SOSG itself behaves under light was required. Fluorescence is sensitive to the physical−chemical properties of the environment in which the fluorophore is included. Citric acid is reported as the main organic acid in milk, in a concentration around 1.8 g/L (approximately 0.01 M).[23] Citrate buffer (0.01 M) was therefore selected to fix the acidity of the medium for control solutions. Solutions containing 1.88 μM SOSG were prepared in 0.01 M citrate buffer at pH 6.7. Aliquots of 10 mL of this control solution as well as milk containing SOSG in the same concentration contained in vials (Ø = 22 mm) were exposed to white, blue, and orange light for 20 h (intensities at the solution surfaces about 1.6 W/m^2 under the HMI lamp). Also, control samples and milk with SOSG were stored in the dark. The green fluorescence of the SOSG solutions was monitored throughout the exposure period (on 9 mL aliquots). Five measurements were performed: (i) immediately before starting the light exposure; (ii−iv) during light exposure (at 5, 8, and 14.5 h); and (v) immediately after 20 h of exposure.

Sensory Analysis. The pasteurized milk was evaluated by a trained sensory panel at Nofima Mat AS (Ås, Norway) using a modified quantitative method as described in ISO standard 6564.[24] The panel comprised nine trained people. The panelists were selected and trained according to the recommendations in ISO standard 8586-1.[25] The sensory laboratory was designed according to guidelines in ISO standard 8589,[26] with separate booths and electronic data registration (CSA, Compusense Five, version 4.80, Guelph, ON, Canada). Prior to the assessments, the panel went through a training session with two samples, one fresh and one exposed to orange light for 20 h, to agree on the definition of each attribute and variation in attribute intensity on the 15 cm scale. Six attributes were selected to describe the sensory properties of the stored milk: sour odor and flavor (high intensity in these attributes indicates freshness), sunlight odor and flavor, which are related to oxidation of proteins, and rancid odor and flavor, including all odors and flavors associated with rancidity (grass, hay, candle, and paint), as described in ISO standard 22935-2.[27] The attributes are defined in Table 1.

Table 1. Definition of Sensory Attributes

sensory attribute		description
odors	sour	odor of freshness; sour and sweet odor
	sunlight	odor of sunlight related to oxidized protein; training reference milk exposed to sun
	rancid	intensity of rancid odors such as grass, hay, candle, paint
flavors	sour	flavor of freshness; sour and sweet flavor
	sunlight	flavor of sunlight related to oxidized protein; training reference milk exposed to sun
	rancid	intensity of rancid odors such as grass, hay, candle, paint

Samples (20 mL aliquots) were served in plastic cups (tested to be free from interfering odors and flavors), and all samples were served at room temperature (20 °C). Unsalted crackers and lukewarm water were available for rinsing the palate between samples. The coded samples were served in a randomized order by sample, assessors, and replicate. The samples were evaluated for all six attributes by each assessor. Each assessor was allowed to work at an individual pace. The panelists recorded their results on a 15 cm, nonstructured, continuous scale, with the left side of the scale corresponding to the lowest intensity and the right side of the scale corresponding to the highest intensity. The computer transformed the responses into numbers between 1.0 (low intensity) and 9.0 (high intensity). The sensory evaluation was completed within one day for each sample set (storage under HMI lamp or fluorescent light tubes).

Fluorescence Spectroscopy. Fluorescence emission spectra were measured on intact milk samples using a spectroscopic system previously described by Wold et al.[28] Aliquots (15 mL) of each sample were filled into sample cuvettes, which exposed a flat, circular surface with a diameter of 5 cm for measurement. The fluorescence emission spectra were measured for excitation at 382 nm (10 nm bandwidth interference filter, Oriel 59920; Oriel Corp., Stratford, CT) and 410 nm (10 nm bandwidth interference filter, Oriel 59285), using cutoff filters at 400 nm (Melles Griot 03FCG049; Melles Griot, Rochester, NY) and 475 nm (Melles Griot 03FCG065), respectively. Excitation at 382 nm was chosen because it produces emission from fluorescent tertiary oxidation products,[17,28] whereas 410 nm excitation was used to maximize fluorescence from tetrapyrroles. Riboflavin has excitation maxima at 370 and 450 nm; however, the emission for excitation at 410 nm is also strong. Exposure time was 1 s for all measurements.

To monitor the green fluorescence in the set of samples containing SOSG, emission spectra were registered at an excitation wavelength of 460 nm (10 nm bandwidth interference filter, Oriel 54321), using a cutoff filter at 475 nm (Melles Griot 03FCG065). Measurements were performed on samples from the HMI lamp exposure experiment (on 15 mL aliquots), and samples from the control experiment. Recording time for the fluorescence spectra was 0.2 s for all samples.

Measurement of Absorption Spectra. For clarity of presentation and discussion of the results, absorption spectra of riboflavin, chlorophyll *a*, and the SOSG agent were recorded. Chlorophyll *a* was obtained from Sigma (Steinheim, Germany). A stock solution containing 100 μg/mL of chlorophyll *a* (1.12×10^{-4} M) was prepared in absolute ethanol and stored in the dark at −80 °C. Riboflavin was obtained from Merck (Darmstadt, Germany). A stock solution containing 100 μg/mL (2.66×10^{-4} M) of riboflavin was prepared in absolute ethanol. Diluted solutions of riboflavin, chlorophyll *a*, and SOSG were prepared in water, methanol, and ethanol, respectively, containing 3.75×10^{-6}, 1.68×10^{-9}, and 9.98×10^{-6} M, respectively. Absorption spectra of these solutions were recorded in the range of 300−750 nm by a photodiode array (Agilent 8453 UV−visible spectrophotometer, Agilent Technologies, Waldbronn, Germany). The UV−visible ChemStation software (rev. A.09.01[76]) was used for data acquisition.

The absorption spectrum of β-carotene in hexane was adapted from the database PhotochemCAD[29] for a concentration of 3.72×10^{-7} M.

The absorption spectrum of a 3.9% fat milk sample was measured in reflection mode (XDS Rapid Content Analyzer, Foss NIRSystems, Hillerød, Denmark) covering the region from 400 to 800 nm with readings every 0.5 nm. The sample was filled in a round, 10 mm deep sample cup with quartz cell bottom positioned stationary inside the instrument.

Statistical Analysis and Data Processing. Significance testing of the sensory analysis was performed by General Analysis of Variance (General AOV/AOCV) using Statistic 9 (Analytical Software, Tallahassee, FL) to establish significant differences, followed by Tukey's multiple-comparisons test.

To ease interpretation and analysis of the fluorescence spectra with regard to PpIX and chlorophyllic compounds, an iterative mathematical algorithm was applied to remove the large fluorescence signal from riboflavin. This was done by polynomial fitting, a routine originally introduced to remove background fluorescence from Raman spectra.[30] In the present study a polynomial degree of 3 was chosen and an iteration number of 50 was used for the fitting procedure. The algorithm was applied on the 550−750 nm region of the emission spectra for excitation at 410 nm.

RESULTS AND DISCUSSION

Gas Composition Analysis. The oxygen concentrations in the headspace for samples packed in air, for the experiment carried out under the HMI lamp, immediately after packing and after 20 h of light exposure were 20.9% (SD 0.1%) and 20.5% (SD 0.1%), respectively. With regard to the samples packed in anaerobium atmosphere, the corresponding percentages of oxygen were 0.09% (SD 0.03%) and 0.29% (SD 0.23%). Similar values were found within the samples stored under the fluorescent light tubes. No significant changes in oxygen concentrations were observed between samples subjected to different light exposure conditions.

Sensory Analysis. Table 2, parts A and B, shows the sensory results for the samples stored under HMI lamps, packed with air and nitrogen in the headspace, respectively. Sour and sunlight odors and flavors represent positive and negative sensory attributes, respectively. Significant differences were always observed between exposed and nonexposed samples for these four attributes, independent of the color of the film, the intensity of light, or the atmosphere in the headspace. It could therefore be concluded that the light exposure conditions induced significant changes in the sensory properties of milk. With regard to rancid odor and flavor, the lowest scores for these attributes were always given to the nonexposed samples; however, the differences with a few of the exposed ones were not statistically significant.

For samples stored in air atmosphere and exposed to about 1.6 W/m^2 at the milk surface (Table 2), the observed trend is that orange light induced more intense off-odors and -flavors, compared to white and blue light. Significant differences were

Table 2. Average Sensory Scores for Milk Stored in (A) Air Atmosphere or (B) Nitrogen Atmosphere and Exposed to HMI Lamp for 20 h at 4°C under Different Film Colors[a]

film color (transmitted wavelengths)	light intensity at milk surface (300−750 nm) (W/m^2)	sour odor	sunlight odor	rancid odor	sour flavor	sunlight flavor	rancid flavor
			(A) Air Atmosphere				
dark	0.0	6.38 A	1.57 F	1.00 E	6.62 A	1.75 F	1.00 D
transparent (300−750 nm)	1.6[b]	2.41 BCD	5.85 CD	2.01 BCDE	2.03 BCD	6.36 CD	2.60 BC
blue (300−580 nm)	1.6[b]	1.95 CD	6.49 BCD	2.23 BCD	1.85 CD	6.57 BCD	2.27 BC
orange (520−750 nm)	1.5[b]	1.37 CD	7.49 AB	2.97 AB	1.29 CD	7.83 AB	3.40 AB
transparent (300−750 nm)	1.6[c]	1.50 CD	6.78 BCD	2.58 BC	1.50 CD	6.99 BCD	2.80 B
blue (300−580 nm)	0.5[c]	2.71 BC	5.53 DE	1.69 CDE	2.67 BC	5.70 DE	1.54 CD
orange (520−750 nm)	0.8[c]	2.24 BCD	5.95 CD	1.67 CDE	2.25 BCD	6.31 CD	2.34 BC
			(B) Nitrogen Atmosphere				
dark	0.0	6.05 A	1.47 F	1.00 E	6.46 A	1.50 F	1.00 D
transparent (300−750 nm)	1.6[b]	1.96 CD	6.19 BCD	2.20 BCD	1.95 BCD	6.15 CDE	2.33 BC
blue (300−580 nm)	1.6[b]	1.63 CD	6.69 BCD	1.99 BCDE	1.44 CD	6.88 BCD	2.44 BC
orange (520−750 nm)	1.5[b]	1.00 D	8.25 A	3.77 A	1.00 D	8.45 A	4.24 A
transparent (300−750 nm)	1.6[c]	1.99 CD	6.40 BCD	2.02 BCDE	2.07 BCD	6.43 CD	2.47 BC
blue (300−580 nm)	0.5[c]	3.58 B	4.38 E	1.45 DE	3.32 B	4.81 E	1.56 CD
orange (520−750 nm)	0.8[c]	1.62 CD	7.09 ABC	2.58 BC	1.54 CD	7.25 ABC	3.35 AB

[a] Reference samples stored in the dark are also included. The same letters cover groups of storage conditions where the means are not significantly ($\alpha = 0.05$) different from each other. [b] Light intensity was adjusted to about 1.6 W/m^2 below the filter. [c] Light intensity was adjusted to about 1.6 W/m^2 above the filter.

obtained for the attributes sunlight odor and flavor between samples exposed under transparent and orange films. Orange light at 1.5 W/m^2 also induced the greatest sensory degradation in samples stored in anaerobium atmosphere (Table 2). Significantly higher values were observed for sunlight and rancid odors and flavors for samples exposed under orange compared to both transparent and blue, so when milk is exposed to the same intensity of blue, white, or orange light, the orange light induces the highest sensory degradation. This result corresponds to the observations of Josephson in 1946.[4]

When the light intensity was adjusted to 1.6 W/m^2 above the filters, the intensity at the milk surface varied according to the filter properties. The highest intensity was measured under the clear film (1.6 W/m^2) and the lowest for the blue (0.5 W/m^2). For storage in air atmosphere (Table 2), samples exposed under clear and blue films were the most and least sensory degraded, respectively. However, it is noteworthy that the score for rancid flavor for the samples exposed to orange light was almost as high as for the samples exposed to white light, even though the intensity of the orange light at the sample surface was about half compared to the white light intensity. Within samples packed with nitrogen in the headspace (Table 2), those exposed to orange light were the most sensory degraded, showing statistically significant differences for all of the assessed attributes compared to the samples exposed to blue light. The scores given for samples exposed to orange and white light were not statistically different; however, all of the assessed attributes indicate slightly higher quality deterioration of the samples exposed to orange light, although they received much less light intensity than the ones exposed to white light. Again, these results indicate that wavelengths longer than 550 nm are more harmful than those below.

A third feature to highlight is that within samples exposed to orange light, those stored with anaerobium headspace were always more oxidized (higher scores in off-odors/-flavors and lower scores in sour odor/flavor) than the ones packed in air. For blue and white light this was not the case, because the air atmosphere often induced worse sensory properties. This interaction effect between exposure wavelength and atmosphere was also observed by Intawiwat et al.,[10] and it suggests that the oxidation reaction pathways might vary with wavelength.

The sensory results for milk exposed under fluorescent light tubes followed the same trends as described for the experiment performed with the HMI lamps; however, the differences were less pronounced. A reason for this might be that it was more difficult to perform the light intensity adjustment under this light source due to large intensity variations along the tubes. Figure 3 shows responses for sunlight and rancid flavors for samples exposed to 1.6 W/m^2 at the milk surface under the different films. Again, it can be noted that orange light and nitrogen atmosphere resulted in the highest score for rancid flavor, significantly higher than, for instance, blue light in air atmosphere.

There are not many studies that clearly support our findings except for that of Josephson.[4] Studies on cheese, butter, and milk by our own group have been reported, where light in the orange and red region clearly induced photooxidation at the same level as violet and blue light, but seldom worse.[10,31,32] In reported studies, the exposure conditions vary a lot, making comparisons difficult. The idea of normalizing the intensity of light of different colors/wavelengths at sample surface ("below" adjustment) is

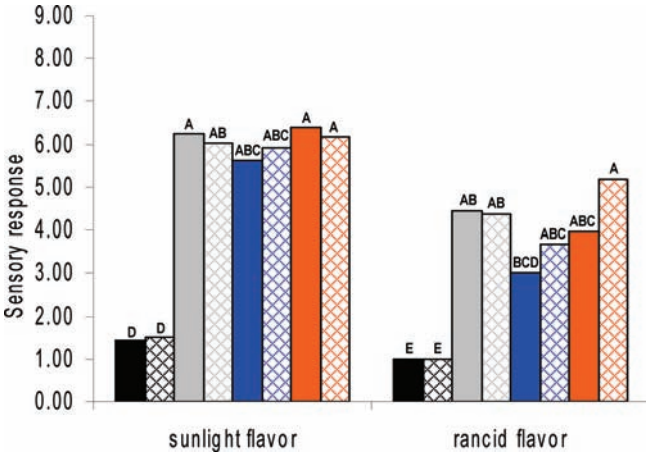

Figure 3. Sensory responses for sunlight and rancid flavor for milk packed in air and nitrogen and exposed to light from fluorescent light tubes (1.6 W/m^2) under different colored films. Storage was maintained under blue (blue bars), orange (orange bars), and white (gray bars) light or in the dark (black bars). Solid bars indicate storage under air and cross-hatched bars, storage under nitrogen. The same letters cover groups of storage conditions for which the means were not significantly ($\alpha = 0.05$) different.

not widely applied, although this approach is the one required to compare the effects of different wavelength regions. Standardization of light intensity above the applied filters is more commonly used, but differences in intensity at the actual sample surface then tend to obscure the impact of the wavelength regions. The effects of the specific combinations of filters and light sources are then evaluated, which in many cases is of practical interest.

Measurement of Formation of Singlet Oxygen by SOSG. It has recently been asserted that the probe SOSG is able to produce singlet oxygen by itself under exposure to ultraviolet and visible radiation.[22,33] The photoinduced production of $^1O_2$ by SOSG is reported to be wavelength-dependent, and different reaction pathways have been proposed.[22] These properties have to be taken into account when using SOSG for evaluation of photooxidation. The first step in this work was therefore to study the behavior of SOSG itself, in the absence of milk, when it was exposed to light of the assayed wavelength regions.

The development of green fluorescence at $\lambda_{ex/em}$ 460/527 nm for control samples is shown in Figure 4A. No green fluorescence was developed by SOSG kept in the dark or when exposed to orange light for 20 h. This makes sense because the absorption of light by the agent is almost negligible for wavelengths longer than 550 nm (Figure 5), which was the transmission region for the orange filter. A nearly linear increase in green fluorescence was observed with time for samples exposed to white and blue light. The slope versus time for the blue light was approximately double the one obtained for white light (0.20 vs 0.11). The difference in slopes can be explained by the absorption spectrum of SOSG, as well as the light intensity wavelength profiles transmitted by the blue and clear filters. The integrated intensities under these profiles below 520 nm were 1.26 and 0.70 W/m^2 for the blue and clear films, respectively. This makes a ratio of 1.80, very close to the slope ratio of blue/clear (1.82). This suggests that SOSG acts as a $^1O_2$ photosensitizer for light below 520 nm in a dose-dependent manner. The main conclusion of these findings is that the SOSG agent can be used reliably under orange/red light, but

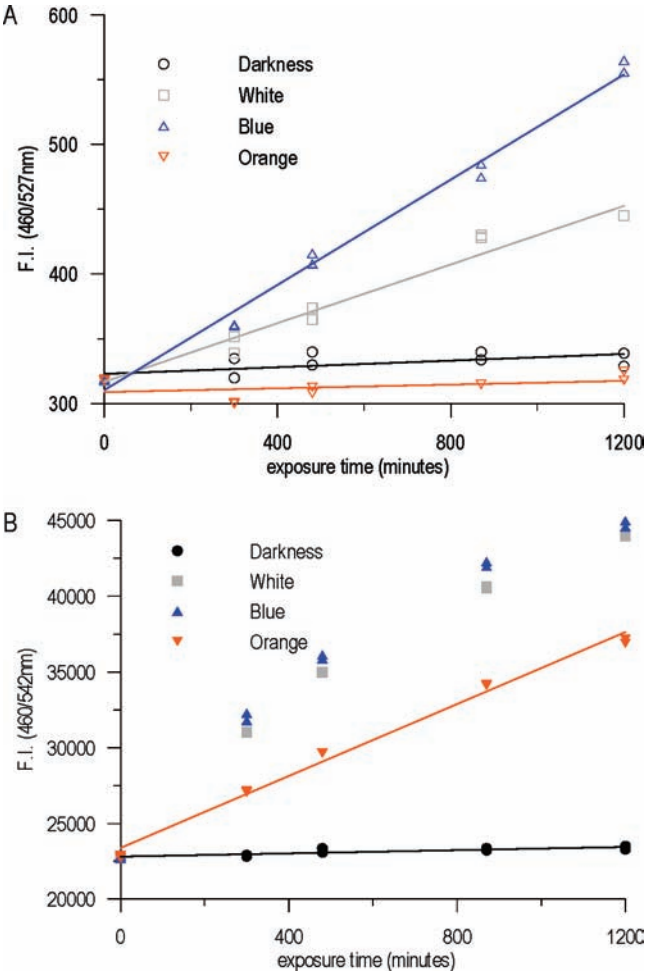

Figure 4. SOSG fluorescence over time in 0.01 M citrate buffer pH 6.7 (control conditions) (A) and added in milk (B) exposed to light of different colors and stored in the dark.

not under UV, violet, and blue light because the agent is then an active photosensitizer itself.

Figure 4B shows the green fluorescence ($\lambda_{ex/em}$ 460/542 nm) for milk with SOSG. The maximum of the fluorescence emission for the probe in milk was bathochromically shifted from 527 to 542 nm. Again, no green fluorescence was developed for the samples stored in the dark. The green fluorescence of samples exposed to blue and white light showed a very similar and slightly curved increase during the exposure period. Both blue and white light are absorbed by different photosensitizers: riboflavin, chloropyllic compounds, and PpIX, as well as SOSG (Figures 2 and 5), which all can generate $^1O_2$. A portion of the light would also be absorbed by β-carotene, which then would inhibit the production of $^1O_2$. All of these possible effects make it difficult to interpret the obtained results for blue and white light.

For exposure to orange light the fluorescent signal increased close to linearly during the 20 h of exposure. $^1O_2$ was in this case not produced by the SOSG itself, so clearly, it was formed by naturally occurring compounds in milk. Chlorophyllic and porphyrinic compounds are the only photosensitizers known to absorb light in this spectral region, so they are most likely responsible for this production of $^1O_2$.

Figure 6 shows the fluorescence spectra from milk with SOSG after exposure to light of different colors stored with oxygen or

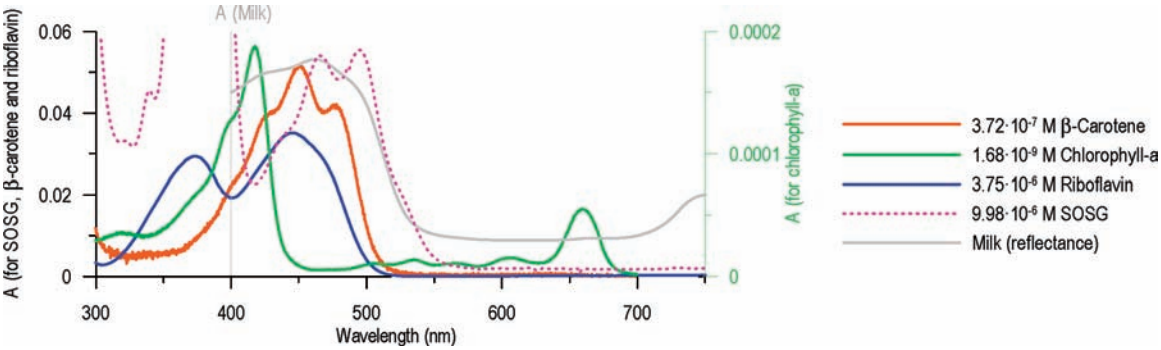

Figure 5. Absorption spectra for β-carotene in hexane, chlorophyll *a* in methanol, and riboflavin in water at the reported typical concentrations in milk and SOSG in ethanol. Note that the scale of the *A*-axis for chlorophyll *a* is zoomed. Absorption spectrum measured for milk (gray curve and axis).

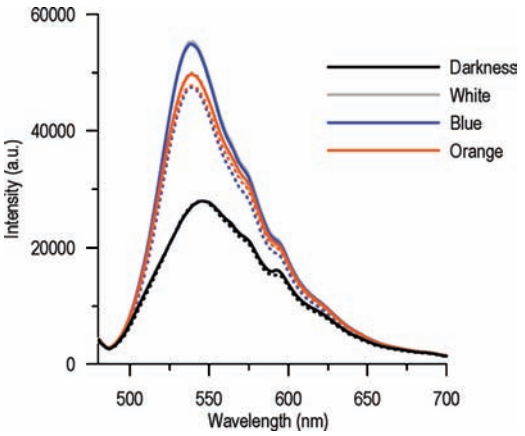

Figure 6. Emission spectra (λ_{ex} = 460 nm) of milk containing SOSG stored in the dark and under different colors of light, packed under air (solid lines) or nitrogen (dotted lines).

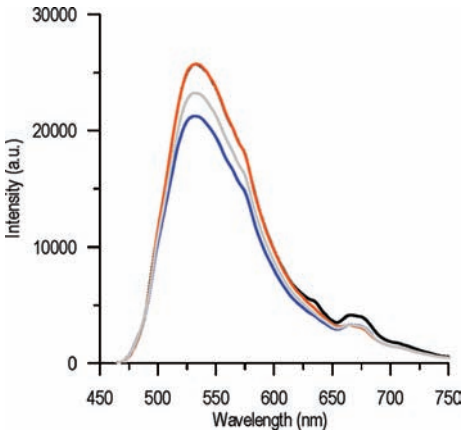

Figure 7. Fluorescence emission spectra (λ_{ex} = 410 nm) from milk samples stored with air in the headspace and exposed to 1.6 W/m^2 of blue (blue line), white (gray line), or orange (orange line) light. Sample stored in the dark (black line).

air. The spectra suggest that the amount of $^1O_2$ formed in samples stored in nitrogen was lower than in those stored in air. This is reasonable because less oxygen was then available. The concentration of dissolved oxygen in milk packed with nitrogen in the headspace was approximately one-fifth of the concentration for samples packed in air;[10] however, this was sufficient to produce $^1O_2$ at slightly lower levels. Thus, in the light-induced oxidation of milk, type II reactions take place to a high degree also under nitrogen atmosphere because free oxygen is dissolved in the milk.

The sensory results (Table 2) indicate that samples stored under nitrogen and orange light were mostly oxidized, whereas slightly less $^1O_2$ was produced in milk under nitrogen. This result suggests that type I reactions give a substantial contribution to the formation of off-flavors in milk, which is in accordance with previously reported results.[14]

Fluorescence Analysis. Front-face fluorescence spectroscopy is a nondestructive, rapid, and sensitive method, which can be used to detect and monitor photosensitizers in dairy products. Very low concentrations (probably below 1−1.5 ng/g) of different tetrapyrroles can be detected.[10,34] When photosensitizers are involved in photoreactions, either as part of type I reactions or when reacting with $^1O_2$ after type II reactions, the molecules are degraded. The degraded molecular bonds correspond to the fluorescent ones. Photodegradation of the molecules will therefore result in a decrease in fluorescence intensity, which means that the initiation and the extent of photooxidation can be indirectly measured. In this section we present the fluorescence spectra from samples without SOSG; only intrinsic fluorophores are studied. Figure 7 shows the fluorescence emission spectra from milk samples after excitation at 410 nm. The large peak at 531 nm stems from riboflavin. The smaller peaks at 635 and 662−680 nm originate from PpIX and chlorphyllic compounds, respectively.[16] It can be seen that riboflavin was degraded mostly by blue light and less by white light, because a large portion of the white light was of wavelengths longer than 500 nm. Negligible degradation of riboflavin was induced by orange light (the spectrum corresponding to exposure to orange light in Figure 7 overlaps the one for dark storage in the riboflavin region).

For a light intensity of 1.6 W/m^2 at the milk surface, approximately 86% and 92% of the riboflavin was left after exposure to blue and white light, respectively. Approximately 100% was left after exposure to orange light. When the intensity was adjusted to 1.6 W/m^2 above the filters, we got the same result for white and orange light, whereas 94% of the riboflavin was left after exposure to blue light, because the intensity was reduced to 0.5 W/m^2.

PpIX and the chlorophyllic compounds were degraded under all light exposure conditions, except in the dark. This makes sense because these compounds absorb throughout the entire visible region, mostly in the violet (the Soret band) but pronounced also in the green and red.[35] Degradation of PpIX and chlorophyllic compounds is shown in Figure 8. In this figure the background

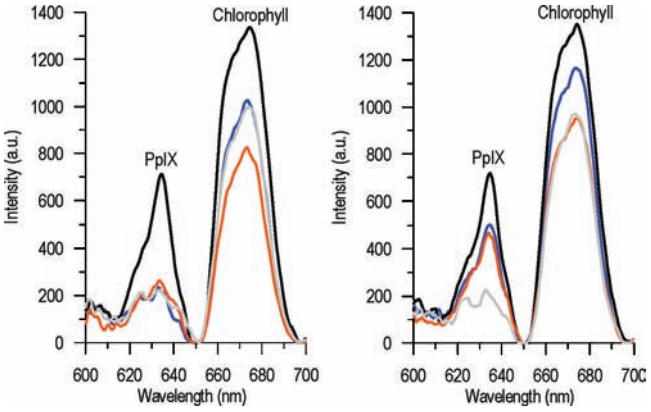

Figure 8. Background-corrected fluorescence spectra (λ_{ex} = 410 nm) from milk stored under air atmosphere with the light intensity adjusted to about 1.6 W/m^2 at the milk surface (left) and 1.6 W/m^2 above the film (right). Sample was exposed to blue light (blue line), white light (gray line), orange light (orange line). Sample stored in the dark (black line).

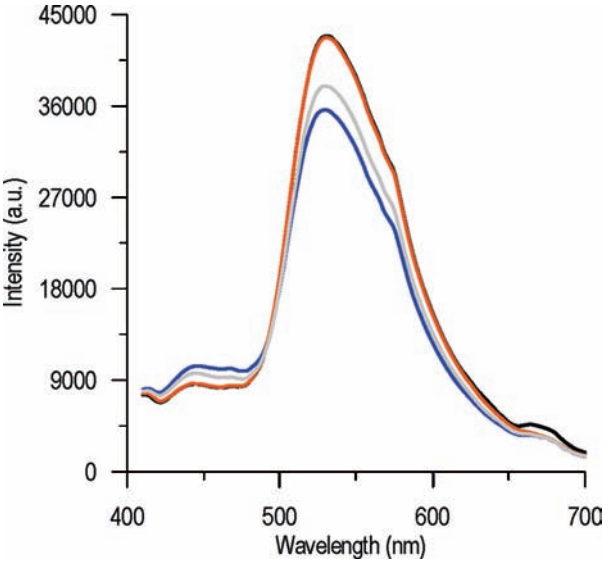

Figure 9. Fluorescence emission spectra (λ_{ex} = 382 nm) from milk samples stored with air in the headspace and exposed to 1.6 W/m^2 of blue (blue line), white (gray line), or orange (orange line) light. Sample stored in the dark (black line).

fluorescence from riboflavin has been subtracted from the spectrum, leaving only the spectral contributions from PpIX and chlorophyll. Only data for samples stored in air are shown, because spectra from those stored in nitrogen were very similar. When the intensity was adjusted to 1.6 W/m^2 at milk surface, orange light degraded chlorophyll the most. White light and blue light induced the same degree of degradation. PpIX was degraded equally by all light exposures. When the intensity was adjusted to 1.6 W/m^2 above the filters, that is, the actual intensity at the milk surface was 0.5, 0.8, and 1.6 W/m^2 for blue, orange, and white light, respectively, chlorophyll was equally degraded by orange and white light and less degraded by blue light. PpIX was in this case most degraded by white light and less so by blue and orange light. No degradation occurred in the dark-stored samples.

These results are interesting. Chlorophyllic compounds have their absorption maxima around 410−420 nm (Figure 5), so one should assume that most degradation would occur under the blue filter. Also, white light at 1.6 W/m^2, which covers the entire visible region, degraded less of the chlorophyll than orange light at 1.5 W/m^2. It is also noteworthy that 1.6 W/m^2 of white light did not degrade chlorophyll more than only 0.8 W/m^2 of orange light; the white light contains all of the orange light as well as the blue light.

Intawiwat et al.[10] showed that the degree of degradation of chlorophyllic compounds correlated well with the sensory properties of light-exposed milk. In this study the simple correlations between, for example, sunlight flavor and the chlorophyll fluorescence peak were 0.92 and 0.91 for milk stored in air and N_2, respectively. The corresponding correlations with the riboflavin peak were 0.08 and 0.21, so there is no doubt that the chlorophyll peak is a good marker for photooxidation in milk, and it is therefore likely that chlorophyll plays an active role in the photoreactions leading to off-flavors.

There are at least two reasons for why chlorophyll is degraded more by orange light than by white or blue. Light absorption in milk occurs in a competitive manner. The molecules with the highest extinction coefficients at certain wavelengths absorb most of the light at these wavelengths. Figure 5 shows spectra that characterize some main absorption properties in milk. The absorption spectrum of milk shows that the main absorption in the visible is in the blue region from 400 to 525 nm. This broad peak is due to the absorbance of riboflavin and β-carotene. The narrow peaks in the β-carotene spectrum can be distinguished as small shoulders in the milk spectrum. According to normal concentrations in milk and the individual extinction coefficients, riboflavin and β-carotene can be assumed to contribute about the same amount of absorbance in the violet/blue region. The absorption spectrum of milk also indicates chemical absorption in the 550−700 nm region; however, most of this stable level can be regarded as an offset due to light scattering. Compared to riboflavin and β-carotene, chlorophyllic compounds have much lower absorbance in milk, and it is difficult to detect these features in the milk spectrum. On the other hand, these compounds are among the few absorbers we know about in the 550−750 nm region for milk. When milk is exposed to violet and blue light, a large portion of the photons will be absorbed by β-carotene, which will act as a protective filter and reduce the amount of light reaching the active photosensitizers. In this region, riboflavin will also absorb much light, and less light will be available for tetrapyrrollic compounds. In the 550−750 nm region, β-carotene and riboflavin do not absorb, and much more of the incident light will be available for reactions with chlorophyllic and porphyrinic compounds. We have clear indications from studies on milk and other dairy products that these compounds play a significant role as photosensitizers despite the relatively low concentrations compared to, for instance, riboflavin.[10,17,31]

Josephson[4] reported in 1946 that orange light penetrated deeper into the milk than did blue light, and this can be an additional reason why orange light degrades chlorophyll more than other light. A larger volume is exposed, and this will also affect the sensory properties.

It is well-known that some tertiary oxidation products formed in dairy products are fluorescent. Under excitation of 380 nm they have an emission peak around 470 nm.[36] In the present study, the formation of this fluorescence was not very pronounced; however, it was systematic enough to present and interpret. Figure 9 shows that most of these oxidation products were created under blue light, fewer under white light, and none under orange light. A similar trend was observed

by Intawiwat et al.[10] The shown spectra are from samples stored in air, and corresponding spectra from nitrogen-stored samples were almost identical. The results indicate that the oxidation products responsible for off-flavors induced by orange light do not contribute to the formation of fluorescent oxidation products. They are formed under blue light and might be connected to only riboflavin-sensitized oxidation. Again, this suggests that different photoreactions occur under different wavelengths and also illustrates that instrumental methods for monitoring photooxidation in dairy products do not always pick up the relevant (sensory) properties.

In this experiment we have studied the effect of light in the wavelength region from 300 to 750 nm. There was a slight overlap between the orange and blue filters between 525 and 575 nm, but this does not obscure the results much because the most important was that the orange light did not affect the riboflavin. The blue light affected all of the sensitizers anyway. There was also an overlap in the 700−750 nm region. Figure 5 shows that chlorophyll absorbs up to 700 nm, and we do not know of any photosensitizers in milk that absorb between 700 and 750 nm, so we expect little contribution to photooxidation from this light. Josephson,[4] however, observed that all light of wavelengths shorter than 750 nm induced off-flavors. Then off-flavors created by the blue filter might have gotten a small contribution from the 700−750 nm region. If this is the case, then the differences between the orange light and pure blue light without this contribution would have been even bigger, and the main conclusions from this study are still valid.

In conclusion, in this study we have tried to separate the effect of riboflavin and tetrapyrroles in the photooxidation of milk. This is not a simple task because all sensitizers absorb in the violet and blue region. However, the effect of riboflavin can be excluded by using wavelengths longer than about 500 nm.

The sensory responses, which we consider as the most relevant measure of photooxidation, clearly showed that light of wavelengths longer than 550 nm induced more off-flavors than those below. This result contradicts that riboflavin is the main cause of photooxidation in milk.

By using the SOSG agent we could verify that singlet oxygen was indeed formed in milk under exposure to orange light, presumably with minor or no involvement of riboflavin. We also experienced that this agent is a useful and rather simple to use method for monitoring the formation of singlet oxygen, as long as one is aware of its limitations under UV, violet, and blue light.

Finally, by fluorescence spectroscopy we have shown that tetrapyrroles, in particular, chlorophyllic compounds, were degraded more by orange light than by blue and that the degree of degradation correlated closely with the formation of sensory off-flavors. This does not *prove* that cholorophyllic compounds are responsible for a major part of photooxidation in milk, but the results point strongly in that direction.

In many scientific papers dealing with photooxidation in milk, riboflavin is assumed to be, or concluded to be, the responsible photosensitizer. These conclusions are seldom actively verified (or falsified) by performing the same experiments with light that is not absorbed by riboflavin. More care should be taken in discussions of the causes of photooxidation in milk, and possible contributions from tetrapyrroles should not be ignored or underestimated. It is hoped that such an approach can contribute to increased understanding of the complex photoreactions in milk.

AUTHOR INFORMATION

Corresponding Author

*Phone: +47 64 97 01 00. Fax: +47 64 97 03 33. E-mail: diego.airado@nofima.no.

Funding Sources

The Agricultural Food Research Foundation (Oslo, Norway) and The Norwegian Research Council (Project 195614/V11) funded this research. We also thank Junta de Extremadura (DOE 154, 08/08/08).

ACKNOWLEDGMENT

We thank Per Lea for statistical analysis and fruitful discussions. We also thank Siljie Ottestad for her help with reflectance measurements. We gratefully acknowledge skillful technical assistance from Frank Lundby and Aud Espedal.

REFERENCES

(1) Borle, F.; Sieber, R.; Bosset, J. O. Photo-oxidation and photoprotection of foods, with particular reference to dairy products — an update of a review article (1993−2000). *Sci. Aliments* **2001**, *21*, 571–590.

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

(3) Webster, J. B.; Duncan, S. E.; Marcy, J. E.; O'Keefe, S. F. Controlling light oxidation flavour in milk by blocking riboflavin excitation wavelengths by interference. *J. Food Sci.* **2009**, *74*, S390–S398.

(4) Josephson, D. V. Some observations regarding the effect of various wavelengths of light on the riboflavin content and flavor of milk. *J. Dairy Sci.* **1946**, *29*, 508–510.

(5) Herreid, E. O.; Ruskin, B.; Clark, G. L.; Parks, T. B. Ascorbic acid and riboflavin destruction and flavor development in milk exposed to the sun in amber, clear, paper and ruby bottles. *J. Dairy Sci.* **1952**, *35*, 772–778.

(6) Hansen, A. P.; Turner, L. G.; Aurand, L. W. Fluorescent light-activated flavor in milk. *J. Milk Food Technol.* **1975**, *38*, 388–392.

(7) Hoskin, J. C.; Dimick, P. S. Evaluation of fluorescent light on flavour and riboflavin content of milk held in gallon returnable containers. *J. Food Prot.* **1979**, *42*, 105–109.

(8) Cladman, W.; Scheffer, S.; Goodrich, N.; Griffiths, W. Shelf-life of milk packaged in plastic containers with and without treatment to reduce light transmission. *Int. Dairy J.* **1998**, *8*, 629–636.

(9) van Aardt, M.; Duncan, S. E.; Marcy, J. E.; Long, T. E.; Hackney, C. R. Effectiveness of poly(ethylene terephthalate) and high-density polyethylene in protection of milk flavour. *J. Dairy Sci.* **2001**, *84*, 1341–1347.

(10) Intawiwat, N.; Pettersen, M. K.; Rukke, E. O.; Meier, M. A.; Dahl, A. V.; Skaret, J.; Keller, D.; Wold, J. P. Effect of different colored filters on photooxidation in pasteurized milk. *J. Dairy Sci.* **2010**, *93*, 1372–1382.

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

(12) Frankel, E. N. *Lipid Oxidation*; The Oily Press: Davis, CA, 2005.

(13) Spikes, J. D. Photosensitization. In *The Science of Photobiology*, 2nd ed.; Smith, K. C., Ed.; Plenum Press: New York, 1988; pp 79−110.

(14) Lee, J. H.; Min, D. B. Changes of headspace volatiles in milk with riboflavin photosensitization. *J. Food Sci.* **2009**, *74*, 563–568.

(15) He, Y. Y.; An, J. Y.; Jiang, L. J. EPR and spectrophotometric studies on free radicals and singlet oxygen generated by irradiation of cysteamine substituted hypocrellin B. *Int. J. Radiat. Biol.* **1998**, *74*, 647–654.

(16) Wold, J. P.; Bro, R.; Veberg, A.; Lundby, F.; Nilsen, A. N.; Moan, J. Active photosensitizers in butter detected by fluorescence

spectroscopy and multivariate curve resolution. *J. Agric. Food Chem.* **2006**, *54*, 10197–10204.

(17) Wold, J. P.; Veberg, A.; Nilsen, A.; Iani, V.; Juzenas, P.; Moan, J. The role of naturally occurring chlorophyll and porphyrins in light-induced oxidation of dairy products. A study based on fluorescence spectroscopy and sensory analysis. *Int. Dairy J.* **2005**, *15*, 343–353.

(18) Lindmark-Månsson, H.; Fondén, R.; Petterson, H. E. Composition of Swedish dairy milk. *Int. Dairy J.* **2003**, *13*, 409–425.

(19) Krinsky, N. I. In *Oxygen and Oxy-Radicals in Chemistry and Biology*; Rogers, M. A. J., Powers, E. L., Eds.; Academic Press, New York, 1981; p 221.

(20) Molecular Probes (2004). Product information, http://probes.invitrogen.com/media/pis/mp36002.pdf?id=mp36002.

(21) Flors, C.; Fryer, M. J.; Waring, J.; Reeder, B.; Bechtold, U.; Mullineaux, P. M.; Nonell, S.; Wilson, M. T.; Baker, N. R. Imaging the production of singlet oxygen In Vivo using a new fluorescent sensor, Singlet Oxygen Sensor Green®. *J. Exp. Bot.* **2006**, *57*, 1725–1734.

(22) Ragás, X.; Jiménez-Banzo, A.; Sánchez-García, D.; Batllori, X.; Nonell, S. Singlet oxygen photosensitization by the fluorescent probe Singlet Oxygen Sensor Green. *Chem. Commun.* **2009**, 2920–2922.

(23) Belitz, H. D.; Grosch, W.; Schieberle, P. Milk and dairy products. In *Food Chemistry*, 4th ed.; Springer-Verlag: Berlin, Germany, 2009; p 515.

(24) ISO. International standard 6564. *Sensory Analysis Methodology — Flavor Profile Methods*; International Organization for Standardization: Geneva, Switzerland, 1985.

(25) ISO. International standard 8586-1. *Sensory Analysis Methodology — General Guidance for the Selection, Training, and Monitoring of Assessors — Part 1: Selected Assessors*, 1st ed.; International Organization for Standardization: Geneva, Switzerland, 1993.

(26) ISO. International standard 8589. *Sensory Analysis Methodology — General Guidance for the Design of Test Rooms*; International Organization for Standardization: Geneva, Switzerland, 1988.

(27) ISO. International standard 22935-2. *Milk and Milk Products — Sensory Analysis — Part 2: Recommended Methods for Sensory Evaluation*; International Organization for Standardization: Geneva, Switzerland, 2009.

(28) Wold, J. P.; Jorgensen, K.; Lundby, F. Nondestructive measurement of light-induced oxidation in dairy products by fluorescence spectroscopy and imaging. *J. Dairy Sci.* **2002**, *85*, 1693–1704.

(29) Du, H.; Fuh, R.-C. A.; Li, J.; Corkan, L. A.; Lindsey, J. S. PhotochemCAD: a computer-aided design and research tool in photochemistry. *Photochem. Photobiol.* **1998**, *68*, 141–142.

(30) Lieber, C. A.; Mahadevan-Jansen, A. Automated method for subtraction of fluorescence from biological Raman spectra. *Appl. Spectrosc.* **2003**, *57*, 1363–1367.

(31) Wold, J. P.; Veberg, A.; Lundby, F.; Nilsen, A. N.; Moan, J. Influence of storage time and color of light on photooxidation in cheese: a study based on sensory analysis and fluorescence spectroscopy. *Int. Dairy J.* **2006**, *16*, 1218–1226.

(32) Wold, J. P.; Dahl, A. V.; Lundby, F.; Nilsen, A. N.; Juzeniene, A.; Moan, J. Effect of oxygen concentration on photo-oxidation and photosensitizer bleaching in butter. *Photochem. Photobiol.* **2009**, *85*, 669–676.

(33) Hideg, E. A comparative study of fluorescent singlet oxygen probes in plant leaves. *Cent. Eur. J. Biol.* **2008**, *3*, 273–284.

(34) Wold, J. P.; Lundby, F. Approximate non-destructive quantification of porphyrins in butter by front face fluorescence spectroscopy. *J. Anim. Feed Sci.* **2007**, *16*, 190–194.

(35) Juzenas, P.; Iani, V.; Bagdonas, S.; Rotomskis, R.; Moan, J. Fluorescence spectroscopy of normal mouse skin exposed to 5-aminolaevulinic acid and red light. *J. Photochem. Photobiol. B: Biol.* **2001**, *61*, 78–86.

(36) Veberg, A.; Vogt, G.; Wold, J. P. Fluorescence in aldehyde model systems related to lipid oxidation. *LWT — Food Sci. Technol.* **2006**, *39*, 562–570.