Effect of different colored filters on photooxidation in pasteurized milk

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ABSTRACT

The effect of different colored filters and atmospheres on photooxidation and quality in milk was studied. Pasteurized bovine milk (3.9% fat) was packed in 2 different atmospheres (air and N₂) and exposed to light for 20 h at 4°C under 8 transparent filters with different light transmission properties. The following transparent, noncolored, and colored filters based on polyethylene terephthalate (PET) were used: noncolored (PET), noncolored with 2 different UV-block regions, yellow, green, amber, orange, and red. Control samples were stored in darkness and in a carton. Sensory evaluation showed off flavors significantly increased in milk stored under all filters compared with the control samples. Variation in atmosphere resulted in significant differences in formation of rancid flavor in milk stored under different filters. Milk samples stored in N₂ underwent the most sensory deterioration under orange and red filters, whereas milk samples stored in air were most deteriorated under noncolored filters. According to the oxidation compounds measured by gas chromatography, milk samples stored under noncolored and orange filters were highly oxidized, whereas red, green, and amber filters offered better protection against photooxidation. Fluorescence spectroscopy was used to examine the degradation of photosensitizers (riboflavin, protoporphyrin, and chlorophyllic compounds) in the milk samples. Degradation of protoporphyrin and chlorophyllic compounds in N₂ correlated well with sensory properties related to photooxidation ($R^2 = 0.75\text{--}0.95$). The study indicates that protoporphyrin and chlorophyllic compounds were effective photosensitizers in milk. To avoid photooxidation in milk, it is therefore important to protect it against light from the UV spectrum as well as light from the entire visible region.

Key words: milk, photooxidation, packaging, photosensitizer

INTRODUCTION

Photooxidation is a challenge to dairy products as they are exposed to light during transportation, storage, and display in grocery stores. Light induces degradation of lipids, proteins, and vitamins and causes a decrease in both the nutritional value and the sensory characteristics of products. The oxidation of unsaturated lipids leads to the formation of volatile compounds, off flavors, and off odors (Sattar and deMan, 1975).

The packaging is important for preserving and maintaining product quality. Using opaque, nontransparent packaging is one way to protect milk from photooxidation. However, milk is often packed in transparent or semitransparent plastic bottles or in cartons that do not appear transparent but still transmit fractions of incident light.

Studies on the effect of light can be carried out by varying both the spectral emission of light sources and the transmission characteristics of the packaging material (Sattar and deMan, 1975). Many researchers have studied the effect of different colored light on quality deterioration in milk. Bosset et al. (1994) used light sources or packaging materials with broad spectral properties described as cold light (mainly in violet and blue light), which was more harmful than warm light (dominated by yellow, orange, and red light). Hansen et al. (1975) studied homogenized milk packaged in transparent polyethylene containers, which were exposed to different colored light by covering the light source with color filters. Yellow and green filters gave the best protection against formation of off flavors and ascorbic acid (vitamin C) deterioration, whereas the pink filter, for instance, gave less protection.

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 (Cladman et al., 1998; van Aardt et al., 2001; Moyssiadi et al., 2004; Papachristou et al., 2006; Saffert et al., 2006). Corresponding studies have also been performed for UHT milk (Mestdagh et al., 2005; Saffert et al., 2008). The overall finding in

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these studies is that UV block reduces the formation of oxidation somewhat but not sufficiently to avoid oxidation. Visible light, including yellow and red light, apparently induces photooxidation in milk; however, no satisfactory explanation for this has been given.

Riboflavin (vitamin B₂) has been regarded as the photosensitizer responsible for photooxidation in milk (Borle et al., 2001). Recently, it was found that dairy products contain 6 different photosensitizers: riboflavin, protoporphyrin, hematoporphyrin, a chlorophyll a-like compound, and 2 unidentified tetrapyrroles (Wold et al., 2006a). Concentrations of these compounds seem to vary with fat content. According to fluorescence spectroscopy, butter has higher concentrations than cheese and sour cream and considerably higher concentrations than milk (Wold et al., 2005). All these sensitizers absorb light in the UV and violet region, whereas some of the tetrapyrroles absorb throughout the whole visible region, meaning that any wavelength in the visible region can initiate photooxidation in dairy products. The tetrapyrroles generally absorb strongest in the UV, violet, and blue regions, less in the red region, and least in the green region (Wold et al., 2009). This corresponds well with experiments on cheese (Wold et al., 2006b) and milk (Hansen et al., 1975) in which off flavors have been reported to form correspondingly after exposure to light (i.e., green light has tended to give least adverse effects).

Several possible methods, such as peroxide value, sensory analysis, gas chromatography, and fluorescence spectroscopy, can be used to analyze photooxidation in dairy products (Veberg et al., 2007). Gas chromatography-mass spectroscopy, which measures the presence of secondary oxidation products (Sunesen et al., 2002; Mortensen et al., 2003), is very specific in regard to volatile compounds; however, on qualitative measurements, it does not always correlate well with sensory analysis (Veberg et al., 2007). By using fluorescence spectroscopy, it is possible to measure the amount of different light-sensitive compounds in dairy products and, hence, follow their degradation as an effect of light exposure (Wold et al., 2005). This serves as an indirect measurement of the actual initiation of the oxidation process. Several studies have shown that the degradation of photosensitizers by using fluorescence spectroscopy had high correlation with sensory analysis for photooxidation in cheese (Wold et al., 2005) and butter (Veberg et al., 2007). Fluorescence spectroscopy can also measure the formation of tertiary oxidation products (Kikugawa, 1986; Veberg et al., 2007).

Experiments have been performed on cheese and butter in order to study the effect of different light wavelengths with regard to photooxidation (Wold et al., 2005, 2006b; Veberg et al., 2007). For these products,

green light tended to cause less quality degradation compared with light of other colors, leading to the conclusion that tetrapyrroles (porphyrins and chlorophyllic compounds) contribute effectively to the progression of photooxidation. To date, no studies have reported or taken into account the photosensitizing effect of tetrapyrroles in milk. The concentrations of these compounds are probably much lower in milk than in cheese and butter, so the practical effect on photooxidation is to date unknown. Knowledge about this, however, is of high priority for understanding how to maximize shelf life for milk.

The purpose of this study was to evaluate and understand the effect of different colored filters on photooxidation and quality of pasteurized milk. Samples were stored in different atmospheres (air and N₂) and exposed to light for 20 h at 4°C. The level of photooxidation was measured by GC-MS and sensory evaluation, and fluorescence spectroscopy was used to explain the results by studying degradation of the photosensitizers in the milk.

MATERIALS AND METHODS

Materials

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 darkness before being repacked in plastic trays.

Packaging Materials and Preparation

Milk from the cartons was blended to obtain a homogenous set of samples. The milk (230 mL), measured with sterilized gradual flasks, was packed in white, sterilized, high-density polyethylene (HDPE) trays (5.3 × 9.2 × 9.2 cm; Promens AS, Kristiansand, Norway). Two white trays were 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 top web consisting of PET/PE/ethylene vinyl alcohol/PE (Wipak, Nastola, Finland) using a tray-sealing machine (Dyno model 511 VG, Promens AS, Kristiansand, Norway). Samples were divided into 2 sets, one set stored in air and the other set stored in N₂ (99%).

Eight transparent filters based on PET (Ciba Specialty Inc., Basel, Switzerland) with different optical transmission properties in the visible region were used:

Table 1. Description of sensory attributes used in the experiment

Attribute	Definition
Odor	
Fresh	Odor of freshness; sour and sweet odor
Sweet	Basic odor sweetness (sugar)
Plastic	Odor of plastic
Sunlight	Odor of sunlight related to oxidized protein (training reference: milk exposed to sun)
Rancid	Intensity of rancid odors such as grass, hay, candle, and paint
Flavor or taste	
Fresh	Odor of freshness; sour and sweet flavor
Sweet	Basic taste sweetness (sugar)
Bitter	Basic taste bitter (caffeine)
Metallic	Flavor of metal
Pungent	Causes a sharp sensation of the buccal and nasal mucous membranes
Sunlight	Flavor of sunlight related to oxidized protein (training reference: milk exposed to sun)
Rancid	Intensity of rancid flavors such as grass, hay, candle, and paint
Cardboard	Flavor of cardboard
Plastic	Flavor of plastic
Texture	
Fatness	Surface textural attribute relating to the perception of the quantity of fat in the product (mouth feel)
Body	Richness of flavor or impression of consistency
Mouth feel	
Astringency	Organoleptic attribute of pure substances or mixtures that produces the astringent sensation

noncolored (transmission in 300–800 nm), noncolored with UV-block 1 (**UV1**; 310–800 nm) and UV-block 2 (**UV2**; 360–800 nm), yellow (460–800 nm), green (500–800 nm), amber (500–800 nm), orange (520–800 nm), and red (570–800 nm). Both green and amber had transmission around 500 to 800 nm, but the shapes of the transmission spectra were different. The filters were placed on the tops of the packages. In addition, 2 reference samples were included in the design: milk stored in darkness and milk stored in a commercial opaque carton (fresh milk), which was also stored in darkness.

Light Exposure

The pasteurized milk samples were stored at 4°C under 2 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. The light intensity was measured by a calibrated spectrometer (Apogee Spectroradiometer, Apogee Instruments Inc., Roseville, CA), which was integrated in the 300 to 800 nm region. The intensity of light was adjusted on top of the filters to be 2.0 ± 1 W/m² for every sample by adjusting with gray filters over the filters. The intensity of light was measured in the cooling room in which the experiment was performed. Light exposure time for all samples was 20 h and the milk was analyzed immediately after light exposure.

Sensory Analysis

The pasteurized milk was evaluated by a trained sensory panel at Nofima Mat (Ås, Norway) using

a modified quantitative method as described in ISO (1985) standard 6564. The panel comprised 9 subjects employed exclusively to work as sensory assessors at Nofima Mat. The panelists were selected and trained according to the recommendations in ISO (1993) standard 8586-1. The sensory laboratory at Nofima Mat was designed according to guidelines in ISO (1988) standard 8589, with separate booths and electronic data registration (CSA, Compusense Five, version 4.80, Guelph, Ontario, Canada). Prior to the assessments, the panel went through a training session with 2 samples for the purpose of agreement on the definition of each attribute and variation in attribute intensity on the 15-cm scale. Seventeen attributes were selected (Table 1). Fresh odor and flavor refer to the attributes sour and sweet. Empirically, high intensity of these attributes indicates fresh samples. Sunlight odor and flavor are related to oxidation of fat. These are the characteristic attributes of milk that has been exposed to sunlight. Rancid odor and flavor include all odors and flavors associated with rancidity (grass, hay, candle, and paint) as described in ISO (2009) standard 22935-2.

The 2 samples used in the training session were 1 fresh milk sample and 1 light-exposed milk sample. The samples 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 in all attributes by each assessor. Each assessor was allowed to work at an individual pace. The panelists evaluated the samples

in duplicate during 6 sessions on 2 consecutive days. The panelists recorded their results on a 15-cm, non-structured, 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).

Dynamic Headspace/GC–MS Analysis of Volatile Compounds

Volatile compounds were analyzed by a dynamic headspace method (Olsen et al., 2005; Veberg et al., 2006). Milk (20 mL) was weighed into closed Erlenmeyer flasks (500 mL) with glass stoppers. Ethyl heptanoate (>99%; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in methanol (2 μ L) (pro analysis; Merck GmbH, Darmstadt, Germany) was injected into the flasks as an internal standard. The samples were placed in a water bath, heated to 70°C, and purged with purified nitrogen at 100 mL/min through a Drechsel head for 20 min. Volatiles were adsorbed on Tenax GR (mesh size 60/80; Alltech Associates Inc., Deerfield, IL). Water was removed from the absorber by N₂ flushing (50 mL/min) for 5 min in the opposite direction of sampling. Trapped compounds were desorbed at 280°C for 5 min in a Markes Ultra-Unity thermal desorption unit (Markes International Ltd., Llantrisant, UK) and transferred to an Agilent 6890 GC System (Agilent, Santa Clara, CA) with an Agilent 5973 mass spectrometer (quadrupole) operated in electron ionization mode at 70 eV. The compounds were separated on a DB-WAXetr column (0.25 mm i.d., 0.5 μ m film, 30 m length; Agilent) with helium (99.9999%) as the carrier gas. The temperature program started at 30°C for 10 min, increased 1°/min to 40°C, 3°/min to 70°C, and 5°/min to 230°C. Final holding time was 5 min. Integration of peaks and tentative identification of compounds were performed with HP Chemstation (G1701CA version C.00.00, Agilent), Wiley 130K Mass Spectral Database (HP 61030A MS Chemstation, Agilent), and NIST Mass Spectral Library (version 2.0, US Secretary of Commerce; Agilent). Identities of several of the components were confirmed by comparison of retention times and mass spectra of the sample peaks with those of pure standards. The concentrations of the individual volatiles were calculated as nanograms per gram of sample based on the internal standard. The analysis was performed in duplicate for all samples.

Fluorescence Spectroscopy

Fluorescence emission spectra were measured on intact milk samples. The samples were filled into sample

cuvettes that exposed a flat, circular surface with a diameter of 5 cm for the measurements. The fluorescence emission spectra were measured for excitation at 382 nm (Oriel 59920, Oriel Corporation, Stratford, CT) and 410 nm (Oriel 59285) using cut-off filters at 400 nm (Melles Griot 03FCG049, Melles Griot Inc., Irvine, CA) and 475 nm (Melles Griot 03FCG065, Melles Griot Inc.), respectively. Excitation at 382 nm was chosen because it has been shown to give good results for measuring tertiary oxidation products (Wold et al., 2002,2005), whereas 410 nm excitation was used to maximize fluorescence for the tetrapyrroles. Riboflavin has excitation maxima at 370 and 450 nm; however, the emission for excitation at 410 nm is also strong. The spectra were collected by a spectrograph (Acton SP-150, Acton Research Corp., Acton, MA) connected to a charge coupled device camera (Roper Scientific NTE/CCD-1340/400-EMB, Roper Scientific, Trenton, NJ). Exposure time was 0.5 s for all measurements. The spectrograph and detector were controlled by the software WinSpec (version 1.4.3.4, Roper Scientific). The spectra were not subjected to any kind of preprocessing before analysis.

Oxygen Content

The oxygen content in milk was measured by using high-resolution respirometry (Oroboros Oxygraph, Innsbruck, Austria). Milk was divided into 3 sets: carton, milk packed in a tray with N₂, and air atmosphere. Samples were stored for 20 h in darkness at 4°C. The barometric pressure measured by the instrument was 100.90 kPa. Partial oxygen pressure was 20.964 kPa. Samples were measured at 4°C. The oxygen concentration was determined by Datlab software (Innsbruck, Austria).

Statistical Analysis

Principal component analysis (PCA) was applied to the GC-MS and fluorescence data to get the best possible view of the data structure. Loading plots were used to interpret the variation contained in each principal component (PC), whereas score plots were used to visualize the relationship between samples in the corresponding PC. Partial least-squares regression was used to find correlations between fluorescence spectra and sensory-assessed attributes of the milk. Full cross-validation was used to determine the optimal number of partial least squares factors. Principal component analysis and partial least-squares regression (PLSR) analyses were performed by using Unscrambler software (version 7.5, Camo AS, Oslo, Norway).

Figure 1. Mean sensory score for milk stored in N₂ and air atmosphere and light exposed for 20 h at 4°C under 8 different filters, in addition to reference samples stored in darkness (unexposed) and in a carton (fresh). The 3 attributes shown are rancid flavor (A), rancid odor (B), and sunlight odor (C). Samples with the same letter are not significantly different ($P > 0.05$). Samples were served to 9 assessors at room temperature (20°C). Rancid is described as the intensity of rancid flavors and odors such as grass, hay, candle, and paint. PET = polyethylene terephthalate; UV1 = noncolored with UV-block 1; UV2 = noncolored with UV-block 2.

Significance testing of the sensory analysis was performed by the linear models/ANOVA module Statistic 8.1 (Analytical Software, Tallahassee, FL). The effects in the model were filter–gas combination, plus samples stored in darkness and in a carton. The filter–gas combination effect was taken to be a fixed effect, whereas the assessor effect and the assessor by filter–gas combination were taken to be random effects. The error term

was interaction between assessor and filter–gas. The sensory replicates constituted a nested, random effect. The analysis was done on each attribute separately (i.e., a univariate model was employed). Where a significant effect ($P \leq 0.05$) was found, the data were analyzed further by Tukey's multiple pairwise comparison test to get detailed insight into which filter–gas combinations were significantly different.

RESULTS AND DISCUSSION

Sensory Analysis

The 17 evaluated sensory attributes consisted of 5 odors, 9 flavors, and 3 textures. Significant differences were found only for rancid flavor, rancid odor, and sunlight odor; the average sensory scores for these are shown in Figure 1.

Light exposure had a clear effect on the sensory properties; milk stored under all transparent filters (colored and noncolored) had significantly higher rancid flavor and rancid odor than milk stored in darkness and in a carton (fresh milk) (Figure 1). These results are as expected and in accordance with previous studies. For instance, milk packed in a carton was sensory acceptable after storage for 10 d, but was unacceptable because of off flavors when packed in clear PET (Papachristou et al., 2006). For Havarti cheese and cream cheese, nontransparent packaging gave the best protection, whereas the transparent packaging caused rapid quality deterioration (Mortensen et al., 2002; Pettersen et al., 2005).

There were also significant differences in sensory properties as an effect of the different filters. For milk stored in air, significantly lower rancid flavor was observed for samples under a red filter compared with samples under all of the noncolored filters (Figure 1A). In addition, both red and green filters resulted in lower rancid odor than the PET and UV1 filters (Figure 1B). Otherwise, no significant differences were obtained between the different filters for milk stored in air, meaning that typical warm wavelengths (yellow and orange) induced off flavors at the same level as clear transparent filters, even without UV-block. For the sensory attribute sunlight odor, which is often connected to light-induced oxidation, significant differences were detected between only storage in darkness and storage under a yellow filter (in both atmospheres). However, the level of sunlight odor was generally higher for all filters compared with storage in darkness and in a carton. These results were in slight contrast to earlier results for milk (Hansen et al., 1975) and Havarti cheese (Mortensen et al., 2003), where fewer off flavors for yellow filters were obtained.

These results can be partly explained by the intensity of light, which was transmitted by the different filters (Figure 2). The red and green filters resulted in lowest light intensity, which would induce less photooxidation. For samples stored in air, there was a trend that a decrease in overall intensity of the incident light resulted in less rancid flavors. In that respect, it is of interest to note that, for example, orange and amber filters, which transmitted only about 60% and 40% of the light, respectively, did not give significantly less oxidation compared with PET. The wavelength differences are probably also important. Green and amber filters had transmission bands in the region 500 to 800 nm and red filters had a transmission band from 570 to 800 nm, whereas the other colored filters transmitted about 90% of the light from 450 to 800 nm. Cladman et al. (1998) also reported that green PET bottles were able to block out much of the harmful visible and UV light.

Storage in N₂ gave a different result. The overall level of oxidation was not very different from samples stored in air, although one would expect so with less oxygen available. The other notable feature was that the colored filters, especially red and orange, resulted in higher levels of rancid flavor and odor than did the noncolored filters. Considering that the light intensity transmitted by the red and orange filters was less than that transmitted by the noncolored filters (Figure 2), this was a puzzling result.

After storage, we found oxygen levels of 502 μ M and 102 μ M in milk stored in air and in N₂, respectively. This means that there was oxygen in the N₂-stored samples for the oxidation processes to proceed. However, lower oxygen concentration usually leads to a

correspondingly lower oxidation level, as was reported for butter (Veberg et al., 2006; Wold et al., 2009). The results might suggest that there was an interaction effect between the wavelength of exposed light and the oxygen concentration. This will be further discussed with the fluorescence analysis.

Dynamic Headspace/GC-MS Analysis of Volatile Compounds

Gas chromatography was used to measure the content of volatile oxidation products after light exposure. Many volatile compounds were detected, and some important oxidation products were selected for analysis: 2-heptanone, 2-hexanal, 2-heptenal, 2-butanal, 2-nonanal, pentanal, propanal, and 1-octen-3-ol. All these compounds have been reported as important oxidation compounds in milk (Kim and Morr, 1996; van Aardt et al., 2005; Havemose et al., 2007). Dimethyldisulfide usually constitutes a major compound during photooxidation of dairy products (Kim and Morr, 1996; Jung et al., 1998; Veberg et al., 2006), but dimethyldisulfide was not detected in this experiment. Aldehydes were the dominant compounds. A PCA was performed on selected volatile components, and a PC biplot for the first 2 PC is shown in Figure 3, where the first PC represents the variation in oxidation.

The color of the filters affected the content and composition of oxidation volatile products; the concentration of propanal, 2-hexanal, 2-heptenal, and 2-nonanal were higher in milk packed under noncolored filters compared with that packed under most of the colored filters. Samples stored under the orange filter showed higher concentrations of the oxidation compounds compared with those stored under the amber, green, and red filters.

Storage atmosphere seemed to have an effect also on the volatile oxidation compounds. As for the sensory properties, there was a trend that samples stored under the noncolored filters in air had higher concentrations of oxidation products compared with those stored in N₂. The opposite was the case for many samples stored under colored filters; N₂ headspace resulted in more oxidation products.

Fluorescence Analysis

Figure 4A shows the fluorescence emission spectra of light-exposed milk stored in N₂. Spectra of some selected samples are included to illustrate the spectral variation. The region 410 to 480 nm typically shows formation of tertiary oxidation products (Kikugawa, 1986; Veberg et al., 2007). The broad peak around 530 nm stems

Figure 2. Transmitted light (%) for the different filters used in the experiment. The intensity was calculated by integrating the area under the transmission curve for each filter. PET = polyethylene terephthalate; UV1 = noncolored with UV-block 1; UV2 = noncolored with UV-block 2.

Figure 3. Principal component analysis biplot (scores and loadings) for the first 2 principal components (PC) of milk stored in N_2 and air atmosphere after light exposure for 20 h at 4°C under 8 different filters in addition to reference samples stored in darkness (unexposed) and in a carton (fresh) with the gas chromatographic peak areas of selected volatile compounds as described by scores (filters and control) in black and loading (volatile compounds) in gray. PET = polyethylene terephthalate; UV1 = noncolored with UV-block 1; UV2 = noncolored with UV-block 2.

from riboflavin (Wold et al., 2002; Miquel Becker et al., 2003), whereas the tiny peaks in the 630 to 680 nm region are from protoporphyrins and chlorophyllic compounds. The latter range is plotted separately in Figure 5 for clarity. The approximate amount of riboflavin in milk is 1.5 $\mu\text{g/g}$. The amount of protoporphyrin in milk has not been reported, whereas the amount in butter is approximately 0.02 to 0.03 $\mu\text{g/g}$ (Wold and Lundby, 2007). Because protoporphyrin and chlorophyllic compounds seem to be located in fat, we can assume that the amount in milk is much lower, around 1 to 1.5 ng/g according to the amount of fat. This explains the huge difference in fluorescence intensity between riboflavin and these compounds. Compared with butter and cheese, the fluorescence peaks in milk are considerably less intense because of these low concentrations.

The tertiary oxidation product region (Figure 4B) indicates that milk samples stored in darkness in N_2 atmosphere were least oxidized, followed by samples stored under green, red, yellow, noncolored UV1 filters, and noncolored PET. The same result was found for samples stored in air. The variation in fluorescence intensity between the samples was small, so these results are only indicative.

Riboflavin was mostly degraded in milk stored under noncolored filters because these filters transmit UV

and blue light, wavelengths that are known to degrade riboflavin (Bosset et al., 1994). This corresponds with several studies that report riboflavin loss in milk after exposure to UV or cool white light (Hansen et al., 1975; Mestdagh et al., 2005; Papachristou et al., 2006; Saffert et al., 2006). Negligible degradation of riboflavin was observed for samples stored under the colored filters. These results correspond to similar studies performed on, for example, cheese (Mortensen et al., 2002; Wold et al., 2005). Different storage atmosphere did not affect the degradation of riboflavin, which was also observed in butter by Wold et al. (2009). The variation in the riboflavin peak intensity had low correlation with rancid flavor, both for milk stored in air ($R^2 = 0.44$) and in N_2 atmosphere ($R^2 = 0.56$). According to Saffert et al. (2006), 10 to 15% reduction of light transmittance in pigmented PET bottles at 450 nm could protect degradation of riboflavin in milk but could not prevent light-induced sensory changes.

In Figure 5, protoporphyrin can be discerned as peaks at 635 nm and 705 nm (Juzenas et al., 2001). The peak at 705 nm is difficult to see in the spectra because of the low concentration. The peaks at 661 and 672 nm have spectral characteristics for chlorines and are probably caused by chlorophyll residues (Merzlyak et al., 1996). In other dairy products, a peak at 620 nm,

Figure 4. Fluorescence emission spectra (excitation at 382 nm) of milk stored under different filters in N_2 atmosphere and light-exposed for 20 h at 4°C. The spectra shown are from the region 400 to 700 nm (A). Inset is the tertiary oxidation product region from 420 to 480 nm (B). PET = polyethylene terephthalate; UV2 = noncolored with UV-block 2.

ascribed to hematoporphyrin, has also been observed, but this could not be detected in these milk spectra. Milk stored in darkness had, as expected, the highest protoporphyrin and chlorophyll concentrations.

The different filters affected the photodegradation of protoporphyrin and chlorophyllic compounds differently. In general, photodegradation of a photosensitizer would indicate onset of photooxidation. A high degree of degradation (resulting in low concentration) would indicate more progressed photooxidation. For samples stored in darkness, no photodegradation will occur. A detailed study of the spectra revealed the approximate order of degradation for the different filters. For protoporphyrin, the order, sorted from the highest to lowest concentration, was carton $\approx$ dark $>$ red $>$ green $\approx$ amber $\approx$ PET(N_2) $\approx$ UV1(N_2) $\approx$ UV2(N_2) $>$ yellow $>$ orange $\approx$ UV1(air) $\approx$ UV2(air). For the chlorophyllic compounds, the order was carton $\approx$ dark $>$ green $\approx$ amber $\approx$ PET(N_2) $\approx$ UV1(N_2) $\approx$ UV2(N_2) $>$ red $\approx$ yellow $\approx$ orange $\approx$ UV1(air) $\approx$ UV2(air). For the colored filters, the degradation of sensitizers was quite similar in air and N_2 . The results correspond well with what was observed for the same compounds in butter (Wold et al., 2009).

The effect of the different filters was most likely a combination of wavelength of the light and the overall light intensity (Figure 2), and the degradation of protoporphyrin and the chlorophyllic compounds can be explained by the absorption characteristics of these compounds. However, it is not clear why, for instance, light from yellow and orange filters degraded protoporphyrin more than light from the noncolored filters in N_2 because the noncolored filters did indeed also transmit light in the same wavelength region as yellow and orange.

The atmosphere did to some extent affect the degradation of the photosensitizers. For both protoporphyrin and the chlorophyllic compounds, there was a tendency that storage in air gave more degradation than storage in N_2 , but only for the noncolored filters. For yellow and orange filters, we observed the opposite effect for protoporphyrin: slightly more degradation in N_2 . The chlorophyllic compounds were equally degraded by yellow and orange light in air and N_2 . Again, it is notable that these photosensitizers in N_2 were more degraded under yellow and orange filters when the light intensity was lower than for the noncolored filters (Figure 2). It has been shown that the rate of degradation of the

Figure 5. Fluorescence emission spectra (excitation at 410 nm) of milk stored under different filters in N_2 atmosphere and light-exposed for 20 h at 4°C. The spectra shown are from the region 600 to 720 nm. PET = polyethylene terephthalate; UV2 = noncolored with UV-block 2.

different photosensitizers varies with oxygen concentration, but the exact reasons for this is not yet identified (Wold et al., 2009). The explanation is probably related to Type I and Type II photoreactions. Type II reactions generally dominate in high-oxygen environments and involve the formation singlet oxygen, whereas Type I reactions are likely to occur at lower oxygen concentrations. Type I reaction occurs directly between photosensitizers and fatty acids through a free radical mechanism (Frankel, 2005). The observed difference in oxygen concentration between samples stored in air and N_2 was probably big enough to affect the balance of Type I and Type II photoreactions, and the level of oxygen in the N_2 -packed milk was high enough for the oxidative processes to take place.

Photodegradation of the photosensitizers generally indicates onset of photooxidation processes. We can therefore assume that there should be a correlation between the degree of degradation and the formation of off flavors. Variation in the protoporphyrin and chlorophyll peaks correlated well with rancid odor and flavor ($R^2 = 0.75\text{--}0.94$) for milk stored in N_2 , whereas the corresponding correlations for milk stored in air were lower, as shown in Table 2. Similar results have been presented for butter and cheese (Wold et al., 2006a) and have been discussed in Wold et al. (2009).

CONCLUSIONS

Three different methods (sensory evaluation, GC-MS, and fluorescence spectroscopy) were used for measuring the effect of light exposure on milk. Sensory evaluation and GC-MS can detect and quantities of off flavors and off odors can indicate the secondary oxidation products, whereas fluorescence spectroscopy detects the tertiary oxidation products in addition to the photodegradation of photosensitizers. As expected, clear differences were observed for samples stored in darkness compared with light-exposed samples in all performed analyses (sensory evaluation, GC-MS, and fluorescence spectroscopy). High levels of oxidation were measured for all samples stored under transparent filters and also for samples stored under the colored filters, which protect riboflavin from degradation and its photosensitizing abilities. Protoporphyrin and the chlorophyllic compounds were therefore most likely the effective photosensitizers when milk was exposed to light of wavelengths longer than 500 nm. The fact that the degradation of protoporphyrin and chlorophyllic compounds in the samples correlated very well with the sensory evaluation supports this hypothesis. For samples with high oxygen concentration (packed with air in headspace), the red and green filters induced the least adverse effects. This was

Table 2. Results from partial least squares regression for fluorescence spectra (600–720 nm) against sensory-assessed attributes in milk samples stored in N₂ and in air atmosphere

Sensory attribute	N ₂			Air		
	R ²	#F ¹	RMSCV ²	R ²	#F	RMSCV
Sunlight odor	0.75	3	0.5660	0.77	2	0.4371
Rancid odor ³	0.94	4	0.4811	0.57	2	1.2803
Fresh odor	0.93	2	0.3771	0.67	2	0.7506
Sunlight flavor	0.79	2	0.4985	0.82	2	0.4090
Rancid flavor ³	0.89	2	0.7031	0.60	2	1.2753
Fresh flavor	0.95	2	0.3485	0.70	3	0.7627

¹Number of partial least squares regression factors used in the model.²RMSCV = root mean square error of cross-validation.³Rancid is described as the intensity of rancid flavors and odors such as grass, hay, candle, and paint.

probably because of lower light transmission in these filters compared with the others. For samples with low oxygen concentration (packed with N₂) an unexpected, opposite trend was observed: red and orange filters induced the highest oxidation levels. This observation was supported by fluorescence spectroscopy, which showed that the degradation of the chlorophyllic compounds in low-oxygen samples was more severe under red, yellow, and orange filters than under noncolored filters. We cannot at present explain this phenomenon. However, it might be a starting point to understand why, with regard to photooxidation in milk, there apparently is an interaction between color of the light and the oxygen concentration in the samples. A clear conclusion of the study was that complete blocking of light in the UV and the entire visible region is needed to maximize shelf life for pasteurized milk.

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