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Minimizing photooxidation in dairy products by tailor made light barrier properties in packaging materials

Reduksjon av fotooksidasjon i meieriprodukter med skreddersydd lys barriere egenskaper i emballasje

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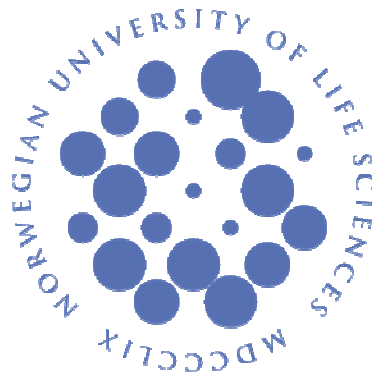
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Acknowledgements

Abstract

Photooxidation is one of the major causes of food quality deterioration. Packaging is a common tool to protect against light and consequently prolong the shelf life of food. Transparent packaging materials are being widely used in the food industry so that the consumers can see the product prior to purchase. It is therefore a challenge to come up with innovative transparent packaging solutions that still have optimal light barrier properties.

The aim of this thesis was to study the effect of different wavelengths of light and storage conditions on photooxidation in dairy products. The knowledge obtained from these photooxidation studies was then used to design appropriate light barrier packaging materials for preventing photooxidation in dairy products.

Descriptive sensory evaluation has been the main method for measuring photooxidation in this study since it is sensitive and relevant. Fluorescence spectroscopy has been an important tool for studying and understanding the photobleaching going on in dairy products. The method allows monitoring of the photodegradation of the photosensitizers during light exposure, and can help to understand differences caused by different wavelengths of exposure light. Singlet Oxygen Sensor Green® (SOSG) reagent was applied for the first time in a food study. It was used to measure the amount of singlet oxygen formed in photooxidation Type II reaction.

In these studies, we have observed that long wavelengths (> 550 nm) in the visible region (orange light) tended to give more photooxidation in milk than did shorter wavelengths (< 500 nm) of blue light. The photooxidation from light above 550 nm was most likely due to the naturally occurring photosensitizers chlorophyll and protoporphyrin IX which absorb light in this region. Additionally, β-carotene will probably act as an optical filter in the blue region (< 500 nm) reducing the amount of light to react with the photosensitizers absorbing in this region (riboflavin, chlorophyll and protoporphyrin IX).

It was also documented that with regard to photooxidation there was an interaction effect between wavelength of light and the storage atmosphere. Wavelengths in the red region resulted in stronger photooxidation in low oxygen atmosphere, whereas blue light gave

stronger phtooxidation in high oxygen atmosphere. This indicates that different photooxidation pathways are active for different wavelengths.

In a study on cheese, it was shown that longer wavelengths also penetrated deeper into the food matrix, generating off-flavors further into the cheese than shorter wavelengths. This might also explain why longer wavelengths resulted in more photooxidation, and it also illustrated the importance of proper sampling with regard to studies of photooxidation.

New transparent materials with tailor made light barrier were developed by using different pigments and additives and combinations of them. All films had a shade of green and transmitted light in the green region to avoid the main absorption areas of known photosensitizers in the blue and red regions. The best protection of photooxidation in pasteurized milk was obtained by blocking wavelengths below 450 nm and also minimizing light at wavelengths longer than 600 nm.

The approach of first elucidating the photooxidation properties and then, based on this, design optimized packaging materials is a general methodology that can be used for other food products in the future.

Sammendrag (Norwegian abstract)

Lys-indusert oksidasjon er en av de viktigste årsakene til kvalitetsforringelse av mat. Emballasje er vanlig å bruke for å beskytte mot lys og dermed forlenge holdbarheten av matvarer. Transparente emballasje er mye brukt i næringsmiddelindustrien slik at forbrukerne kan se produktet før kjøp. Det er dermed en utfordring å komme opp med innovative, gjennomsiktig emballasjeløsninger som allikevel har optimale lysbarriereegenskaper.

Målet med denne avhandlingen var å studere effekten av ulike bølgelengder av lys og lagringsbetingelser på fotooksidasjon i meieriprodukter. Resultatene fra disse forsøkene ble så benyttet som basis for å utforme emballasjematerialer med lysbarrierer for å hindre fotooksidasjon.

Sensorisk evaluering var hovedmetoden for å måle graden av fotooksidasjon under ulike lagringsforhold fordi den er sensitiv og relevant. Fluorescensspektroskopi har vært et viktig verktøy for å studere lysets innvirkning på meieriprodukter. Metoden gjør det mulig å studere nedbrytningen av fotosensitisere i produkter utsatt for lys og kan gjøre det lettere å forstå forskjeller son skyldes ulike bølgelengder av lys. Singlet oxygen sensor green® (SOSG) reagens ble for første gang brukt i forsøk med matvarer. Reagenset ble brukt til å måle hvor mye singlet oksygen som ble dannet i fotoreaksjon Type II.

Vi observerte at lengre bølgelengder (> 550 nm, oransje lys) resulterte i høyere fotooksidasjon i melk enn kortere bølgelengder (< 500 nm, blått lys). Fotooksidasjonen fra lys over 550 nm skyldtes sannsynligvis de naturlig forekommende fotosensitiserne klorofyll og protoporfyrin IX som absorberer lys i dette området. I tillegg vil β-karoten kunne fungere som et filter i det blå området og dermed redusere mengden lys som kan reagere med fotosensitiserne i dette området (riboflavin, klorofyll, protoporphyrin IX).

Det ble også dokumentert en interaksjonseffekt mellom bølgelengde og atmosfære i forbindelse med fotooksidasjonen. Bølgelengder i det røde området forårsaket mer fotooksidasjon når oksygennivået var lavt mens bølgelengder i det blå området forårsaket derimot mer fotooksidasjon når oksygennivået var høyt. Dette indikerer at fotooksidasjonen benytter forskjellige reaksjonsveier ettersom lysets bølgelengde endres.

Det ble påvist i ost at lengre bølgelengder trengte lengre ned i osten og forårsaket uønsket smak lengre inn enn kortere bølgelengder. Dette kan også forklare hvorfor det ble observert mer fotooksidasjon med lengre bølgelengder og viser viktigheten av riktig prøveinnsamling i studier på fotooksidasjon.

Nye gjennomsiktige materialer med skreddersydde lysbarriereegenskaper ble utviklet ved bruk av forskjellige pigmenter og tilsetningsstoffer (og kombinasjoner av disse). Alle filmene var i forskjellige nyanser av grønt for å unngå absorbsjonsområdene til kjente fotosensitisere i de blå og røde områdene. Den beste beskyttelsen av pasteurisert melk ble oppnådd ved å blokkere bølgelengder under 450 nm og minimere lys med bølgelengder over 600 nm. Denne nye tilnærmingen kan justeres for å optimalisere emballasje for andre matvarer i fremtiden.

Fremgangsmåten med først å bestemme fotooksidasjonsegenskapene og deretter, basert på dette, fremstille optimal emballasje er en generell metode som også kan bli benyttet for andre produkter.

List of papers

I.   N. Intawiwat, M. K. Pettersen, E. O. Rukke, M. A. Meier, G. Vogt, A. V. Dahl, J. Skaret, D. Keller and J. P. Wold. 2010. Effect of different colored filters on photooxidation in pasteurized milk. Journal of Dairy Science. 93:1372–1382.

II.   N. Intawiwat, A. V. Dahl, M. K. Pettersen, J. Skaret, E. O. Rukke and J. P. Wold. 2011. Effect of different wavelength of light on the formation of photo-oxidation in Gouda-like cheese. International Dairy Journal. 21: 531-539

III.   D. Airado-Rodríguez, N. Intawiwat, J. Skaret and J. P. Wold. 2011. Effect of Naturally Occurring Tetrapyrroles on Photooxidation in Cow's Milk. Journal of Agricultural and Food Chemistry. 59: 3905-3914.

IV.   N. Intawiwat, E. Myhre, H. Øysæd, S. H. Jamtvedt and M. K. Pettersen. Packaging materials with tailor made light transmission properties for food protection. Polymer Engineering & Science, (submitted June, 2011).

V.   N. Intawiwat, J. P. Wold, J. Skaret, E. O. Rukke and M. K. Pettersen. Minimizing photooxidation in pasteurized milk by optimizing light transmission of green polyethylene films. Journal of Food Science, (manuscript).

In the introduction, results and discussion, the papers are referred to their roman numerals

1. Aim of study

The main purpose of this thesis was to study the effect of light and storage conditions on photooxidation in dairy products in order to design appropriate packaging materials for extended shelf life. Dairy products, milk and cheese, were selected as case food products. This was because they are important product worldwide susceptible to photooxidation and also our good knowledge about the photooxidation properties in these products.

The aim of the study was divided into three sub-objectives:

1. Study photooxidation in milk as a function of wavelength and intensity of light exposure as well as storage atmospheres.

2. Study effects of light penetration on photooxidation in cheese.

3. Design optimized tailor made packaging films with alter light transmission properties in visible wavelength region in order to minimize photooxidation in dairy products.

2. Background and theory

2.1 Light induced photooxidation in dairy products

Photooxidation is one of the major causes of quality deterioration in dairy products. Avoiding light exposure from both sunlight and commercial light is difficult. This is because dairy products are exposed to light during processing, storage, display in retail stores and during distribution to the consumer.

To better understand photooxidation in order to optimize solutions preventing this phenomenon, this study covers the following issues:

  i)   Identification of photosensitizers in dairy products and understand their effects on product quality.
  ii)   Determine factors of storage conditions e.g. the intensity of light, wavelength of light, light exposure duration and storage atmosphere.
  iii) Design and selection of proper packaging material with absorption characteristics for prevention of photooxidation.
  iv)   Practical storage experiments with evaluation of the products quality.

Factors affecting photooxidation in dairy products used in this thesis are briefly explained in Figure 1. They are classified into three main groups: light exposure, packaging and product characteristics. Some of the factors are also relevant to more than one group. Light exposure includes light intensity, wavelength of light and light exposure time. The effect of packaging varies according to color, light transmission property and optical properties. The product characteristics are chemical composition and in particular the presence of photosensitizers. Additionally, the gas headspace inside the packaging also affects photooxidation and light penetration is affected both by the light and the product. Sensory analysis and fluorescence spectroscopy are techniques for measuring the food quality.

When light is exposed to a package containing a product, some light passes through the packaging material into the product. Light is then absorbed by photosensitive compounds initiating the oxidation mechanism. The photooxidation reaction is mainly occurs because of

the presence of oxygen and photosensitizers (Skibsted, 2000). The quality deterioration of dairy products caused by photooxidation includes loss of vitamin and nutrition value, reduction of sensory quality and discoloration (Sattar and deMan, 1975; Bosset et al., 1994). In addition, the degradation of photosensitizer compounds leads to formation of oxidation volatile compounds related to sensory off-flavors and off-odors.
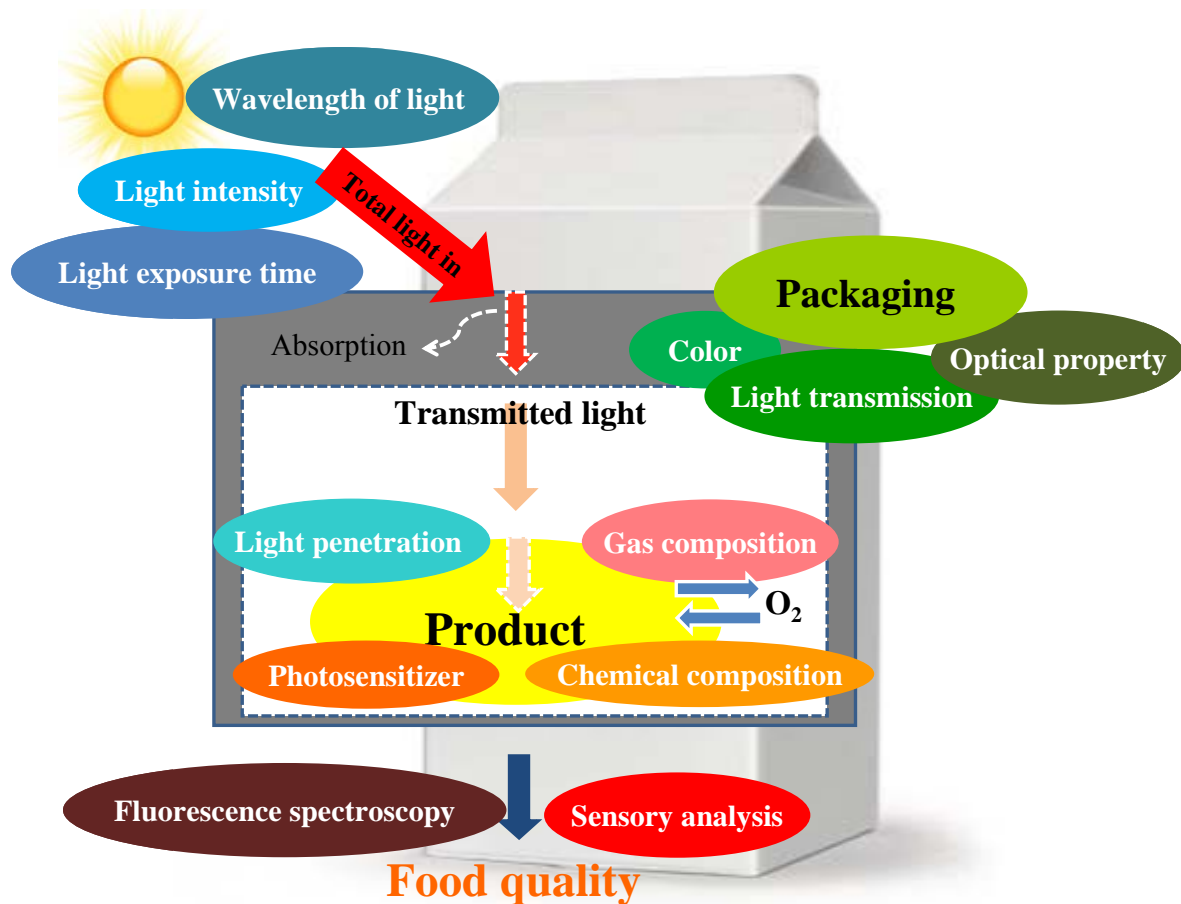
Figure 1. Overview of the different parameters used in this study on photooxidation in dairy products.

2.1.1 Lipid oxidation

Lipid oxidation is one of the major causes of food quality deterioration. Lipids are important components in dairy products and contain polyunsaturated fatty acids susceptible to oxidation. The oxidation reactions may develop secondary oxidation products and lead to the production

of unpleasant off-flavors. Lipid oxidation is divided into three types: autooxidation, photooxidation and enzymatic lipid oxidation (Frankel, 2005). However, the autooxidation and photosensitized oxidation are discussed in this thesis. More information on lipid oxidation can be found in the references (Frankel, 2005; Nawar, 1985).

Autooxidation

The autooxidation is divided into three stages: initiation, propagation and termination. Autooxidation is usually initiated by the presence of oxygen and unsaturated fatty acids. This oxidation proceeds through a free radical chain reaction.

The initiation process starts when the unsaturated fatty acid loses a hydrogen atom and produces a free radical. This reaction is catalyzed by heat, metals and light. In the propagation process, free radicals (R·) react with oxygen, and then peroxyl radicals (ROO·) are formed. Peroxyl radicals react with hydrogen atom from unsaturated fatty acid (RH) to generate unstable hydroperoxide (ROOH) and free radical (R·). Hydroperoxides are commonly known as primary oxidation products. These unstable compounds will be degraded and turn to secondary oxidation products. Hydroperoxides are usually tasteless and odorless, but their secondary oxidation products such as aldehydes, alcohols and ketones contribute to produce off-flavors and off-odors (Bekbolet, 1990).

Photooxidation

Photooxidation can proceed through autooxidation and photosensitized oxidation. Autooxidation has been mentioned previously, thus photosensitized oxidation is presented in the following section.

The main factors in the photosensitized oxidation are light, oxygen and photosensitizers in food products (Bradley and Min, 1992). The sensitizers have conjugated double bonds which can absorb light in the UV and visible light regions. When they absorb light, they are excited from ground state (^{0}Sens) to the higher energy level (excited state). Sensitizers have two unstable excited states called singlet (^{1}Sens) and triplet (^{3}Sens). The singlet sensitizer has a very short life time (1-100 ns). Thus, mostly the oxidation process is initiated by triplet sensitizer since it has longer lifetime (Spikes, 1989). In the termination process, triplet

sensitizers are decayed or converted to the ground state by chemical quencher products. Photosensitizer oxidation can process through two different photoreaction: Type I and Type II reactions.

Type I reaction proceeds directly from the excited state sensitizers activated free radical initiator. The triplet sensitizers (^{3}Sens) react with unsaturated fatty acids forming free radicals. Free radicals are very reactive chemically. The free radicals react with oxygen, and then hydroperoxides are produced as shown below. The peroxide products react further to initiate the free radical chain in autooxidation process (Spikes, 1989).

$$^3\text{Sens} + \text{RH} \xrightarrow{h\nu} [\text{intermediate}] + O_2 \longrightarrow \text{hydroperoxides} + {}^0\text{Sens}$$
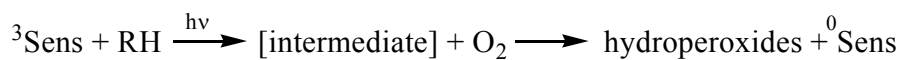
In Type II reaction, the triplet sensitizers (^{3}Sens) react with oxygen, and transfer energy to produce an excited state of oxygen so called singlet oxygen ($^1O_2$). The reactive singlet oxygen rapidly interacts with unsaturated fatty acid to form hydroperoxides as shown below.

$$^3\text{Sens} + O_2 \xrightarrow{h\nu} [\text{intermediate}] + {}^1\text{Sens} \longrightarrow {}^1O_2 \xrightarrow{\text{RH}} \text{hydroperoxides}$$
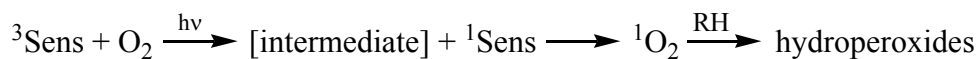
Type I and Type II reactions can proceed simultaneously in a competitive fashion (Spikes, 1989). Most photosensitizers are degraded in the processes that involve the presence of oxygen. However, photosensitized reactions can occur without oxygen as well. Type I reaction is more efficient under low oxygen concentrations (He et al., 1998).

2.1.2 The formation of sensory off-flavors and off-odors

The degradation of unsaturated fatty acids, proteins and vitamins produces secondary oxidation products which are responsible for unpleasant off-flavors and off-odors. The sensory attributes related to photooxidation can be classified into three main groups: sunlight, oxidized and activated flavors (Shipe et al., 1978). Sunlight flavor is a characteristic of milk exposed to sunlight. Oxidized flavor is caused by oxidation of unsaturated fatty acids.

Activated flavor is described as burnt, burnt feather etc. and occurs from the oxidation of sulphur-containing proteins and amino acids e.g. methionine.

The volatile compounds produced from milk fat oxidation can be detected at very low concentration (part per billion), thus off-flavors are very easily generated. Aldehydes and ketones are typical volatiles produced by lipid oxidation which are derived from the hydroperoxides of polyunsaturated fatty acid. The flavors of these carbonyl compounds are described as oxidized, fishy, metallic, card board, painty and tallowy (Frankel, 2005).

The important volatile compounds measured by gas chromatography mass spectroscopy (GC-MS) in milk exposed to light are 2-heptanone, hexanal, heptenal, 2-butanal, 2-nonenal, pentanal, propanal, 1-octen-3-ol and dimethyl disulfide (Kim and Morr, 1996; Frankel, 2005; van Aardt et al., 2001). For Havarti cheese exposed to fluorescent light, the common secondary oxidation products are 1-pentanol, 1-hexanol, nonanal and benzaldehyde (Mortensen et al., 2002a, b; 2003a, b).

2.1.3 Known photosensitizers in dairy products

Riboflavin

Riboflavin (vitamin B2) is a water soluble photosensitizer in milk (Figure 2). It has a conjugated double bond system which absorbs light (Bradley and Min, 1992). The amount of riboflavin varies between different dairy products. The concentration of riboflavin is approximately 0.17 mg per 100 g in whole milk (Westermann, 2009) and 0.30-0.60 mg per 100 g in cheese (National Food Institute, 2009). Moreover, different fat concentrations in milk also effect the photo degradation of riboflavin. The degradation of riboflavin was found to be greater in skimmed milk compared to whole milk (Allen and Parks, 1979; Lee et al., 1998). The riboflavin photooxidation mechanism is well described in Skibsted (2000) and Westermann (2009).
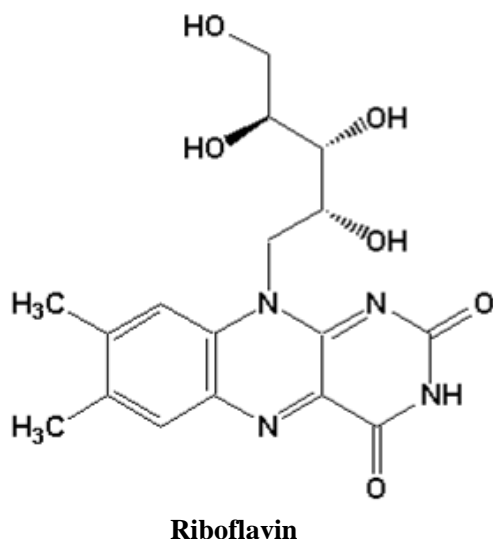
Riboflavin

Figure 2. Chemical structure of riboflavin

Source: Mathews, et al. (2000)

The photosensitizing property of riboflavin was the first reported marker correlated to the formation of off-flavors and off-odors in dairy products (Aurand et al., 1966; Sattar and deMan, 1975; Allen and Parks, 1979; Mortensen et al., 2004). Recently, the light sensitive compounds chlorophyll and porphyrins have also been shown to be responsible for formation of off-flavors in milk and dairy products (Wold et al., 2005).

Chlorophyll and porphyrins

The chemical structure of chlorophyll and porphyrin contains a tetrapyrrole ring conjugation. Porphyrin is a completely conjugated tetrapyrrole, whereas chlorophyll-a has a magnesium chelated tetrapyrrole structure with methyl group substitutions. Cholorphyll-b has the same configuration as chlorophyll-a, except it has a formyl group instead of a methyl group.

Chlorophyll is described as a green pigment which relates to photosynthesis of plants. Chlorophyll is found in various types named as chlorophyll-a, b, c and d. However, chlorophyll-a and b are the main types found in foods. Chlorophyll and porphyrins are naturally present in milk and dairy products (Wold et al., 2005). The presence of chlorophyll in milk is derived from the cow's diet. Porphyrin is well-known as hemes, pigment in red cell (Francis, 1985) and may come from cell of the lacteal organs in the cow (Wold et al., 2005). Chlorophyll and porphyrins are fat soluble and present in the lipid phase in milk. The amounts

of chlorophyll and porphyrins are much lower than riboflavin, with chlorophyll reported 0.02-0.03 ppm in butter (Wold and Lundby, 2007). The concentration of chlorophyll is lower in milk because milk has a lower fat content than butter.

The chemical structures of porphyrin and protoporphyrin IX are presented in Figure 3. The photosensitized oxidation based on chlorophyll and protoporphyrin IX proceeds via Type II reactions where singlet oxygen reacts with unsaturated fatty acids, or when the oxygen concentration is low, it can proceed through Type I reactions. Chlorophyll and porphyrins have also been reported to degrade in low oxygen content. It has been suggested that this photodegradation proceeds through Type I reaction (Wold et al., 2006a, 2009; Veberg et al., 2007).

Porphyrin
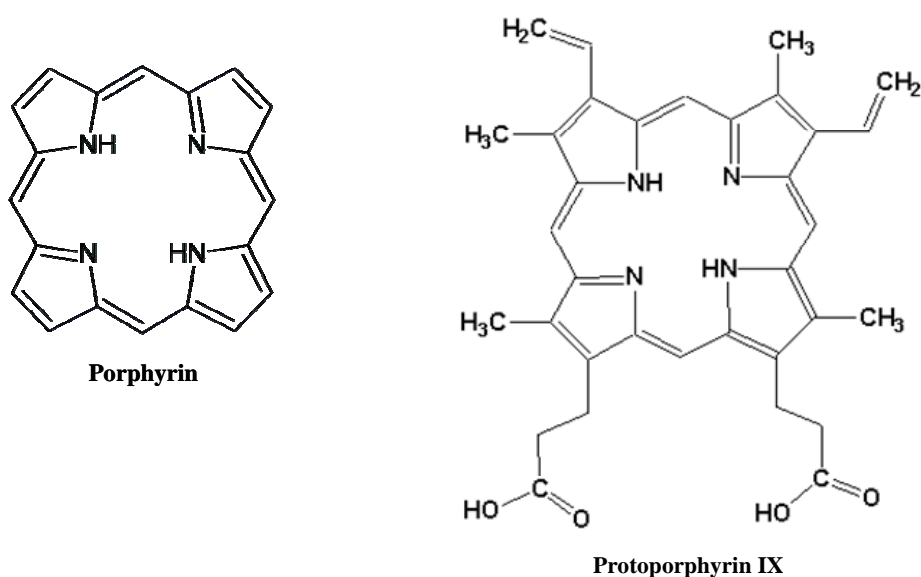
Protoporphyrin IX

Figure 3. Chemical structure of porphyrin and protoporphyrin IX.
Source: Mathews et al. (2000) and Pushpan et al. (2002)

Absorption spectra of light sensitive compounds

Riboflavin has absorption bands in UV light and a maximum peak around 450 nm in visible light, whereas chlorophyll has absorption peaks around 420 and above 600 nm (Figure 4). With regards to absorption, riboflavin is an active compound in the UV and blue regions,

while chlorophyll is active in both blue and red regions. Protoporphyrin IX has maximum peak at 410 nm and has several small absorption peaks above 500 nm. ß-carotene, as riboflavin, absorbs light in the near UV and blue visible wavelength regions. Therefore, it will absorb some of the light and reduce the negative effect of light in the 400-500 nm region.
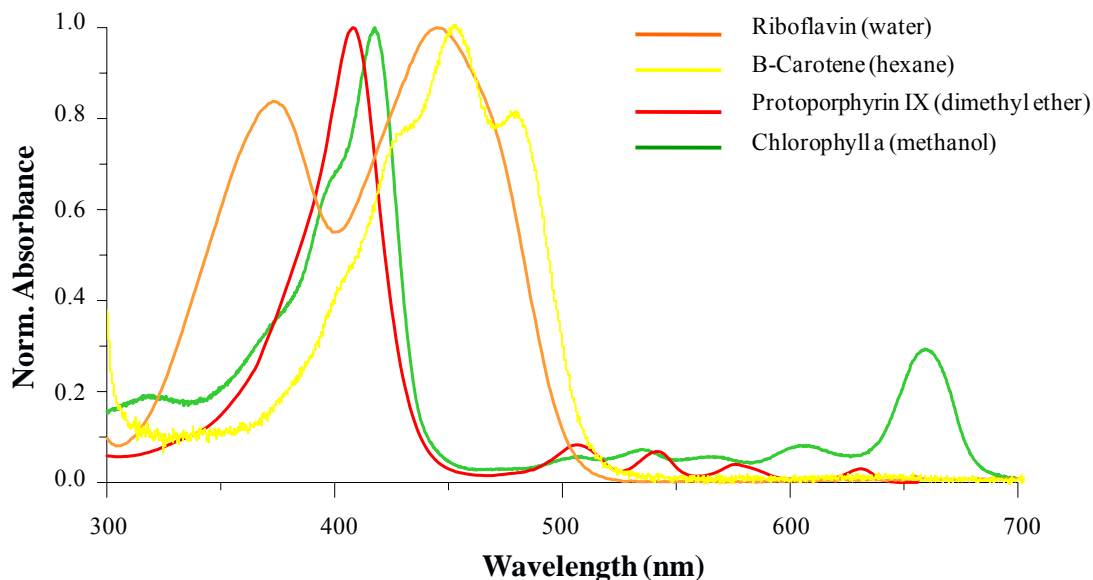
Figure 4. Absorption normalized spectra for riboflavin in water (orange line), β-carotene in hexane (yellow line), protoporphyrin IX (red line) in dimethyl ether and chlorophyll-a (green line) in methanol.

2.1.4 The inhibition of photosensitized oxidation

ß-carotene, ascorbic acid and α-tocopherol are singlet oxygen quenchers in milk and dairy products which can prevent the formation of oxidation products. Carotenoids are used as antioxidant and coloring agents for dairy products. They are important inhibitors involved in photooxidation Type II reactions, since they react rapidly with singlet oxygen and inhibit photosensitized oxidation. The quencher effect occurs when electron is transferred from singlet oxygen to carotene and form stable products (Frankel, 2005). ß-carotene has been used to protect against the degradation of riboflavin in dairy products, since it absorbs light in the same area as riboflavin (Hansen and Skibsted, 2000). Adding ß-carotene has been reported as

an efficient quencher to reduce oxidation related to rancid flavor in high presence of oxygen concentration (Veberg et al., 2007).

Antioxidants, such as ascorbic acid and α-tocopherol, can be used to protect or delay the formation of oxidation products (Borle et al., 2001). Ascorbic acid is an active oxygen quencher. It is used for inhibiting riboflavin degradation in milk (Lee et al., 1998). However, the effect of the inhibition of photosensitized oxidation was not studied in this thesis.

2.2 Effect of storage condition parameters on photooxidation in dairy products

Storage conditions are very important in order to protect against photooxidation in food products. The light source, wavelength of light, light intensity, and duration of light exposure together with gas composition in headspace are factors affecting on photooxidation. Those factors are briefly presented in the following chapter.

2.2.1 Light exposure

Light source

Light has a major effect on food quality deterioration. Different light sources may provide different wavelengths of light. Sunlight has a broad emission spectrum wavelength in UV (200-380 nm) and visible light (380-700 nm). Nowadays, the photooxidation is mainly caused by artificial light. Fluorescent light is commonly used in commercial light in production processes and in the refrigerator cabinet in retail stores. The fluorescent tubes provide both narrow spectral lines and a broad emission so called polychromatic (white) light (Borle et al., 2001). Fluorescent produced light can be traditionally classified into two groups, so called cool white light and warm white light (Bosset et al., 1994). Fluorescent "cool white light" tubes provide a strong violet, blue and green emission wavelength of light. The "warm white light" provides more in yellow, orange and red emission wavelength of light.

Fluorescent light is used in retail stores mostly for sale and presentation of product purposes. Different types of fluorescent light can be used to improve the appearance of food products. For example warm white light is normally used in the meat and bakery departments in order to make the meat seem redder and fresher to the consumer, whereas cool white light is used in display cabinets for dairy products. Fluorescent tubes used in retail stores emit light in harmful wavelength region which sometimes are not always suitable for maintain the food quality. The suitable type of fluorescent light source for food products should be taken in consideration in addition to light intensity etc.

Light intensity

The light intensity is also an important factor regarding food quality (Bosset et al., 1994). It is well-known that a higher light intensity may induce more photooxidation.

The light intensity may vary dependant on different factors. For instance, large differences in light intensity have been reported in three different supermarkets depending on distance from product to light source (Haisman et al., 1992). Large deviation of light intensity in a dairy display cabinet has also been observed (Chapman et al., 2002). It should be noted that measuring light intensity in lux unit is not an ideal measurement with regard to photooxidation in foods. This is because it measures the intensity as perceived by the human eye which is more sensitive to some wavelengths (green light) than others. The intensity of light should be measured with equal weight on all wavelengths in term of power incident per surface area as presented in W/m^2.

To minimize photoreaction light intensity should be as low as possible. This can be achieved by increasing the distance between light source and product or container, and/or use light source e.g. light-emitting diode (LED) light. The LED light provides lower radiated heat compared to fluorescent tubes. Furthermore, the wavelengths of light emitted from LED lamps can be adjusted to specific regions optimal for the food properties.

In studies of photooxidation in foods, two different ways of normalization of light intensity can be adjusted by two approaches as shown in Figure 5. First approach, adjustment of light intensity *above* films is commonly used for practical interest applied for food packaging industry. Mostly researches have studied the efficient of films or filters to absorb or surpass energy of the light. Second approach, light intensity adjusted to be equal *below* color films for getting the same intensity at the surface samples. This adjustment makes it possible to compare the effect of different wavelength regions of light on photooxidation.
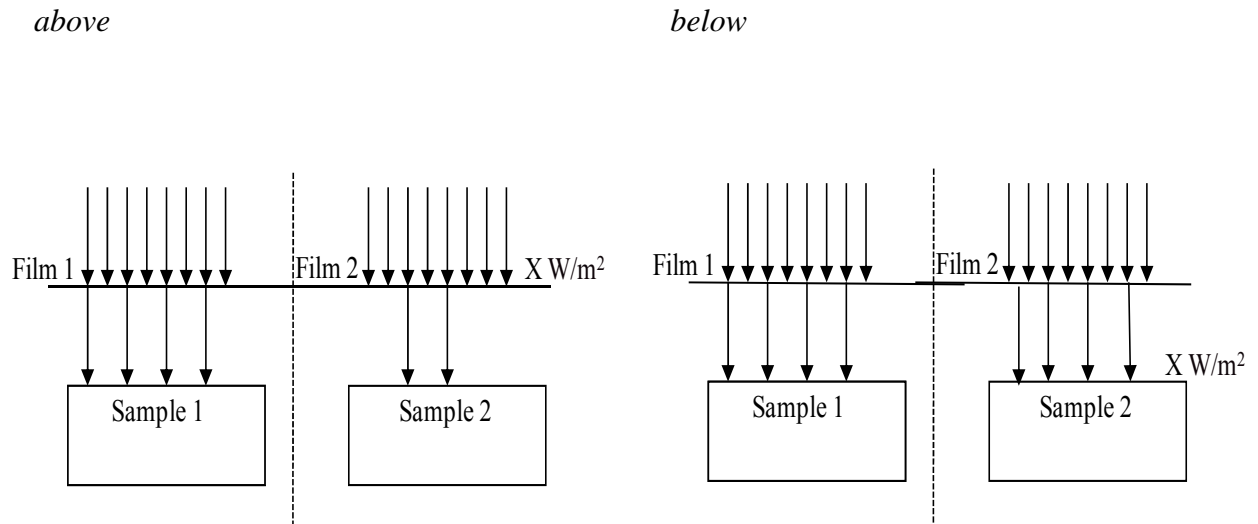
above   *below*

Figure 5. Light intensity adjusted to specific intensity (X W/m^2) either *above* the films or *below* the films. In the *above* case the intensity at the sample surface is X W/m^2 or less, depending on the film properties. In the *below* case, the intensity at the sample surface adjusted to X W/m^2 for each film.

Light penetration

Light penetration is one factors of light-induced photooxidation. Different wavelengths of light have different energy to penetrate into food matrices and also different scattering properties affecting the distance of penetration. Product characteristics influence light penetration e.g. type, fat content etc. Some products are more light-scattering than others. The fat globules in whole milk make it more scattering than skimmed milk, thus the light penetrated deeper into skimmed milk than in whole milk (Allen and Parks, 1979). Light penetration depends on the wavelength of light and light scattering. Light at longer wavelengths generally penetrates deeper into food matrices than shorter wavelengths due to less scattering at this wavelengths (Josephson, 1946; Westermann et al., 2009). Even though the shorter wavelengths have high energy, they are more scattered according to the rule of wavelength dependent Rayleigh scattering. Few studies have investigated light penetration on photooxidation in dairy products. The white light has been reported to induce off-flavors up to 6 mm into cheese (Wold et al., 2002a).

Wavelength of light

Many photooxidation studies focus on the effect of different wavelengths on food quality deterioration. The wavelength of light is a very important factor for photooxidation in dairy products since the photosensitizers are active under different wavelengths.

UV and blue light have been suggested as the most harmful to dairy product since they are absorbed by riboflavin. Using only a UV filter in packaging material is not enough to prevent light induced deterioration in milk (Cladman et al., 1998; Mestdagh et al., 2005; Karatapanis et al., 2006). They suggested that it was because this material did not exclude wavelengths at the absorption of riboflavin. Thus, excluding wavelengths below 500 nm has been recommended in order to protect against riboflavin sensitized oxidation.

It has been observed that the wavelengths in red region also induce off-flavors in dairy products (Josephson, 1946; Webster et al., 2009; Wold et al., 2005, 2006b). The major light-induced volatile compound, pentanal, has been detected in high levels under light exposure at 610 nm (Webster et al., 2011). The production of pentanal is initiated by other photosensitizers than riboflavin, thus there are other compounds involved in the photooxidation which absorb light at longer wavelengths (above 550 nm). Chlorophyll and porphyrins are sensitizers in this region since they absorb light in red region. Moreover, photodegradation of chlorophyll and protoporphyrin IX have showed higher correlation to sensory properties compared to riboflavin (Wold et al., 2005, 2006a, b).

Comparison of the wavelength of light to absorption peaks of photosensitizers shows that violet and blue light degrade riboflavin, protoporphyrin IX and chlorophyll (Wold et al., 2005, 2006a), while red light only degrades chlorophyll and porphyrins (Wold et al., 2005, 2006a, b, 2009) and green light does not target any of these. Green light has been shown to cause less adverse effects in regards to photooxidation of milk (Hansen et al., 1975) and Norvegia cheese (Wold et al., 2006 b). Thus, avoiding violet and blue light to protect riboflavin as well as red light to protect chlorophyll has been recommended. Blocking wavelengths from UV to 500 nm and above 600 nm should be used for preserving acceptable milk qualities (Webster et al., 2011).

2.2.2 Gas composition in the headspace

Oxygen is well-known as an important factor for oxidation and discoloration in milk and dairy products. In general, the oxidation is more intense in the presence of high amounts of oxygen concentration in the headspace. Modified atmosphere packaging (100% N_2 or 50%N_2 and 50%CO_2) has been found to give a significantly lower degree of lipid oxidation compared to aerobic atmosphere packaging under light exposure for 4 weeks (Trobetas et al., 2008). It has been reported that even small amounts of oxygen (0.2%) in the headspace were able to produce oxidation products and detectable off-flavors (Mortensen et al., 2003a). Degradation of photosensitizers varies according to the concentration of oxygen as described previously under photoreaction mechanisms.

2.3 Effect of packaging materials on photooxidation in dairy products

2.3.1 The function of packaging

The function of packaging is generally divided into two main purposes: technology and marketing. In terms of technology packaging is defined as containment, protection, and preservation in addition to convenience for handling, distribution, transportation and storage. The basic requirement of packaging is to protect against physical damage and mechanical damage. Physical damage is caused by environmental storage conditions such as light, moisture, gas, aroma, microbiological and temperature. Thus, packaging should provide a suitable barrier based on food product requirements. Additionally, the interaction between packaging material and food may affect sensory quality e.g. odors and flavors. The migration of toxic compounds or chemical substances such as monomers, additives, or break down products of polymer, from packaging materials into food products is also should be concerned. Packaging is used to protect against mechanical force such as compression, drop and impact which mostly occurs during handling, transportation and distribution.

The packaging can also be used as a function of marketing to identify products, display product information, self-promotion and recognize. Furthermore, packaging is used as a tool for either increasing the value of products or changing marketing strategy to increase the market share.

However, sometimes there is conflict between marketing strategy based on consumer requirements and packaging technologies based on food protection. Thus, the optimal solution has to consider both product characteristics and consumer demands. The protection of food products e.g. milk and dairy products against light during transportation, storage and display in stores is one of the biggest concerns.

The packaging materials commonly used for liquid milk are glass, carton or plastic. In Norway milk is commonly packed in cartons which are made from paper board coated and/or laminated with many layer of polyethylene or other plastic films in order to increase barrier properties against water and oxygen. However, in other countries pasteurized milk is usually packed in plastic bottles, either transparent or partly transparent (translucent).

Cheeses are packed in different types of packaging materials. Different techniques are needed for different types of products for example thermoforming, skin packed, vacuum packed, and modified atmosphere packaging etc. Transparent plastic materials are often used, some combined with printed label on top of the package. The storage time for cheese is longer than for milk liquid products. Thus, the packaging materials have to provide good light, water and gas barrier properties.

2.3.2 Light transmission

Packaging material absorbs and reflects some of the light, while some light transmits through the material and into the product. Light transmission is measured as the percent of incident light through a film and reported as the wavelength spectra in visible region or an integration of entire wavelengths or specific wavelength regions. Light transmission of packaging material can be modified by changing the absorption characteristic of the packaging material. This is useful technique to avoid the harmful wavelength inducing photooxidation.

The reflection and absorption varies according to type of packaging materials, colors and thickness. These factors make different level of protection against photooxidation even when exposed to the same light source (Mortensen et al., 2004). Regarding to the variation of packaging materials, it is maybe difficult to compare results with other studies. Additionally, the material processing, polymer structure, crystallinity, orientation, additives and pigments

also have an impact on the light transmission properties. Light transmission is related to wavelength of light so that light transmission of material has often been presented together with wavelength of light.

2.3.3 Oxygen transmission rate (OTR)

Oxygen transmission is one of the most important properties for selective appropriate packaging material suitable for food products. The OTR is the quantity of oxygen gas passing through a unit area of material per time under controlled conditions. It is related to permeability of material based on a solubility-diffusion process. The gas passes through the polymer material by dissolving in the polymer at one surface and diffuses through the polymer by a partial pressure difference across the film known as permeation. The OTR depends on the difference between partial pressure of oxygen inside and outside the packaging, and on the relative humidity and storage temperature (Robertson, 1993). Thus, the transmission rate has to be given with pressure or the concentration of the gas and thickness of material under the measurement conditions. Further information about this measurement applied on flexible films is presented in ASTM D3985-05 (2010).

2.4 Methods used for developing packaging materials to improve the light barrier

Packaging is well-known as one of the alternative methods to prevent light induced oxidation in food products. The factors influencing light protection of packaging material that should be considered are (i) light absorption of material, (ii) thickness of material, (iii) processing of material, and (iv) coloration of material (Mortensen et al., 2004). This has to be combined with a basic knowledge of photooxidation in the food. A good packaging design contributes to product protection, prolonging shelf life, and cost efficiency (Borle et al., 2001).

2.4.1 Packaging material combined with color pigments

Pigments are commonly used in polymer, but mostly for design and decoration purposes. However, colored packaging material can be applied for protection and preservation of food

quality. Adding pigments to the polymer can modify light transmission wavelength profiles without disturbing other functionality of the material, and even improve the light barrier in specific wavelength regions.

Packaging material with black pigment is the best protection against light induced oxidation, and the transparent packaging provides the worst protection (Sattar and deMan, 1975; Mortensen et al., 2002b; Pettersen et al., 2005). Regarding to the harmful wavelengths of light is reported previously, packaging material with green color has also been reported as providing a good barrier to photooxidation in milk and dairy products (Hansen et al, 1975: Cladman et al., 1998; van Aardt et al., 2001; Wold et al., 2005, 2006b). According to this knowledge, the optimal packaging design in order to protect against photooxidation in milk has been investigated in this thesis.

2.4.2 Additives

Metal oxide is added into material to increase the properties of e.g. UV absorption, mechanical strength, abrasion resistance and antimicrobial property (Garland, 2004). The light barrier property of packaging material is a main focus of this thesis.

There are several metal oxides that could be used as additives in packaging material. Titanium oxide (TiO_2) and zinc oxide (ZnO) are commonly used as additives in order to absorb and reflect UV light (Garland, 2004). Incorporating those additives into plastic material can reduce light transmittance in wavelength of light below 400 nm. Particles at nanoscale are smaller than the wavelength of visible light so that they still provide transparency property. Normally, adding metal particle diameter below 40 nm provides transparent nanocomposites (Althues et al., 2007).

Blocking UV light by using TiO_2 in polyethylene packaging has been reported to inhibit the development of light induced oxidized flavors in milk (Hoskin and Dimick, 1979). However, UV barrier commercial films are commonly available in the packaging market, thus this topic was not studied in this thesis.

Silver nanoparticle

Metal additives such as silver (Ag) and gold (Au) are applied in materials due to the unique optical property of having specific absorption which can be used to adjust transmission wavelengths of materials (Kamat, 2002; Medda et al., 2005). Absorption spectra of Au and Ag nanoparticles are at 520 nm and 418 nm, respectively (He et al., 2002). In this thesis only silver was used because it has absorption spectra close to chlorophyll absorption.

In addition to the absorption properties, silver nanoparticles are used to incorporate into polymer nanocomposites for food packaging to inhibit growth of microorganisms (Liau et al. 1997; Damm et al. 2008). Moreover, silver nanoparticles can be applied in packaging as an ethylene absorber for extending shelf life of fruits and vegetables (An et al., 2008). However, the antimicrobial property has not been taken into account in this thesis.

3. Methodology

Sensory evaluation (Paper I, II, III and V) and gas chromatography mass spectroscopy (GS-MS) (Paper I) were used to monitor the level of photooxidation which related to off-odors and off-flavors. Fluorescence spectroscopy (Paper I, II, III and V) was used to measure photo degradation of photosensitzers. The fluorescent agent Singlet Oxygen Sensor Green® (SOSG) was used to measure the amount of singlet oxygen formed in photoreaction Type II (Paper III). The material properties measurements were light transmission (Paper I-V), optical properties e.g. total transmittance, haze, gloss, clarity and surface morphology (Paper IV).

3.1 Determination of food quality

3.1.1 Sensory descriptive analysis

Sensory evaluation is defined as "a scientific method used to evoke, measure, analyze, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste, and hearing" (Stone and Sidel, 2004).

Sensory analysis is widely used in many food companies for providing information in terms of human perception. This method is often used as an instrument for measuring lipid oxidation related to sensory attributes. A sensory panel can detect oxidation volatile compounds in term of off-flavors and off-odors, even if those compounds can not be detected by other chemical and instrument analysis (Frankel, 2005). However, the accuracy of the sensory results depends on the training of the panel.

The sensory evaluation is divided into 3 methods: discrimination, descriptive and affective. Each method is selected according to purpose, number of participants, and selected criteria. The descriptive analysis with a trained panel is used in this thesis (Paper I, II, III and V). This method measures the intensity of sensory characteristics of products and is able to define variation of products compare to the reference standards. Before analyzing the samples, the panelists create a list of attributes based on reference standards. The results from this method can be related to instrumental measurement by statistical methods such as regression and

correlation (Lawless and Heymann, 2010). The sensory attributes used in this thesis are defined in Table 1.

Table 1. Definition of sensory attributes presented in this thesis.

Sensory attribute		Description
Odors	Sour/fresh	Odor of freshness; sour and sweet odor.
	Sunlight	Odor of sunlight related to oxidation of proteins.
	Rancid	Odor of rancid such as grass, hay, candle, paint.
Flavors	Sour/fresh	Flavor of freshness; sour and sweet flavor.
	Sunlight	Flavor of sunlight related to oxidation of proteins.
	Rancid	Flavor of rancid such as grass, hay, candle, paint.

3.1.2 Gas chromatography mass spectroscopy (GC-MS)

GC-MS is an analytical technique for the identification and quantification of volatile compounds. The gas chromatography separates the component in the mixtures and mass spectroscopy provides information of each component. The volatile compounds that can be measured by GC-MS are for instance esters, fatty acids, alcohols, aldehydes, ketones and hydrocarbons. GC-MS can be used to indicate lipid oxidation related to sensory analyses (Frankel, 2005).

The dynamic headspace GC-MS method was used on milk (Paper I). The samples in liquid phase are heated and purged with nitrogen as carrier gas in glass vessel in order to evaporate volatile compounds. The vaporized volatile compounds are trapped in a short column containing an absorbent such as porous polymer or charcoal. These compounds are transferred by back-flushing carrier gas into the column in gas chromatograph for separating components of the mixtures. The molecules appear at the so called retention time. The molecules transfer to the mass spectrometer in a downstream unit which detects these fragment ions and reports

them in term of the mass –to-charge ratio (m/z). The ion signals are displayed as a mass spectrum. Further introduction of GC-MS can be found in Frankel (2005).

3.1.3 Front face fluorescence spectroscopy

Traditionally, food quality is determined by using different wet chemical analyses which mostly are time consuming. For this reason, new techniques for measuring food quality are developed. Optical spectroscopy is widely used in many food research areas. This method is used to determine quality of the food and to identify structures in order to be able to predict components in food products. Some main techniques are for instance near infrared (NIR), mid infrared (MIR), fluorescence spectroscopy and Raman spectroscopy. Fluorescence spectroscopy was used in this thesis due to it is sensitivity to several photosensitizers as well as oxidation products.

Fluorescence spectroscopy is well-known as an analytical technique. It is widely used to investigate molecular structure and function in biochemistry and chemistry (Strasburg and Ludescher, 1995). This technique has high sensitivity, versatility, accuracy in addition to being rapid and rather low cost. Moreover, it collects large amounts of information in a non-destructive way. Fluorescence spectroscopy also has a potential to be implemented as an on-line measurement technique.

One commonly used method is front face fluorescence which is used in this thesis. The front face technique is different from the traditional technique by changing the angle of incidence of radiation on the sample, from an angle of 90 degrees in the traditional technique to an angle close to 30 degrees. Then, it measures the radiation emitted directly from the surface of sample as the incident radiation without passing through the solution of the sample. Sample characteristics and sample preparation methods are major concerns for using this technique. Samples should be homogeneous and surface is used to represent the rest of the sample (Veberg, 2006). This is because the signals are only collected from a surface of a sample to 2-3 mm deep inside. The method is sensitive to environmental changes such as temperature, pH and color. Thus, sample preparation and measurement should be well controlled.

Front face fluorescence spectroscopy has been used to measure lipid oxidation in solid samples e.g. meat and turkey (Wold and Mielnik, 2000, Wold et al., 2002b, Veberg et al., 2006) and recently in cod caviar (Airado, et al., 2010). Furthermore, it has been used to measure the amount of photosensitizer compounds and their degradation in dairy products such as butter (Veberg et al., 2007, Wold et al., 2006a, 2009), cheese (Wold et al., 2005, 2006b), and cream cheese (Wold et al., 2002a). A very interesting property is that the method can measure and quantify all known photosensitizers in dairy products. This can be used to monitor the photodegradation of these sensitizers in dairy products during or after light exposure. In this thesis, front face fluorescence was used to investigate photooxidation in milk. It also was used to study how sensitizers are photodegraded in depth of cheese.

Principle of fluorescence spectroscopy

The theory of fluorescence spectroscopy is well presented in the reference (Lakowicz, 1986). However, a brief description is presented as following.

Incident light is absorbed by light sensitive compounds in food products. The light excites molecules from their ground state to an excited state. At the excited state, the molecule can emit light to release energy and then goes back to its ground state. The emitted energy is usually lower than the absorbed energy, so that the fluorescence emission light is found at longer wavelengths than the excitation light. A measure of the energy difference between absorbed and emitted light is called the Stokes'shift (Strasburg and Ludescher, 1995).

The characteristic of fluorescent molecules is presented as fluorescence excitation and emission spectra (fluorescence intensity versus wavelength in nanometer). The emission is the intensity of light emitted from the fluorophore, measured with a single constant excitation wavelength. Fluorophores are light sensitive compounds which contain conjugated double bonds ready to absorb light and emit light. Fluorescence spectroscopy can be used to determine the composition of mixed compounds based on their specific excitation and emission spectra. In case two compounds have similar emission spectra, but different excitation spectra, they can be measured together in the same sample by changing excitation wavelength (Wold, 2000).

The excitation and emission spectra of the common well-known fluorophores are summarized by Lakowicz, (1986) and Ramanujam, (2000). For example tryptophan (ex 280 nm /em 350 nm), porphyrins (ex 400-450 nm /em 630 and 690 nm) , chlorophyll (ex 410 nm /em 680 nm), vitamin A (ex 327 nm /em 510 nm) and riboflavin (ex 450 nm /em 530 nm).

The front face fluorescence system used in this thesis was used in two different modes: for front face fluorescence spectra and for fluorescence imaging (Figure 6). The different excitation filters are placed between light source (xenon lamp) and sample holder. A cut-off filter is placed in front of the spectrograph for suppressing excitation light reflected from surface of the sample. The fluorescence is measured with a sensitive charge coupled device (CCD) camera. Besides measuring fluorescence spectra, this system has also been adjusted to be able to capture fluorescence images (Wold and Kvaal, 2000). For this system, a photographic lens and stray light shield with a filter holder is placed on the spectrograph (Veberg, 2006).
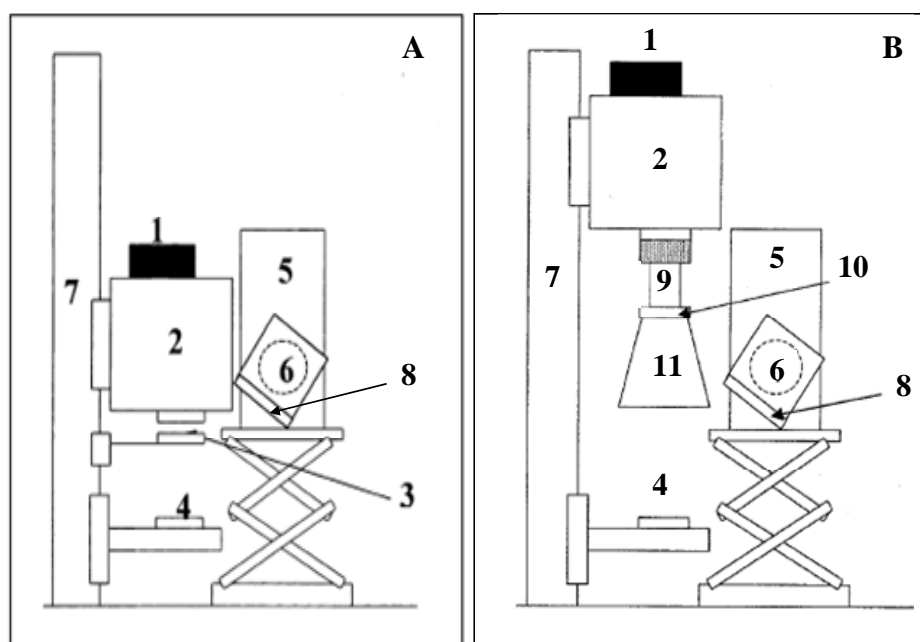
Figure 6. Fluorescence spectroscopy instrument. A) Fluorescence system. 1. CCD camera, 2. spectrograph, 3. cut-off filter, 4. sample holder, 5. lamp housing with Xenon arc lam, 6. beam turning assembly, 7. vertical optical bench. 8. excitation filter holder. B) Fluorescence imaging system. 9. photographic lens, 10. stray light shield, 11. filter holder
Source: Wold et al. (2002a) and Wold and Kvaal, (2000)

Fluorescence imaging is used for giving information about degradation of photosensitizers and formation of fluorescent oxidation products caused by light exposure. This method can be used as a marker for lipid oxidation in dairy products e.g. cream cheese, sour cream (Wold et al., 2002a), chicken (Wold and Kvaal, 2000), turkey (Veberg et al., 2006) and cod caviar (Airado et al., 2010). The images and fluorescence spectra are collected on the same CCD camera, thus those two obtained data are relevant information (Wold et al., 2002a).

The relationship between fluorescence spectra and images is shown in Figure 7. Fluorescence image capture of three major compounds: riboflavin (at 530 nm), chlorophyll (at 660 nm) and chlorophyll residues (670 nm) are in accordance with the fluorescence spectra. The gradients seen in the cross sections are caused by photosensitizer degradation after exposure to different colors of light.
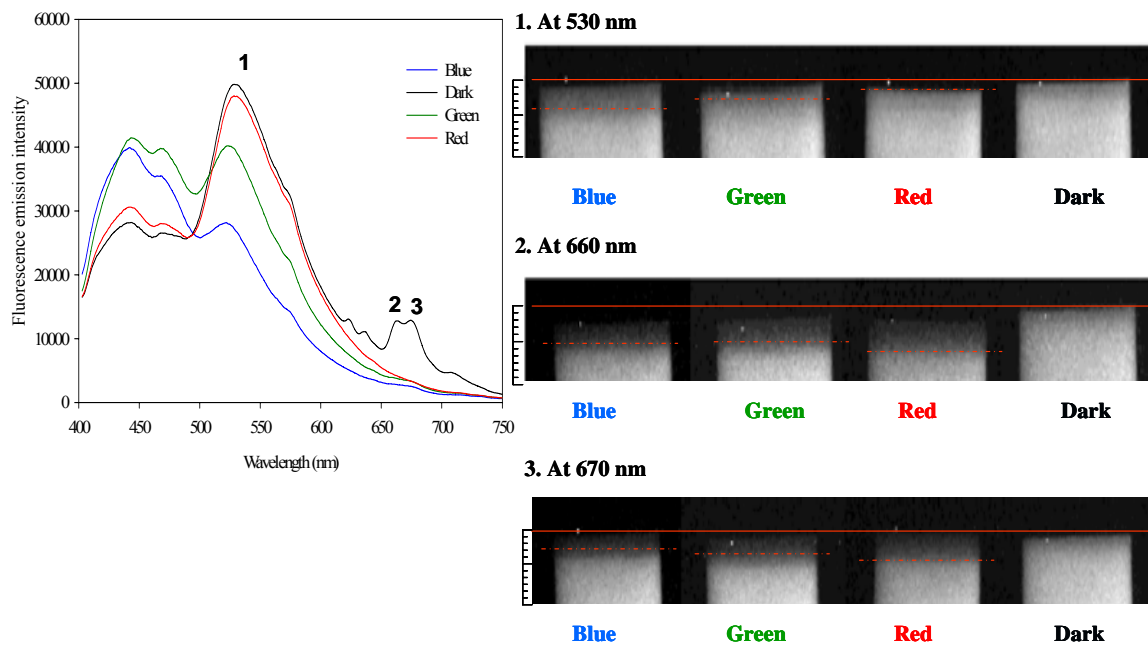
Figure 7. Fluorescence spectra (left side) and image captures cross sections (right side) of cheeses exposed to light of different colors for 7 days at 4 °C in air atmosphere.

3.1.4 Singlet Oxygen Sensor Green® (SOSG)

A novel method for monitoring the formation of singlet oxygen after light exposure is using a $^1O_2$ fluorescent sensor probe, the so called Singlet Oxygen Sensor Green® (SOSG) reagent (Molecular probes, 2004). The SOSG is a detection reagent which is highly selective for singlet oxygen. This indicator emits a weak blue fluorescence peaks at 395 and 416 nm for excitation at 372 and 393 nm, respectively, whereas after reaction with $^1O_2$ emits a green fluorescence peak at 525 nm for excitation around 504 nm (Molecular probes, 2004). SOSG has been used in biological cell research in order to measure $^1O_2$ generated in plant tissue during photo-oxidative stress, pathogen attack and wounding (Flors et al., 2006), and photo bleaching in human tissue (Tam et al., 2009). The photometric and fluorescent spectral properties of SOSG before and after reaction with $^1O_2$ have been reported by Flors et al. (2006). It should be noted that this probe has limitations with regard to photooxidation studies. This is because it might act as a $^1O_2$ photosensitizer itself under exposure to UV and light in the blue regions (Ragás et al., 2009). In this thesis SOSG was used to detect the function of $^1O_2$ in milk during light exposure.

3.2 Determination of material properties

3.2.1 Optical properties

The optical properties of packaging films are very important in food application. It can be used to design materials to meet consumer requirements. The optical properties clarity, haze, total light transmittance, and gloss were measured in this thesis (Paper IV). The haze, clarity and total transmittance were measured according to ASTM D 1003-00 (1992) and the gloss was measured according to ASTM D 2457-03 (1997).

The clarity indicates the degree of distortion of an object when viewed through the film (Robertson, 1993). Clarity is sometime used in the same meaning as transparency. The transparency of material depends on the basic polymer structure. Generally polymers have a crystalline structure, which appears as translucent. However, a polymer can be transparent if their crystals are smaller than the wavelength of light.

High haze gives an appearance as cloudy, foggy, or translucent. The appearance of haze is caused by light scattering on the surface or by non-homogeneous polymer matrices. The non-homogeneities are caused by large crystallites and/or incomplete dissolved additives (Briston, 1988). Haze is defined as the percentage of transmitted light passing through a film, deviating by more than 2.5° on average from an incident parallel beam (Robertson, 1993).

Light transmittance is the percentage of incident light that passes through the material (Robertson, 1993). It is determined by the intensity of absorption and scattering effects. The light might be reflected at the surface, and some transmitted through inside, and some is absorbed by the material. When the light scattering is zero, the material is transparent. An opaque material has low transmittance, so that it has high scattering. The light scattering is caused by morphology, non-homogenous sample, and the presence of crystallinity in the material (Hernandez et al., 2000).

Gloss is related to surface smoothness defined as a measure of the ratio between reflected intensity and incident light intensity of samples (Smith et al., 1996). A surface with high reflectance has high gloss value and gives a shiny sparkle appearance. The gloss is reduced by surface roughness, irregularities, and scratches (Hernandez et al., 2000). The gloss is measured as the incident light at a specific angle to the film surface by glossmeter. The light reflected at the same angle is collected and measured (Shah, 1998). The glossmeter is calibrated by using a flat highly polished plate as a reference standard. The three basic angle of light incident (20°, 60° and 85°) are used. The 20° is normally used for a high gloss surfaces, the 60° for medium gloss surfaces, and the 85° for low gloss surfaces.

3.2.2 Microscopy techniques

Light microscopy (LM) and scanning electron microscopy (SEM) were used in this thesis for analyzing surface morphology of the materials (Paper IV). The difference between those two techniques is that light microscopy gives structure morphology, whereas the SEM may provide both structure morphology and substances identification in the samples. Additionally, the SEM provides a higher magnification and detects greater depths into the surface of the sample than does LM.

The SEM usually operates similar as a LM. The difference between them is SEM uses electrons for imaging instead of visible light as in LM. The surface information is obtained by an electron beam scan across the regions of interest (Poole and Owens, 2003). The electron source is generated by the tungsten filament and accelerated energy. The emitted electron is detected by electric and magnetic field for each position of scanning area and reflected beam to electron detector for getting an image. Samples need to have conductivity to increase the interaction with the electron beam (Atkins et al., 2006). Further information about SEM is well described in Goodhew et al. (2001).

3.3 Statistical analysis

3.3.1 Analysis of variance (ANOVA)

ANOVA is a set of statistical methods based on comparing two variance estimates with each other (Johnson and Bhattacharyya, 2006). ANOVA is used to analyze and interpret observations from several populations. The populations are compared based on the difference between means of population and variation within population (error). In this thesis, the populations were milk exposed to different color lights and the variables were sensory attributes. The general linear model (GLM) was applied to analyze the complex results obtained from descriptive sensory data. The significant differences were obtained by Tukey's honestly significant difference (HSD) test. The ANOVA was applied for sensory analysis in Paper I, II, III and V.

3.3.2 Multivariate data analysis

Multivariate analysis techniques are statistical tools suitable for analysis and interpretation of large and complex data sets, for example fluorescence spectroscopy data. Chemometrics method is used as a tool for multivariate data to improve understanding of chemical information by statistical and mathematical methods. It is widely used in spectroscopic area to interpret data and to make regression calibration against quality parameters. The methods can provide a good overview of complex data with easy interpreted correlation between samples and variables.

The data in this thesis was analyzed using principal component analysis (PCA), partial least squares regression (PLSR), and multivariate curve resolution (MCR). An introduction to multivariate data analysis and its applications are presented in Esbensen (2006).

Principle component analysis (PCA)

The PCA is a method used to study the variation in a multivariate data set. It is used to give information of main variability in the data set, find relations between different parameters, and clusters within samples. The matrix (X) of original variables is compressed down to a set of fewer variables, so called principal components (PCs). The first principal component explains the main variation of the data. The second component is orthogonal to the first component and explains the second largest variation, and so on (Næs et al., 2002). PCA is well suited for analysis of fluorescence emission spectra. The score plot presents the samples projected down on each PC and shows the differences and similarities between samples which can identify groups of samples and sample outliers. The commonly used plot is a score plot of vector PC1 versus vector PC2. The loading plot presents the variables on each PC. For spectroscopic data, the loading plot is usually presented as a line for each PC. The bi-plot is a combination of score plot and loading plot in order to determine the relation between variables and samples. PCA was used to visualize the effect of different color filters on pasteurized milk, and relationship (bi-plot) between fluorescence spectra of those samples and photooxidation volatile compounds obtained by GC-MS (Paper I). In paper IV, PCA bi plot showed the relation between the different films and the optical properties measurements.

Partial Least Square Regression (PLSR)

PLSR is widely used in multivariate data analysis to find correlation between a set of X-variables and Y-variables (Martens and Næs, 1989). This regression method can be used for relating variations in one or several variables (Y-variable) to the variations of several predictors (X-variable). A calibration model is developed based on data from a test set for determining how to predict new samples of interest based on available measurement. This method is validated on validation set. The first PLS component contains the variation in the data matrix X, which is most relevant for the variation in the data matrix Y. The second PLS component contains the variation in data matrix X, which is second most relevant for the data matrix Y, and so on.

The PLSR model performance is usually given by the correlation coefficient r, which is the linear correlation between y and ŷ, where y is the reference value and ŷ is the prediction value. The r value range is in the range between +1 and -1, with +1 being a perfect fit. The root mean square error of prediction/ cross validation (RMSEP/ RMSECV) expresses the variability of the difference between predicted and measured values of validation sample set. In this thesis, PLSR was used to investigate the correlation between fluorescence spectra with sensory attributes (Paper I, II, and V).

Multivariate Curve Resolution (MCR)

MCR is used to resolve the unknown mixtures by determining the number of components, estimating pure spectra profiles and relative concentrations of the pure components. For example it has been used to analyze the fluorescence spectra data with containing several fluorophores. The two important points for using MCR are that the each component should have peaks at the same position and have same shape in each experiment run (Tauler, 1995). Thus, wavelength regions selection is necessary to be able to determine underlying spectra. MCR is applied to two dimensional data analysis based on to the assumption of a linear model Lambert-Beer' Law. MCR decomposes the experimental data matrix of the spectra D into the product of two smaller matrices C and S^T as in the following equation

$$D = CS^T + E$$

Where C is a matrix with concentration profiles for each modeled component in the system and S is the matrix of the corresponding pure spectra. E is residual error. The initial estimation of C and S are optimized by alternating least squares optimization which is carried out until convergence is achieved. The algorithm for MCR has been described in Tauler (1995) and Tauler et al. (1995). MCR was performed on baseline corrected fluorescent spectra to classify the mixture components in Paper II. The baseline correction was done by polynomial fitting a routine original used for removing background fluorescence from Raman spectra (Lieber and Mahadevan-Jansen, 2003). This correction was applied to fluorescence spectra in order to remove the riboflavin peak (Paper II, III and V).

4. Paper summaries

Paper I: Effect of different colored filters on photooxidation in pasteurized milk

Different transparent, non-colored and colored filters with different light transmission properties and different atmospheres (air and nitrogen) were investigated effect on quality of milk. Interaction between colors of light and atmospheres was found. The milk stored in nitrogen had high sensory off-flavors under orange, red and non-colored transparent filters, whereas milk stored in air had most sensory deterioration under non-colored transparent filters. Milk stored under green and amber filters was less effected by photooxidation. The chlorophyllic compounds were less degradation in milk stored under green filters.

Paper II: Effect of different wavelength of light on the formation of photooxidation in Gouda-like cheese

The effect of different colors of light penetration on photooxidation as a function of depth and atmospheres was investigated for Norvegia cheese. Three different color lights were obtained by colored films; blue (350-560 nm), green (450-620 nm) and red (580-700 nm). Red and green light penetrated deeper into the cheese than did blue light. Blue light degraded riboflavin at the two upper layers (0-6 mm), whereas red and green light degraded hematoporpyrin, protoporphyrin IX and chlorophyll further down to 21 mm. The conclusion was that riboflavin was sensitized at the surface, whereas tetrapyrroles were sensitized from surface and further down into cheese. The degradation of chlorophyll was higher correlation to sensory off-flavors and off-odors than riboflavin did.

Paper III: Effect of naturally occurring tetrapyrroles on photooxidation in Cow's Milk

This paper continues the study from Paper I to give a better understanding of the photosensitizing effect of riboflavin versus chlorophyll compounds in photooxidation of pasteurized milk. Milk was exposed to blue light (400-500 nm), orange light (575-750 nm) and white light (300-750 nm). The light intensity was standardized both above films and below films (at sample surface). Milk exposed to orange light had high formation of sensory off-flavors and off-odors. They were at the same level as milk exposed to white light, even

though samples exposed to orange light received only half the light intensity compared to white light. The formation of singlet oxygen measured by SOSG® reagent was found to be high in milk exposed to orange light. Orange light degraded chlorophyll more than white and blue light. The degradation of chlorophyll correlated well with the function of sensory off-flavors. Thus, the tetrapyrroles, in particular chlorophyll compounds seem to be responsible for photooxidation in milk.

Paper IV: Packaging materials with tailor made light transmission properties for food protection

As shown in Paper I, II and III chlorophyll was a good marker for measuring photooxidation related to sensory off-flavors, this paper aimed to develop a green film in order to improve light barrier properties for preventing chlorophyll degradation. The goal was to exclude light in specific wavelength regions of 400-450 nm and 600-660 nm. Color pigments and additives at different concentrations and combinations were applied to produce the films. Samples containing high concentrations of green and yellow pigment had the lowest gloss value and total light transmittance compared to other samples. This film was able to block all wavelengths below 450 nm and transmit 10% light at 600-650 nm. The film with silver additive increased light transmission in blue region (380-500 nm) and decreased light transmission in red region (600-700 nm) compared to film without silver additive. These results showed that although the films were all green, they had different light transmission profiles, amount of light transmittances and optical properties. Thus, designing appropriate packaging material for protection of food containing chlorophyll compounds should be concerned of these effects.

Paper V: Minimizing photooxidation in pasteurized milk by optimizing light transmission of green polyethylene films

This paper connects to Paper I, III and IV. The new tailors made green films and different light exposure time were used to investigate their abilities to protect against photooxidation in pasteurized milk at different exposure times. The best protecting green film (420-600 nm) was also compared with non-colored transparent film (300-700 nm) and orange film (520-700 nm). Milk exposed to green light had significantly less sunlight flavor and rancid flavor compared to those exposed to orange light and white light. In milk stored under

transparent and orange film, off-flavors were found after 14 hours of light exposure, whereas those stored under green film showed good sensory quality after 26 hours of light exposure. The overall best film for protection against sensory deterioration and photosensitizers degradation in milk blocked wavelengths below 450 nm and reduced light transmission to below 5 % above 600 nm.

The relationship between the papers in this thesis

The effect of different wavelength of lights on photooxidation in dairy products was studied in Paper I, II, III and V. In Paper IV, packaging materials were developed according to the results based on Paper I, II and III. The connection between all papers and different films and storage conditions parameters used in studies is shown in Figure 8. Several different wavelengths of light were studied to determine their effect on photooxidation (Paper I). Three different wavelengths of light were chosen in accordance with photosensitizers absorption in order to verify their effect on photooxidation (Paper II, III and V). The differences within green films were investigated on the effect of photooxidation in addition to comparing the best selected green film with two color lights (Paper V).

The light intensity was adjusted on the top of films to compare the effect of different light transmission properties of films (Paper I, III and V), whereas light intensity was adjusted at the surface of sample for investigating wavelength of lights effect (Paper II and III).
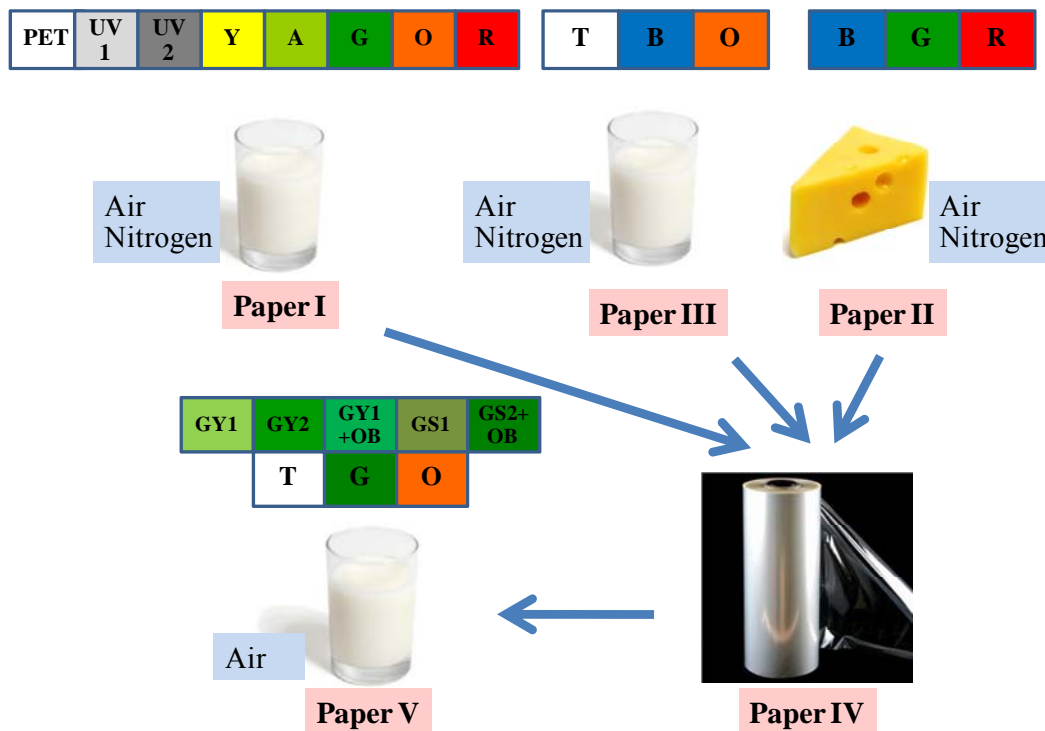
Figure 8. The relationship between the papers. Different transparent, non-colored and colored films and different storage conditions were used in the experiments.

5. Discussion

5.1 The influence of wavelength of light on photooxidation in dairy products

Different wavelengths of light have different effects on photooxidation because absorption properties of photosensitizers vary along the UV-visible regions. The selected transparent, non-colored and colored films with different light transmission properties used in this thesis are presented in Figure 9.

Samples exposed to wavelengths longer than 550 nm induced more off-flavors than those exposed to shorter wavelengths (< 500 nm) (Paper I, II and III). Samples stored in nitrogen under orange film (520-700 nm) had the same level of off-flavors as samples stored under non-colored transparent films (Paper I). In another experiment, the orange light induced significantly more intense off-odors and off-flavors than those exposed to white and blue light (Paper III). The results from these experiments were in accordance with the results reported by Josephson (1946).

These results are in contrast to several reported studies where shorter wavelengths were shown to be more harmful to dairy products (Bekbolet, 1990; Bosset et al., 1994; Mortensen et al., 2004). It is an interesting question why during more than fifty years, very limited research has been published on photooxidation caused by longer wavelengths. One explanation is that most researchers focused on the effect of riboflavin on photooxidation, since riboflavin was considered to be the main photosensitizer in dairy products. However, when considering the effect of other naturally occurring photosensitizers like chlorophyll and porphyrins, the picture looks different.

Recently, several papers have reported formation of sensory off-flavors and volatile lipid oxidation products caused by wavelengths longer than 500 nm (Webster et al., 2009; Wold et al., 2005, 2006a, b). In most of the studies on cheese and butter, the photooxidation effect of shorter wavelengths had been stronger than of the longer wavelengths. For milk (Paper I, III and V), this is not the case. This might be due to the chemical composition in milk compared to the other studied samples.

The SOSG reagent was used to measure the formation of singlet oxygen caused by photoreaction Type II. The results from SOSG in Paper III showed that singlet oxygen did increase in milk exposed to orange light. Due to some limitations with the SOSG sensor it was not possible to compare these results with the formation of singlet oxygen under exposure to blue light.

Even though chlorophyll have a higher absorption peak in the blue region, it was most degraded in samples exposed to wavelengths longer than 550 nm (red or orange light) compared to wavelengths shorter than 550 nm (Paper I, II, III and V). This can be due to the fact that riboflavin and ß-carotene also absorbs light in the blue region at the same wavelengths as chlorophyll. Riboflavin and ß-carotene are present in higher amounts in milk compared to chlorophyll (Paper I) and will therefore absorb more of the light in this region. While ß-carotene is not a photosensitizer, it acts as a protective filter absorbing light in a competitive manner with sensitizers, thus both riboflavin and chlorophyll receive less energy. In the red region however, chlorophyll has less competition from other sensitizers.

Green light had less harmful effect on sensory properties related to photooxidation in dairy products compared to other colors of light (Paper I, II and V). Similar results have been presented in Wold et al. (2005, 2006b). Samples exposed to green light had more chlorophyll compounds left than samples exposed to other colors. It should be noted that there was variation in the effect of photooxidation within the different green films in accordance with their light transmission spectra and light transmittance (Paper V). Green films with higher transmitted light in the red region gave more photooxidation compared to the films with less transmitted light in the red region. This result showed that even within the green films there is room for an improvement of light barrier properties. Also, it is not enough to know just the color or wavelength regions of a filter or light source, the whole transmission profile is needed.

The best way to protect against sensory deterioration and photosensitizer degradation in dairy products is to avoid both the wavelengths below 450 nm (violet-blue region) and longer wavelength than 600 nm (red region).

Because both the intensity and the wavelength of light affect the photooxidation, experiments looking at just the wavelength have to normalize the light intensity at the sample surface. This

was done in Paper II and III to isolate the effect of wavelength. Adjusting the light intensity above the film is more comparable to practical applications, but the resulting photooxidation will be a combination of both the light intensity and wavelength. The last approach was used in Papers I, III and V.
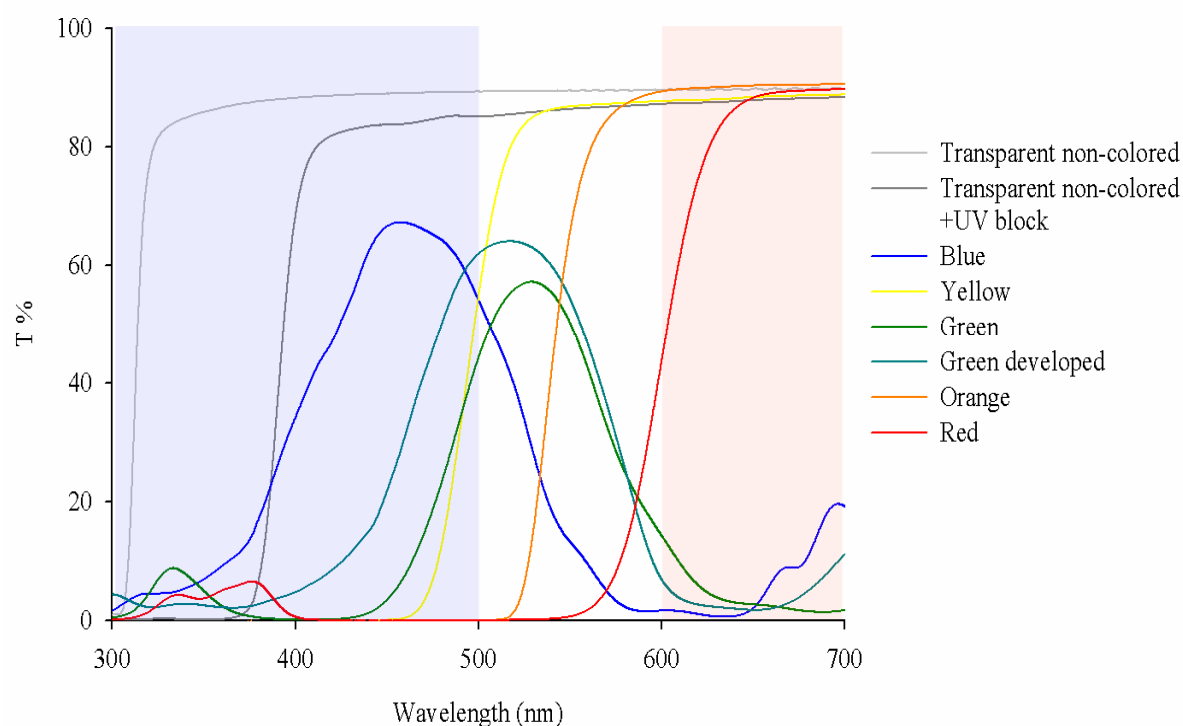
Figure 9. The light transmission profiles of different transparent non colored and colored films/filters used in this study (Figure does not show all films used). The eight selected films were non-colored transparent film, non-colored transparent with UV additive, blue, yellow, green, green new developed which gave the best results against photooxidation, orange and red. The absorption of riboflavin, β-carotene and chlorophyll are located in wavelengths in blue region (blue box), whereas chlorophyll is located in wavelengths in red region (red box).

5.2 Penetration of different wavelengths of light and its effect on photooxidation

Overall results showed that the longer wavelengths caused photooxidation deeper into the cheese than shorter wavelengths (Paper II). The same results have been observed in milk (Josephson, 1946) and cream cheese (Westermann et al., 2009). Blue light (350-560 nm) had less effect compared to green light (450-620 nm) and red light (580-700 nm) on photooxidation with increasing depth (Figure 10). This is partly because blue light with shorter wavelengths is more scattered than red light due to Raleigh scattering. This can help explain the effect observed in Paper I and III where longer wavelengths (orange light) caused more photooxidation. In addition to the effect of wavelengths of light, the longer wavelengths penetrated deeper into milk causing photooxidation in a larger volume compared to the shorter wavelengths. Another factor limiting the penetration of blue light compared to red is that it is absorbed by riboflavin and β-carotene, which are present in relatively high concentration in cheese.

By observing the degradation of protoporphyrin IX and chlorophyll compounds, we concluded that red and green light were able to penetrate down to 21 mm into cheese (Figure 10). Riboflavin was only degraded from the surface down to 3 mm. There were two possible reasons to explain why riboflavin did not degrade further down. First, blue light might not penetrate further down, due to increased light scattering compared to red light. Second, the oxygen concentration was likely lower further down in the cheese. Thus, there might not be enough oxygen to produce photooxidation by Type II reaction.

This study showed that different wavelengths of light had different penetration effect on photooxidation. Thus, sampling should be performed with this in mind when measuring in food quality studies in order to get accurate results. For example, sampling only the top 6 mm in this experiment would miss out on the degradation caused by green and red light from 6 mm to 21 mm.
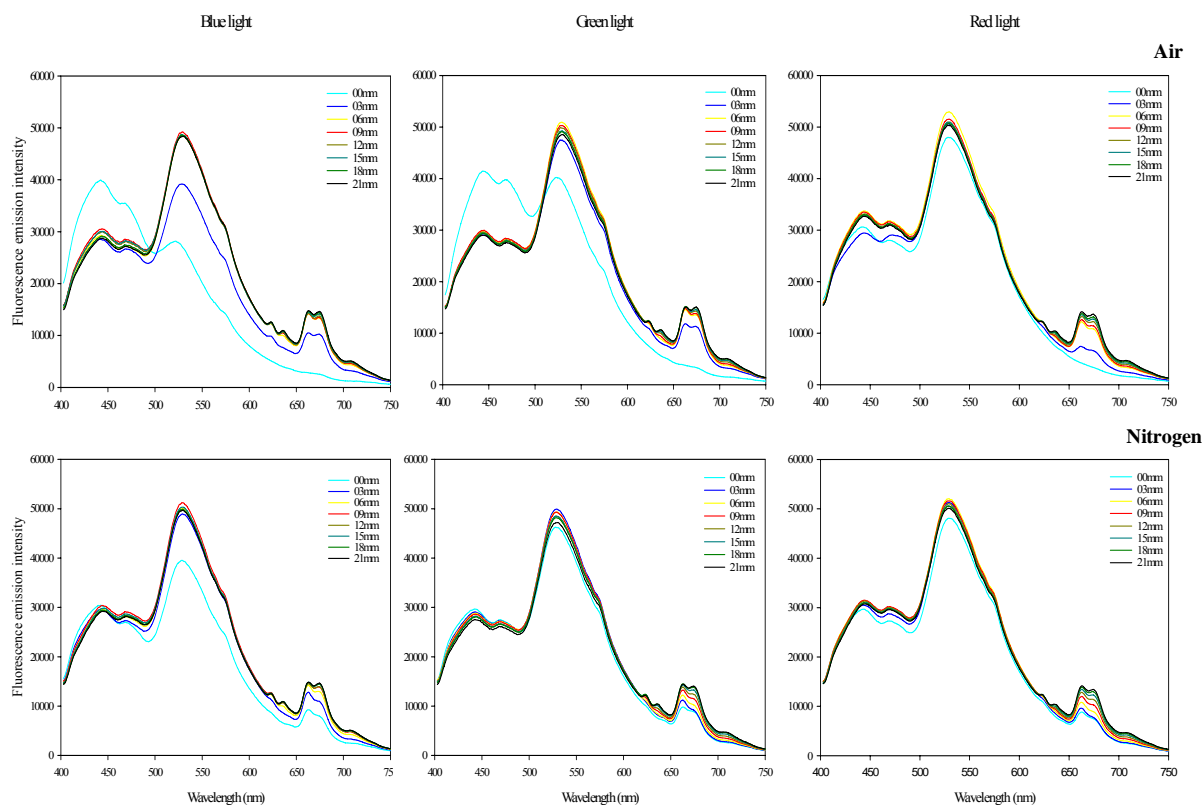
Figure 10. Fluorescence emission spectra (λex =382 nm) at different depths of cheese after light exposure for 7 days at 4 °C with different colors of light; blue (left), green light (middle) and red light (right). Samples were stored in air atmosphere (above) and nitrogen atmosphere (below).

5.3 The influence of storage atmosphere on photooxidation

The storage atmospheres were found to have a different effect on the sensory properties depending on the product. Cheese stored in an air atmosphere showed significantly higher off-flavor than those in nitrogen atmospheres (Paper II). The amount of oxygen in cheese was not measured and might have been equal between the two atmospheres due to low amount of dissolved oxygen in the cheese. On the other hand, for pasteurized milk (Paper I and III), the effect of storage atmospheres were dependent on the wavelengths of light.

Photooxidation in samples stored in a nitrogen atmosphere occurs because of a Type I reaction which is dominant at low oxygen (He et al., 1998). This photooxidation is produced

through a free radical mechanism (Frankel, 2005). For samples stored in an air atmosphere, the degradation of photosensitizers is caused by singlet oxygen in a Type II reaction.

There was an interaction between colors of light and the oxygen concentration in milk (Paper I and III). Samples stored under non-colored transparent films in air atmosphere gave more degradation of protoporphyrin and chlorophyllic compounds than those stored in nitrogen. The opposite results were found in milk stored under orange films, where sensitizers were more degraded in nitrogen. One conclusion is that the shorter wavelengths (UV-blue light) are more likely to induce photooxidation through a Type II reaction with high levels of oxygen, whereas the longer wavelength (orange-red light) are more likely to induce photooxidation through Type I reaction with low oxygen. The interaction between colors of light and oxygen concentration has also been reported for butter (Wold et al., 2009).

5.4 Optimal light transmission for protection against photooxidation in pasteurized milk

Films were produced with several combinations of pigment and additive to exclude specific wavelengths at 400-450 and 600-650 nm (Paper IV). In addition to the pigments and additives presented in this study, nanoclay has also been investigated due to the increased interest for nanotechnology and many reports that it provides high transparency. However, the nanoclay film transmitted UV-visible light and did not exclude the target wavelengths. Thus, these films were not used for further study. Only the surface structure of the sample was studied by microscopy techniques, whereas the information of dispersion and exfoliation which is importance to the optical properties was not investigated. Silver additive was used due to its absorption around 420 nm which is the same area of chlorophyll absorption. However, film with pure silver additive transmitted light in UV-visible region, thus we had to combine silver additive with green pigment to obtain the wavelength target. Optical brightener was used because it absorbs UV light and emits blue light. It reduced the yellow color and gave more intense green color appearance. However, adding optical brightener to the film did not increase light transmission. No study has been reported dealing with relationship between optical brightener and pigments and additives.

The different green colored films were investigated for their ability to prolong shelf life of dairy products (Paper V). Significant differences were found in sensory evaluation of off-

flavors and off-odors in milk and in degradation of chlorophyll compounds. Several studies report that green light causes less off-flavors and lower degradation of photosensitizers compared with other lights in visible wavelength (Wold, et al. 2005, 2006b; Webster et al., 2009). However, according to our knowledge it has not been reported any studies on the differences within green light. Therefore, the green films were made to have different shapes of the light transmission profiles and different amount of light transmittances and optical properties e.g. haze, gloss due to different concentrations of pigments and additives (Paper IV). The study in Paper V identified that green light was less harmful, but light transmittance of 30 % through the film in the green wavelength region was still high enough to produce photooxidation in milk after light exposure for 20 hours. It was also important how much light was transmitted at the various wavelengths within the green region.

6. Conclusions

In this thesis we have studied the effect of the wavelengths of light and storage conditions on photooxidation in dairy products in order to be able to design packaging materials with optimized light transmission property to protect against photooxidation.

A significant effect on photooxidation in dairy products was observed when using different wavelengths of light. Orange light induced higher levels of sensory off-flavors and off-odors in milk compared to blue light, and at the same level as white light. This was explained by showing that degradation of photosensitizers absorbing orange and red light had higher correlation to sensory off-flavors and that light with longer wavelengths penetrated deeper than shorter wavelengths and induced photooxidation in a larger volume.

Although chlorophyll has a higher absorption peak in the blue region, it was still shown to be more degraded by red light. One reason is that there are more light absorbing compounds in the blue region, for instance β-carotene, which act as a filter for chlorophyll. In the red region, however, chlorophyll is the main photosensitizer and therefore has no filter.

An interaction effect between wavelengths of light and storage atmospheres was found.
The longer wavelengths (orange and red light) induced higher intensity of off-odors and off-flavors in nitrogen atmosphere than air atmosphere, whereas the shorter wavelengths (blue light) and white light induced more severe effect in air atmosphere.

Chlorophyll and porphyrins showed good correlation to sensory properties related to photooxidation despite being present at very low concentration. Thus, these compounds should be taken into account when studying photooxidation in dairy products.

Different tailor made films were designed to transmit light mainly in the green region to avoid the main absorption peaks of photosensitizers in the blue and red regions. The green films provided different light transmittance in accordance with the amount of pigments and additives. The new material blocking wavelength below 450 nm and above 600 nm was the most effective to prevent against sensory deterioration and photosensitizers degradation in pasteurized milk.

The development of a new green commercial film with altered light transmission in order to avoid harmful wavelengths of light on photooxidation in pasteurized milk was a good prototype for other dairy products. This new approach can be used to optimize proper packaging materials for other food products.

7. Challenges and future perspectives

7.1 Fluorescence spectroscopy is used to investigate photooxidation in dairy products combined with other analytical techniques as following the interesting topics:

- Verify the different lipid oxidation compounds generated under exposure to blue light and orange light related to sensory off-flavors and off-odors. The sensory analysis could detect off-flavors occurring under samples exposed to blue light, but not to orange light. There might also be different mechanisms between those two lights.

- The effect of colored light penetration as a function of depth in liquid metric products should be studied. The solubility and dispersion have to be taken into account regarding study in liquid products.

- A better understanding of the reaction or mechanisms of photosensitizers related to photooxidation Type I and Type II reaction under wavelength dependent in specific blue vs. red regions is required.

- It has been shown that milk with a low fat concentration (skimmed milk) is more sensitive to light than milk with a high fat content (whole milk). Thus, the effect of different fat concentrations and colored light on photooxidation should be investigated in milk and other dairy products.

- It may be desirable to study in more detail how β-carotene works as a protective filter to inhibit the photooxidation in dairy products.

- The relation between the degradation of photosensitizers and the formation of free radicals by using electron spin resonance (ESR) spectroscopy to better understand the photo reaction in dairy products is also a possible future study topic.

7.2 Developing innovative light barrier packaging material and the optimization storage conditions effect on photooxidation in dairy products.

Mostly milk and cheese are packed in transparent or partly transparent containers. Thus the packaging design and suitable storage conditions in order to protect against photooxidation should be considered as following;

- Evaluate the possibility to use nanotechnology, surface reflection and surface treatment to develop transparent packaging which exclude or partly exclude harmful visible wavelengths

- Design or adjust emitted wavelengths of the light sources/light tubes in display cabinet to provide less harmful light to food products.
- Design and plan to minimize direct light exposure during transportation, handling, storage area and display cabinets or shelves.

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Paper I

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 N_2) 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 N_2 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 N_2 correlated well with sensory properties related to photooxidation ($R^2 = 0.75$–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

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 B_2) 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 N_2) 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 gabletop 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 N_2 (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/m^2 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, 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).

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 µL) (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 N_2 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 desorbtion 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 µm 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 N_2, 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).
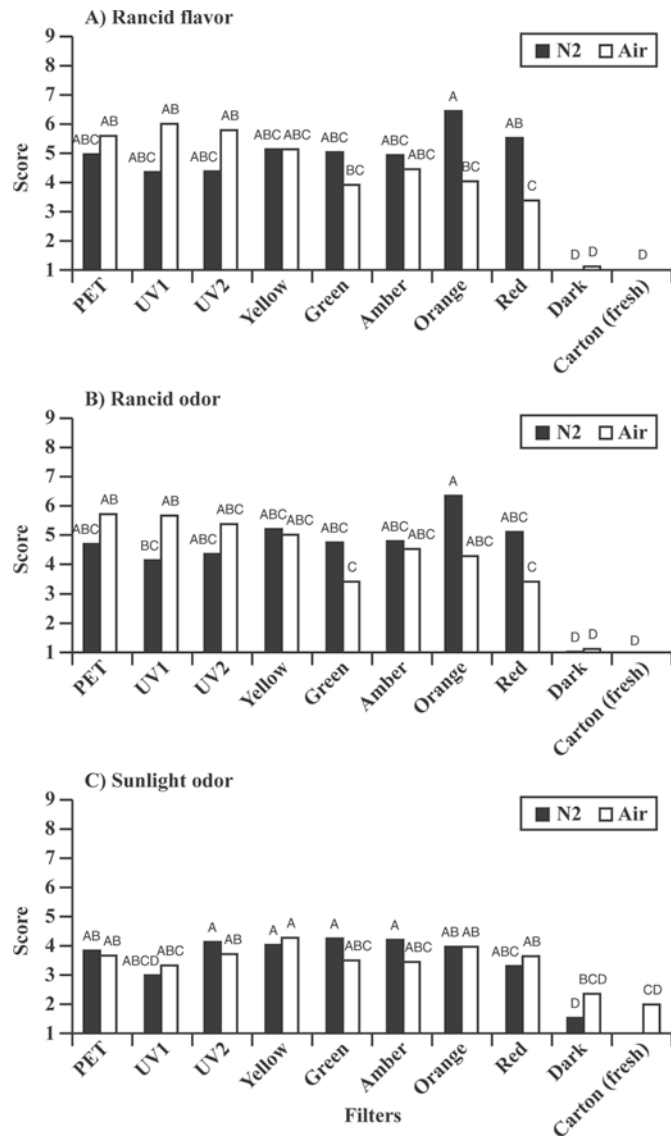
Figure 1. Mean sensory score for milk stored in N_2 and air atmosphere and light exposed for 20 h at 4°C under 8 different filters, in addition to reference samples stored in darkness (unexposed) and in a carton (fresh). The 3 attributes shown are rancid flavor (A), rancid odor (B), and sunlight odor (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 N_2 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 μM and 102 μM in milk stored in air and in N_2, respectively. This means that there was oxygen in the N_2-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-nonenal, 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-nonenal 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 N_2. The opposite was the case for many samples stored under colored filters; N_2 headspace resulted in more oxidation products.

Fluorescence Analysis

Figure 4A shows the fluorescence emission spectra of light-exposed milk stored in N_2. 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
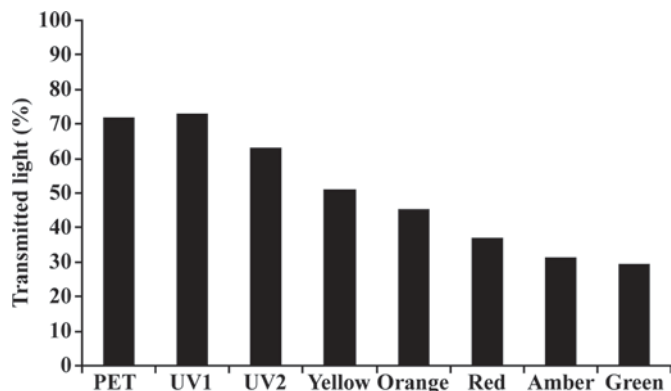
Figure 2. Transmitted light (%) for the different filters used in the experiment. The intensity was calculated by integrating the area under the transmission curve for each filter. PET = polyethylene terephthalate; UV1 = noncolored with UV-block 1; UV2 = noncolored with UV-block 2.
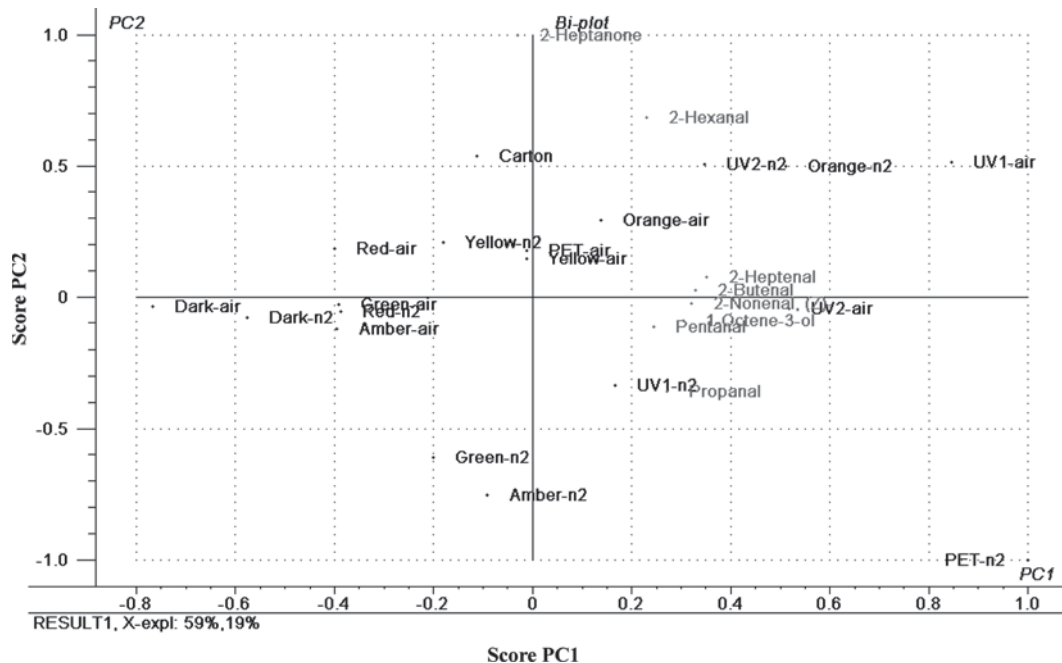
Figure 3. Principal component analysis biplot (scores and loadings) for the first 2 principal components (PC) of milk stored in N_2 and air atmosphere after light exposure for 20 h at 4°C under 8 different filters in addition to reference samples stored in darkness (unexposed) and in a carton (fresh) 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 µg/g. The amount of protoporphyrin in milk has not been reported, whereas the amount in butter is approximately 0.02 to 0.03 µg/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,
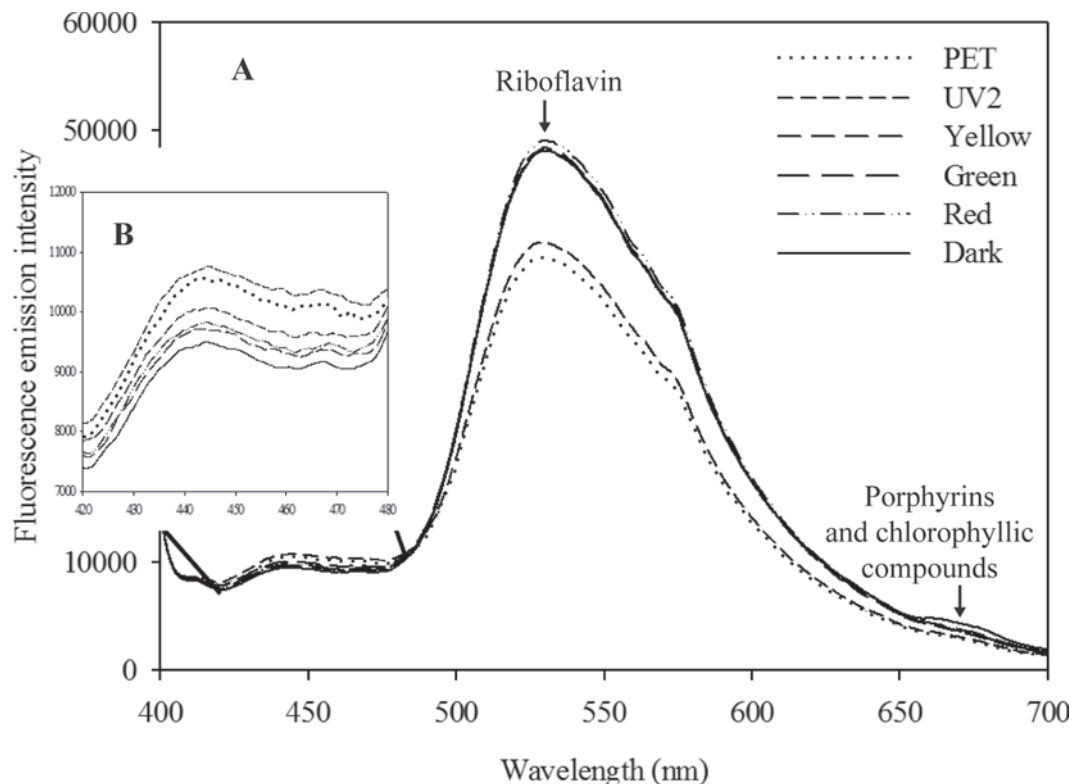
Figure 4. Fluorescence emission spectra (excitation at 382 nm) of milk stored under different filters in N_2 atmosphere and light-exposed for 20 h at 4°C. The spectra shown are from the region 400 to 700 nm (A). Inset is the tertiary oxidation product region from 420 to 480 nm (B). PET = polyethylene 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 ≈ dark > red > green ≈ amber ≈ PET(N_2) ≈ UV1(N_2) ≈ UV2(N_2) > yellow > orange ≈ UV1(air) ≈ UV2(air). For the chlorophyllic compounds, the order was carton ≈ dark > green ≈ amber ≈ PET(N_2) ≈ UV1(N_2) ≈ UV2(N_2) > red ≈ yellow ≈ orange ≈ UV1(air) ≈ 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
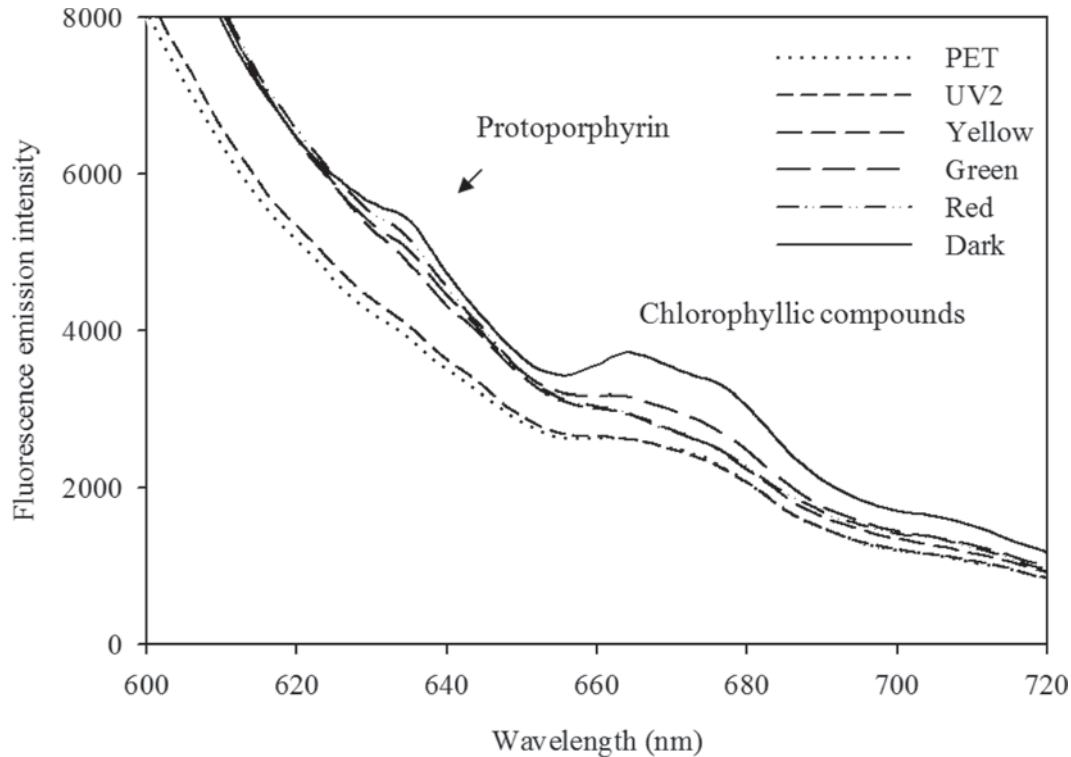
Figure 5. Fluorescence emission spectra (excitation at 410 nm) of milk stored under different filters in N_2 atmosphere and light-exposed for 20 h at 4°C. The spectra shown are from the region 600 to 720 nm. PET = polyethylene terephthalate; UV2 = noncolored with UV-block 2.

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 ($R^2 = 0.75$–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 N_2 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

[1]Number of partial least squares regression factors used in the model.

[2]RMSCV = root mean square error of cross-validation.

[3]Rancid 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 N_2) 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.

ACKNOWLEDGMENTS

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Paper II

Effect of different wavelength of light on the formation of photo-oxidation in Gouda-like cheese

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ARTICLE INFO

ABSTRACT

Norvegia cheese samples were packed in air and nitrogen atmospheres and exposed to light of different colours, blue (350–560 nm), green (450–620 nm) and red (580–700 nm). After 7 days of light exposure, each cheese was sliced, from the exposure surface and down, into eight slices of 3 mm thickness. The slices were analyzed by sensory analysis and fluorescence spectroscopy, enabling studies of how photo-oxidation progressed as a function of depth of the cheese. Green light gave the most oxidation at the surface for air stored samples. Oxidized flavours at depths down to 9 mm were more intense for exposure to red and green light. Blue light degraded riboflavin in the two upper layers (0–6 mm), whereas red and green light affected hematoporphyrin, protoporphyrin IX and chlorophyll as far as 21 mm into the cheese. The results suggest that tetrapyrroles are responsible for photo-oxidation at the surface and the interior of the cheese.

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1. Introduction

Light is a major cause of reduction of quality and shelf-life of food products. Dairy products are often exposed to light during processing, transportation and display in the grocery store. Light induces degradation of nutritional quality such as proteins, lipids, and vitamins, and it also causes formation of off-flavours and odours, as well as colour changes. The light transmission properties of packaging materials can be designed to provide an optimal protection of dairy products. To do this we need information about the different wavelengths effect on photo-oxidation.

Riboflavin, porphyrins and chlorophyll derivatives are naturally present as photo-sensitizers in dairy products such as cheese (Wold et al., 2005, 2006a), butter (Wold & Lundby, 2007) and milk (Intawiwat et al., 2010). These photo-sensitizers can initiate photo-oxidation when exposed to visible light. Photo-sensitized oxidation can proceed through Type I or Type II reactions, and these reactions can occur at the same time in a competitive manner (Spikes, 1989). Type I reactions proceed through a free radical mechanism and the reactions tend to be most efficient at low oxygen concentrations (He, An, & Jiang, 1998). In Type II reactions, energy from the excited

sensitizer is transferred to triplet oxygen, and the very reactive singlet oxygen is formed (Foote, 1991).

Many researchers have investigated the effect of different colours of light on photo-oxidation in dairy products. Sattar and deMan (1975) reported that wavelengths below 455 nm effectively induced fat oxidation in milk. They made this conclusion based on the measurement of peroxide value. Bosset, Gallmann, and Sieber (1994) used fluorescent white light tubes with two different spectral properties, referred to as cold light (more violet and blue light), and warm light (rich in yellow, orange and red light). The results indicated that cold light yielded a more severe effect on the quality of milk and dairy products than did warm light. These results are supported by the results from studies on the colour of light in relation to photo-oxidation in cheese and butter (Wold, Veberg, Lundby, Nilsen, & Moan, 2006b; Wold et al., 2005, 2009). In these studies, violet and blue light (300–500 nm) gave the highest sensory degradation, whereas green light (450–600 nm) caused less adverse effects. Recently, Wold et al. (2009) studied the effect of oxygen concentration and light colour on photo-oxidation in butter, where higher oxygen concentration generally resulted in more sensory degradation. Violet light (300–500 nm) resulted in a slightly higher degree of photo-oxidation compared with green (450–600 nm) and red light (580–700 nm) at low oxygen concentrations, whereas at high oxygen concentrations, minor differences were found between the wavelength regions. There appears to be an interacting effect

between the wavelength of exposure light and the storage atmosphere.

Many studies have been carried out to study the effect of different packaging materials on photo-oxidation in dairy products (Bosset et al., 1994; Cladman, Scheffer, Goodrich, & Griffiths, 1998; Intawiwat et al., 2010; Mortensen, Sorensen, & Stapelfeldt, 2002a; Webster, Duncan, Marcy, & O'Keefe, 2009; Wold et al., 2005, 2006a, 2006b). It is well documented that transparent packaging provides poor protection against photo-oxidation, reported for instance for Harvarti cheese (Mortensen et al., 2002a) and cream cheese (Pettersen, Eie, & Nilsson, 2005). Andersen, Andersen, Hansen, Skibsted, and Petersen (2008) studied the effect of three specific wavelengths on the degradation of photo-sensitizers in cheese. Riboflavin was degraded by 436 nm light, whereas chlorophylls and porphyrins were affected by 436 and 546 nm light. In Havarti cheese, lipid oxidation occurred during exposure to white light, but also to yellow light without any corresponding degradation of riboflavin. It was then suggested that photo-oxidation might be initiated by other mechanisms than riboflavin sensitization (Andersen, Wold, & Mortensen, 2006; Mortensen, Sorensen, & Stapelfeldt, 2003). Wold et al. (2005) reported other compounds in addition to riboflavin, which seem to contribute to light induced oxidation in dairy products. Chlorophylls and porphyrins are among these compounds, and this can explain the formation of off-flavours in dairy products exposed to light of wavelengths longer than 500 nm. These compounds absorb strongly in the violet region (the Soret band), but also throughout the visible region.

Few studies have been reported on the topic of light penetration and photo-oxidation as a function of depth in dairy products. Allen and Parks (1979) reported that cool white light penetrated deeper into skimmed milk (low fat) than in whole milk, probably due to a higher degree of light scattering in the whole milk. Westermann, Bruggemann, Olsen, and Skibsted (2009) studied the effect of light exposure of cream cheese of different fat contents in relation to light penetration and formation of lipid oxidation by using electron spin resonance (ESR) spectroscopy. The results showed that after 30 min of light exposure, the low fat cream cheese had a high content of oxidation products at the surface layer whereas the second layer (1−2 mm depth) was significantly less affected. They also observed that a larger fraction of light of wavelengths longer than 500 nm were transmitted to deeper regions of the cream cheese, compared with violet and blue light (<500 nm). This was ascribed to the absorption of riboflavin; however, the absorption of β-carotene would probably also be pronounced. They suggested that riboflavin is the most active photo-sensitizer at the surface of the cream cheese, whereas porphyrins and chlorophyllic compounds might be more important deeper into the cream cheese. Wold, Jorgensen, and Lundby (2002) studied the depth effect of white light in Jarlsberg cheese with regard to sensory properties. The results showed that light induced a significant increase in off-flavours as deep as about 6 mm into the cheese after 4 days of light exposure. These results corresponded well with the photo-degradation of riboflavin, as well as protoporphyrin IX.

The objective of this study was to investigate the effect of different coloured light and atmospheres on the sensory properties and degradation of photo-sensitizers as a function of depth in Gouda-like cheese. Blue (350−560 nm), green (450−620 nm) and red (580−700 nm) light adjusted to an intensity of 3.5 ± 0.1 W m^{-2} at the cheese surface was used to compare the different regions. The spectral properties of the three exposure conditions were chosen to excite different groups of photo-sensitizers. The concentrations of photo-sensitizers (riboflavin, protoporphyrin IX, hematoporphyrin as well as non-characterized chlorophyllic compounds) were measured by the means of front face fluorescence spectroscopy. Multivariate curve resolution (MCR) applied on

the fluorescence spectra were used to extract pure spectral components of the photo-sensitizers, and enabled monitoring of how each of them were photo-degraded inwards in the cheese. The sensory properties were compared with, and explained by, the degradation of photo-sensitizers.

2. Materials and methods

2.1. Materials

Rind-free Norvegia cheese is a well-known as a semi-hard cheese in Norway. The cheese samples (Gouda-like; 27% fat, 59% dry matter, 5 kg packages) were obtained from Tine BA (Oslo, Norway). The four cheeses of 5 kg package came from the same batch to obtain a fairly homogeneous set of samples.

2.2. Packaging method

The cheese was cut into samples of $14 \times 7.5 \times 5$ cm (length × width × height). The sides of the cheese were covered with aluminium foil and placed into amorphous-polyethylene terephthalate/polyethylene (A-PET/PE) thermoformed trays. A-PET/PE sheets were manufactured by Wipak (Nastola, Finland) thermoformed by Jihå Plast AB (Karlskoga, Sweden). The thermo-formed trays ($14.5 \times 20.5 \times 7.5$ cm) were sealed with top a web consisting of PET/PE/ethylene vinyl alcohol/PE (PET/PE/EVOH/PE, oxygen transmission rate (OTR) < 5 cc m^{-2}, 24 h, 1 atm; Wipak) by using a tray-sealing machine (DYNO model 511 VG, Promens AS, Kristiansand, Norway). To assure proper sealing and no leakages during light exposure/storage, the gas composition (O$_2$ and CO$_2$) was measured using a CheckMate 9900 (PBI Dansensor AS, Ringsted, Denmark). The gas composition was measured directly after sealing and after 7 days of storage.

2.3. Sample preparation

The cheese samples were packed in 16 trays with one sample of cheese in each tray. Samples were divided into two sets of eight: one set for storage in air, and one for storage in nitrogen (99%). In the nitrogen set, two FreshPax oxygen absorbers (R-100 Multisorb Technologies Ltd, Buffalo, NY, USA) were packed in each tray to assure an oxygen-free atmosphere. The different coloured filters were placed on the top of the trays. For the eight samples in one group, two were covered with blue filters, two with green filters and two with red filters. The two last were stored in the dark. Samples covered with the same filter were treated as parallels.

After light exposure, each cheese sample was cut into slices of 3 mm from the surface layer and down to a depth of 21 mm, as illustrated in Fig. 1. The upper six slices (0, 3, 6, 9, 12 and 15 mm) were subjected to sensory analysis, whereas for the dark stored samples, only the three upper slices (0, 3 and 6 mm) were evaluated. Fluorescence emission spectra were collected from the upper surface of all eight slices (0, 3, 6, 9, 12, 15, 18 and 21 mm).

2.4. Light exposure

The cheese samples were exposed and stored for 7 days under two broad band 575 W metal halide lamps (OSRAM HMI 575 W/SE), which has a relatively flat emission spectrum in the visible and near UV region. Coloured filters (Rosco, Stamford, CT, USA) were used to define the three different spectral regions used for light exposure. The filters used were blue (69 Super Brilliant blue; 350−560 nm, λ_{max} 450 nm), green (89 Moss green; 450−620 nm, λ_{max} 530 nm), and red (19 Super Fire red; 580−700 nm, λ_{max} 620 nm). Light transmission properties of these filters are shown in Fig. 2. The
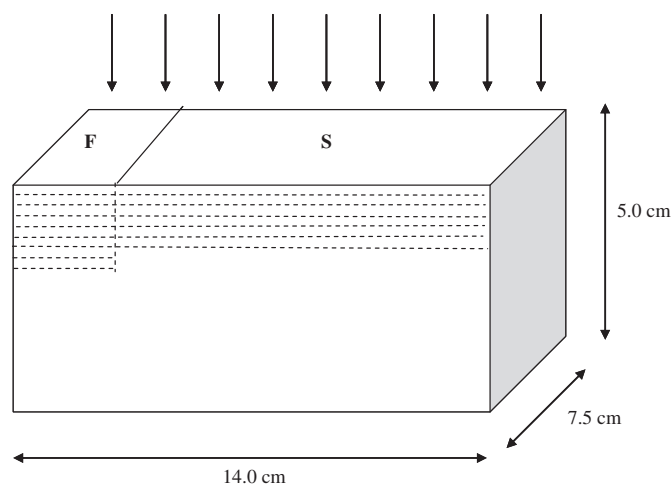
Fig. 1. Schematic description of sub-sampling showing how each cheese sample was sliced into sub-samples at different layers (each 3 mm thick to a depth of 21 mm). The eight slices of sub-sample F were analyzed by fluorescence spectroscopy whereas the six slices of sub-sample S were analyzed by the sensory panel. Each slice of cheese was cut into smaller pieces for the 10 sensory assessors.

spectral regions of the filters were defined by where the transmission was above 10% of maximum transmission. The light intensity at the cheese surface was adjusted to 3.5 ± 0.1 W m^{-2} for each filter. This means that we studied the effect of wavelength and not differences in filter transmission. The light intensity was measured by a calibrated spectrometer (Apogee Spectroradiometer, Apogee Instruments Inc., Logan, UT, USA) for the wavelength region 300–700 nm as described by Wold et al. (2005).

The packages with cheese were placed on the floor in a refrigerated room where the temperature was 3–4 °C. Sensory evaluation and fluorescence measurements were carried out the day the light exposure period ended.

2.5. Sensory evaluation

Sensory properties were evaluated by a trained sensory panel at Nofima Mat (Ås, Norway). The attributes sour, sunlight, and rancid flavours and odours according to ISO 5492 (1999) were evaluated. A high intensity of sour odour and flavour indicates freshness.
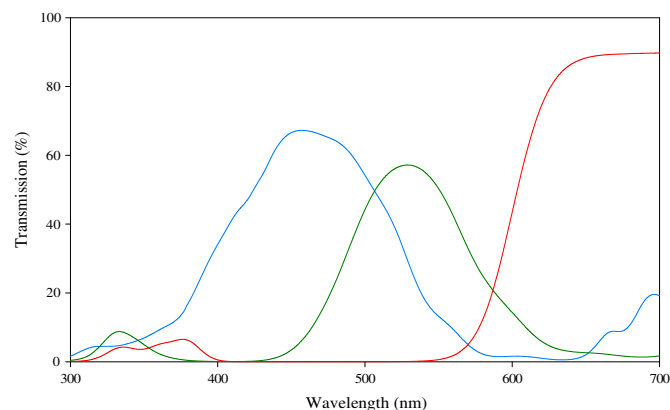

Fig. 2. Light transmission (percent transmitted light per wavelength) through the coloured filters used. Colours of the spectral curves resemble those of the filters used. Blue filter: 350–560 nm, green filter: 450–620 nm and red filter: 580–700 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Sunlight odour and flavour are related to oxidation of proteins. Rancid odour and flavour related to intensity of rancidity (grass, hay, candle and paint) as described in ISO (2009) standard 22935-2. The sensory panel consisted of 10 assessors, who were selected, trained, and monitored according to guidelines in ISO 6658 standards (ISO, 2005) and ISO 3972 standards (ISO, 1991). The sensory facilities also fulfilled the ISO 8589 standards (ISO, 1988). Prior to the analysis, the panel was trained in the definition and intensities of each of the attributes using cheese with varying sensory properties (a dark stored sample packed in nitrogen and a sample, which was light-exposed for 7 days under blue filter). Each 3 mm slice of samples was cut into 10 smaller pieces (2×2 cm^2) for serving 10 assessors. Each assessor was served each sample on a cardboard plate. The different samples were served at room temperature (20 °C) and served twice as replicates where the serving order was randomized with respect to sample, assessor and replicate. Intensity of odours and flavours were evaluated and graded on a continuous non-structured scale ranging from the lowest intensity of each attribute (value 1.0) to the highest intensity (value 9.0). The evaluation was carried out and data recorded by a computerized system (CSA, Compusense, Version 4.6, Compusense Inc., Guelph, ON, Canada) as described in Wold et al. (2005, 2009). The sensory intensities for each sample of cheese were obtained by averaging the individual intensities from the 10 assessors. In the main experiment, 84 samples were evaluated (12 light-exposed samples × 6 layers + 4 dark stored samples × 3 layers). The sensory measurements therefore had to be carried out over 3 days. The first day, 18 samples were measured, 33 samples the second day and 33 samples the third day. Samples were randomized over the 3 days to avoid a block effect. The light exposure of the samples was carried out to correspond in time with the three sensory trials.

2.6. Front face fluorescence measurements

Front face fluorescence was measured by a specially designed bench-top system described in detail by Wold et al. (2002). The samples were illuminated by 382 nm excitation light, and fluorescence emission spectra were measured in the range 410–750 nm. Excitation at 382 nm was chosen, as this had earlier shown to give good results for measuring tertiary oxidation products as well as degradation of riboflavin, chlorophyll and porphyrin compounds (Wold et al., 2002, 2005). Exposure time was 0.5 s for all spectroscopic measurements. The temperature of the samples was 4 °C. Two replicate samples were measured and each replicate was measured twice. Samples were rotated 90° between the two exposure samples to average out possible non-homogenous field of illumination.

2.7. Data analysis

Multivariate curve resolution (MCR) was used to study the photo-degradation of each sensitizer as function of colour of the filters, atmosphere and depth in cheese. MCR was used to extract pure compound spectra from the composed fluorescence spectra. For each MCR component, a set of scores was obtained which represents the relative concentration of the respective compound in each sample. The algorithm for MCR has been described by Tauler (1995) and Tauler, Smilde, and Kowalski (1995). Before applying the MCR algorithm on the spectra, a mathematical algorithm was applied to remove the large fluorescence signal from riboflavin. This was done by polynomial fitting, an iterative routine originally introduced to remove background fluorescence from Raman spectra (Lieber & Mahadevan-Jansen, 2003). In the present study, a polynomial degree of 4 was chosen and an iteration

number of 50 were used for the fitting procedure. The procedure was written in Matlab code (The MathWorks, Inc., Natick, MA, USA). This transformation was only applied on the part of spectrum from 575 to 750 nm, which was then used for MCR analysis. Relative concentrations of riboflavin were measured as the emission intensity at 580 nm, where negligible overlap of other spectral bands was expected.

Partial least-squares regression (PLSR) was used to find correlations between fluorescence spectra and sensory assessed attributes of the cheese. Full cross-validation was used to determine the optimal number of PLS factors. MCR and PLSR were carried out by using The Unscrambler ver. 7.5 (Camo AS, Oslo, Norway). Significance testing of the sensory analysis was carried out using a general linear model (GLM) using SAS 9.1.3 (SAS Institute Inc, Cary, NC, USA) within each treatment to establish significant differences followed by Tukey's honestly significantly different (HSD) Test.

3. Results and discussion

3.1. Gas composition analysis

The oxygen content in the nitrogen atmosphere packages directly after packing was 0.06–0.10%, and after 7 d storage at 4 °C, the oxygen had decreased to 0% and CO_2 content had increased to 10–14%. These changes in gas composition were as expected since the cheese ripening will continue during storage, consume O_2 and produce CO_2 (Mortensen, Sorensen, & Stapelfeldt, 2002b). In packages with air atmosphere, the oxygen and CO_2 content was stable during the whole storage time. In addition, no significant differences in gas composition were found between samples exposed to light and those kept in darkness.

3.2. Sensory analysis

The attributes sour flavour and rancid flavour were chosen to present the results for sensory analysis since they spanned the main variation related to photo-oxidation. Sunlight flavour is a commonly used property for describing light induced oxidation, however, no significant differences between samples exposed to blue, green and red light were detected. Cheese stored in air received significantly higher off-flavour scores compared with samples stored in the dark, whereas cheese stored in a nitrogen atmosphere did not differ significantly from the samples stored in the dark. The same results for light-exposed butter stored in nitrogen with oxygen absorbers have been reported (Veberg, Olsen, Nilsen, & Wold, 2007; Wold et al., 2009).

The average sensory scores for sour flavour and rancid flavour in cheese stored under different coloured filters in air atmosphere are shown in Fig. 3. Clear differences in sensory scores for sour flavour and rancid flavour between the light-exposed samples and samples stored in darkness were found, as expected. The sour flavour shows scores with an opposing trend to rancid flavour since this attribute refers to freshness of the cheese. Samples stored in the dark were considered as fresh with correspondingly high scores for sour flavour and low scores for rancid flavour. Similar results have been reported for Havarti cheese and cream cheese; samples stored in black laminated and white laminated (non-transparent) packaging were better protected against photo-oxidation than samples stored under transparent packaging (Mortensen et al., 2002a; Pettersen et al., 2005). Different colours of light gave some significant differences in sensory properties. Samples exposed to green light received significantly higher rancid scores at the surface and for the third layer (6–9 mm) compared with exposure to blue light. There was a trend that cheese stored under blue light received lower scores for rancidity deeper into the cheese (up to 6–9 mm)
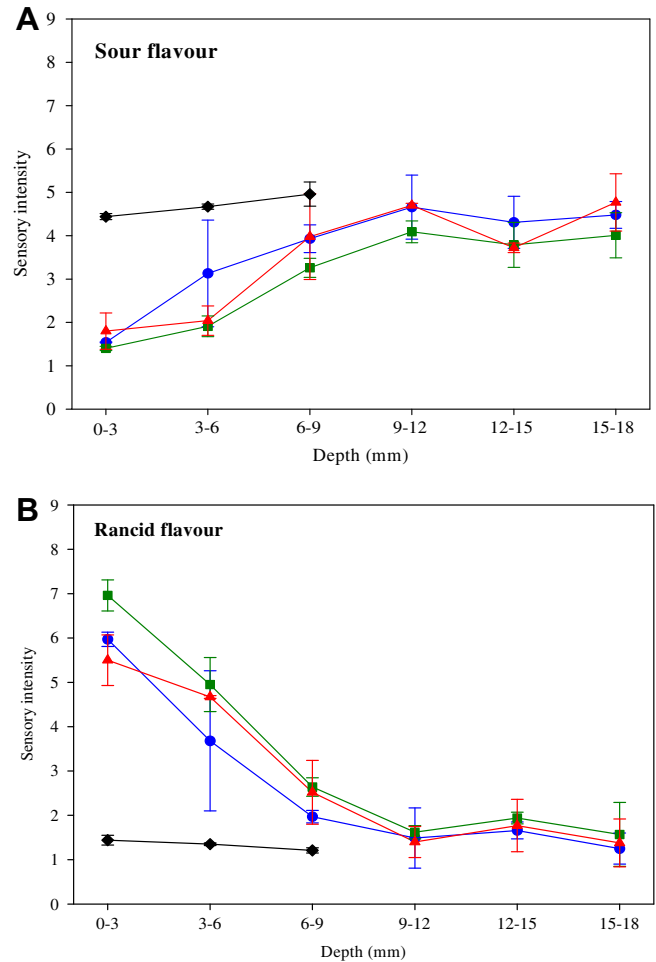

Fig. 3. Sensory responses for sour (A) and rancid flavour (B) of cheese stored under different coloured filters exposed to light for 7 d at 4 °C in an air atmosphere. The control samples were cheese stored in the dark. The values are means of duplicates, with bars indicating standard deviations. For samples exposed to blue, green and red light, the upper six slices were measured, whereas for the dark stored samples, only the three upper slices were evaluated.

compared with samples stored under red and green light. No significant differences were found between different colours of light deeper than the third layer (6–9 mm). The dark stored samples were not measured by the sensory panel for depths deeper than 9 mm, since it was assumed that the sensory scores were stable between 9 and 18 mm.

The fact that green light induced the highest scores for rancidity at the cheese surface was not expected. In a similar study on Jarlsberg cheese, green light gave less adverse effects compared with both blue and red light (Wold et al., 2006b). A main difference between the two experiments was the storage atmosphere. In the present study we used air (21% oxygen), whereas in the Jarlsberg cheese study, a commercial gas with a minimum of oxygen was employed. The commercial packaging method of Jarlsberg cheese is flow-packed with minimum oxygen, whereas Norvegia cheese is packed in vacuum packaging using a thermoformer. Both cheeses are packed under transparent film material with the printed label on top.

It had earlier been shown for butter that green light induced less off-flavours compared with blue light at low oxygen concentrations, whereas at high concentrations (5–21%) the level of off-flavours were not significantly different (Wold et al., 2009). The

green light in this study covered the spectral region 450–620 nm where protoporphyrin IX is a very active photo-sensitizer. The sensitizing effect of protoporphyrin IX increases with the oxygen concentration (Dysart & Patterson, 2006). Another possible reason why green light induced relatively more off-flavours in the present study compared with other studies, might be due to sampling. In this study, only the upper 3 mm of the cheese was evaluated by the sensory panel, whereas in the butter study, the samples were 10 mm thick. Green light might give the largest effect at the surface where the oxygen concentration is high; the effect would be less distinct for thicker samples.

It should be mentioned that Jarlsberg cheese has characteristic such as nutty, sweet and acetic flavours (Frolich-Wyder & Bachmann, 2004), and also more intense flavours than Norvegia cheese, which might affect the sensory results. Jarlsberg cheese contains an adjunct culture of propionic acid bacteria (Kraggerud, Skeie, Hoy, Rokke, & Abrahamsen, 2008). Due to the propionic bacteria, the Jarlsberg cheese develops a more bitter, pungent and intense flavour with increasing age.

The main point, however, is that all three colours induced high levels of off-flavours at the top layer of the cheese. The depth and intensity of the generated off-flavours resembled earlier results obtained with white light (Wold et al., 2002).

The sensory properties of sour and rancid flavours of cheese stored in an air atmosphere and exposed to blue, green and red lights are shown in Table 1. Significance tests were carried out and compared within each column. Significant differences for rancid flavour were found between the first layer (0–3 mm) and the second layer (3–6 mm) in samples exposed to green light. Red light induced a significant difference in rancid flavour between the second layer (3–6 mm) and the third layer (6–9 mm). The differences between green and red light was that green light gave a more gradual change in the rancid flavour score than the red light. Samples exposed to blue light also showed a gradual change in the rancid score, and a significant difference was found between the first and third layers.

The occurrence of rancid flavour regarding to different colour lights penetration can be explained by two main reasons. First, there is a general tendency that longer wavelengths in the visible region penetrate deeper into biomaterials than do shorter wavelengths. Red light (580–700 nm) penetrates deeper than blue light (350–560 nm), since blue light is scattered more according to the general rule of the wavelength dependent Rayleigh scattering. Second, riboflavin and β-carotene are present in relatively high concentrations in cheese. Blue light is absorbed by riboflavin and β-carotene, whereas wavelengths longer than about 500 nm are not absorbed by any high concentration compounds, to the best of our knowledge. Reported transmittance spectra for cream cheese confirm that visible light of wavelengths longer than 500 nm

Fig. 4. Fluorescence spectra measured at the surface of cheese stored in an air or nitrogen atmosphere after light exposure for 7 d. Colours of the spectral curves resemble those of the filters used. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

penetrate deeper into the cheese than blue and violet light (Westermann et al., 2009). This means that porphyrins and chlorophyllic compounds, which absorb in the entire visible region, are exposed to relatively more light since green to red light penetrates deeper and is not absorbed by β-carotene. This might explain why green and red light had a worse effect on sensory properties deeper into the cheese compared with blue light. This will be further discussed in the next section.

3.3. Fluorescence spectroscopy

The level of photo-oxidation can be indicated both by formation of fluorescent oxidation products (Kikugawa, 1986; Veberg et al., 2007) and more indirectly by the breakdown of photo-sensitizers (Wold et al., 2005). Fig. 4 shows the fluorescence spectra measured at the surface of the cheese stored in air or nitrogen atmospheres after light exposure for 7 days at 4 °C. In the 400–490 nm region the formation of fluorescent oxidation products is indicated by an increase in the peak intensity (Veberg et al., 2007; Wold et al., 2002). Riboflavin shows the most intense fluorescence with a broad peak at 530 nm (Miquel Becker, Christensen, Frederiksen, & Haugaard, 2003; Wold et al., 2002). The tetrapyrroles generate smaller peaks in the 600–750 nm region with hematoporphyrin at 620 nm, protoporphyrin IX at 635 nm and 705 nm (Juzenas, Iani, Bagdonas, Rotomskis, & Moan, 2001; Wold et al., 2006a), and the

Table 1
Sensory evaluation of cheeses stored in an air atmosphere with blue filters (350–560 nm), green filters (450–620 nm) or red filters (580–700 nm) and exposed under light for 7 days at 4 °C[a].

Depth (mm)	Sour flavour			Rancid flavour		
	Blue	Green	Red	Blue	Green	Red
0–3	1.54[b]	1.40[c]	1.80[c]	5.97[a]	6.96[a]	5.50[a]
3–6	3.13[ab]	1.91[bc]	2.04[bc]	3.68[ab]	4.95[b]	4.67[a]
6–9	3.93[ab]	3.26[ab]	3.98[ab]	1.97[b]	2.64[c]	2.52[b]
9–12	4.66[a]	4.09[a]	4.70[a]	1.49[b]	1.62[c]	1.40[b]
12–15	4.31[ab]	3.79[a]	3.72[abc]	1.66[b]	1.94[c]	1.77[b]
15–18	4.48[a]	4.01[a]	4.77[a]	1.25[b]	1.57[c]	1.38[b]

[a] Mean values within each column with the same superscript letter are not significantly different ($P > 0.05$). The sensory attributes were ranged from 1 to 9, where 1 was lowest intensity.
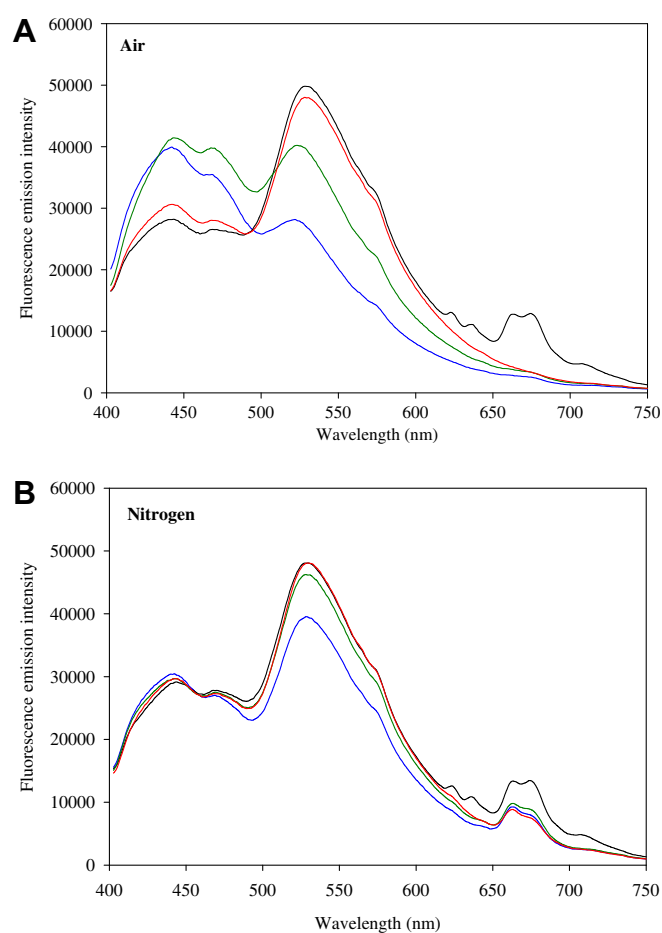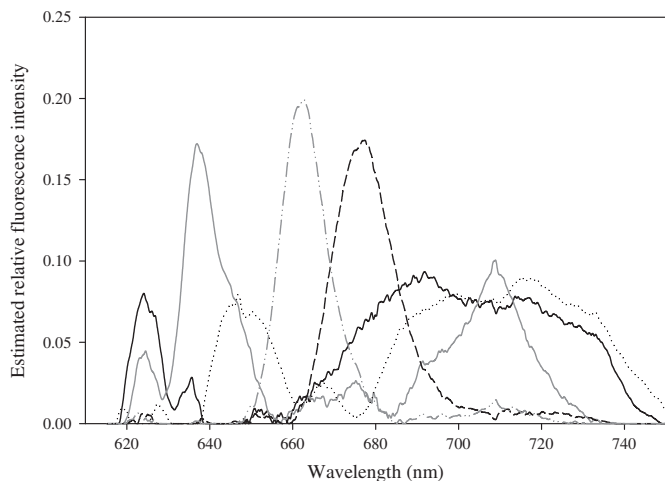
Fig. 5. The estimated fluorescence emission spectra of tetrapyrroles by multivariate curve resolution. A multivariate curve resolution was applied to background-corrected fluorescence spectra in the region 600–750 nm. The five spectra compounds were hematoporphyrin (black-solid line), protoporphyrin IX (grey-solid line), chlorophyll (black-dash line); two unidentified tetrapyrroles compound are named compound X1 (grey-dash-dot-dot line) and compound X2 (black-dotted line).

double peak at 661 and 672 nm are identified as chlorophyll residues (Merzlyak et al., 1996). Similar spectra have previously been obtained from dairy products (Intawiwat et al., 2010; Wold et al., 2005, 2006a).

The fluorescent oxidation products were formed in samples stored in air, but not in nitrogen. It should be noted that the fluorescence intensity in the 400–490 nm region has a large background contribution from the broad riboflavin emission band. When this background is considered, it can be seen that most fluorescent oxidation products were formed under blue light, less under green, and least under red light. It is reasonable to believe that the high concentration of fluorescent oxidation products in samples stored under blue and green filters was caused by riboflavin sensitized oxidation. Nevertheless, the sensory panel detected high scores for off-flavours also for the samples stored under the red filter. This result indicates that the oxidation products formed under red light and parts of the green light were not fluorescent. Exactly the same mechanism has been observed for light-exposed milk (Intawiwat et al., 2010). Negligible formation of fluorescent oxidation products in nitrogen-stored cheese is in accordance with similar results on light-exposed butter (Veberg et al., 2007). The fluorescent oxidation product is an interesting marker of photo-oxidation, but not always relevant since it is wavelength-dependant and does not necessarily correspond well with sensory properties. It should be noted that pores in the cheese can affect the rate of dissolved oxygen and also affect the oxidative process. However, the effect of dissolved oxygen in cheese was not evaluated.

Riboflavin was most degraded in cheeses stored under blue filters, less under green, and least under red filters. It was also more degraded in an air atmosphere than in a nitrogen atmosphere. These results correspond well to reported results on cheese (Mortensen et al., 2002a; Wold et al., 2005) and pasteurized milk (Intawiwat et al., 2010).

Multivariate curve resolution (MCR) was applied to the background-corrected fluorescence spectra in the region 600–750 nm to extract the pure spectral components of the different fluorescent compounds. The loading plot of the calculated MCR obtained spectral profiles of each compound is shown in Fig. 5. The spectral compounds were identified as hematoporphyrin (peak at 623 nm),

protoporphyrin IX (637 and 709 nm), a chlorophyll a-like compound (677 nm) and two unidentified tetrapyrroles termed compound X1 (662 nm) and compound X2 (647 nm). The MCR solution is very similar to a spectral curve resolution model obtained on fluorescence excitation emission matrices from butter by the use of parallel factor analysis (PARAFAC; Wold et al., 2006a). The specific photo-sensitizing effect of these different compounds is, to date, not clear, but it has been shown that they are more or less active in dairy products (Wold et al., 2006a, 2009).

The estimated relative concentrations as a function of depth in the cheese are shown for riboflavin, hematoporphyrin, protoporphyrin IX and chlorophyll (Fig. 6). The concentration of sensitizers in cheese stored in the dark was more or less constant at greater depths in the samples. As already seen in Fig. 4, riboflavin was degraded in samples stored under blue and green filters, but only in the two upper layers as shown in Fig. 6. In samples stored in a nitrogen atmosphere under a blue filter, degradation of riboflavin was detected only at the surface layer. This indicates that riboflavin would be an active photo-sensitizer only where close to the surface of the cheese.

Hematoporphyrin, protoporphyrin IX and chlorophyll were degraded under all coloured filters, however, in general more by green and red light than by blue light. It is also clear that green and red light affected the compounds deeper into the cheese than did blue light. Protoporphyrin IX and chlorophyll were affected all the way down to 21 mm. Chlorophyll was mostly degraded in cheese stored under the red filter. This is in accordance to Wold et al. (2006b) where it was stated that red light causes more degradation of chlorophyll compounds than does blue and green light.

The storage atmospheres had notable effects on the degradation of photo-sensitizers. For protoporphyrin XI, there were differences at the surface of the cheese, but then very similar deeper into the cheese. For chlorophyll there were large differences at the surface, and then especially for green and red light, there were differences all the way into about 12 mm. This suggests that the atmosphere affects the amount of dissolved oxygen in the cheese, and that there was a gradient in oxygen concentration in the cheese, high at the surface and lower further in. Differences in oxygen concentration will affect which of the Type I and II photo-reactions that are dominating (Wold et al., 2009). The photo-degradation of chlorophyll (and other photo-sensitizers) in samples stored in nitrogen is caused by Type I reactions. This photo-reaction occurs directly through a free radical mechanism (Frankel, 2005) and can produce different oxidation products (Veberg et al., 2007). During Type II reactions, the degradation of photo-sensitizers is usually due to reactions with singlet oxygen. Even though some sensitizers were photo-degraded as far as 21 mm into the cheese, off-flavours were not registered at these depths. Both the light intensity and oxygen concentration at these depths were low, and less off-flavours would be formed.

It was a little surprising that the sensory panel did not find any differences in rancid flavour in samples stored under nitrogen atmosphere, since the photo-sensitizers were heavily degraded. The same was observed for light exposure of butter (Wold et al., 2009) where storage in nitrogen with oxygen absorbers did not give any formation of off-flavours, even though many of the photo-sensitizers were degraded. The reason is probably that the possible radicals formed depend on oxygen for further reactions and formation of off-flavours.

This study shows that the photo-reactions in cheese are rather complex and probably involve many different photo-sensitizers. In our case, where we have six photo-sensitizers in the same system, it is difficult to interpret and understand the results in depth. However, some main conclusion can be made. Westermann et al.
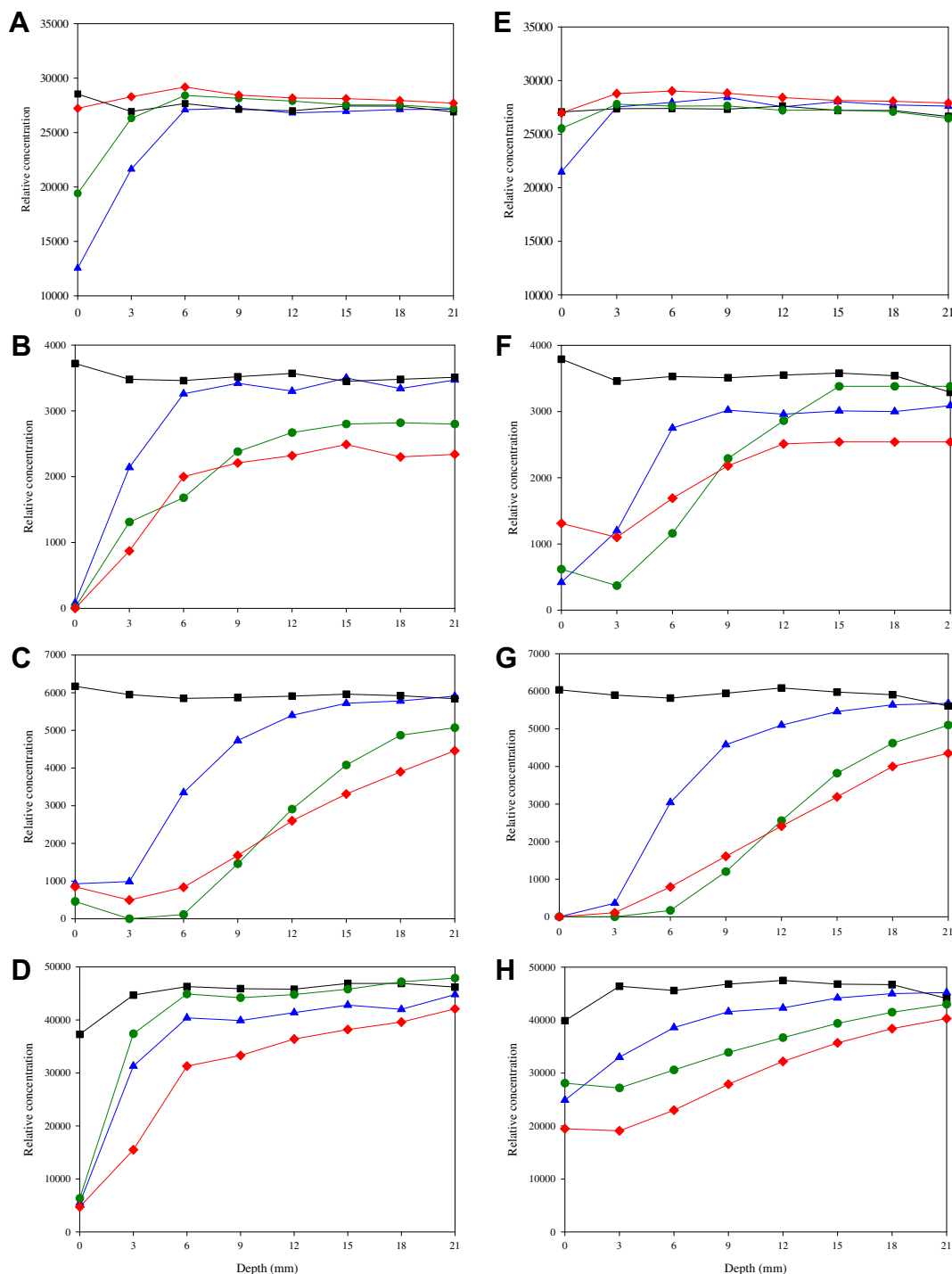
Fig. 6. Measured relative concentrations of the photo-sensitizers, riboflavin (A, E), hematoporphyrin (B, F), protoporphyrin IX (C, G) and chlorophyll (D, H) in cheese after light exposure for 7 d at 4 °C. Measurements were carried out on the upper surface of the slices (0, 3, 6, 9, 12, 15, 18 and 21 mm). Colours of the spectral curves resemble those of the filters used. Blue filter: 350–560 nm, green filter: 450–620 nm and red filter: 580–700 nm. Storage was in an air atmosphere (left side, A–D) or a nitrogen atmosphere (right side, E–H). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(2009) suggested that riboflavin is the most important sensitizer in the upper layers of cheese, and that tetrapyrroles might be more active at deeper layers. In the present study, riboflavin was most active under the blue light, and less active under green light. This is indicated by the degradation of riboflavin, as shown in Fig. 4. But green light still caused more intense off-flavours at the cheese surface than did blue light. It is then reasonable to believe that the tetrapyrroles were responsible for this extra formation of off-

flavours. The oxidation at the surface generated by red light cannot be ascribed to riboflavin sensitization. It is therefore likely that the tetrapyrroles are active at the surface also under blue light, even though the concentration of these compounds is less than that of riboflavin. The yellowish colour of Norvegia cheese and other cheeses based on bovine milk comes mainly from β-carotene. β-carotene absorbs wavelengths below about 500 nm, and will thus function as a protective filter in the blue region, whereas no

Table 2
Correlation between relative concentrations of photo-sensitizers obtained by MCR against sensory assessed attributes.

Sensory attributes	Riboflavin[a]	Hematoporphyrin	Protoporphyrin IX	Chlorophyll	Compound X1	Compound X2
Sour odour	0.61	0.92	0.71	0.84	0.84	0.78
Sunlight odour	0.41	0.75	0.62	0.68	0.69	0.70
Rancid odour	0.68	0.91	0.68	0.88	0.89	0.73
Sour flavour	0.60	0.91	0.74	0.81	0.81	0.80
Sunlight flavour	0.40	0.69	0.57	0.66	0.68	0.64
Rancid flavour	0.66	0.92	0.70	0.87	0.88	0.75

[a] Relative concentrations of riboflavin were measured as the emission intensity at 580 nm.

comparable filter exists for wavelengths longer than 500 nm in the yellow and red region.

One empirical way to get a hint of which sensitizers contribute to the sensory off-flavours is to study correlations between the sensory properties and the degree of photo-sensitizer degradation. Table 2 shows the simple correlations between the sensitizer concentrations and some sensory attributes. It can be seen that, for instance, hematoporphyrin, chlorophyll and compound X1 were highly correlated with sensory properties, whereas riboflavin and protoporphyrin IX were less correlated with the sensory data. Multivariate regression (PLSR) of the variation in porphyrins and chlorophyllic fluorescence spectra onto the sensory scores gave models with high correlations. These compounds correlated well with sour flavour ($R^2 = 0.85$) and rancid flavour ($R^2 = 0.90$) for cheese stored in air, whereas the corresponding correlations for cheese stored in nitrogen were low since there was little variation in sensory properties. All six photo-sensitizers seemed to contribute to the variation in sensory properties.

4. Conclusions

We have investigated how the progression of photo-oxidation in cheese depends upon different wavelengths in the visible region. The wavelength region was divided in three parts by optical filters; blue (350–560 nm), green (450–620 nm) and red (580–700 nm), and cheese samples were exposed to one of the colours. After 7 days of storage in light, all colours affected sensory properties down to about 6–9 mm depth, but green and red light induced greater changes than did blue light. Riboflavin was photo-degraded only in the upper 0–6 mm region by blue light, whereas other photo-sensitizers, such as hematoporphyrin, protoporphyrin IX and chlorophyll, were degraded as deep as 21 mm into the cheese. This suggests that green and red light penetrates deeper into the cheese than blue light. Tetrapyrroles seem to be the responsible photo-sensitizers, at least when light exposure wavelengths are longer than about 500 nm and for off-flavours formed deeper than 6 mm. The less adverse effects by blue light might be explained by a protective filter effect by β-carotene, which will absorb much of the incident exposure light. The results should be taken into consideration when protective packaging materials for cheese is designed.

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Paper III

ARTICLE

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

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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:

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 $\sim$ 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
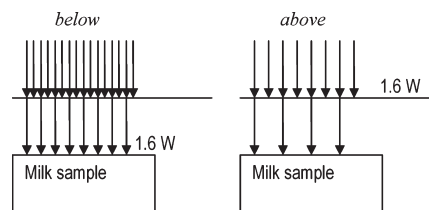

Figure 1. Light intensity was adjusted to about 1.6 W/m^2 either below the filters or above the filters. In the case of adjustment below, the intensity at the milk surface was around 1.6 W/m^2. In the case of adjustment above, the intensity at the milk surface was approximately 1.6 W/m^2 or less, depending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

wavelengths. In this section an overview of the experimental design is given. Details on materials and methods follow in the next sections.

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
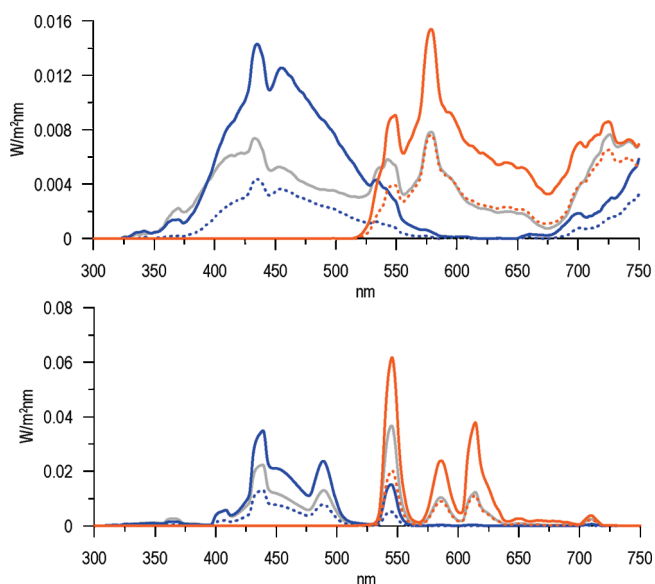
Figure 2. Transmitted light intensity as a function of wavelength for clear (gray line), blue (blue line), and orange (orange line) films, under metal halide (HMI) lamp (top) and fluorescent light tubes (bottom). Solid and dashed lines correspond to the different light intensity adjustments, below or 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 Chem-Station 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/m²)	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/m² below the filter. [c] Light intensity was adjusted to about 1.6 W/m² 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/m² 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/m² 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/m²) and the lowest for the blue (0.5 W/m²). 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/m² 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
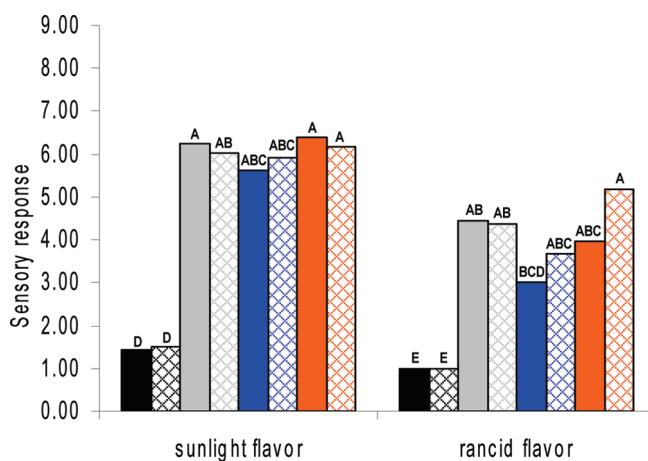
Figure 3. Sensory responses for sunlight and rancid flavor for milk packed in air and nitrogen and exposed to light from fluorescent light tubes ($1.6 \, W/m^2$) under different colored films. Storage was maintained under blue (blue bars), orange (orange bars), and white (gray bars) light or in the dark (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
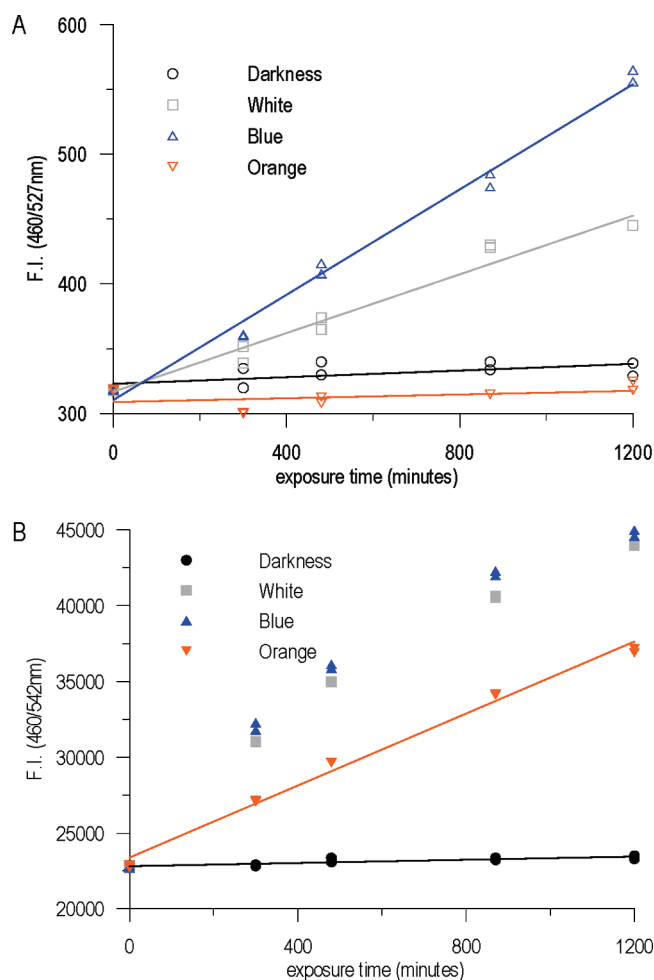

Figure 4. SOSG fluorescence over time in 0.01 M citrate buffer pH 6.7 (control conditions) (A) and added in milk (B) exposed to light of different colors and stored in the dark.

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

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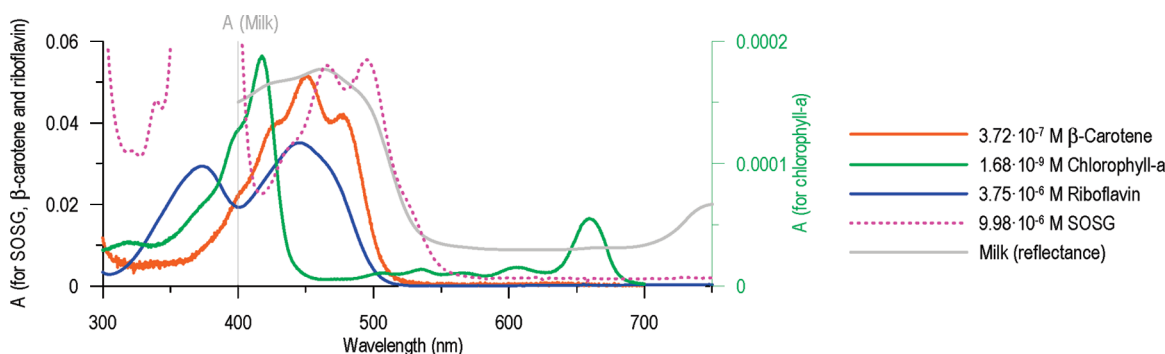
dx.doi.org/10.1021/jf104259p |*J. Agric. Food Chem.* 2011, 59, 3905–3914

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).
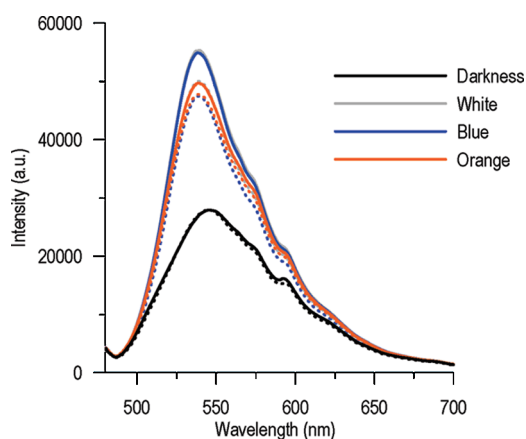

Figure 6. Emission spectra (λ_{ex} = 460 nm) of milk containing SOSG stored in the dark and under different colors of light, packed under air (solid lines) or nitrogen (dotted lines).

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,
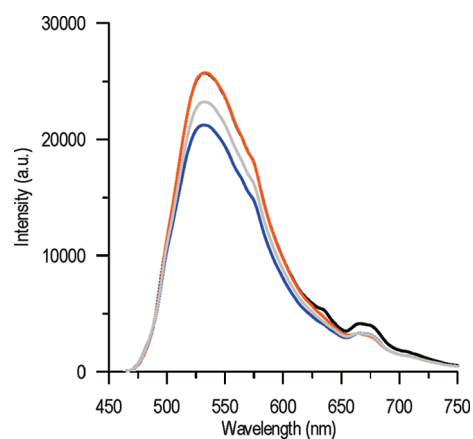
which means that the initiation and the extent of photooxidation can be indirectly measured. In this section we present the fluorescence spectra from samples without SOSG; only intrinsic fluorophores are studied. Figure 7 shows the fluorescence emission spectra from milk samples after excitation at 410 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
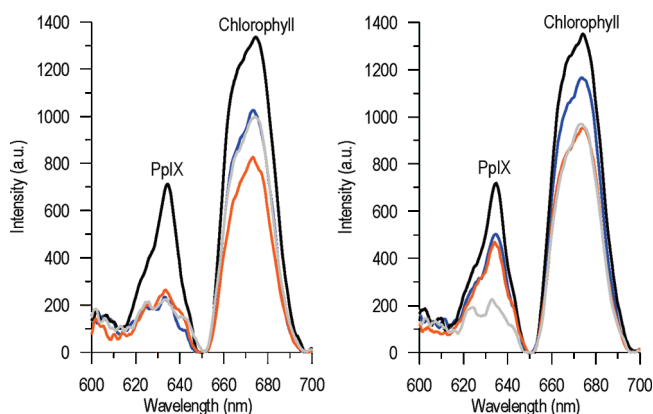
Figure 8. Background-corrected fluorescence spectra (λ_{ex} = 410 nm) from milk stored under air atmosphere with the light intensity adjusted to about 1.6 W/m^2 at the milk surface (left) and 1.6 W/m^2 above the film (right). Sample was exposed to blue light (blue line), white light (gray line), orange light (orange line). Sample stored in the dark (black line).

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.
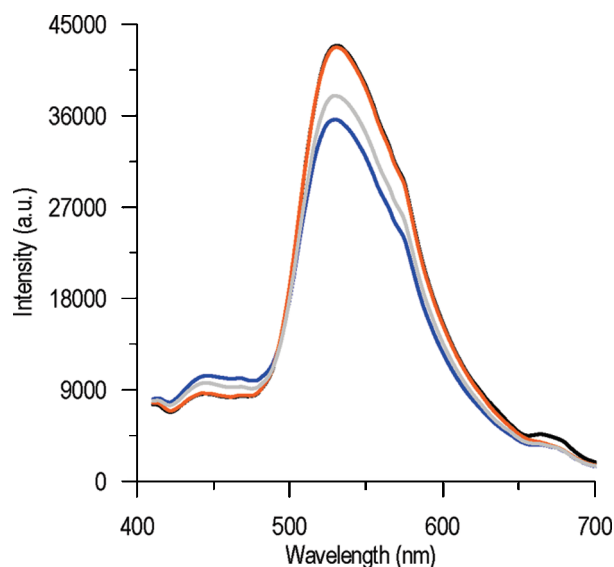

Figure 9. Fluorescence emission spectra (λ_{ex} = 382 nm) from milk samples stored with air in the headspace and exposed to 1.6 W/m^2 of blue (blue line), white (gray line), or orange (orange line) light. Sample stored in the dark (black line).

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 chlorophyllic 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.

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Paper IV

Packaging materials with tailor made light transmission properties for food protection

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ABSTRACT

The altering light transmission properties of packaging films in order to exclude specific visible wavelengths (400-450 nm and 600-650 nm) was studied. Six low density polyethylene blown films were formulated with the combination of four different pigments and additives; green, yellow, silver additives and optical brightener. In addition, the three films containing pure pigment and additive and one film without those ones were reference samples. Optical properties, light transmission and microscopy analysis were used to measure characteristics of the produced materials. The six films appeared as different shades of green according to different concentrations of pigments and additives. The higher concentration of green pigment resulted in darker green color. Films containing silver additives were more intense green than those without. The sample containing high concentration of both green and yellow pigment had the lowest value in gloss and transmittance. Samples in combination of green and yellow pigments transmitted 20 % of light from 450 to 600 nm. Samples containing silver additive gave a higher light transmission in blue regions (380-500 nm) and lower in red regions (600-700 nm) compare to samples without silver. The high concentration of both green and yellow pigment film blocked the light below 450 nm and transmitted 10 % at 600-650 nm.

INTRODUCTION

Many food products are exposed to light during processing, packaging, distribution retailing and storing. Light can induce oxidation processes which can decrease food quality. Packaging is one of the best solutions to protect against photooxidation. Opaque and non transparent materials such as aluminum foil are recommended to use against the light. Aluminum foil has been used as one layer of multilayer film in several packaging materials. Although aluminum layer gives good protection from the light [1], it has some environmental concerns. Nowadays transparent packaging materials have been widely used in many food industries. This is due to the increasing demand from consumers to better appraise the product prior to purchase. The use of transparent packaging with high light transmission is a big challenge. The main goal is to protect the product quality, while the packaging still remains transparent. Thus, the design of the packaging material has to be taken to account in order to obtain the desired properties.

Dairy products contain many photosensitizers such as riboflavin, porphyrin and chlorophyll [2-4]. These active sensitizers are responsible for photooxidation in dairy products. Wold et al. [2] reported that the degradation of porphyrin and chlorophyll correlated well to the sensory properties. The light transmission properties of packaging materials can be used in order to provide an optimal protection of dairy products. Several transparent or translucent packaging materials with color pigments and sometimes with UV block have been studied on the effect of different color of lights in relation to their light transmittance properties and prevention of photooxidation in dairy products [2, 3, 5, 6]. Up to date the studies have concluded that blocking UV light and blue light below 500 nm can reduce formation of oxidation products. However, complete prevention of formation of oxidation products has not been obtained. Recently, Webster et al. [7] have reported that using UV block and excluding

the blue light below 500 nm is not sufficient to avoid oxidation in milk. Yellow (460-800 nm), orange (520-800 nm) and red (570-800 nm) light have been reported to also cause off flavors in dairy products [3, 6]. Green light (500-620 nm) has been shown to give less deteriorate to sensory quality and nutrition value in dairy products compared with other colors in visible light [3, 5, 6, 8].

Altering the light transmission property of packaging material is one solution that can be used for preventing photooxidation in dairy products. Many packaging companies have produced flexible films with different transmission properties, for instance UV blocking. However, most transparent packaging materials do not exclude wavelengths of light in the visible regions, which can be harmful to several light sensitive compounds in food products. Traditionally colored materials have been selected for the design purpose as a marketing strategy tool in order to increase product sale.

Several methods e.g. adding pigments and additives are used in the packaging film production in order to change light barrier properties. Adding pigments into the polymer can modify light transmission wavelength profiles without disturbing the functionality of materials. It also can be used to improve material light barrier properties in specific wavelength regions. Pigments, as organic and inorganic pigment, in packaging application are used for multifunctional purposes. Good pigment-polymer product must provide high purity of tone, high coloring, high light transmittance, heat stability, chemical reagent resistance and insolubility in polymer carrier [9]. Previous studies using color pigments have focused on the effects of color pigments on the morphology and crystallization of polymer [9, 10]. No studies have been focusing on light transmission profiles and percentage of light transmittance in the specific wavelength of e.g. green light.

High transparency can be achieved by using inorganic nanoparticles with size below the wavelength of visible light. Metal oxide nanoparticles are commonly applied into polymer

matrix in order to provide mechanical strength, abrasion resistance and UV absorption. Additives of metal nanoparticles of silver (Ag) and gold (Au) have unique optical properties [11-13]. Silver and gold additives can be used for adjusting transmission properties of material regarding to the specific absorption of those metal particles. The absorption of the metal nanoparticle depends on the type, geometry and size of the particles, and the environment surrounding the metal particles [12]. He et al. [14] have studied Au-Ag alloy nanocrystals. They have reported that monometallic Au and Ag have absorption peaks at 517 and 418 nm, respectively. In addition, silver nanoparticles have been used as additives for antibacterial/antifungal food packaging applications [15-17].

Optical brighteners work by absorbing light from the ultra-violet end of the spectrum and emitting light in the visible blue/white range of the spectrum. This shift in the frequency of light energy, results in a whiter and brighter appearance of the treated product. Optical brightener has been studied in relation to the effect of whitening properties of the polymer [18]. Although, optical brighteners are commonly used in materials, few studies have been reported on this topic.

Traditionally, different types of pigments and additives have mainly been studied separately in order to alter specific properties. Up to date, no studies have investigated the effect of the combination of pigment, silver additive and optical brightener effect on light transmission properties. The purpose of this study was to evaluate the effect of different pigments and additives, separately and in combination to develop packaging material with specific light barrier properties; transparent and partly colored material which can exclude the harmful light for chlorophyll compound including both wavelength area 400-450 and 600-660 nm. The different percent light transmission and transmission profiles of produced films were also investigated. This novel packaging could be useful as a prototype as it provides transparency while still providing light protection of the dairy products.

MATERIALS AND METHODS

1. Polymer, pigments and additives

Additives and pigments have been selected according to blocking the desired wavelengths and in dialog with the suppliers in order to achieve the objectives. The characteristics, commercial availability and food contact approvals have also been taken into account for the additive and pigment selection. Commercially available grade of low density polyethylene (LDPE), (FT5236, Borealis, Austria) was used in this study. Polymer, selected additives and pigments, and their properties used in this study are presented in Table 1.

2. Experimental design

The pigments and different additives, optical brighteners and silver additives with specific absorption properties, were used individually and in combination with different concentrations in the polymer. Six films, in addition to four references were prepared by mixing the four different additives and pigments in different concentrations. The different compositions are shown in Table 2. Reference sample (REF) was LDPE without adding additives or pigments. Samples with pure single silver additive (S) and pigment (Y or G) as the other three reference samples were prepared in order to compare with samples using combinations of those substances. The total weight of each sample was 3 kg.

3. Compounding and film conversion

3.1 Compounding process

Low density polyethylene, additives and pigments were pre-mixed by Forberg mixer (Forberg AS, Larvik, Norway) before the polymer compounding process. To increase the dispersion of these substances into the polymer matrix during the compounding process isododecane solution was used for coating the surface of the LDPE pellets in the mixing process. Microlen Yellow concentrate was grinded by pulverizing mill machine (Powder-King, USA) before mixing in the Forberg machine.

Compounding was done by a twin screw co-rotating extruder (TSE PRISM 24, Thermo Fischer, Stone Staffordshire, UK) (die diameter = 24 mm, L/D = 28, screw speed = 300 rpm) using a feed speed of 9 kg/h. The following temperature setting was used: in first zone 190°, in the middle zones 200° and at the extruder die 190°. The melt temperature was measured to ca. 220°. The premix of LDPE and the pigments and additives were added to the feeder unit of the extruder. The polymer samples were extruded, water cooled and pelletized. The reference samples were processed under the same conditions.

3.2 Film blowing process

Samples were converted into film (100 $\pm$ 10 µm thickness), by a film blowing process with a lab scale Collin blown film line (Collin, Ebersberg,Germany). The melt temperature was set at 180°. Die gap was 1.5mm and die diameter 50 mm.   The machine conditions were set to: blow-up ratio (BUR) 3.00, screw speed 90 rpm, pressure 100 bars, and take off speed 1.7 m/min. The film layflat was 235 mm.

The film thickness was measured by micrometer and was also confirmed by Gauge Profile Analyzer MAC100 GAA (Octagon Process Technology, Würzburg, Germany) All samples were stored at room temperature for 24 hrs after film blowing process.

4. <u>Analyses</u>

4.1 Optical properties

4.1.1 Gloss

Gloss was measured by using a gloss meter (Sheen Micro – TRI Gloss 20-60-85, Sheen, Gerelsried, Germany). The gloss value was measured at 20° angle according to ASTM D 2457-03 [19]. Six replications were performed.

4.1.2 Haze, clarity and transmittance

Haze, clarity and total transmittance were measured according to ASTM D 1003-00 [20] by using Haze Gard plus instrument (Haze-Gard plus, M-4260, BYK Garder, Gerelsried, Germany). The samples were cut in the machine direction (MD) and placed into the sample holder (diameter 12.5 cm). The measurements were taken at 3 positions per sample. Six replications were measured.

4.2 UV-Visible Spectroscopy

Light transmission was measured by using a Perkin-Elmer Lambda 800, UV/Vis Spectrometer (Perkin-Elmer Ltd.,Buckinghamshire, UK). The scanned wavelength range was 200 – 900 nm. The light transmission was measured on flat sample sheets. Two replications per sample were measured.

4.3 Microscopy technique

The cross section of the film samples were investigated by transmitted light microscope Leica DM6000 (Leica Microsystems, Wetzlar, Germany) with contrast techniques bright field, dark field, polarized light and phase contrast. Cross section of the film (5 µm thickness) was

prepared by microtome cutting device Leica RM2165, (Leica Microsystems, Wetzlar, Germany).

The surface topography of the film samples was investigated by scanning electron microscope (Philips XL-30 ESEM, FEI Company, Eindhoven, the Netherlands) with secondary electrons, operating at a voltage of 15 KeV. The amplification ratios 150x was used. Two replications per sample were measured.

5. Statistical analysis

Principal component analysis (PCA) was applied to the data obtained from gloss, haze, clarity and transmittance in order to get the best possible view of all the samples related to measurement parameters. Loading plots were used to interpret the variation contained in each PC. Score plots were used to visualize the relation between samples in the corresponding PCs. PCA analyses was performed by using Unscrambler ver. 7.5 (Camo AS, Oslo, Norway).

RESULTS AND DISCUSSION

1. Visual appearance

Six blown films with different combination of pigments and additives were produced, in addition to the 4 reference films. All pigments and additives had good dispersion in the polymer matrix. All samples were transparent. The reference film (S) containing pure silver pigment was transparent due to the diameter size of the silver additive was ca. 50 nm. Normally, particle diameter less than 50 nm are recommended in order to obtain transparency [21, 22]. The mean particle size of the green and yellow pigments was 100-200 nm. Green pigment gave a dominant color in samples compared to yellow pigment, thus the sample containing both of these pigments appeared green. It should be noted that green and yellow pigment tended to form agglomerates during processing which may increase the particle size of pigment more than 1 micron.

The samples had different green colors in accordance with the different compositions of pigments and additives (Figure 1). Films containing silver additive (GS1, GS2 and GS2+OB) resulted in darker green films compared to the samples without the silver additive (GY1, GY2 and GY1+OB). This could be explained by the fact the silver additive gave brownish color in the films.

Discoloration of plastics may be altered by adding small amount (normally 5 to 100 ppm) of an optical brightener (also called a fluorescent whitening agent). The samples containing optical brightener had lighter green color compared to those without optical brightener. The reason for this behavior is that the optical brightener absorbs invisible UV radiation in the wavelength range of about 360 to 380 nm, converting it to longer wavelength and re-emitting it as a visible blue or violet light. As a result, the unwanted yellowish

appearance of the substrate is offset and, in addition, more visible light in the range of 400 to 600 nm is reflected than without the use of optical brightener.

2. Optical properties

The optical properties were measured in terms of gloss, clarity, haze and transparency. Gloss has been described as surface smoothness which is measured by reflected intensity of samples compared to a standard [23]. Significant differences were found between samples containing pigment and the reference samples without adding pigments and additives (REF). The reference samples containing pure green pigment (G) and silver additive (S) had significant higher gloss value than samples containing combination of pigments and additives (GY2, GY1+OB, GS2 and GS2+OB).

The concentration of the pigments and additives affected the gloss value of the samples. Samples with high concentrations of green and yellow pigments (GY2) had lower gloss compared to the samples at low concentration (GY1). The same result was found in the samples containing the combination of green pigment and silver additive. Samples containing high concentration of both green and yellow pigments (GY2) had the lowest gloss value and also dark green colored appearance. This is because gloss may affect the color perception of the material. Our results were in contrast to reported by Arino et al. [24]. They studied the effect of gloss on color of injection-molded pigmented plastic and reported that high glossy surface perceived much intense colored (less lightness). However, the differences could partly be explained by two difference parameters: type of material and material processing. They have studied effect of gloss on injection mold of rigid material, acrylonitrile butadiene styrene polymer (ABS), while we have studied on flexible LDPE blown film. Moreover, the thickness of sample also has affect on the gloss in term of light diffusion (scattered) and reflection and this information was not given in the study of Arino et al. [24].

Using optical brightener in combination with pigments did not alter the gloss compared to the samples without optical brightener (Figure 2). Similar result was found when adding optical brightener in the samples containing silver additive. The optical brightener is mainly used for enhanced color appearance of material. To this date, the study effect of optical brightener and adding in combination with other additives in term of gloss values is still limited.

Figure 3 shows light transmittance, haze and clarity of the different blown film samples. Light transmittance and haze showed big differences among the samples, while small variations in clarity were observed for all the samples (91%-95%). Clarity is defined as the percentage of light which in passing through the film, deviates from the incident beam less than 2.5° on the average. The clarity is often difficult to judge by the naked eye. This is because the perceived clarity depends on the thickness of the sample. However in this study, all samples had the same thickness (100 µm).

The reference samples REF, S and Y had significantly higher light transmittance than the sample with pure green pigment (G), and the samples containing pigments and additives (Figure 3A). The light transmittance was lower in the samples containing high amount of the combination of green pigment together with silver additive (GS2 and GS2+OB), and lowest in the sample with high amount of green and yellow pigments (GY2). The total transmittance of visible light through the polymer film is equal to the incident light minus the absorbed and reflected light. The absorption and reflection of light increases by increasing concentration of pigments, resulting in reduced total transmission. These results in transmittance are supported by the results in gloss (Figure 2). The gloss value was the lowest in sample GY2 because of the lower light transmittance.

Haze is defined as the percentage of total transmitted light which in passing through a sample, deviates from the incident beam greater than 2.5° on the average [20]. The haze value

showed results in the opposite direction to transparency property. This is because haze is scattering of light by some medium, which results in cloudy appearance, and poorer clarity of the polymer film. Sample GY2 showed significantly highest value of haze followed by GS2+OB, Y and GS2 (Figure 3B). It should be noted that the GY2 resulted in highest haze, while it had high clarity (>94 %). This is because haze occurs from light scattering both from the surface and the interior of the film. Thus, the high haze is not necessarily increasing the clarity [25].

Addition of silver additive resulted in significant reduction in transmittance and increase in haze independent of the presence of other pigments or additives. There was a trend that samples with high amount of silver additive (GS2 and GS2+OB) had lower transmittance and clarity than all other samples (Figure 3). Although there was a small difference in clarity, the sample containing high green pigment and silver additive with optical brightener (GS2+OB) had the lowest clarity. It might be because of the high level of haze in this particular sample. However, the clarity results did not affect to the transparency perception of the films.

Principle component analysis (PCA) was used to analyze the samples in order to get a good overall view of the samples related to the measured parameters. Figure 4 shows PCA bi plot (loading and score plot) of first 2 PCs. First PC (PC1) explained the variation of transmittance properties from high (right axis) to low transmittance (left axis). The PCA results support the previous discussion in total light transmittance results. Second PC (PC2) explained the variation of haze value to gloss. The PCA also showed that the sample containing pure yellow pigment (Y) was located close to haze value in the plot, whereas pure green pigment (G) was located nearly by gloss value in the plot. It can be assumed that adding yellow pigment in high concentration could increase the haze value. This is also evident in Figure 3B where the haze value in the reference sample with pure yellow pigment (Y) was higher than sample with pure green pigment (G).

The concentration of the pigments and additives had effect on the transmittance, haze and clarity of the samples. Significant difference in total light transmittance (Figure 3A) was found between samples containing yellow and green pigments at high concentrations (GY2) and those samples at low concentration (GY1). Adding high amount of pigments resulted in increased colored appearance of the materials and decreased transmittance. The same result was found in the sample containing high amount of silver additive (GS2).

According to Figure 3, addition of optical brightener had no significant effect on transmittance, haze and clarity for the samples containing yellow and green pigment (GY1). On the other hand, the addition of optical brightener significantly increased haze, and reduced light transmittance and clarity in the samples containing green pigment and silver additive (GS2). These results can be partly explained by the light absorption of optical brightener. It can be clearly seen in the samples with added optical brightener in combination with silver additive. The optical brightener emits light in blue regions and reduces yellow appearance of material. Adding optical brightener reduced the yellow color and gave more intense green color appearance in these samples. The darker green color of sample had the lower total light transmittance and clarity. However, the interaction between silver additive and optical brightener is not clear and studies dealing with the relation between these two additives are not reported so far.

The optical properties of films are very important for food packaging application. It can be used to design proper materials regarding consumer requirements. The key requirement for transparent film is low haze and high clarity. This is because it allows the consumer to see the product contained in the packaging. The clarity indicates the degree of how the appearance of the object changes when viewed through the film [26]. It should be noted that the see-through clarity as determined by the naked eye might be different from light transmission measured by instrument. This is because the eye is more sensitive to the contrast than the instrument [27].

The appearance of haze is caused by light scattering on the surface and insufficient homogenization of additives dissolved in the polymer matrix [26, 28]. High haze gives appearance as cloudy, foggy, or translucent. As we know that haze is the opposite of transparency and surface haze is in contrast with gloss [25]. A matt surface is sometimes described as high hazy and not shiny. As mentioned, gloss is defined as a measure of the ratio between reflected intensity and incident light intensity, thus gloss is associated with surface roughness of material [29]. However, in this study all samples had smooth surface and the effect of surface roughness is not discussed.

3. Light transmission

The transmission properties of 10 different blown films are shown in Figure 5. Light transmission was obtained by measuring transmitted light through film samples. The reference samples (REF) and (S) transmitted light in UV- and visible regions, 80-90 %T and 60-80% T, respectively. The reference sample containing only silver additive (S) transmitted light in visible wavelength area in less amount compared to reference sample without adding pigments and additives (REF). Silver additive is capable to blocking light to some extend in the wavelengths 400-450 nm, but is not efficient in the 600-660 nm area. According to the transmission spectra of this sample, it did absorb some light around 420 nm, however it still had high transmission (around 60 %T). Using pure silver additive did not block wavelength in UV and visible area.

The reference sample containing pure green pigment (G) had light transmission at 400-600 nm with a maximum peak at 520 nm, whereas the reference sample containing high yellow pigment (Y) had light transmission at 450-700 nm with a maximum at 550-700 nm. Both these samples transmitted 85 % of light at the highest peak.

Samples containing the combination green and yellow pigment (GY1, GY2 and GY1+OB) transmitted light from 450 to 600 nm with maximum peak at 550 nm. This could be expected because of the combination of pure green and yellow spectral wavelengths. Those samples can be used to block wavelength below 450 nm (blue regions) and above 600 nm (red regions) which have been reported as harmful regions for dairy products. Supporting results have been reported by Cladman et al. [5], who found that green colored PET bottles, which prevent light below 500 nm, gave less lipid oxidation and vitamin A degradation compared to clear PET bottles. However, they did not give information of wavelength regions of those green PET bottles. Our study showed that green films had different shades of green color depending on the concentration and the combination of pigments and additives. Those films showed differences in the light transmission and the optical properties. Furthermore, this present study showed that the sample containing high concentration of green and yellow pigments (GY2) was able to block all wavelengths below 450 nm. This is a very useful advantage, and means that we can avoid using UV block additives or UV filters in the PE film.

The samples containing green pigment combined with silver additive (GS1, GS2 and GS2+OB) transmitted light from 400 to 600 nm with maximum peak at 520 nm. Those samples transmitted more light (around 10% T) in violet-blue regions (250-380 nm) than samples containing both green and yellow pigments. Addition of the silver additive decreased the UV transmittance. It should be mentioned that GS1, GS2 and GS2+OB had transmission spectra similar to spectra of the reference sample containing pure green pigment (G). This is due to the green pigment being dominant.

The different concentration of additives had effect on the light transmission of the samples. The sample containing pigments with the high concentration (GY2) had less light transmission than the sample with low concentration (GY1) as expected. Similar result was found in green pigment combination with silver additive between GS2 and GS1.

This present study showed that optical brightener with pigment (GY1+OB) did not increase the light transmission, similar to the resulted in gloss, total light transmittance and clarity. No effect of optical brightener was found in the sample containing silver additive and green pigment (GS2 and GS2+OB). It can be assumed that color pigment and silver additive had dominant effect compared to optical brightener.

4. Microscopy

Surface topology of films can be measured by a variety of methods. Optical light microscopy (LM) and scanning electron microscopy (SEM) are widely used both for surface characterization as well as investigation of structures and crystal morphology within the bulk of materials.

Figure 6 shows cross sections of the samples viewed in an optical light microscope with polarized light as contrast technique. The results show typical structures of polyethylene, which has a finer crystal structure than structure of e.g. polypropylene. The microscopy pictures of the outer layers show different colors compared to the inner layer. For somewhat thicker products this is normal due to the difference in cooling temperature during processing. For thin films, a so called edge effect of the light beam will be the obvious reason for this phenomenon. In general, the different color patterns occurring with polarized light are caused by small thickness variations in the cross-section, anisotropy in the sample, rotation of the cross-section and other circumstances.

All samples show that the pigments and additives were well dispersed into the polymer matrices. Furthermore, these pigments and additives resulted in colored appearances compared to the reference sample as expected. With bright field light microscopy, the green and yellow colors in the images represent the green and yellow pigments in the samples GY2. Similar

result was obtained for GY1 (Results not shown). However, the green pigment seems to have higher particle size than the yellow pigment. The samples containing silver additive GS2 had black colored particles dispersed into the polymer. This is caused by the silver additive. The same result was found in GS1 and GS2+OB (Results not shown).

The images from light microscopy can not give quantitative measurement of pigments and additives, but the scanning electron microscope (SEM) may provide such information. The SEM is traditionally a technique used to look at the surface morphology and not as an analytical tool, however a SEM equipped with an energy dispersive x-ray spectrometer (EDS) can be used to detect and quantify element heavier than beryllium down to a concentration of approximately 0.5%. However, in this case the SEM was used solely as a surface morphology technique. The SEM results showed that the samples surfaces in all samples were smooth (Figure 6). The particles were well distributed and dispersed throughout the polymer matrix.

CONCLUSIONS

Packaging films with altered light barrier properties have been developed in order to exclude the wavelength region around 400-450 nm and 600-660 nm. Tailored light transmission properties have been done by addition of the combination of pigments and additives. The combination of those pigments and additives provided varied intense green colors resulting in the different light transmission and optical properties. Samples containing a combination of green and yellow pigment appeared green in color. The color varied from light green to dark green according to the different concentrations of pigments.

Samples containing high amount of silver additives transmitted light in UV region and visible wavelength, however some light absorption around 420 nm was observed. When adding

silver pigments in combination with green colored pigment, the sample got a darker green color. The light transmission spectra of samples containing silver additive together with green pigment was mainly influenced by the transmission spectra of the green pigment.

The optical brightener was used for improving color visual appearance. Optical brightener had no effect in increasing light transmission in samples containing colored pigments, and neither in samples containing green pigment together with silver additives.

We have found that the green films showed differences in light transmission depending on the different combinations of pigments and additives. This should be taken into account in order to design the packaging for preserving and extending shelf-life of food products in future.

ACKNOWLEDGEMENTS

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Table 1. Polymer, additives and pigments used in the study

Polymer, additives and pigments	The available commercial name	Chemical Abstracts Service (CAS) number.	Products description	The supplier or the producer company
Low density polyethylene (LDPE)	FT5236		Standard film extrusion grade containing synthetic silica (antiblock) and erucamide slip agent. Density 923 kg/m3, MFR (190°C/2.16 kg) = 0.75 g/10min	Borealis, Austria
UV/Vis light absorber	STSS-10	3507-99-1	Based on silver stearate	Stabilization Technologies LLC , USA
Transparent green pigment [a]	Eupolen PE Green K87-3001	1328-53-6	65% concentrate in polyethylene of Heliogen Green K87-3001 (Pigment Green 7)	BASF, Ludwigshafen, Germany
Transparent yellow pigment [a]	Microlen Yellow GR-MC	5280-80-8	50% concentrate in polyethylene of Chromophtal Yellow GRP (Pigment Yellow 95)	BASF, Ludwigshafen , Germany
Optical Brightener [b]	Hitex-OB	7128-64-5		HPL Additives Limited, New Delhi, India

[a] 1 % of the pigment is the maximum limit for food contact approvals

[b] Food approved in EU with a Specific Migration Limits (SML) of 0.6 mg/kg food

Table 2. Sample coding and formula compositions of pigments, optical brightener and silver additive. The pigments and additives are used individually and in combination with different concentrations. All samples are produced based on low density polyethylene (LDPE) polymer.

Sample	Sample code	Microlen Yellow GR-MC	Eupolen Green K87-3001	Hitex-OB	STSS-10
1	REF				
2	GY1	1,0 %	0,8%		
3	GY2	3,0%	1,5%		
4	GY1+OB	1,0%	0,8%	0,005%	
5	GS1		0,8%		0,1%
6	GS2		1,5%		0,2%
7	GS2+OB		1,5%	0,01%	0,2%
9*	Y	2,0%			
10*	G		1,5%		
11*	S				0,2%

*Sample 9 and 10 used pure single pigment-concentrates and sample 11 used pure single silver additive into the substrate polymer.
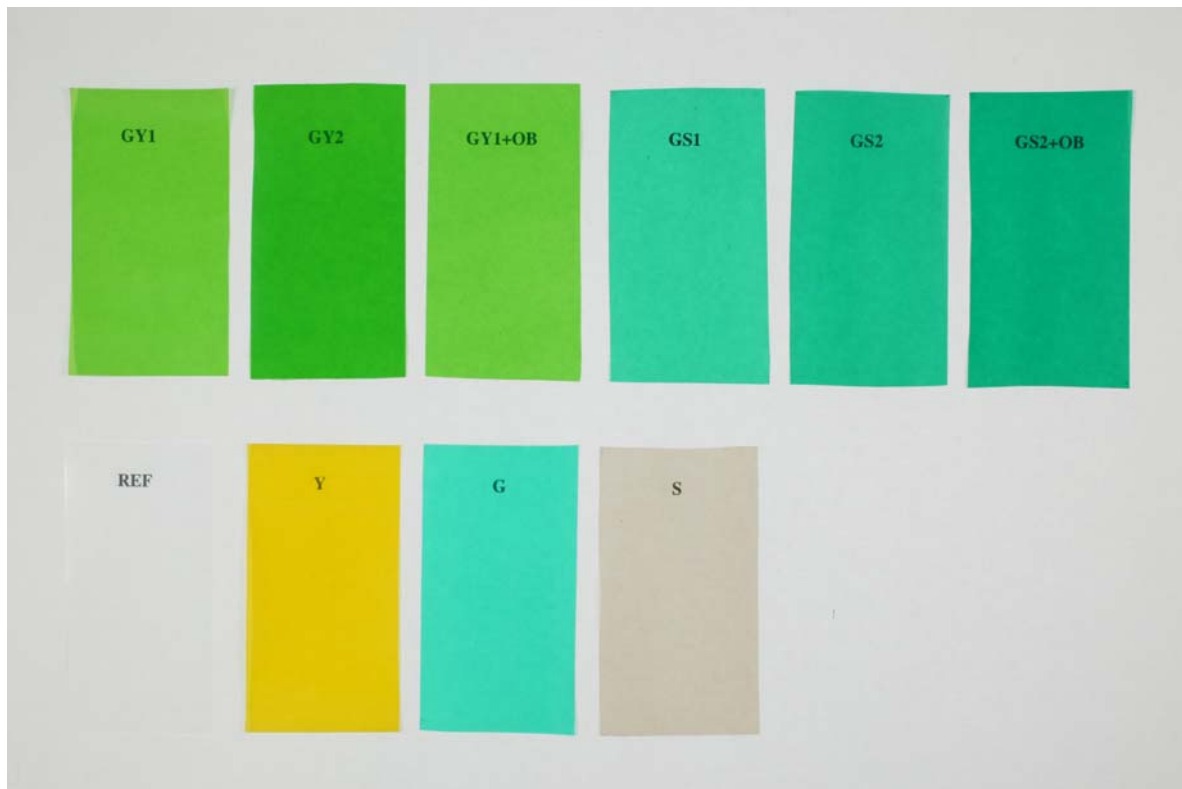
Figure captions

Figure 1. Visual and color appearance of the 10 different blown films. The 6 different combinations of additives and pigments compounds (above row), in addition to references, low density polyethylene without adding pigments and additives (REF), reference samples with pure pigments (Y) and (G) and with pure silver additives (S) (below row).
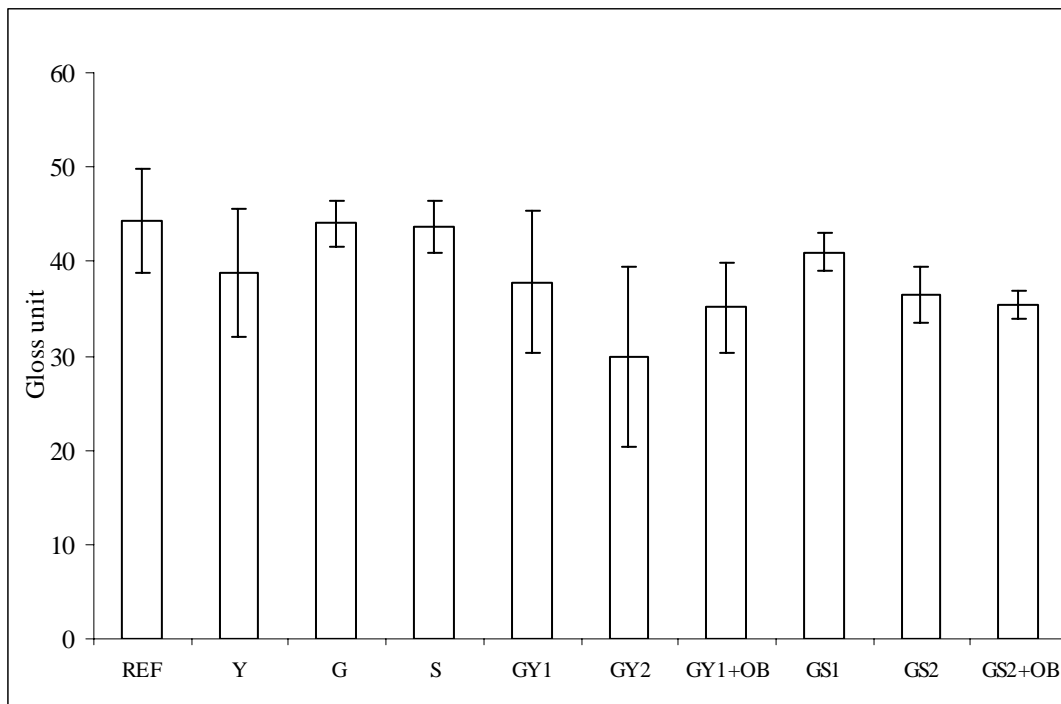
Figure 2. Gloss value as measured of the 10 different blown film samples. The 6 different combinations of additives and pigments compounds, in addition to the references, low density polyethylene without adding pigments and additives (REF), reference samples with pure pigments (Y) and (G) and with pure silver additives (S). Error bar presents standard deviation of 6 replications.

Figure 3. Optical properties measured in 10 different blown films. The 6 different combinations of additives and pigments compounds, in addition to the references, low density polyethylene without adding pigments and additives (REF), reference samples with pure pigments (Y) and (G) and with pure silver additives (S). A) total transmittance, B) haze and C) clarity. Error bar presents standard deviation of 6 replications. See table 2 for abbreviations explanation.

Figure 4. Principal component analysis bi plot (scores and loadings) for the first 2 principal components (PC) of different 10 blown films. Samples with data of optical properties (transmittance, haze, clarity and gloss) as described by scores (samples and references) in blue and loading (optical properties) in black.

Figure 5. Light Transmission (%) of 10 different blown films. The 6 different combinations of additives and pigments compounds: GY1 (solid-amber), GY1+OB (dash-amber), GY2 (solid-green), GS1 (solid-violet), GS2 (solid-blue green), GY+OB (dash-blue green). In addition to the references, low density polyethylene without adding pigments and additives (REF: solid-blue), reference samples with pure yellow pigments (Y: solid: yellow), with pure green pigment (G: solid-light green) and reference samples with pure silver additives (S: solid: dark red). The measurement was performed in two replications.

Figure 6. Light Microscopy (above row) and Scanning Electron Microscope (SEM) with x150 amplified (below row) of references low density polyethylene without adding pigments and additives (REF), high concentration of yellow and green pigments (GY2) and silver additive combination with green pigment (GS2).

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A

B

C

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Figure 6. Light Microscopy (above row) and Scanning Electron Microscope (SEM) with x150 amplified (below row) of references low density polyethylene without adding pigments and additives (REF), high concentration of yellow and green pigments (GY2) and silver additive combination with green pigment (GS2).

Paper V

Minimizing photooxidation in pasteurized milk by optimizing light transmission of green polyethylene films

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Short title: Green film preventing photooxidation in milk.

ABSTRACT

Effect of different amount of transmitted green light on photooxidation in pasteurized milk was studied. Five green films were produced with the combination of pigments and additives in order to exclude harmful wavelength for chlorophyll compounds (400-450 nm and 600-650 nm). In addition, a non-colored transparent and an orange film were compared with a selected green film. Pasteurized milk (3.9% fat) was packed in air atmosphere and exposed to light for 14, 20, 26 and 32 h at 4°C under the different films. Samples stored in dark were control samples. The results showed that the best green film has low overall light transmission, and also nearly blocked light wavelength shorter than 450 nm and wavelength longer than 600 nm, which prevented photooxidation of riboflavin and chlorophyllic compounds. The chlorophyll was a light sensitive compound in the longer wavelength regions (above 600 nm). The chlorophyll degradation had highly correlated with sensory properties (R^2= 0.80-0.94). Films with light transmittance <5-10 % below 450 nm and <5% at 600-650 nm are suggested to use in order to preserve the milk quality. The new developed green film can be used as a prototype for protection of dairy products in order to reduce the degradation of photosensitizers.

INTRODUCTION

Light is well-known for inducing chemical changes in food products and leading to formation of off-flavors and off-odors. Protection against photooxidation can be done by light blocking packaging materials. Complete blocking of all visible light, black or opaque packaging, is not always feasible because there is sometimes a need to see the actual product through the packaging material. In order to design a transparent material with optimal protection properties it is important to know the photosensitive properties of food products. For dairy products, these main properties have been elucidated the last years. Based on these finding, it is possible to suggest how transparent films can be optimized for extended shelf life.

Dairy products contain several photosensitizers e.g. riboflavin, porphyrins and chlorophyllic compounds, which absorb visible light in different wavelength regions. Riboflavin absorbs at wavelengths below 500 nm, which means that it absorbs UV and blue light, whereas chlorophyll absorbs light around 420 nm and above 600 nm. Porphyrins also absorb strongly around 400-420 nm and have many small absorption peaks throughout the visible light.

The wavelengths below 500 nm have been reported to degrade riboflavin and cause quality deterioration in dairy products (Bekbolet 1990; Bosset and others 1994; Mortensen and others 2004). Josephson (1946) reported that the wavelengths below 500 nm degraded riboflavin the most, but that longer visible wavelengths (590-630 nm) induced the formation of sunlight flavor in milk. Recently, Airado and others (2011) showed that milk exposed to wavelengths longer than 575 nm induced significantly higher amount of off-flavors than wavelengths shorter than 500 nm. Thus, excluding the ultra violet (UV) and blue light below

500 nm does not avoid the oxidation (Webster and others 2009, 2011; Intawiwat and others 2010, 2011; Airado and others 2011).

Photosensitizers absorbing light in the red regions do most likely contribute to initiate the photooxidation. Wold and others (2005, 2006a) showed that photosensitation of chlorophylls and porphyrins contributes to produce off-odors and off-flavors in dairy products exposed to wavelengths longer than 500 nm. Furthermore, the photodegradation of chlorophyll correlated well the formation of sensory off-flavors and off-odors (Wold and others, 2005; Intawiwat and others, 2010, 2011; Airado and others, 2011).

Using packaging material with light barrier is one alternative to protect against photooxidation. Transparent packaging materials with different transmission properties, and sometimes together with pigment have already been applied in the market. Traditionally, colored packaging materials are used mostly for design and to promote sales, and not specific for protecting food products. Thus, most packaging materials in the market do not block specific visible wavelengths, for instance those harmful to chlorophyll compounds.

In many studies, green light has been shown to give the least severe effect on photooxidation in dairy products (Hansen and others 1976; Cladman and others 1998; Wold and others 2005, 2006b; Intawiwat and others 2010). The green light is the regions that is least absorbed by photosensitizers in dairy products and green film is likely to give longer shelf life compared to blue or red films.

The purpose of this study was to investigate the effect of different green films on photooxidation in pasteurized milk. Because they have different optical properties, they might offer different degree of protection. We also compared the performance of the best green film with a transparent non-colored film transmitting white light, and an orange film. The evaluation of the films was performed by sensory analysis of the milk after light exposure. Fluorescence spectroscopy was used to measure the degradation of photosensitizers. Films

used in this study were produced with different concentration and combination of pigments and additives in order to provide the different light transmission profiles in specific wavelength regions.

MATERIAL AND METHODS

Milk samples

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 came from the same batch to obtain a fairly homogeneous set of samples. All milk was stored at 4°C in darkness before sample preparation.

Packaging method and sample preparation

Milk from the cartons was blended to obtain a homogenous set of samples. 230-mL milk aliquots, measured with sterilized gradual flasks, were placed in white, high-density polyethylene (HDPE) trays (5.3 × 9.2 × 9.2 cm; Promens AS, Kristiansand, Norway). Two of HDPE trays were packed in black amorphous polyethylene terephthalate (A-PET)/PE thermoformed trays. A-PET/PE sheets were manufactured by Wipak (Nastola, Finland) thermoformed by Jihå Plast AB (Karlskoga, Sweden). The thermoformed trays (14.5 x 20.5 x 7.5 cm) were sealed with a top web consisting of PET/PE/EVOH/PE (Oxygen transmission rate (OTR) < 5 cc.m^{-2} 24 hr 1 atm; Wipak) by using a tray-sealing machine (DYNO model 511 VG, Promens AS, Kristiansand, Norway). The samples were stored in air atmosphere.

Packaging material

Seven different films were used in this study as shown in Table 1. Five transparent green LDPE blown films were produced by Norner Innovation AS (Stathelle, Norway) with different light transmission profiles. The films were produced with the different concentration and the combination of pigments, silver additive and optical brightener. Additives and pigments have been selected according to their specific absorption in order to block the desired wavelengths areas 400-450 and 600-660 nm. Low density polyethylene (LDPE) commercial grade, (FT5236, Borealis, Austria) was used as polymer base. In addition, orange and transparent non-colored films were evaluated. The transparent film (PET/PE/ethylene vinyl alcohol/PE (PET/PE/EVOH/PE) was obtained from Wipak (Nastola, Finland). Orange transparent film based on PET was manufactured by Ciba (Ciba Specialty Inc., Basel, Switzerland). To compare with these films, the best green film was selected based on giving the best protection against photooxidation.

Light transmission properties of these films are shown in Figure 1. The spectral regions of the films were defined by where the transmission was above 10%. The total light transmittance through each film was calculated by integrating the area under light transmission spectra from 300-700 nm (Figure 2).

Experimental Design

Five green films were used to investigate the effect of different light transmission properties of green films on photooxidation. After that, the best green film was compared to one non-colored transparent film and one orange film.

Samples were stored under air atmospheres and exposed to light for four different exposure times (14, 20, 26 and 32 h) at 4°C. The samples were analyzed immediately after ending light exposure. Sensory analysis was used to measure the formation of off-flavors and

off-odors, while fluorescence spectroscopy was used to study the degradation of photosensitizers. Two parallels were used. In addition to samples exposed to light, two samples were stored in the dark as reference samples. Milk samples are named according to codes in Table 1.

Light Exposure

The milk samples were placed on the floor in a refrigerated room (3-4ºC) under two broadband 575 W metal halide lamps (Osram HMI 575W/SE, Munchen, Germany). The light intensity was measured by a calibrated spectrometer (Apogee Spectroradiometer, Apogee Instruments Inc., Logan, UT, USA) for the integrated wavelength region 300-700 nm as described by Wold and others (2005).

The intensity of light was measured on top of the films and adjusted to 2.0 ± 0.1 W/m^2 for every sample by the use of grey filters. The intensity of light was measured in the cooling room in which the experiment was performed.

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 (1985) standard 6564. The sensory panel consisted of 10 assessors. The details of the sensory analysis methodology are presented in Intawiwat and others (2010). Prior to the analysis, the panel was trained in order to agree on the definition and intensities of each of the attributes. The training session used two different milk samples with varying sensory properties (one was a dark stored sample, another was light-exposed for 32 hour under film GY1 in first experiment and under transparent non-colored film in the second experiment).

Six attributes were selected; sour, rancid and sunlight odors and flavors. Sour odor and flavor refer to the attributes sour and sweet. A high intensity of these attributes indicates freshness. Sunlight odor and flavor, which are related to oxidation of proteins, rancid odor and flavour, including all odors and flavors associated with rancidity (grass, hay, candle, and paint) as described in ISO (2009) standard 22935-2. 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). The different samples were served twice as replicates where the serving order was randomized with respect to sample, assessor and replicate. Unsalted crackers and lukewarm water were available for rinsing the palate between samples.

Intensity of odors and flavors were evaluated and graded on a continuous non-structured scale ranging from the lowest intensity of each attribute (value 1.0) to the highest intensity (value 9.0). The samples were evaluated in all attributes by each assessor. The sensory intensities for each sample were obtained by averaging the individual intensities from the 10 assessors. The evaluation was carried out and data recorded by a computerized system (CSA, Compusense, Version 4.6, Compusense Inc., Guelph, ON, Canada).

In the study effect of the five different green films, 42 samples were evaluated ((4 light exposure times × 5 films) x 2 parallels + 2 dark stored samples). The sensory measurements therefore had to be carried out over 2 days due to capacity limitation. Samples were randomized over the 2 days in order to avoid block effects. To study the effect of the non-colored transparent, orange and green films, 26 samples were evaluated ((4 light exposure times × 3 films) x 2 parallels + 2 dark stored samples). The sensory measurements were done within one day.

Fluorescence Spectroscopy

Milk (20 mL) from each sample was poured into sample cuvettes that exposed a flat, circular surface with a diameter of 5 cm for the measurements. Fluorescence emission spectra were measured on the milk surface. The front face fluorescence set up is described in detail by Wold and others (2005). The fluorescence emission spectra were measured for excitation at 410 nm (Oriel 59285) and using cut-off filters at 475 nm (Melles Griot 03FCG065, Melles Griot Inc.). Excitation at 410 nm excitation was used to maximize fluorescence for the tetrapyrroles (Wold and others 2002, 2005). Moreover, excitation at 410 nm can be used to measure riboflavin due to riboflavin has excitation maxima at 370 and 450 nm.

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 temperature of the samples was 4°C. Samples were measured twice. Samples were rotated 90° between each measurement in order to average out possible non-homogenous field of illumination. The spectrograph and detector were controlled by the software WinSpec (version 1.4.3.4, Roper Scientific).

To improve interpretation of spectral response for protoporphyrin IX and chlorophyll compounds, the large background signal from riboflavin was removed from the fluorescence emission spectra. This was performed by polynomial fitting, an iterative routine originally introduced to remove background fluorescence from Raman spectra (Lieber and Mahadevan-Jansen, 2003). The final polynomial fit is subtracted from the original spectrum. In the present study, a polynomial degree of 5 was chosen and an iteration number of 50 were used for the fitting procedure. The correction was only applied to the part of spectrum between 575-750 nm. The procedure was written in Matlab code (The MathWorks, Inc., Natick, MA, USA).

Data analysis

Partial least-squares regression (PLSR) was used to find correlations between fluorescence spectra and sensory assessed attributes. Full cross-validation was used to determine the optimal number of PLS factors. The PLSR was carried out by using The Unscrambler ver. 7.5 (Camo AS, Oslo, Norway).

Significance testing of the sensory analysis was carried out within each treatment using a general linear model (GLM) using SAS 9.1.3 (SAS Institute Inc, Cary, NC, USA) to establish significant differences followed by Tukey's honestly significantly different (HSD) test.

RESULTS AND DISCUSSIONS

Sensory analysis

The effect of the different green films is shown by average sensory scores for sour, sunlight and rancid flavors (Figure 3). Large variation in flavour scores were observed compared to odor scores. This might be because the taste of samples gave more intense perception than smell. Thus, the flavors are used for presenting the sensory results. Sour refers to freshness of milk samples and it is a positive attribute, whereas sunlight and rancid are negative attributes.

Significant difference in sour flavor at 14 h was only found between sample stored in dark and film with low concentration of green and yellow pigment (GY1) (Figure 3A). At 20 h, samples GY1, low concentration of green and yellow pigment and optical brightener (GY1+OB) and low concentration of green pigment and silver (GS1) also had significant lower sour scores than dark storage sample. At 26 h all exposed samples were significantly

different from dark sample. However, after light exposure to 32 h, sample with high concentration of green and yellow pigment (GY2) was again not significantly different from dark.

Figure 3B shows the results for sunlight flavor. Milk stored under film GY1, GY1+OB and GS1 had significantly higher level of sunlight flavor compared to the reference at light exposure 20 to 32 h. These films also had the highest percent transmitted light with around 30 % (Figure 2). Next was the film GS2+OB, (20%) and last the film GY2 (15%). These results showed that light transmittance in green wavelength regions of 30% were enough to initiate the photo reaction in milk samples at 20 h light exposure time.

Significant difference in rancid flavor between dark storage and light exposure samples was only found in sample GY1 from 20 h to 32 h (Figure 3C). No significant difference was found between samples stored under different green films. Film GY1 transmitted the same amount of total light (30%) as GY1+OB and GS1 (Figure 2), however the light transmission profile was different. Film GS1 transmitted more light in the violet and blue wavelength regions (300-500 nm) than film GY1 and GY1+OB (Figure 1). On the other hand, film GY1 transmitted light in red regions (600-700 nm) three times higher than film GS1. The fact that sample GY1 had higher off-flavor scores than sample GS1, suggests that orange and red light causes more photooxidation than blue light. This corresponds with Airado and others (2011) and Intawiwat and others (2011).

Overall, the samples GY2 and GS2+OB had less off-flavors compared to the other samples after 20 h light exposure. Meanwhile, sample GY1 had significantly higher sunlight flavor at 14 h and rancid flavor at 20 h compared to sample stored in dark. An important reason that film GY2 gave better protection against off-flavors was because the total transmitted light of this film was the lowest (Figure 2). Film GS2+OB transmitted light below 5 % in both blue and red regions. These results indicate that reducing transmitted light

in both wavelengths regions (blue and red light regions) provide good protection against light induced oxidation in milk.

The results for non-colored transparent, orange and green films was presented by average sensory scores in three attributes; sour, sunlight and rancid flavors (Figure 4). The film GS2+OB was selected to compare with the non-colored transparent (T) film and the orange (O) film. Significant difference was found between samples under non-colored transparent film and GS2+OB after light exposure at 14 to 26 h (Figure 4A). Additionally, at 26 h light exposure, significant difference was also found between samples under orange film and GS2+OB. Sample GS2+OB had significantly less sunlight flavor compared to transparent film at 14 to 26 h of exposure (Figure 4B). Difference between sample GS2+OB and orange film was only found at 26 h. However, no differences were observed in sour flavor and sunlight flavor between the samples at 32 h. Figure 4C shows the rancid flavor score after exposure to different colored light at different exposure time. Samples GS2+OB had significant lower intensity of rancid flavor than transparent and orange at 20 and 26 h. At 32 h, the only significant difference was between GS2+OB and transparent.

The new developed film, GS2+OB, gave overall significantly less off-flavors until 26 h of exposure compared to samples stored under transparent and orange films. Similar results have been presented for green filter compared with other colors before (Intawiwat and others 2010). No significant differences in off-flavor were found between exposure to light under transparent and orange films, although the transparent film had a much higher total light transmittance (85 %) compared to the orange film (35%) (Figure 2). Airado and others (2011) showed that in milk samples exposed to orange light of only half of the total intensity compared to white light, the formation of off-favors was at the same level for the two exposure conditions.

Fluorescence spectroscopy

Fluorescence spectroscopy has been used to monitor the amount of photosensitizer compounds and their degradation after light exposure in dairy products (Wold and others 2005). The photooxidative processes in the product starts when the photosensitizers are photobleached. The decrease of fluorescence intensity of the sensitizers can therefore be used as marker for photo-oxidation measured by sensory analysis (Wold and others 2005). A detailed study of how photosensitizers are degraded under different exposure conditions can be used to understand and interpret how the light affects the products.

The degradation of riboflavin is not presented in this study because the main interest was to investigate the effect on chlorophyll compounds. In addition, all green films transmitted light mostly in green wavelength regions and less in blue regions, thus little effect was observed on the degradation of riboflavin.

The fluorescence emission spectra of protoporphyrin IX and chlorophyll are presented for samples exposed to light for 32 h (Figure 5). The peak at 635 is generated by protoporphyrin IX (Juzenas and others 2001; Wold and others 2006a). The broad peak at 661-680 originates from chlorophyll residues (Merzlyak and others 1996) and has previously been observed in pasteurized milk (Intawiwat and others 2010; Airado and others 2011).

The degradation of protoporphyrin IX and chlorophyll compounds is closely correlated to the duration of light exposure while dark storage prevented photo bleaching of chlorophyll compounds. Comparing samples exposed to light, sample GS2+OB had the highest concentration of chlorophyll followed by GY2, GS1, and GY1 $\simeq$ GY1+OB. These results were in accordance with sensory results where sample GS2+OB and GY2 had lower off-flavors scores compare to other samples.

Our results showed that film GS2+OB gave better protection of chlorophyll than GY1, although film GS2+OB transmitted more light than film GY1 in region 400-500 nm (Figure

1). However, the film GS2+OB had the lowest light transmittance in 600-700 nm, whereas film GY1 had the highest light transmittance. The results indicated that exposure to longer wavelengths (above 600 nm) is more harmful the chlorophyll compounds than shorter wavelengths.

Airado and others (2011) suggested two reasons for why chlorophyll is more degraded by longer wavelength (red light) than shorter wavelength (blue light). First, longer wavelengths in visible regions penetrate deeper into products matrix than the shorter wavelengths. Intawiwat and others (2011) recently reported that red light penetrated and degraded protoporphyrin and chlorophyll compounds deeper into the cheese than did blue light. Second, most of the blue light is absorbed by ß-carotene and riboflavin, so a smaller source of the light would be absorbed by chlorophyll compounds. In the red light regions, however, chlorophyll is the main absorption compound. This explains why chlorophyll can be the active compound at the longer wavelength.

The estimated relative concentrations of chlorophyll and protoporphyrin in milk stored under different films and different exposure times are presented in Figure 6. These relative concentrations are obtained from the fluorescence emission peaks of chlorophyll at 676 nm and protoporphyrin IX at 643 nm. The concentration of chlorophyll was stable in samples stored in the dark, while it decreased in samples exposed to light (Figure 6A). Chlorophyll compounds were less degraded in samples GY2 and GS2+OB, and the most degraded in sample GY1+OB. Similar results were observed for protoporphyrin (Figure 6B). This was because film GY1+OB transmitted 25% light, but films GS2+OB and GY2 transmitted less than 5% and 10% light respectively at 600-650 nm (Figure 1). The results showed that an increase in light transmittance of 15-20 % in red regions was efficient enough to degrade chlorophyll.

The fluorescence emission spectra of pasteurized milk exposed to white, orange and green light for 32 h are shown in Figure 7. Chlorophyll in samples stored under orange was the most degraded, less under non-colored transparent, and the least under GS2+OB.

The degradation of protoporphyrin and chlorophyll compounds showed high correlation with the sensory properties (R^2= 0.80-0.94). The correlation between the peak of protoporphyrin and chlorophyll compound in regions (620-750 nm) and sunlight and rancid flavor were 0.85 and 0.90. Similar results have been reported for milk (Intawiwat and others 2010; Airado and others 2011), butter (Wold and others 2006a) and cheese (Intawiwat and others 2011).

CONCLUSION

The effect of different light transmission properties of green films on the photooxidation in pasteurized milk has been studied. Blocking or minimizing transmission of wavelengths below 450 nm and above 600-650 nm increases stability towards photooxidation. The main absorption bands of chlorophyll and riboflavin are then protected.

The chlorophyllic compounds in milk are apparently responsible for photooxidation induced by the longer wavelengths above 600 nm. The fluorescence from this compound was a good indicator for sensory off-flavors.

The specially designed green film was able to reduce photooxidation of the pasteurized milk compared to non-colored transparent and orange films. Blocking specific harmful wavelength regions by tailor-made packaging can be a relevant approach to minimize photooxidation with low cost films. The approach can also be used for other light sensitive products. However, it is then important to know the photosensitive properties of the products.

ACKNOWLEDGMENT

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Table 1. The description of the seven different films and different combination of pigments and additives concentration used in the experiment.

No.	Film code[1]	Microlen Yellow GR-MC [2]	Eupolen Green K87-3001[2]	Hitex-OB[3]	STSS-10 [4]
1	GY1	1,0 %	0,8%		
2	GY2	3,0%	1,5%		
3	GY1+OB	1,0%	0,8%	0,005%	
4	GS1		0,8%		0,1%
5	GS2+OB		1,5%	0,01%	0,2%
6	T[5]				
7	O[6]				

[1] The film code is named according to different pigments and additives used

    G = Green pigment, Y = Yellow pigment, OB = Optical brightener, S = Silver additive

    T=Transparent non-colored film and O=Transparent orange colored film

[2]BASF, Ludwigshafen, Germany.

[3]HPL Additives Limited, New Delhi, India

[4]Stabilization Technologies LLC, USA

[5]Wipak, Nastola, Finland

[6]Ciba Specialty Inc., Basel, Switzerland
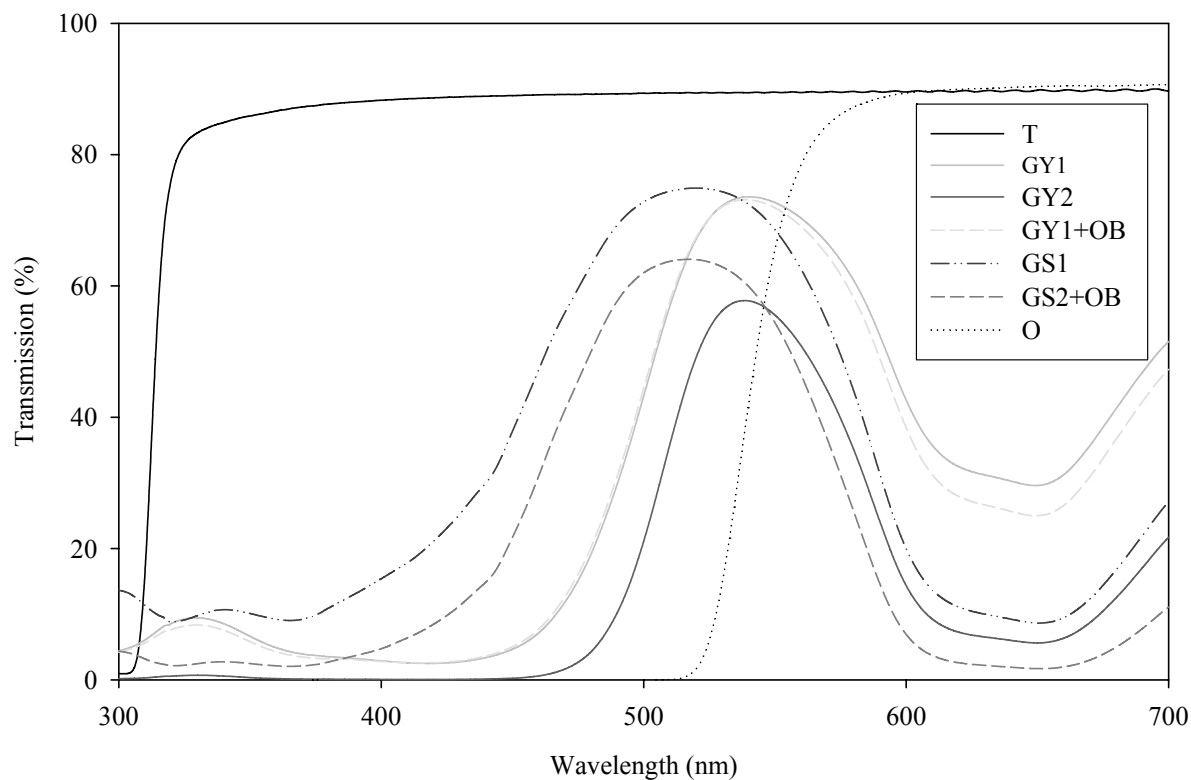
Figure 1. The light transmission (percent transmitted light per wavelength) in the region 300-700 nm through the seven different films used in the experiment (see Table 1). GY1: film with low concentration of green and yellow pigment, GY1+OB: film with low concentration of green and yellow pigment and optical brightener, GY2: film with high concentration of green and yellow pigment, GS1: film with low concentration of green pigment and silver additive, GS2+OB: film with high concentration of green pigment, silver additive and optical brightener in addition to non-colored transparent film (T) and orange film (O).
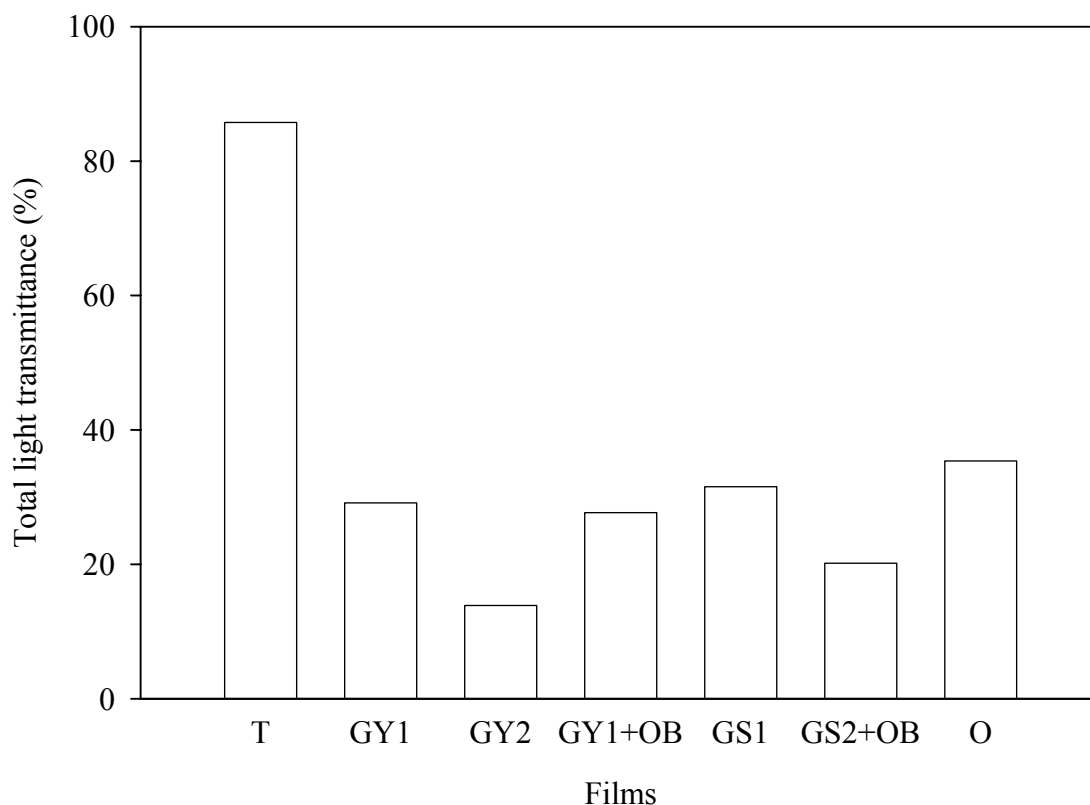
Figure 2. The total transmitted light (%T) of the seven different films used in the experiment. The intensity was calculated by the integrating the area under light transmission spectra curve between 300-700 nm for each film. GY1: film with low concentration of green and yellow pigment, GY1+OB: film with low concentration of green and yellow pigment and optical brightener, GY2: film with high concentration of green and yellow pigment, GS1: film with low concentration of green pigment and silver additive, GS2+OB: film with high concentrat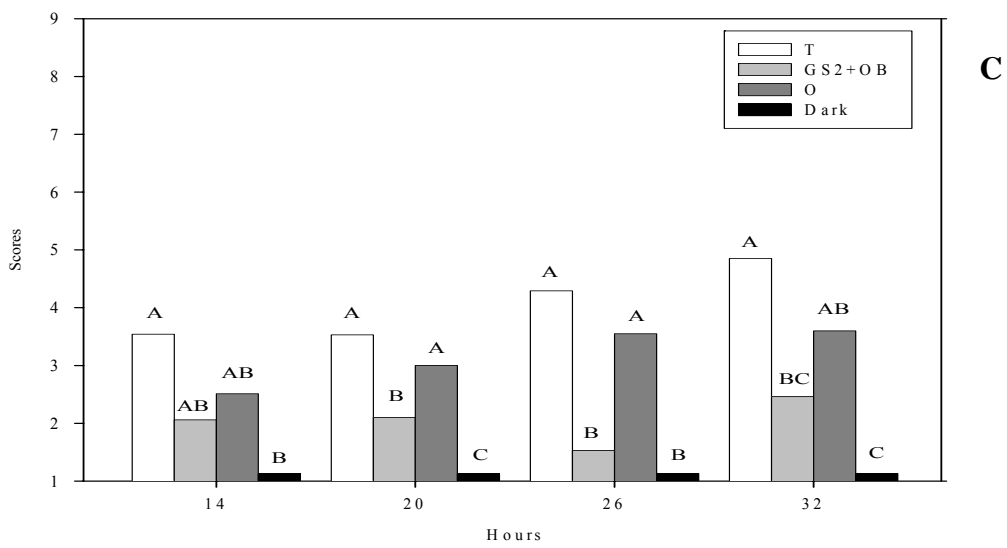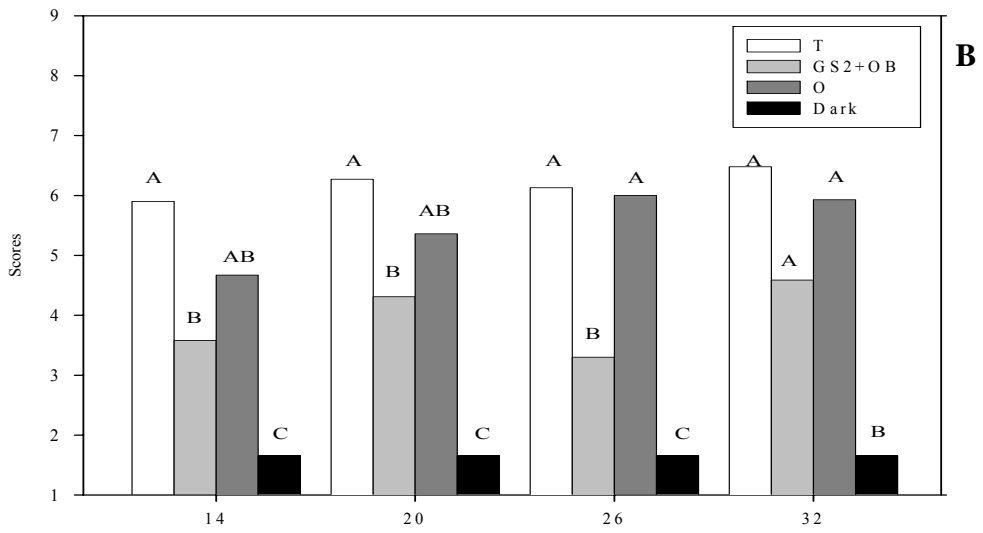i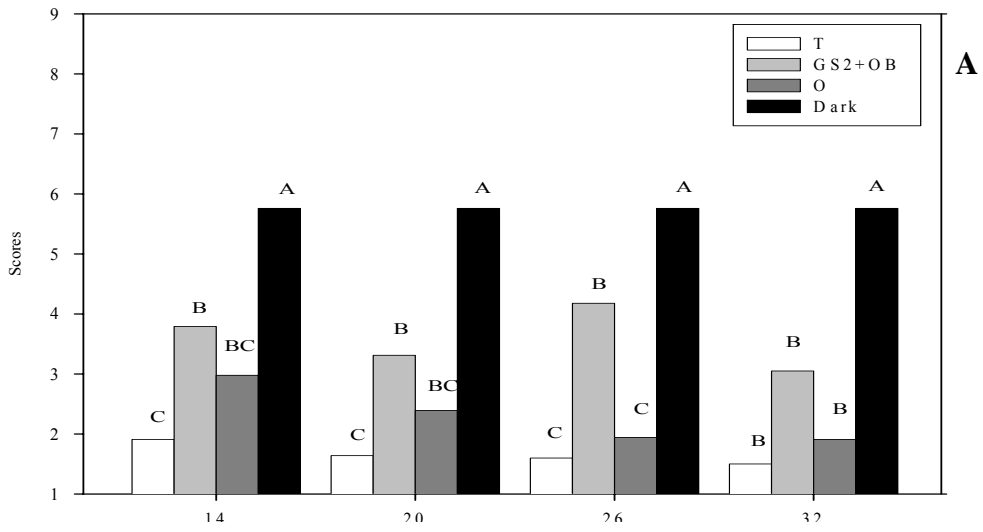on of green pigment, silver additive and optical brightener in addition to non-colored transparent film (T) and orange film (O).
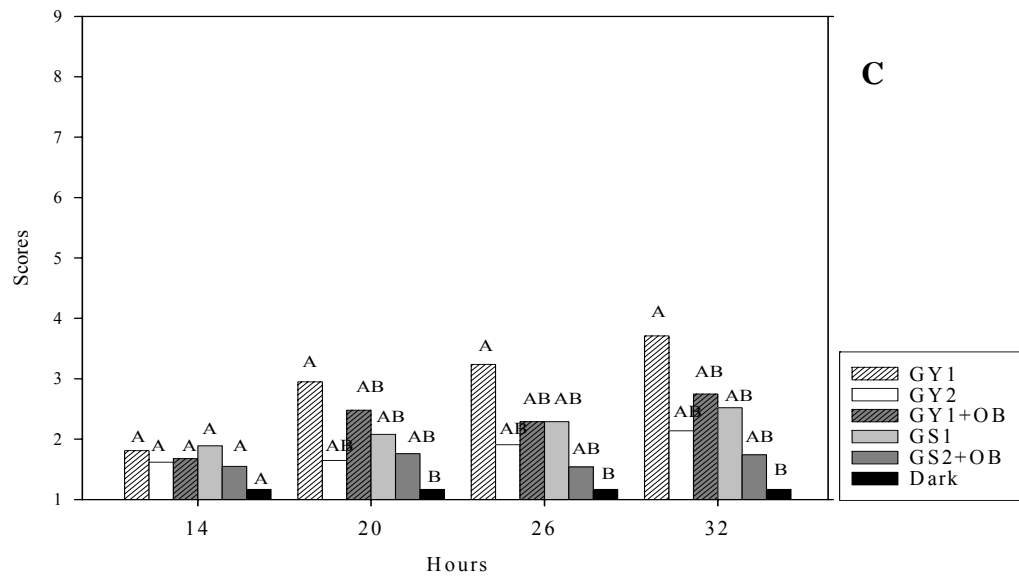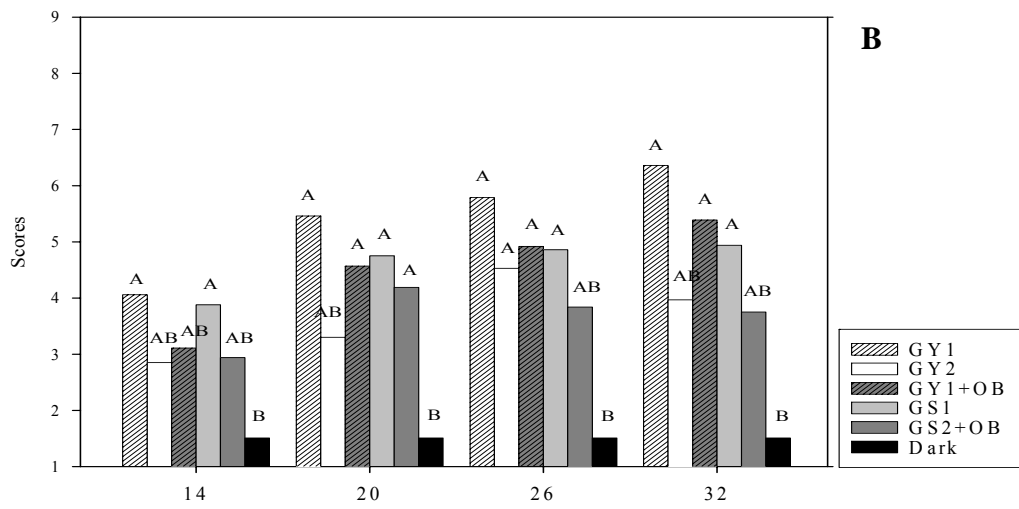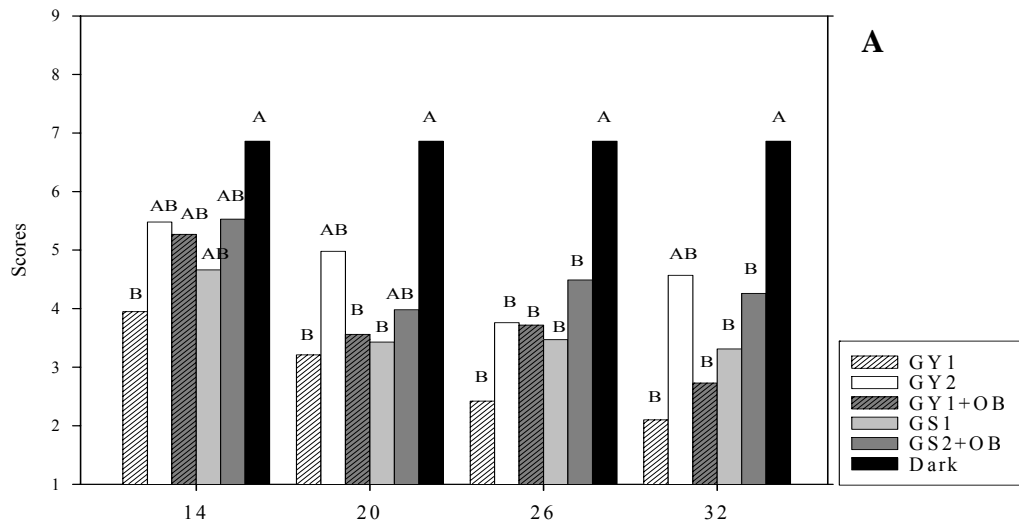
Figure 3. The mean of sensory scores for A) sour flavor, B) sunlight flavor, and C) rancid flavor of milk stored under five different green films and exposed to light at different exposure times, in addition to sample stored in dark as reference sample. Samples were only compared within the same exposure time. Samples wi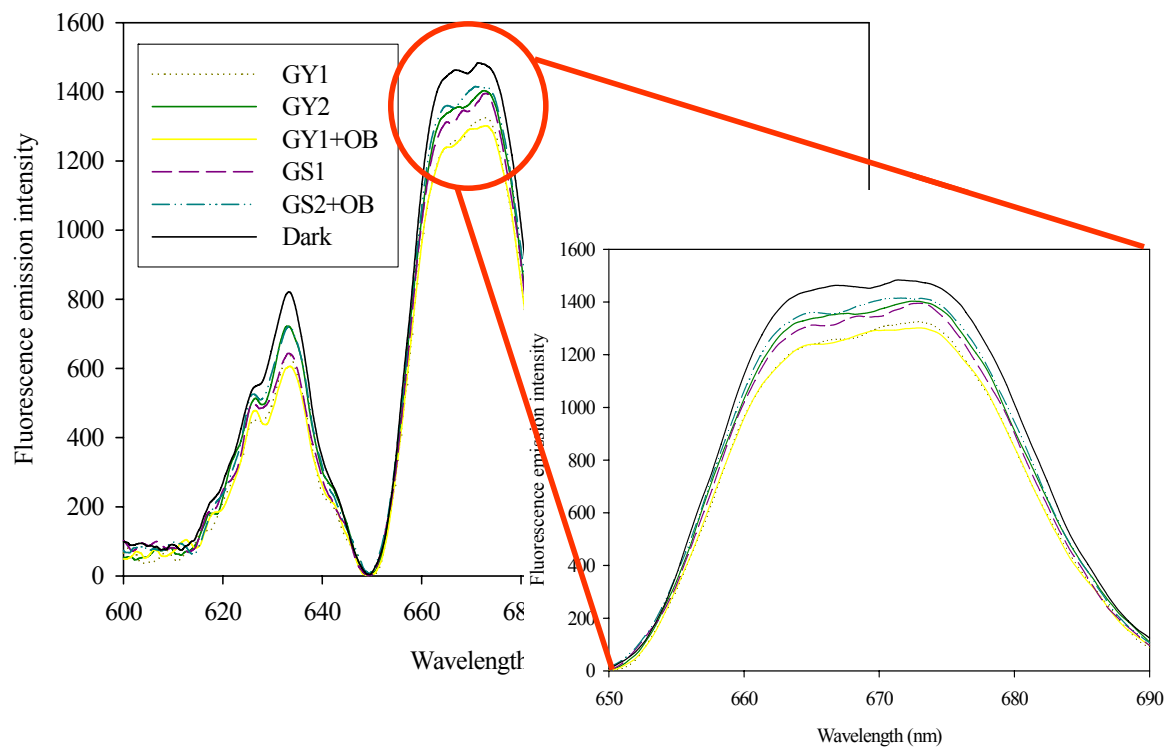th the same letter are not significantly different (P>0.05). GY1: film with low concentration of green and yellow pigment, GY1+OB: film with low concentration of green and yellow pigment and optical brightener, GY2: film with high concentration of green and yellow pigment, GS1: film with low concentration of green pigment and silver additive, GS2+OB: film with high concentration of green pigment, silver additive and optical brightener.

Figure 4. The mean of sensory scores for A) sour flavor, B) sunlight flavor, and C) rancid flavor of milk stored under three different colored films; film with high concentration of green pigment, silver additive and optical brightener (GS2+OB), non-colored transparent film (T) and orange film (O). Samples were exposed to light at different exposure times, in addition to sample stored in dark as reference sample. Samples were only compared within the same exposure time. Samples with the same letter are not significant difference (P>0.05).

Figure 5. The baseline corrected fluorescence emission spectra (λex = 410) obtained from milk stored under five different green films and exposed to light for 32 h. GY1: film with low concentration of green and yellow pigment, GY1+OB: film with low concentration of green and yellow pigment and optical brightener, GY2: film with high concentration of green and yellow pigment, GS1: film with low concentration of green pigment and silver additive, GS2+OB: film with high concentration of green pigment, silver additive and optical brightener in addition to non-colored transparent film (T) and orange film (O). The reference sample was milk stored in dark.
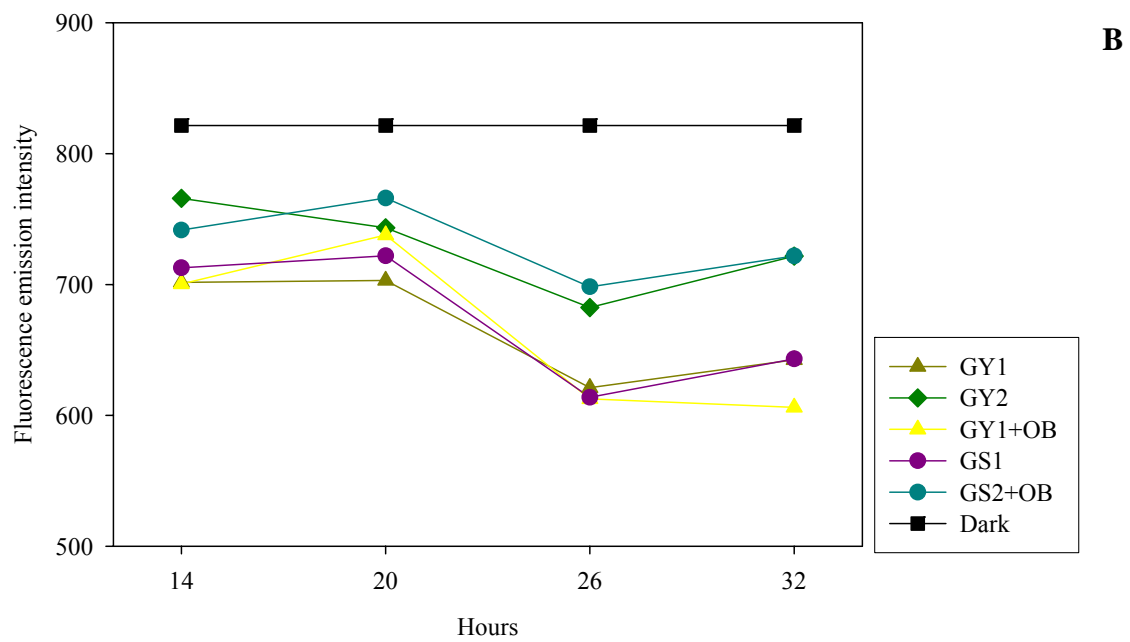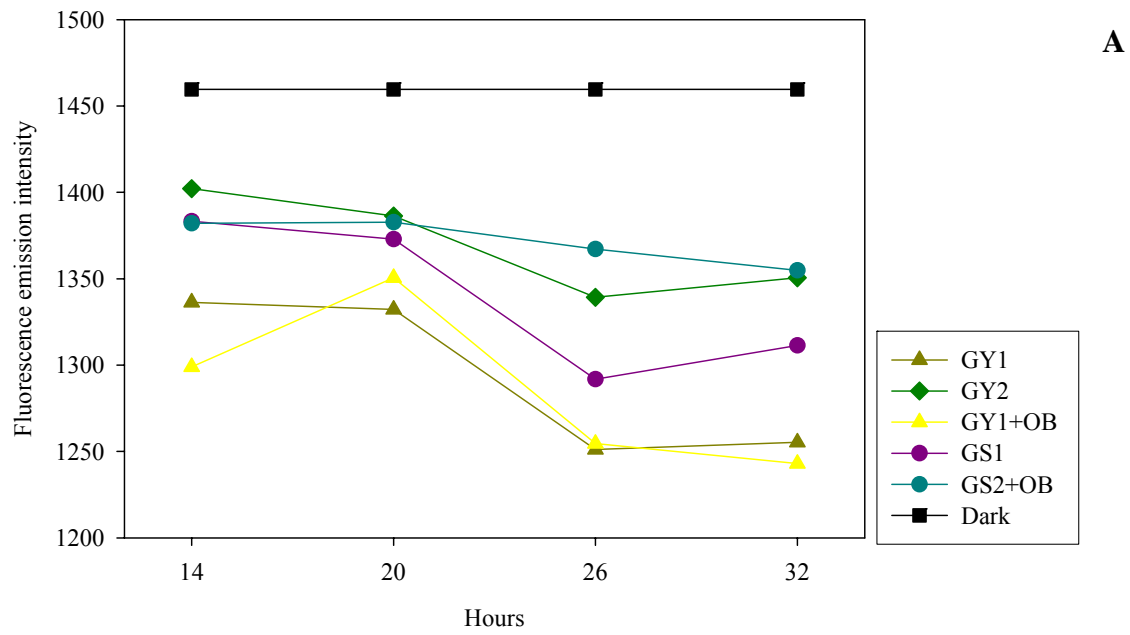
Figure 6. The relative concentration of A) chlorophyll at 676 nm B) protoporphyrin IX at 643 nm in milk stored under five different green films and exposed to different exposure times.

GY1: film with low concentration of green and yellow pigment, GY1+OB: film with low concentration of green and yellow pigment and optical brightener, GY2: film with high concentration of green and yellow pigment, GS1: film with low concentration of green pigment and silver additive, GS2+OB: film with high concentration of green pigment, silver additive and optical brightener. The reference sample was milk stored in dark.
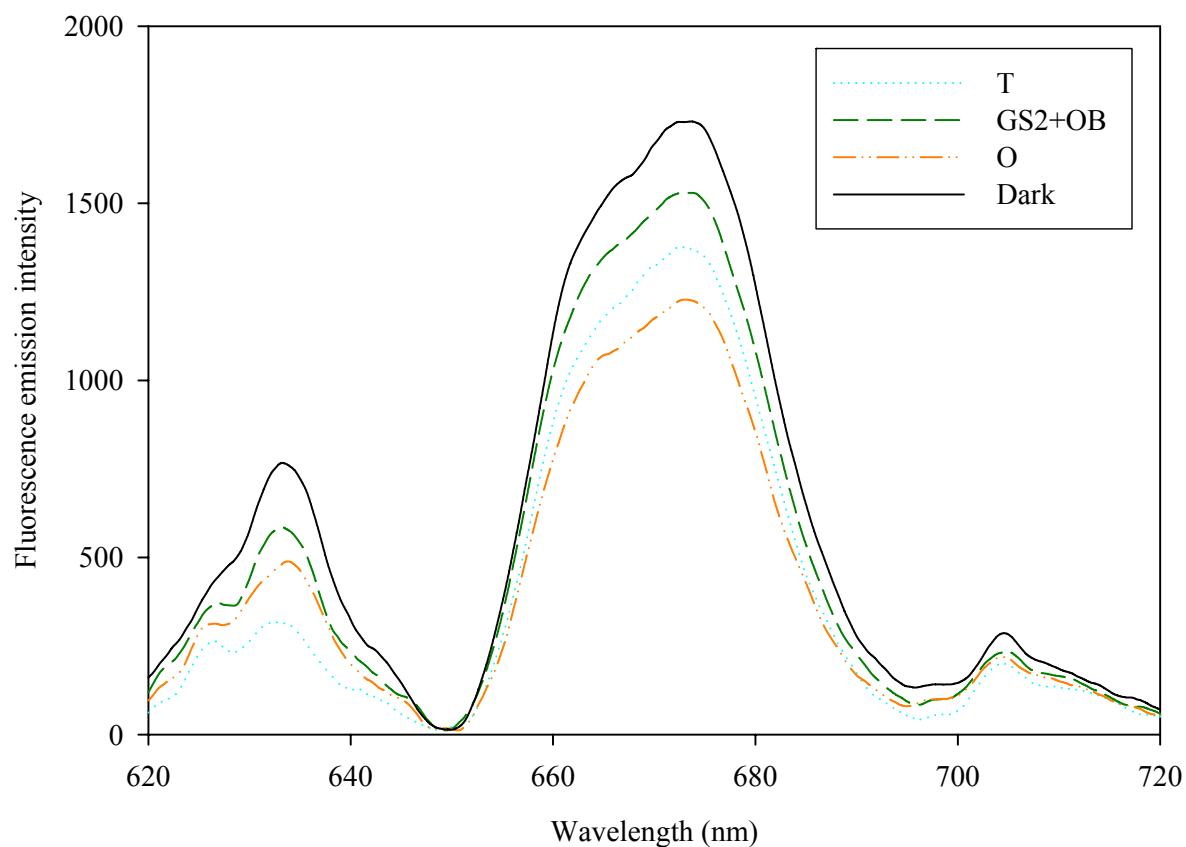
Figure 7. The base line corrected fluorescence emission spectra (λex = 410) obtained from milk stored under three different colored films; film with high concentration of green pigment, silver additive and optical brightener (GS2+OB), non-colored transparent film (T) and orange film (O). Samples were exposed to light under HMI for 32 h. The reference sample was milk stored in dark.